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The Use of Proteomic Techniques to Study the Physiology and Virulence of *Staphylococcus aureus*

by

Frances Rivera

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science
Department of Cellular Molecular Microbiology
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Abstract

Staphylococcus aureus is a bacterial pathogen that is believed to be the most common agent of human infectious disease, causing conditions ranging from common skin lesions to life-threatening illnesses. *S. aureus* has also shown a remarkable ability to develop resistance to antimicrobial treatment, making infections difficult to treat. In the post-genomic era, proteomic studies analyzing the protein complement of a genome in a particular organism at any given time, have gained real significance. This result is largely due to dynamic changes in protein expression profiles which can lead wide alterations in physiology and behavior. For proteomics, it is necessary to maximize protein concentration and to devise a method that can be easily employed and provide reproducible results. Most proteomic studies of *S. aureus* involve 2D gel electrophoresis (2-DE); however, 2-DE has many drawbacks. Proteins that are too large, hydrophobic, acidic, or basic are poorly resolved. Multi-dimensional protein identification (MudPIT) allows complex protein samples to be analyzed in solution. As yet, there has not been a study involving solely 2D liquid chromatography followed by mass spectrometric analysis in *S. aureus*; therefore we sought to catalogue the intracellular proteome and secretome of a commonly used and well-studied lab strain, SH1000. This was conducted during post-exponential and stationary phases of growth so as to understand its adaptation over time by utilizing differential protein synthesis. We found cytoplasmic proteins involved in glycolysis to be highly expressed in post-exponential phase while proteins involved in tricarboxylic acid cycle to be prevalent in stationary phase. We also found

production of *agr*-regulated secreted toxins and proteases to be upregulated in stationary phase. In addition to this we employed proteomic approaches to quantitatively profile the secretomes of leading clinical isolates of *S. aureus*, as such a study is currently lacking. These included the two most common hospital-associated *S. aureus* strains (USA100 and USA200), and the two most common community-associated *S. aureus* strains (USA300 and USA400). We found *agr*-regulated proteins are generally upregulated in CA-MRSA strains USA300 and USA400 and surface-associated proteins to be upregulated in HA-MRSA strains USA100 and USA200. This finding concurs with literature regarding transcriptomic studies showing a hyperactive *agr* in CA-MRSA strains compared to HA-MRSA strains.

Introduction

The Staphylococci. Staphylococcus is a genus of Gram-positive bacteria that have characteristic spherical cells (cocci) in the arrangement of grape-like clusters. Indeed the word *staphylos* is derived from Greek, meaning bunch of grapes. These bacteria are facultative anaerobes and are commonly tolerant to high concentrations of salt that normally inhibit the growth of other bacteria [52, 76]. Staphylococci include bacteria that are a part of the normal skin flora, such as *Staphylococcus epidermidis*, and others who are medically significant human pathogens, such as *Staphylococcus aureus* [13].

Staphylococcus aureus

Staphylococcus aureus is different from other staphylococci due to the rich golden pigmentation of its colonies, as denoted by its name *aureus*, which is Greek for "golden". It can be found anywhere in the environment, from soil, air, water, and sewage; as well as inhabiting the skin and mucous membranes of warm-blooded animals. It is innately resistant to desiccation, and can live on inanimate objects for extended periods of time [105]. *S. aureus* is an opportunistic pathogen, meaning that it is not obligated to this lifestyle, but more commonly causes disease only upon entering the body via wounds or indwelling devices [121]. The genome of *S. aureus* commonly consists of a single, circular chromosome of approximately 2.8 million base pairs [85]. Additionally, strains of *S. aureus* may also possess mobile genetic elements such as prophages, plasmids and transposons that can be transferred between themselves and other gram-positive bacteria [162, 114].

The diseases of Staphylococcus aureus

As a major human pathogen, *S. aureus* is believed to be the most common cause of human disease [51]. It has the ability to cause a wide range of infections that are both nosocomially and community acquired. These diseases vary from common skin lesions, such as wound infections and abscesses, to life-threatening illnesses, such as septicemia and endocarditis [121]. Moreover, localized skin infections can spread from the initial site of colonization to other parts of the body via systemic dissemination. This can lead to metastatic infections at locations such as the heart muscle, joints, brain, lungs, and bones; causing endocarditis, septic arthritis, brain abscesses, pneumonia and osteomyelitis [121]. Another subset of diseases caused by *S. aureus* is toxinoses. These are caused by particular toxins, and include scalded skin syndrome (exfoliative toxins), toxic shock syndrome (toxic-shock syndrome toxin), and gastroenteritis (enterotoxins) caused by food poisoning [5]. Recently, the production of the Panton-Valentine leukocidin (PVL) toxin has been associated with fatal necrotizing pneumonia, leading to death within 36 hours [70].

Toxin production by Staphylococcus aureus

S. aureus is an adaptive and versatile pathogen that possesses a diverse arsenal of virulence factors, which it employs to cause disease in a variety of niches within the host. These virulence factors are, however, considered accessory elements as they are not crucial to survival. The virulence determinants of *S. aureus* include cytolytic toxins, several extracellular proteins [152, 91, 146], major secreted proteases [188, 117, 178], and surface-associated factors [37] that work alone or in concert to facilitate disease.

Alpha-hemolysin is perhaps the major secreted toxin, and is cytolytic in action, forming pores in the membrane of red blood cells, epithelial cells, endothelial cells, platelets, and mononuclear immune cells. Membrane damaged caused by direct action of this toxin often results in apoptosis of the target cell, and necrosis of surrounding tissue [12**,** 53**,** 19**,** 132]. In addition to alpha-hemolysin, *S. aureus* also produces at least 3 other such factors, including the β-, γ-, and δ-hemolysins. The β-hemolysin is another cytolytic toxin that induces hot-cold lysis of red blood cells [61**,** 65], and is one of the most abundant proteins secreted by *S. aureus* into the extracellular medium [450]. δ-hemolysin is a small and heat-stable protein that can be lytic towards several types of membranes, including those of red blood cells, organelles and even bacterial protoplasts [61]. The γ-hemolysin and other toxins produced by *S. aureus* (such as a number of leukocidins), contain two components, commonly termed S and F [75, 98]. In the case of PVL it has the ability to lyse only neutrophils and macrophages [158], whilst the gamma-hemolysin can also lyse red blood cells by forming a pore in their membrane [98, 182]. In addition to toxins that act directly on the membrane of host cells, *S. aureus* also produces pyrogenic toxin superantigens that include staphylococcal enterotoxins A-E and toxic shock syndrome toxin-1 (TSST-1) [17]. These superantigens stimulate T cells and cytokine activity that adversely affects the host by causing fever, shock, and immunosuppression [137]. Exfoliative toxins on the other hand, have a far more specific mode of action, causing scalded-skin syndrome via the targeted destruction of human desmoglein 1, causing blister formation and sloughing of the skin [130]. Additionally, there are a total of seven phenol-soluble modulins (PSMα1-PSMα4, PSMβ1, PSMβ2, and delta-toxin), which display leukocidal activity, and facilitate evasion of the host immune

system. The *psm* genes have been identified in all sequenced strains of *S. aureus*, however, the expression of these genes can differ significantly [194].

In addition to specific toxins, *S. aureus* also produces extracellular enzymes that serve as virulence factors by degrading host tissue, facilitating dissemination, and helping to evade the host immune system. Coagulase, for example, converts fibrinogen to fibrin [102] and is thought to create a clot around localized infections to protect the bacteria from host defenses [172]. Conversely, staphylokinase is an activator of plasminogen [32] and may serve to release bacteria from these fibrin clots to allow spreading to other sites of the body [93]. *S. aureus* also produces a series of extracellular proteases that are commonly produced as a pre-proenzyme which are enzymatically inactive, and require activation in the extracellular millieu [178]. Perhaps the most abundant of these is the SspA or V8 serine protease, which cleaves immunoglobulins, thereby inhibiting host defense mechanisms [157]. It also seems to be important for transition from adhesive to non-adhesive phenotypes via degradation of fibronectin-binding proteins, and other proteins on the surface of *S. aureus* cells [100, 126]. In addition to the V8 protease, there are many other serine protease-like enzymes (SplA through SplF) that have trypsin-like catalytic activity, but are not cleaved by other enzymes for activation. *S. aureus* also secretes two cysteine proteases, known as staphopain A and staphopain B. Staphopain A has strong activity against elastin, suggesting a role in the pathogenesis of *S. aureus* [154], whilst staphopain B (*sspB*) cleaves fibronectin, fibrinogen, and kininogen, suggesting that it may serve to allow the spread of *S. aureus* during infection [35, 178]. The final major proteolytic enzyme is a metalloprotease, known as aureolysin, which has activity against plasma protease inhibitors, suggesting a role in the pathogenesis of *S.*

aureus [154, 155]. A further protease-like enzyme, lipase, exists, which is a glycerol ester hydrolase that cleaves long-chain triacylglycerols [109], and may have a role in the *in vivo* nutrition of *S. aureus* [166].

The regulation of toxin production

The success of *S. aureus* as a pathogen is commonly attributed to its vast array of virulence determinants, which are tightly controlled by a central global regulator called *agr* (for accessory gene regulator) [4, 91**,** 144, 145, 152]. *agr* is a quorum sensing, two-component regulator that is maximally expressed during post-exponential growth, where it represses surface and attachment proteins and induces the transcription of toxins and exoproteins [202]. *agr* is of particular importance to *S. aureus*, as cells which contain a mutated or nonfunctional *agr* gene are attenuated in virulence in animal models of infection [205]. The *agr* effector molecule is a small regulatory RNA, known as RNAIII, which also encodes the delta-hemolysin. RNAIII acts as a regulator of target genes [91**,** 146] by binding to target mRNA molecules and either stabilizing them, or targeting them for destruction [135, 113, 186]. The most significant of these interactions is thought to be for a repressor of toxin production, known as Rot. RNAIII binds to, and inhibits Rot mRNA translation, thereby facilitating the upregulation of extracellular virulence factor synthesis [66, 18]. RNAIII also serves to negatively regulate the synthesis of protein A and the fibronectin-binding proteins, which are used for adhesion [91, 143, 171].

In addition to *agr* there are a number of other important regulators of toxin production in *S. aureus*, with perhaps the next most important being SarA. SarA (for staphylococcal accessory regulator) is a DNA-binding protein that acts as a transcriptional regulator of a

variety of different genes that play a role in pathogenesis and metabolic processes [9, 29, 30, 49]. Specifically, it controls the expression of surface and secreted proteins by binding to the promoter regions of target genes and either repressing them, or bringing about their upregulation. Its targets include other regulatory systems, such as *agr* [30, 160], and virulence genes, such as protein A (*spa*), fibronectin-binding protein A (*fnbA*) and the major extracellular proteases [64, 208, 16]. The final major regulator of toxin production is the two-component system SaeRS (for *S. aureus* exoprotein expression). SaeRS modulates the production of secreted proteins such as coagulase and alpha-hemolysin at the level of transcription [721]. The regulation of these exoproteins by SaeRS overlaps that of the Agr regulon [72], though it has no direct effect on the expression of *agr* or *sarA* [71].

Drug resistance in Staphylococcus aureus

In the early 1940's, penicillin was introduced for the treatment of bacterial infections; however, shortly after *S. aureus* strains appeared that were resistant to this antibiotic. [104]. Since this time, the same has proven true for almost every other antibiotic, including erythromycin, tetracycline, and streptomycin [41, 78]. Methicillin, introduced in 1960, was used as an effective treatment for infections caused by antibiotic resistant strains of *S. aureus*. This antibiotic differs from penicillin as its unique structure blocks the β-lactam ring from inactivation by β-lactamases. However, methicillin-resistant *S. aureus* (MRSA) strains were discovered shortly thereafter, in 1961 [83]. Methicillin resistance is conferred by the *mecA* gene, which is harbored on a mobile genomic island known as the staphylococcal cassette chromosome *mec* (SCC*mec*) [89]. Given the widespread distribution of MRSA strains, and limited therapeutic options, glycopeptide

antibiotics, such as vancomycin (Van), have been commonly used as a last resort drug [84]. It acts by irreversibly binding to D-ala-D-ala residues of N-acetylmuramic acid pentapeptide precursors. Binding of these precursors by Van prevents peptide cross-linking between growing layers of peptidoglycan, and consequently weakens the cell wall of gram-positive bacteria [8]. Expectedly, in 1996, a *S. aureus* isolate in Japan was found to be resistant to increased concentrations of Van [84]. The new strain was termed Van intermediate *S. aureus* (VISA), and cases of VISA soon began making an appearance throughout Europe, Asia, and the USA; and are now present worldwide [153]. Since 2002, nine cases of Van resistant *Staphylococcus aureus* (VRSA) have appeared in the United States [2, 3, 59, 196]. The difference between VISA and VRSA strains can be found at the molecular level, with VISA isolates possessing mutations in existing genes, while VRSA have acquired exogenous genetic material. The intermediary resistance of VISA strains to Van is speculated to be modulated by synthesis of a thicker cell wall that contains an increased number of D-ala-D-ala residues, which trap Van molecules in the outer layers of the cell wall, keeping it from accessing cell wall precursor targets in the cytoplasm [79]. Van resistance in VRSA isolates on the other hand, is attributed to expression of *vanA*, found on a conjugative plasmid that is likely obtained from Van resistant *Enterococcus faecalis* (VRE) by horizontal gene transfer [196]. The Van resistance of VRSA strains is achieved by changing the D-ala-D-ala pentapeptide residues attached to N-acetylmuramic acid to D-ala-D-lac. Van, having a lower affinity for D-ala-D-lac, consequently does not inhibit synthesis of the altered cell wall in VRSA isolates [122].

Trends in S. aureus infections

S. aureus infections have commonly been confined to health care facilities [43]. Historically, such infections affect the immunocompromised, the young or the very old. These hospital-acquired methicillin resistant *S. aureus* (HA-MRSA) strains are highly-resistant to antibiotics, making HA-MRSA infections very difficult to treat. The two most common HA-MRSA strains in the United States are CDC PFGE (pulse-field gel-electrophoresis) types USA100 and USA200 [127]. Recently, community-acquired methicillin resistant *S. aureus* (CA-MRSA) infections have been reported in individuals with no ties to health care facilities [1, 94, 138]. These CA-MRSA strains appear to be far more virulent than HA-MRSA, and are especially significant because they cause infections in young, healthy individuals with no predisposing factors [24, 54]. There are a number of genetic differences between HA-MRSA and CA-MRSA which might explain the vast differences in their transmission, spread and pathogenesis. Specifically, CA-MRSA strains usually possess SCC*mec* types IV, V, or VII [194], whilst HA-MRSA strains commonly harbor SCC*mec* types I-III. There are currently a total of seven SCC*mec* variations, with the common CA-MRSA types being the smallest, probably facilitating the expedient transfer of these elements between *S. aureus* strains [164]. HA-MRSA strains usually possess the larger SCC*mec* types I, II, and III, which are more burdensome energetically, and result in the slower growth of encoding strains [147, 162]. Another distinguishable feature of CA-MRSA is the presence of the bacteriophage-encoded Panton-Valentine leukocidin toxin (PVL) [193]. PVL is an exotoxin that creates pores in the membrane of leukocytes, effectively destroying them, and exposing surrounding cells to their damaging contents, commonly leading to tissue necrosis [36,

189]. Recent work has suggested that PVL is not the only toxin involved in CA-MRSA hypervirulence, as PVL-negative and PVL-positive CA-MRSA strains have been found to be equally virulent in sepsis and abscess models of infection using mice [193]. Supporting this finding, Wardenberg et al. recently demonstrated that hyperexpression of α-hemolysin, not PVL, is the essential factor in necrotizing pneumonia infections [20]. Further to this, a study by Diep et al., showed that CA-MRSA USA300 strains contain another mobile genetic element known as the arginine catabolic mobile element (ACME). This locus has been suggested to contribute to the rapid growth and survival of USA300, giving it a selective advantage in disease causation [116]. Transcriptome studies have demonstrated that despite sharing a core genome of approximately 82%, *S. aureus* strains demonstrate huge variations in gene expression profiles and pathogenesis [116, 120]. Thus the virulence of *S. aureus* is likely not determined by the presence or absence of variable genetic elements, but by alterations in the temporal expression of innate core factors. A key example of this is that the marked hypervirulence of CA-MRSA strains appears to be attributable to differential gene expression resulting from elevated activity of regulators such as *agr*. This regulator mainly controls the expression of secreted toxins and surface-associated proteins that help the organism adhere to the host and become invasive. Despite their increased virulence however, CA-MRSA strains currently have reduced antibiotic resistance compared to HA-MRSA. With that said, CA-MRSA resistance is on the rise, and these strains have recently been reported to be replacing HA-MRSA strain in healthcare facilities [195].

Proteomics. Experimentally, genomics is the study of entire genomes, whilst transcriptomics is the study of mRNA transcription, giving insights into the gene

expression of a particular organism at a given time. Proteomics, the identification of entire protein sets present in biological samples, complements these approaches, and is a relatively recent development in the post-genomic era. Because mRNA levels do not always correlate with the synthesis of proteins, proteomics has become an extremely useful tool for the study of differential protein expression as a result of varying physiological conditions. This discipline has gained significance because proteomes are highly dynamic, and protein expression profiles can change relatively quickly. Understanding changes in a given proteome from a particular organism can provide much insight into its behavior, physiology and interaction with its environment.

Mass spectrometry. A major tool used in proteomic studies is mass spectrometry. This technique uses protein samples cleaved by a site-specific enzyme, such as trypsin, for the identification of proteins present in a biological sample. Following digestion, samples are washed of salts and detergents, which can interfere with the formation of ions in the mass spectrometer, and therefore affect the determination of molecular mass [87]. After de-salting, peptides are fractionated by high-performance liquid chromatography (HPLC) using a strong cation exchange (SCX) column. Each resulting fraction is then ionized by MALDI (matrix assisted laser desorption ionization) or ESI (electrospray ionization), coupled to a mass spectrometer. A MALDI mass spectrometer utilizes a laser to produce ions from a sample that has been mixed with a matrix and crystallized on a sample plate. The most common matrices used for MALDI-TOF peptide analysis include 2,5-dehydroxybenzoic acid (DHB) and α-cyano-4-hydroxycinnamic acid [174, 136]. The sample plate is then placed inside the mass spectrometer in a vacuum at high voltage, and the matrix absorbs laser radiation and rapidly breaks down. This causes the matrix to

expand into gas phase, bringing analyte molecules with it, and the matrix dissociates, leaving a pure and ionized analyte [44]. Mass analysis of the peptides by time-of-flight (TOF) entails accelerating ions, resulting in different ion velocities that are inversely proportional to the square root of the mass-to-charge ratio. These ions are separated in the flight tube and the time of their arrival to the detector is then converted to the mass of the ions [34, 55]. An ESI mass spectrometer involves the spraying of samples from a needle at high potential, into a chamber at atmospheric pressure. Droplets of the sample in solution are formed, and the solvent is evaporated, creating a stream of ions from the sample to be analyzed by the mass spectrometer [55, 56]. Electrospray ionization was introduced in the 1980s as a new method for ionizing and introducing peptides into gas phase, and spraying peptides across a potential difference [43] between a capillary and the opening of a mass spectrometer [56]. Once in the gas phase, peptide fragments enter a collision chamber and are collided with atoms of inert gas, such as argon, producing collision-induced dissociation [197]. As a result, peptides are mainly fragmented at amide linkages, and the resulting fragments are sent to a mass analyzer to detect the fragments according to mass-to-charge ratio. The resulting mass spectrum is made of ions that are characteristic of a sequence of amino acids in a particular peptide. The mass spectral data from these peptides is then compared to protein sequences contained in databases specific to the originating organism [43, 174, 204].

Proteome analysis techniques. In order to generate proteome samples for mass spectrometric analysis, two major approaches have been used. The first, and perhaps most common of these, is two-dimensional gel electrophoresis (2-DE). Using this technique, proteins are first separated by differences in electric charge (isoelectric

focusing), and then perpendicularly by SDS-PAGE. Separated protein spots are then visualized by staining, and those protein spots of interest are excised from the gel and analyzed by mass spectrometry [74, 106]. Though the data obtained from 2-DE is of significant value, it has many drawbacks. Proteins that are very large, hydrophobic, acidic, or basic are poorly resolved [80]. For this reason a newer method, known as multi-dimensional protein identification (MudPIT) [39], has become more commonplace. MudPIT combines multi-dimensional liquid chromatography with tandem mass spectrometry; allowing complex protein samples to be analyzed. Experimentally, proteomes are digested with trypsin to generate peptide fragments. Using HPLC, the peptide fragment mixture is then applied to a column packed with a strong cation exchange (SCX) resin. The eluted peptides collected in fractions are then analyzed using a mass spectrometer. After the mass spectral data are obtained, database searches are performed which identify the proteins from the original samples. This technique is very efficient because it is a relatively expedient method of analysis, and the two-dimensional chromatographic separation of peptides also increases the number of proteins identified [80, 148].

Proteomic research and *Staphylococcus aureus.* The first proteomic study of *S. aureus* protein expression was conducted by Ziebandt et al. almost a decade ago [208]. The study compared extracellular proteins of two closely related strains, RN6390 (laboratory-derived) and COL (clinical isolate) using 2D-gel electrophoresis and N-terminal sequencing, or MALDI-TOF, for the identification of proteins. Eighteen secreted proteins were identified in COL [208], an early hospital-acquired methicillin resistant *S. aureus* isolate [69], of which nine had not yet been found in the laboratory strain

RN6390. Comparing the cytoplasmic proteins of RN6390 and COL showed only a few differences between the two strains, as opposed to the more striking difference in expression of secreted proteins [208]. This was a particularly important finding given the importance of secreted proteins in the virulence of *S. aureus*. This study also compared the secretomes of *S. aureus* mutants lacking the global regulator of virulence, SarA, in an RN6390 background. Five proteins (glycerolester hydrolase, autolysin, β-hemolysin, lipase, and immunodominant antigen A) were found to be positively regulated by SarA, with a further twelve shown to be negatively controlled by this regulator, including secreted proteases such as staphopain, V8 protease, and aureolysin. In this same study, proteomic techniques were employed to understand the role of the major alternative sigma factor, SigmaB. SigmaB is involved in induction of the general stress response of other gram-positive bacteria [81] and is thought to have a role in the virulence of *S. aureus* [25, 142]. In a s*igB* mutant, α-hemolysin, lipase, and thermonuclease were produced at significantly higher levels than in the wild-type [28, 111]. Conversely, nine other secreted proteins were found to be negatively regulated by SigmaB (including enterotoxin B, serine proteases, leukotoxin D, β-hemolysin, and glycerol ester hydrolase) [208].

In 2002, Bernardo et al used different forms of SDS-PAGE, followed by MALDI-TOF analysis, to identify the secreted proteins of three strains: methicillin sensitive ATC29213, methicillin resistant ATC 43300, and a clinical isolate provided from the University Clinic of Cologne in Germany [11]. What was found was that important proteins such as the major autolysin, staphylococcal nuclease, and three hypothetical proteins could not be resolved by 2D-gel electrophoresis. These proteins in particular

have high pI values; 9.65, 9.21, 9.21, 9.17, 9.28, respectively. As mentioned before, 2D-gel electrophoresis is not an effective technique for resolving proteins that are too basic. Subsequently, Bernardo et al found that these proteins could be resolved by conventional 1D-gel electrophoresis (SDS-PAGE) and identified by MALDI-TOF mass spectrometry. Compared to 2D-gel electrophoresis, 1D-gel electrophoresis requires less protein, yielded more reproducible results, and allowed for the detection of some alkaline proteins. 2D-gel electrophoresis, however, had the ability to identify proteins in low abundance when compared to 1D-gel electrophoresis [11]. In a later study by Kohler et al, cytoplasmic proteins of *S. aureus* COL were analyzed using two methods: 2D-gel electrophoresis and multidimensional liquid chromatography followed by mass spectrometry using a matrix-assisted laser ionization-time of flight (MALDI-TOF) mass spectrometer. The combination of these two methods identified a total of 1123 cytoplasmic proteins. Four-hundred and seventy-three proteins were identified by 2D-gel electrophoresis and belonged to the transcriptional and translational machinery, aerobic respiration and fermentation pathways, and some biosynthetic pathways. A gel-free method, followed by MALDI-TOF mass spectrometry, yielded an additional 650 proteins that mainly belonged to metabolic pathways, and included alkaline and hydrophobic proteins that were not seen in the 2D gel electrophoresis analysis [107].

In *S. aureus* the expression of virulence genes is regulated in a highly coordinated manner. Cell surface-associated proteins are expressed during exponential growth, while secreted virulence factors are expressed during post-exponential growth [202]. In another study by Ziebandt et al, again using 2-DE, 70 secreted proteins were found to be affected by a mutation in another major global regulator of toxin production, *agr*. Amongst these

affected proteins, many virulence factors such as proteases, toxins, lipases, and staphylokinase were found to be upregulated by *agr*. Apart from upregulating the expression of secreted proteins in late exponential and stationary phases, *agr* seemingly represses the expression of proteins usually present in cells during exponential growth, such as immunodominant antigen A, autolysins, and protein A [206].

In an attempt to quantify the entire *S. aureus* proteome, Becher et al. analyzed cytoplasmic, membrane-bound, surface-associated, and secreted proteins in growing and non-growing cells of *S. aureus* COL by a combination of 2-DE and mass spectrometric analysis [10]. Cytoplasmic proteins were metabolically labeled, and, using a combination of 2-DE and GeLC-MS/MS (a fusion of 1-DE and LC-MS/MS analysis), a total of 1796 proteins were identified, representing ~67% proteomic coverage of *S. aureus*. In the transition from exponential to stationary phase of growth, it was apparent that ribosomal proteins, translational factors, and some enzymes involved in amino acid synthesis were significantly down-regulated. Proteins that were found to be upregulated in stationary phase include PEP carboxykinase (PckA, a gluconeogenetic enzyme), enzymes involved in the TCA cycle, and members of the phosphate regulon. Membrane proteins were analyzed in this study via a combination of GeLC-MS/MS [45] and trypsin shaving [200]. The GeLC-MS/MS approach resulted in the contamination of membrane proteomes with cytoplasmic proteins, whilst the shaving method, using trypsin, revealed only membrane proteins in the isolated fractions. With the membrane-targeted approach, a total of 125 proteins were identified including integral as well as peripheral proteins, and accounted for ~56% of all predicted membrane proteome. Specifically, proteins associated with phosphotransferase systems (PTS), the glycerol uptake facilitator (GlpF),

and transporters involved in uptake of C_3 carbon sources were found at increased levels during the stationary phase [10]. Membrane proteins found to be up-regulated in stationary phase included ABC transporters involved in the import of amino acids and oligopeptides, whilst those found in decreased levels included high affinity iron-compound ABC transporters. Interestingly, the highly conserved Sec secretion machinery was found to be present at constant levels from the exponential to stationary phases of growth [10]. This study also used a biotinylation approach for surface-exposed cell wall-associated proteins analysis. The advantage of this is that such proteomes can be purified with minimal contamination by other subproteomic fractions. A total of 146 surface-associated proteins were identified, including membrane proteins, proteins that are covalently attached to the cell wall, lipoproteins, cell wall-associated proteins containing signal peptides, and cell wall-associated proteins that were also found to be secreted [10]. Proteins involved in adhesion such as fibronectin-binding proteins (Fnbps) [171] and fibrinogen-binding protein (ClfB) [126] were mainly upregulated in the exponential phase of growth. Conversely, levels of ClfA and immunodominant protein IsaB were significantly increased in stationary phase [10]. The last fraction, secreted proteins, was analyzed using a GeLC-MS/MS approach. With this technique, a total of 57 secreted proteins were identified, mainly during stationary growth. Specifically, there was an observable switch from the production of proteins involved in adhesion, biofilm formation, and cell invasion during exponential growth, to the production of virulence factors such as toxins, enzymes, and superantigens in stationary growth [10]. This study represents the most comprehensive quantification of the *S. aureus* proteome to date.

In 2010, a study by Hempel et al. quantitatively profiled the SigmaB-dependent expression of surface-associated proteins by metabolic labeling, biotinylation, and GeLC-MS/MS. A total of 49 proteins were found to be regulated by SigmaB [82], 21 of which were known to be dependent or influenced by SigmaB at the transcriptional level [14, 149, 206]. The remaining 28 proteins have not been described as being modulated by SigmaB activity. Consistent with the literature, fibrinogen-binding proteins ClfA and ClfB were found to be strongly decreased in the *sigB* mutant, whilst there was an accumulation of surface-associated proteins noted, mainly in stationary phase [82]. This negative influence of SigmaB must be indirect as sigma factors can only act directly as positive regulators [67, 206].

In a 2010 study by Ziebandt et al., the secretomes of 25 clinical isolates of *S. aureus* revealed an extreme heterogeneity of secreted protein expression due to genomic plasticity, and differences in regulation. Of the 63 identified secreted proteins, only 7 proteins (IsaA, Lip, LytM, Nuc, SA0620, SA2097, and SA2437) were commonly produced by clinical isolates of different clonal lineages [207]. This finding is likely explained by the observation that up to 30% of the genomes of different *S. aureus* isolates consist of variable mobile genetic elements, such as pathogenicity islands, lysogenic bacteriophages, plasmids, and transposons [198]. Virulence determinants are commonly encoded on these mobile genetic elements, thus facilitating proteome variability [207].

Finally, recent proteomic analyses in *S. aureus* have been focused on characterization of cell surface proteomes. Cell surface proteins are directly in contact with the extracellular environment and might perhaps serve as the largest group of targets for vaccine or antibody development. These surface-exposed proteins were termed the “surfacome” in a

study by Dreisbach et al. in 2010 [45]. The aim of this study was to refine and optimize the isolation technique of surface proteins to increase the number of proteins identified and reduce the number of contaminating cytoplasmic proteins due to cell lysis. Seemingly, the most effective method for identifying surface-exposed proteins is by shaving these proteins with trypsin followed by MS analysis [176, 180, 185]. The study compared different buffers and trypsin digestion conditions to determine the best method for the isolation of surface-exposed proteins. The trypsin shaving technique was used to analyze *S. aureus* strains from different clonal lineages, including the laboratory strain RN6390, early clinical isolates Newman and COL, and the CA-MRSA strain USA300. After mass spectrometric analysis, 39 surface-exposed proteins were found in RN6390, 59 in Newman, 47 in COL, and 24 in USA300. Taken together, a total of 96 surface-exposed proteins were detected from all four strains, 5 proteins of which contained an LPXTG motif for covalent attachment to the cell wall. A further 17 were predicted to be completely secreted into the extracellular environment, and 2 other secreted proteins also had motifs for cell wall-binding. Intriguingly, there were two toxins that were detected on the surface of the cell that are not predicted to be secreted. Additionally, 16 ribosomal proteins and 17 proteins involved in metabolism or possessing housekeeping functions were also found in the surfacome [45]. The fact that only 7 proteins (including GAPDH and FBA) were common to all four strains illustrates the heterogeneity of *S. aureus* strains and their associated sub-proteomic fractions. In a similar study published at the same time, Ventura et al. analyzed the surface-exposed proteome (terming it the "surfome") of the CA-MRSA USA300 strain known as LAC (LA County Clone) [190]. A total of 113 surface-exposed proteins were identified in LAC during the post-

exponential phase of growth, the most abundant of which was Protein A. Surprisingly, a novel uncharacterized two-component leukotoxin was also detected in the surfome, termed LukGH. This newly identified bipartite toxin was found to have a significant role in pathogenesis, specifically via pore formation in human PMNs [190].

Quantitative proteomic analysis of *S. aureus*. There are several methods for determining relative protein expression within a given organism, the most popular method being 2-DE. Using this technique, proteins are first separated by differences in electric charge (isoelectric focusing), and then perpendicularly by SDS-PAGE. Separated protein spots are then visualized by staining, and those protein spots of interest are excised from the gel and enzymatically digested with trypsin. The resulting peptides are then analyzed by mass spectrometry. This technique, however, has many drawbacks. Proteins that are very large, hydrophobic, acidic, or basic are poorly resolved. Another popular method for determining relative protein expression is known as iTRAQ [168]. iTRAQ (isobaric tag for relative and absolute quantitation) is a peptide-labeling technique used for identification and relative quantification of proteins from different samples in one single experiment. The iTRAQ 4-plex analyzes four different samples in one single experiment after labeling peptides from each sample with an isobaric tag reagent, which includes a reporter moiety and a balance moiety. The mass of the balance moiety from each reagent is different in order for the total mass of the balance and reporter moieties to equal 145 because mass of each reporter moiety is different [168]. A workflow for the iTRAQ-labeling of peptides is outlined in Figure 1.

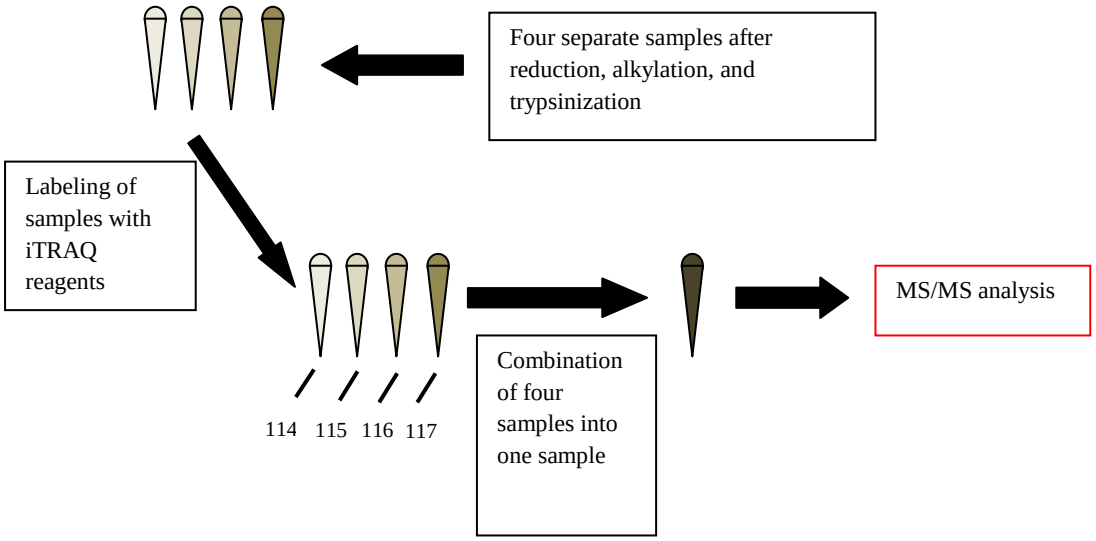

Figure 1. Workflow for iTRAQ-labeling of peptide samples

After MS/MS analysis, which includes high collision induced dissociation, the balance moieties are lost, leaving only the reporter tags. Because of the unique isotopic distribution of each reporter tag (114, 115, 116, and 117), it is possible to determine relative protein expression across the different samples in a single experiment. An example of a mass spectrum resulting from MS/MS analysis of an iTRAQ sample is shown in Figure 2.

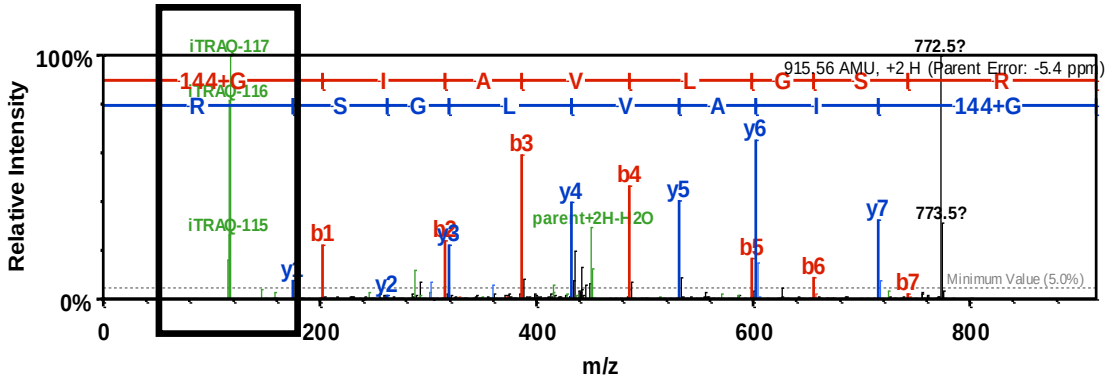

Figure 2. Example of mass spectrum resulting from MS/MS analysis of iTRAQ sample

Differential expression of a particular protein across the four samples can be determined by the relative abundance of each reporter tag resulting from MS/MS analysis. Figure 3 displays an example of the relative intensities of each reporter tag indicating relative protein expression.

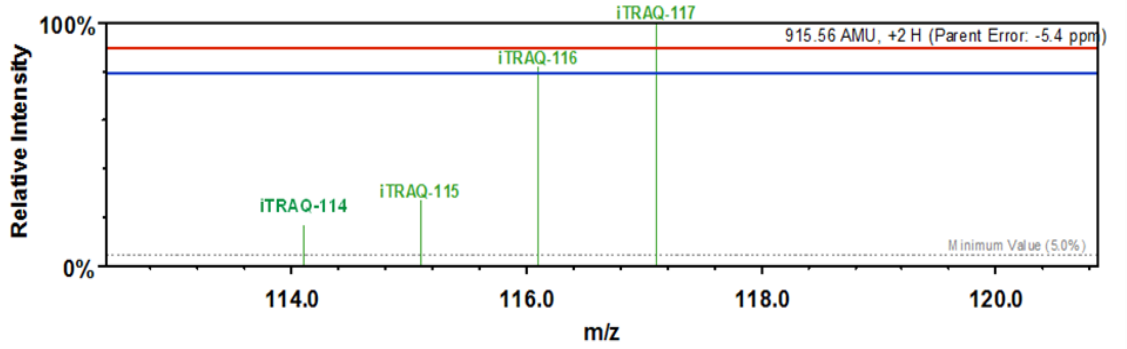

Figure 3. Relative abundance of reporter ion intensity from iTRAQ sample

A 2005 study by Choe et al., compared 2-DE and shotgun proteomic analysis with isobaric-tagged samples (iTRAQ) of *Escherichia coli* for relative protein expression after induction of *rhsA* [31], believed to be involved in biosynthesis and export of capsular polysaccharides [129]. The purpose of this study was to determine the best method for biological reproducibility that minimizes technical variability. It was found that shotgun proteomic analysis with iTRAQ samples provided the best reproducibility with minimal outlier data [31]. In 2006, Wolff et al. studied the heat shock response of exponentially-

growing *Bacillus subtilis* cells by gel-free iTRAQ-labeled samples and by the 2-DE approach for quantitation of proteins involved in this response. It was found that the proteomic analysis of gel-free iTRAQ-labeled protein samples resulted in the identification of more proteins, and was more reproducible [201]. With regards to *S. aureus*, the first such quantitative proteomic analysis was focused on Van resistance. Van has commonly been used as a last resort drug to treat MRSA infections [84]. It acts by preventing peptide cross-linking between growing layers of peptidoglycan, consequently weakening the cell wall [8]. In 1996, a *S. aureus* isolate in Japan was found to be resistant to intermediate concentrations of Van [84], and are now present throughout Europe, Asia, and the USA [153]. Drummelsmith et al. in a 2007 study developed a method for the rapid identification of potential biomarkers of VISA strains with the use of 2-DE and iTRAQ tagging. A VISA strain, Mu50, was compared to a Van-sensitive MRSA strain, CMRSA-2, for relative protein expression of cytoplasmic proteins. It was found that expression of SAV2095, known as SceD-like protein, is consistently and significantly increased in VISA strains. This protein has a potential association with the cell wall which is known to be thickened in such isolates [47].

Project Aim. The studies detailed above reveal a concerted effort to develop the most efficient and productive methods for discovering proteins that are differentially expressed in *S. aureus*. Each provides knowledge and insight into the intricate phenotypic switching mechanisms employed by *S. aureus* that make it such a successful human pathogen. Previous studies have been fraught with problems, including the use of 2D gel electrophoresis that is not efficient for resolving proteins that are too hydrophobic, large, acidic, or basic. To date, there has not been a proteomic study solely based on 2D liquid

chromatography followed by mass spectrometry (2DLC-MS). Studies that do involve some 2DLC-MS analysis use a urea concentration that is too low for solubilization of insoluble proteins, do not include filter sterilization of supernatants to remove bacterial cells and ensure purity of secreted proteins, and centrifuge secreted proteins during precipitation at a speed that is too low to ensure maximal protein recovery [176, 206, 208]. Appropriate method refinement is therefore important for obtaining reproducible data across several biological replicates of a sample. In this work we seek to, through method refinement, catalogue the intracellular proteome and secretome of a commonly used and well-studied lab strain of *S. aureus* SH1000 during post-exponential and stationary phases of growth to provide an insight into its physiology; and how it adapts to its changing environment over time by utilizing differential protein synthesis. Additionally, we aim to profile the secretomes, of clinically significant strains currently afflicting individuals in hospitals (USA100 and USA200) and in the community (USA300 and USA400) settings. A proteomic analysis encompassing the entire secretomes of these clinically relevant strains is lacking. With this proteomic approach we hope to not only identify but also quantify the production of secreted proteins that enable this organism to swiftly infect and cause disease in a patient. It is possible these analyses could lead to the identification of antigens and the development of protective vaccines against *S. aureus.*

Materials and Methods

Buffers.

Phosphate buffered saline

0.8% sodium chloride

0.14% disodium phosphate

0.02% potassium chloride

0.02% potassium dihydrogen phosphate

UDS buffer

6M urea

5mM DTT

1% SDS

50mM Tris-HCl

Strains, media, and growth conditions.

For cataloguing of the *S. aureus* intracellular proteome and secretome, the common lab strain SH1000 was used. For relative toxin expression, the strains USA100 (N315), USA200 (MRSA252), USA300 (LAC), and USA400 (MW2) were used. Overnight cultures of the wild-types (SH1000, USA100, USA200, USA300, and USA400) were grown in 3% tryptic soy broth (TSB).

To obtain a sample from each strain at a specific phase of growth, 1 ml of the overnight culture was added to 100 ml of 3% TSB. The new culture was allowed to grow for 3 hours and then the optical density of the new culture was standardized to 0.05 in 400 ml of TSB. The new synchronous culture was allowed to grow until the desired phase of growth: post-exponential (5 hours) and stationary (15 hours). When incubating, the cultures were grown at 37˚C shaking at 250 rpm with a volume to flask ratio of 1:2.5, ensuring adequate aeration of cultures.

Cataloguing of intracellular proteome and secretome of *S. aureus.*

Cytoplasmic protein extraction

The synchronized cultures of SH1000 were allowed to grow until post-exponential and stationary phases of growth. Cultures were then centrifuged for 10 minutes at a speed of 4,150 rpm. The pellets were washed three times with PBS, pH 7.4, and finally resuspended in 1 ml of UDS buffer, pH 8, with 0.1 mm disruption glass beads. Cells were then lysed using a BioSpec Mini-BeadBeater for a total of 4 minutes, with intermittent cooling phases. The lysed cells were then centrifuged for 10 minutes at 13,300 rpm. The supernatants were collected and centrifuged again, to prevent contamination from non-cytoplasmic proteins and ensure purity of the intracellular protein fractions, for 10 minutes at the same speed and then transferred to new tubes. Protein concentrations were determined using a Pierce 660 nm protein assay kit and then standardized to 1 mg/ml.

Secreted protein extraction

Synchronized cultures of SH1000 were allowed to grow until post-exponential and stationary time points. The cultures were then centrifuged for 10 minutes at a speed of 4,150 rpm. Supernatants were collected and any contaminating bacterial cells were removed by filter sterilization. Clean supernatants were then concentrated using Millipore Centricon Plus-70 centrifugal filter units before being precipitated in a final concentration of 10% trichloroacetic acid overnight at 4˚C. The following day, the precipitated proteins were centrifuged at 8,500 rpm for 70 minutes at 4˚C. The resulting pellets of precipitated secreted proteins were washed with 100% ice cold ethanol and centrifuged for 70 minutes at 8,500 rpm at 4˚C for a total of three times. After air drying the samples and after determination of protein concentrations, samples were standardized to 1 mg/mL.

Trypsin digestion

Protein samples were reduced with 50µl of 200mM dithiothreitol for 1 hour at room temperature followed by alkylation with 200 µl of 200 mM iodoacetamide for another hour in the dark at room temperature. Any remaining alkylating reagent was consumed with 200µl of 200 mM dithiothreitol. The samples were then diluted up to 5ml with 25 mM ammonium bicarbonate and then digested with a ratio of 1:30 by weight of trypsin to protein (33.33µl) overnight at 37˚C. The following day, the digested proteins were de-salted with C-18 Vydac columns. The C-18 Vydac columns were activated with 1 ml of 100% acetonitrile and repeated once. The columns were then equilibrated with 1 ml of 0.1% formic acid in water and repeated once. The samples were applied to the columns and the peptides were washed twice with 1 ml of 0.1% formic acid in water. The

peptides were eluted from the columns with 300 µl of 0.1% formic acid in acetonitrile and repeated for a total of 3 times. Once de-salted, the peptide samples were dried using the SpeedVac centrifuge. The peptides were resuspended in 100 µl of 0.1% formic acid in water and sonicated for 10 minutes.

Mass spectrometric analysis of peptides

The peptide samples were then placed in the autosampler of the LTQ XL mass spectrometer. Each sample was fractionated in the gas phase as opposed to fractionation utilizing multidimensional HPLC. For gas phase fractionation, three separate methods were created. The first method scanned ions with a mass-to-charge ratio within a range of 350-550. The second method scanned ions with a mass-to-charge ratio within a range of 550-750. Finally, the third method scanned ions with a mass-to-charge ratio of 750-1500. There were a total of 6 full scan events with the top five most intense ions in a 120 minute HPLC gradient and CID normalized collision energy of 35.0%.

Identification of proteins

The resulting files from mass spectrometric analysis of the samples were processed using Mascot Daemon software for database searching with a Uniprot database containing sequences specific to the *S. aureus* COL strain. A maximum of 1 missed cleavage by trypsin was allowed. Peptide tolerance was set to ±2.5 Da and the MS/MS tolerance was set to ±0.6 Da. After alkylation with iodoacetamide, the peptides were quantitatively modified at cysteine residues by carbamidomethylation. Variable modifications include acetylation of the protein at the N-terminus, oxidation of methionine, and phosphorylation of serine, threonine, and tyrosine. Proteins were identified and listed

using Scaffold 3 software. A random concatenated database of the *S. aureus* COL strain was created in Scaffold 3 for the detection of false-positive identification of proteins using a decoy database search strategy. A false-positive rate less than 5% was found acceptable.

Relative Toxin Production of *S. aureus* using iTRAQ.

Concentration of secreted proteins

Synchronous cultures of USA100, USA200, USA300, and USA400 were grown to post-exponential phase (5 hours) or to stationary phase (15 hours). Once the cultures had grown to the desired phase, they were centrifuged at 4,150 rpm for 10 minutes. After centrifugation, the supernatants were filter sterilized and then concentrated using Millipore Centricon Plus-70 filter units according to the manufacturer's instructions. Following concentration of the supernatants, the proteins were precipitated with 10% trichloroacetic acid overnight at 4˚C. Following precipitation, the supernatants were centrifuged at 8,500 rpm for 70 minutes at 4˚C. The supernatants were removed, leaving the protein pellet to be washed. The proteins were washed with 100% ice cold ethanol and then centrifuged at 8,500 rpm for 70 minutes at 4˚C. The ethanol washing was repeated a total of 3 times. After the last ethanol wash, the pellets were allowed to air dry.

Trypsin digestion of secreted proteins

Labeling with the iTRAQ reagents was completed according to the manufacturer's instructions. The secreted protein pellets were resuspended in dissolution buffer provided by the iTRAQ kit. The Pierce 660 nm Protein Assay was used to determine the

concentrations of the protein samples. The concentrations were standardized to 100 µg in a final volume of 20 µl of dissolution buffer. One µl of denaturant (SDS) was added to each sample followed by 2 µl of reducing reagent. The samples were incubated at 60˚C for 1 hour. After the incubation period, 1 µl of cysteine blocking reagent was added to the samples followed by a 10 minute incubation period at room temperature. Trypsin was added to the samples in a ratio of 1:30 (3.33µl) and the samples were digested for 12-16 hours at 37˚C.

iTRAQ labeling of peptides

The iTRAQ reagents were allowed to reach room temperature and were mixed with 70 µl of ethanol. The peptide samples were individually labeled with the iTRAQ reagents (USA100-114, USA200-115, USA300-116, USA400-117) for 1 hour at room temperature. After the incubation period, the 4 labeled samples were combined into a new tube. To rid the sample of ethanol, the samples were dried using the SpeedVac centrifuge. The samples were then resuspended in 1 ml of 0.1% formic acid in water. The labeled peptides were then de-salted using C-18 Vydac columns as previously described. After de-salting, the samples were dried using the SpeedVac centrifuge and then resuspended and sonicated in 25 µl of 0.1% formic acid in water.

Mass spectrometric analysis of iTRAQ-labeled peptides

The peptide samples were placed in the autosampler of the LTQ Orbitrap XL mass spectrometer. There were a total of 7 full scan events which included a full survey scan (m/z 350-1500) and subsequent MS/MS of the top 3 most intense ions of a range of 350-1500 m/z in a 180 minute gradient. There were 3 scan events with CID of 35.0%

normalized collision energy in the linear ion trap followed by 3 scan events with HCD of 40.0% normalized collision energy at a mass resolving power of 30,000 full scan MS with 7,500 high collision induced dissociation scan in the Orbitrap mass analyzer.

Identification and Quantitation of iTRAQ-labeled peptides

The resulting files from mass spectrometric analysis of the samples were processed using Mascot Daemon software for database searching with the USA100, USA200, USA300, and USA400 strains. A maximum of 1 missed cleavage by trypsin was allowed. Peptide tolerance was set to ±10 ppm and the MS/MS tolerance was set to ±0.6 Da. A fixed modification of the peptides included methyl methanethiosulfonation of the cysteine residues of trypsin-digested peptides. Variable modifications, on the other hand, included acetylation of the proteins at the N-terminus and oxidation of methionine. The resulting files from database searching using Mascot were then submitted to the HCD merging tool provided by the ExPASy proteomics server (http://www.ex-pasy.ch/tools/HCD_CID_merger.html) to merge the qualitative peptide sequence-ion m/z range of CID with the quantitative reporter-ion m/z range of HCD. Following merging of HCD with CID spectra, the resulting files were re-searched using Mascot Daemon software. Proteins were identified and quantified using Scaffold 3 Q+ software. A random concatenated database of the *S. aureus* USA100, USA200, USA300, and USA400 was created in Scaffold 3 for the detection of false-positive identification of proteins.

Results

The application of mass spectrometry for proteome analysis in *S. aureus.* As a medically significant pathogen, *S. aureus* employs an arsenal of virulence factors to cause and maintain infection in humans. These virulence factors and other proteins central to the survival of *S. aureus* are differentially expressed as the organism progresses through the different phases of growth. When used for the study of different tissues and organisms, mass spectrometry can be a useful tool for the identification and quantitation of protein variations within cells. Therefore in this project we aimed to catalogue the intracellular proteome and secretome of a common lab strain of *S. aureus* SH1000 during post-exponential and stationary phases of growth to provide an insight into its physiology and how it adapts to its changing environment over time by utilizing differential protein synthesis. We also aimed to profile the secretomes of clinically significant strains currently afflicting individuals in hospitals (USA100 and USA200) and in the community (USA300 and USA400) settings as a complete secretomic analysis of these clinically relevant strains is currently lacking.

An improved method for the extraction of intracellular proteomes from *S. aureus* cells. Traditionally, cytoplasmic protein extraction in *S. aureus* was performed by boiling cells; allowing them to burst and release cytoplasmic proteins into the buffer. Initially we followed such a protocol, extracting cytoplasmic proteins from the *S. aureus* laboratory strain SH1000. After many repetitions of this approach, coupled with reading protein concentration using a Nanodrop device, we obtained highly inconsistent results.

As such, it was important to determine if this resulted from inefficient extraction methods or inaccuracies of the Nanodrop device for determining protein concentration. To resolve this, we used a Pierce 660nm Protein Concentration Assay to calculate protein concentration via use of the included protein standards and a BioTek Synergy II plate reader. The subsequent readings obtained revealed far more consistent results from our extraction, however protein yields were consistently low, for example 147.7 µg/ml for a post-exponential phase culture and 152.2 µg/ml for a stationary phase culture of SH1000.

To maximize protein concentrations, different methods for cell lysis were tested. Four different lysis methods were used in quintuplicate: boiling of cells; mechanical shearing of cells by bead-beating with 0.1mm glass beads; treatment of cells with a dedicated lytic agent, lysostaphin; and sonication of cells using a disruptor. For each of these methods, a 100 ml overnight culture of SH1000, grown in a 250 ml Erlenmeyer flask, was centrifuged at 4,150 rpm for 10 minutes. The resulting pellets were washed twice with phosphate buffered saline (PBS) to eliminate any remaining growth medium, before being resuspended in 1 ml of fresh PBS. Each of the resuspended SH1000 cell samples were then subjected to the various cell lysis methods.

For lysis via boiling, samples were placed in a 100C water bath for 10 minutes. Mechanical shearing was achieved with an approximately 0.5 cm depth of 0.1 mm glass disruption beads in the sample tube and a BioSpec Mini BeadBeater programmed for 4 total minutes of lysis, with regular cooling intervals. To enzymatically lyse the cells, 100 µg lysostaphin was added to cells resuspended in PBS, before incubation at 37˚C for 1 hour. Finally, for sonication, a disrupting probe was inserted in tubes containing resuspended cells, which were pulsed at an amplification of 70% for 20 seconds, with 10

seconds rest periods between treatments. Cells were sonicated for a total of 3 minutes. Following each different lysis method, samples were centrifuged, and supernatants removed to new 1.5 ml tubes. These tubes were centrifuged further, and the supernatants were again removed to clean 1.5 ml tubes. This process was performed to remove all cell wall and membrane proteins, and ensure the purity of the cytoplasmic fraction. Using each of these methods for extraction we obtained consistent results for each method, however they varied by process used (Table 1).

Boiling	Bead-beating	Lysostaphin treatment	Sonication
194.6 µg/ml	766.6 µg/ml	853.27 µg/ml	256.6 µg/ml

Table 1. Comparison of different cell lysis techniques for extraction of cytoplasmic proteins. Data presented is the average of 5 samples that showed less than 10% variation.

The protein yields for boiling and sonication were dramatically low when compared to that from bead-beating and lysostaphin treatment. Lysostaphin treatment and bead-beating of the cells yielded comparable protein concentrations, although lysostaphin treatment produced consistently higher yields. For our analyses, bead-beating was selected as the preferred method because of its ease and speed of use, and the low cost associated with it, compared to lysostaphin treatment.

Proteomic analysis of *S. aureus* SH1000 cytoplasmic proteins via 1D SDS-PAGE coupled with LC-MS/MS. For our initial proteomic analysis of cytoplasmic proteins we grew an overnight culture of SH1000 and extracted proteins via the bead-beating method detailed above. These were then resolved via 1D SDS-PAGE. After electrophoresis, the gel was cut into 7 fragments (Figure 4), which were subjected to in-gel digestion with trypsin.

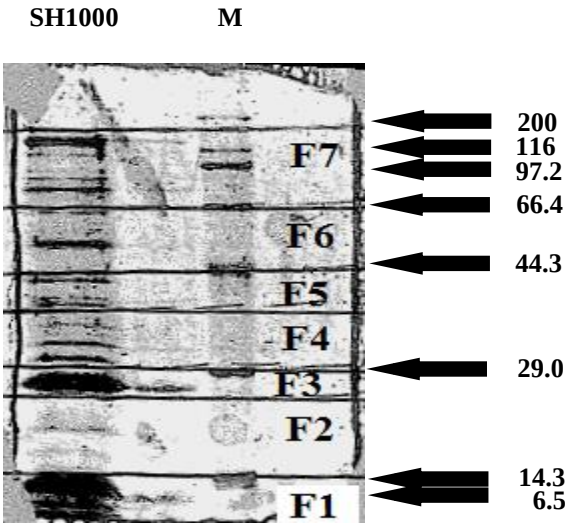

Figure 4. 1D SDS-PAGE analysis of cytoplasmic proteins from an overnight culture of *S. aureus* SH1000. M refers to molecular weight markers in kDa and F1-F7 refer to the excised fragments.

After trypsinization, the fragments were analyzed by a ThermoFinnigan LTQ mass spectrometer. Database searching of the returned results for all 7 fragments identified a total of 380 cytoplasmic proteins (Table 2). The database used for searching the mass spectral data was the general, non-specific Uniprot database (NCBI). This search found many contaminant proteins belonging to organisms other than *S. aureus*. The reason for this is likely that, after trypsin digestion, the resulting *S. aureus* peptides have incidental homology to peptides from organisms unrelated to *S. aureus*, as a result of using a non-specific database.

Identified Proteins (380)	Accession #	Spectral Counts
bifunctional autolysin (atl)	SACOL1062	67
ATP-dependent Clp protease, putative	SACOL2563	56
sdrD protein (sdrD)	SACOL0609	43
N-acetylmuramoyl-L-alanine amidase domain protein	SACOL2666	36
DNA-directed RNA polymerase, beta' subunit (rpoC)	SACOL0589	35
translation elongation factor G (fusA)	SACOL0593	34
chaperonin, 60 kDa (groEL)	SACOL2016	32
ATP synthase F1, beta subunit (atpD)	SACOL2095	32
dnaK protein (dnaK)	SACOL1637	31
transketolase (tkt)	SACOL1377	30

Table 2. The ten most abundant cytoplasmic proteins identified from overnight cultures of *S. aureus* SH1000, separated by 1D SDS-PAGE

Spectral counts measure relative protein quantification by comparing the number of identified MS/MS spectra from the same peptide to the total number of identified MS/MS spectra found in a dataset. The number of spectra matched to peptides from a protein is used as a measure of protein abundance in the sample [118].

Amongst the most abundant proteins identified were bifunctional autolysin (Atl), putative, ATP-dependent Clp protease, SdrD protein, N-acetylmuramoyl-L-alanine amidase domain protein, RNA polymerase (β unit), translation elongation factor G (FusA), GroEL chaperonin, ATP synthase F1 β subunit (AtpD), DnaK protein, and transketolase. Some of the identified proteins in Table 2, however, were not expected to be located in the cytoplasm of *S. aureus*. The bifunctional autolysin, SdrD protein, and N-acetylmuramoyl-L-alanine amidase domain protein were expected to be surface-associated proteins; whereas ATP synthase F1 β subunit (atpD) was expected to be present in the cell membrane. The cytoplasmic proteins found in Table 2 are involved in important biological processes. For example, the ATP-dependent Clp protease, chaperonin GroEL, and DnaK are involved in protein turnover and folding; whilst the RNA polymerase β subunit is involved in mRNA transcription. Additionally, translation elongation factor FusA has a role in protein synthesis and the ATP synthase produces ATP from ADP in a proton gradient present across the cell membrane. Finally, transketolase has a role in the pentose phosphate pathway.

Analysis of the effects of solubilization buffer on protein concentration yield. In addition to lysis methods, we also investigated the buffer used to solubilize proteins during extraction. As hydrophobic proteins can be easily lost during sample preparation, two different resuspension buffers were compared in an attempt to maximize protein

yield in general, and specifically capture the maximum number of hydrophobic proteins. In our analysis above, PBS was used to resuspend cells for the extraction of cytoplasmic proteins described in Table 1; however PBS is not efficient at dissolving insoluble proteins, and may not be able to guarantee maximum proteome coverage. Due to its molecular structure, urea is able to solubilize insoluble proteins by expanding hydrophobic cores via hydrogen bonding, encouraging the solvation of hydrophobic regions and allowing water to compete with intraprotein interactions. UDS buffer (6M urea, 5mM DTT, 1% SDS, 50mM Tris-HCl, pH 8) has been used in other studies for protein extraction [124], and therefore we set out to compare results obtained with this solution, with PBS. Cytoplasmic proteins from overnight cultures of SH1000 were extracted in PBS and in UDS by mechanical shearing of the cells. The concentrations of the cytoplasmic proteins from each sample were determined using a Pierce 660 nm Protein Assay. We found that UDS buffer commonly yielded protein concentrations from SH1000 cultures in stationary phase of around 1256 µg/ml compared to PBS, which was only around 783 µg/ml. UDS appeared to allow for more coverage of the cytoplasmic proteome, including hydrophobic proteins, and was thus chosen as our buffer for future analyses.

The use of complex mixture analysis coupled with HPLC separation to increase the number of proteins identified from overnight cultures of *S. aureus* cells. Given that the *S. aureus* genome contains approximately 2800 genes, our analysis above (Figure 2, Table 2) clearly does not represent the entire intracellular proteome of overnight SH1000 cells. Therefore, whilst 380 was a promising initial result, we employed alternative approaches to maximize the total number of proteins we could identify because proteins

that are very large, hydrophobic, acidic, or basic are poorly resolved by gel electrophoresis. For this reason a newer method was used, known as multi-dimensional protein identification (MudPIT), which combines multi-dimensional liquid chromatography with tandem mass spectrometry; allowing complex protein samples to be analyzed. Experimentally, proteomes are reduced and digested with trypsin to generate peptide fragments. Using HPLC, the peptide fragment mixture is then applied to a column packed with a strong cation exchange (SCX) resin. The eluted peptides collected in fractions are then analyzed using a mass spectrometer. This technique is very efficient because it is a relatively expedient method of analysis and the two-dimensional chromatographic separation of peptides also increases the number of proteins identified [145, 76]. Therefore, our new methodology involved using complex mixture analysis of cytoplasmic proteins coupled with HPLC separation, rather than 1D SDS-PAGE gel resolution. Thus proteins were again extracted from an overnight culture of SH1000 as described above. These were then reduced, alkylated, and digested overnight with trypsin. The resulting peptide fragments were fractionated via HPLC by applying the peptide fragments to a column packed with a strong cation exchange (SCX) resin. The resulting fractions containing peptides were then analyzed using a ThermoFinnigan LTQ mass spectrometer. After obtaining the mass spectral data, we analyzed the returned information using a database specific to *S. aureus* strain COL (a close relative of SH1000). This had the effect of more accurate protein identification and also returned all proteins as belonging to *S. aureus*. This proteomic analysis of overnight *S. aureus* SH1000 cells yielded a total of 747 cytoplasmic proteins identified (Table 3).

Identified Proteins (747)	Accession #	Spectral counts
alkaline shock protein 23	SACOL2173	1129
enolase (eno)	SACOL0842	886
translation elongation factor Tu (tuf)	SACOL0594	873
glyceraldehyde 3-phosphate dehydrogenase (gapA1)	SACOL0838	873
formate acetyltransferase (pflB)	SACOL0204	577
aldehyde dehydrogenase (aldA1)	SACOL0154	372
ATP-dependent Clp protease, putative	SACOL2563	371
DNA-binding protein HU (hup)	SACOL1513	356
pyruvate dehydrogenase complex E3 component, lipoamide dehydrogenase (pdhD)	SACOL1105	341
dnaK protein (dnaK)	SACOL1637	298

Table 3. The ten most abundant cytoplasmic proteins identified from MudPIT analysis of SH1000

Compared to proteins identified from 1D SDS-PAGE found in Table 2, proteins identified by MudPIT analysis in Table 3 were overall more abundant. For example, in Table 1, Clp protease had a total of 56 spectral counts whereas the spectral counts for this particular protein in Table 3 were 371. Also, there was seemingly less contamination by non-cytoplasmic proteins.

Specific cataloging of the *S. aureus* intracellular proteome using MudPIT analysis. Reproducibility using HPLC fractionation proved to be highly inconsistent; therefore a new fractionation method was needed for the cataloging of SH1000. Usually, ions with mass-to-charge ratios, used to determine the masses of peptides, between the values of 350 and 1500 are selected in the mass spectrometer for analysis. With a new gas phase fractionation method, three separate protocols were created in the mass spectrometer. The first method scanned ions with a mass-to-charge ratio within a range of 350-550. The second method scanned ions with a mass-to-charge ratio within a range of 550-750. Finally, the third method scanned ions with a mass-to-charge ratio of 750-1500. Each sample was analyzed using the three methods with three injections each.

Having derived methodologies that were effective and consistent for the intracellular proteomic analysis of *S. aureus*, we undertook a cataloging project using the laboratory strain SH1000. For this analysis, we chose to isolate intracellular proteomes from two different phases of growth: post-exponential (5 hours), and stationary (15 hours). Toxin production begins in the post-exponential phase with toxins accumulating in the stationary phase. For this reason, these two time points are of particular interest when cataloging proteomes of *S. aureus*. Thus, 1 milliliter of an overnight culture of SH1000 was added to a fresh 250 ml flask containing 100 ml of TSB which was incubated for 3 hours at 37˚C. After incubation, these cultures were again used to inoculate fresh TSB (identical conditions) at an optical density of 0.05. These test cultures were then allowed to grow at 37˚C for the appropriate amount of time for each growth phase. Cytoplasmic proteins were extracted by mechanically shearing the cells with glass beads in UDS buffer for a total of 4 minutes. Protein yields were calculated using a Pierce 660 nm Assay and standardized to 1 mg/ml for trypsin digestion. After trypsin digestion, the samples were subsequently analyzed by mass spectrometry for the final identification of cytoplasmic proteins present in the original cultures.

The ten most abundant cytoplasmic proteins identified by MudPIT analysis from SH1000 during post-exponential phase are included in Table 4. A total of 346 proteins were identified including DNA polymerase III β subunit, DNA polymerase I, DNA topoisomerase 4, and DNA ligase. Each of these is involved in the central cellular process of DNA synthesis. There was also a presence of DNA-directed RNA polymerase subunits ω, β, and β' for mRNA transcription. Many aminoacyl tRNA synthetases were also present in SH1000 during post-exponential phase, as were proteins involved in

protein synthesis, including elongation factor Tu, elongation factor G, and numerous 30S and 50S ribosomal proteins. The majority of the proteins identified such as enolase, pyruvate kinase, pyruvate dehydrogenase, fructose bisphosphate aldolase, dihydrolipoyl dehydrogenase, phosphoglycerate mutase, are involved in central carbon metabolism and energy generation. These proteins would be expected in the post-exponential phase because cells in this phase have not yet entered the stationary phase and are metabolically active and still growing. The complete list of proteins identified can be found in the Appendix.

Identified Proteins (346)	Accession Number	Sample 1	Sample 2
Elongation factor Tu	sp|Q5HIC7|EFTU_STAAC	239	119
Probable transglycosylase isaA	sp|Q5HCY1|ISAA_STAAC	81	111
Elongation factor G	sp|Q5HIC8|EFG_STAAC	89	65
Enolase	sp|Q5HHP1|ENO_STAAC	64	39
Pyruvate kinase	sp|Q5HF76|KPYK_STAAC	54	27
Dihydrolipoyl dehydrogenase	sp|Q5HGY8|DLDH_STAAC	39	36
Pyruvate dehydrogenase E1 component subunit beta	sp|Q5HGZ0|ODPB_STAAC	35	38
Bifunctional autolysin	sp|Q5HH31|ATL_STAAC	45	24
50S ribosomal protein L30	sp|Q5HDX6|RL30_STAAC	28	25
50S ribosomal protein L15	sp|Q5HDX7|RL15_STAAC	15	45

Table 4. The ten most abundant cytoplasmic proteins identified from MudPIT analysis of SH1000 during post-exponential phase

The ten most abundant cytoplasmic proteins identified by MudPIT analysis from SH1000 during stationary phase are included in Table 5. A total of 366 proteins were identified, including most of the proteins found in post-exponential phase, although in lesser quantities. As will be mentioned later, the method had to be refined yet again due to poor and inconsistent results using the HPLC. The newer gas phase fractionation method that will be mentioned bypasses the need for the HPLC but sacrifices proteome coverage. Proteins involved in DNA replication, transcription, protein synthesis, and central carbon metabolism were present in stationary phase cultures of SH1000. Though toxins such as

phenol soluble modulins and delta hemolysin are expected to be secreted, they are still present in high quantities intracellularly during stationary growth, when toxins begin to accumulate.

Identified Proteins (366)	Accession Number	Sample 1	Sample2
Elongation factor Tu	sp|Q5HIC7|EFTU_STAAC	246	521
Antibacterial protein (Phenol soluble modulin)	tr|Q5HGQ7|Q5HGQ7_STAAC	115	215
Probable transglycosylase isaA	sp|Q5HCY1|ISAA_STAAC	140	124
Pyruvate kinase	sp|Q5HF76|KPYK_STAAC	115	58
Uracil phosphoribosyltransferase	sp|Q5HE88|UPP_STAAC	66	66
Elongation factor G	sp|Q5HIC8|EFG_STAAC	81	67
Dihydrolipoyl dehydrogenase	sp|Q5HGY8|DLDH_STAAC	78	48
Bifunctional autolysin	sp|Q5HH31|ATL_STAAC	92	31
Cell division protein ftsZ	sp|Q5HGP5|FTSZ_STAAC	68	43
Delta-hemolysin	sp|Q5HEG6|HLD_STAAC	98	18

Table 5. The ten most abundant cytoplasmic proteins identified from MudPIT analysis of SH1000 during stationary phase

Derivation of an improved method for extraction of the *S. aureus* secretome. Given that *S. aureus* secretes a variety of exoproteins and toxins throughout growth, we determined it of significant importance to globally analyze these proteins at a proteomic level. As such, 100 ml of an overnight culture of SH1000 grown in a 250 ml flask was centrifuged for 10 minutes at a speed of 4,150 rpm. Secreted proteins were precipitated from the supernatant by adding 10% trichloroacetic acid and incubating for 1 hour. After the incubation period, the precipitated secreted proteins were washed with room temperature acetone and then centrifuged for 10 minutes at a speed of 13,300 rpm. Washing with acetone was repeated a total of 3 times and the secreted protein pellet resuspended in UDS buffer. This was then fractionated via HPLC and analyzed using a ThermoFinnigan LTQ mass spectrometer. Our analysis revealed a total of 728 proteins identified from the supernatants collected from the overnight cultures of SH1000 which unexpectedly contained many cytoplasmic proteins. Thus it would appear that

centrifuging is not sufficient for the removal of bacterial cells that could contaminate the supernatant fraction with cytoplasmic proteins. In order to improve the purity of secreted proteins in supernatants we inserted a filter sterilization step after centrifugation to remove cell contamination.

Further to this, TCA based precipitation of secretomes is difficult, time consuming and inconsistent, as a result of the large volumes involved (up to 400 ml). In order to maximize efficiency and expedite analysis, we employed Millipore Centricon Plus-70 Centrifugal Filter Units with a 5 kDa cutoff to concentrate supernatants. This method allowed for reduction in culture volumes; however protein concentrations directly from this process were still lower than required for analysis. Protein precipitation using trichloroacetic acid was thus still necessary to be able to resuspend the secreted proteins in UDS in an even smaller volume for subsequent mass spectrometric analysis. Therefore, we investigated a new method for precipitating secreted proteins so as to significantly increase yields and concentrations. After the addition of 10% TCA to the concentrated supernatant, we extended incubation times to overnight at 4˚C. Samples were then centrifuged at 4˚C for 70 minutes at a speed of 8,500 rpm. Supernatant were discarded and protein pellets washed with 100% ice cold ethanol, before centrifugation again at 4˚C for 70 minutes at a speed of 8,500 rpm. The ethanol washing was repeated a total of 3 times to ensure complete removal of TCA. Use of this method resulting in significantly higher proteins yields compared to the former, more rapid method of TCA precipitation. As an average, a stationary phase culture of SH1000 yielded a concentration of 336.6 µg/ml of proteins using the new TCA precipitation method compared to the older precipitation method that yielded an average of only 201.8 µg/ml.

Specific cataloging of the *S. aureus* secretome using MudPIT analysis. As with our intracellular investigations, given that we have determined effective methods for analysis, we set about cataloging the secretome of *S. aureus* SH1000. Because HPLC fractionation proved to give inconsistent results, as with the analysis of the intracellular proteome of *S. aureus*, we resorted to the gas phase fractionation method detailed above.

We again conducted this study for post-exponential and stationary phase cultures to observe the alteration of secreted proteins during growth. Accordingly, we took the supernatants derived from the intracellular cataloging experiments and filter sterilized them. These were then concentrated using Millipore Centricon Plus-70 centrifugal filter units with a 5 kDa cutoff, before precipitation using the TCA method derived above. After precipitation, the concentrations of secreted proteins were calculated using a Pierce 660 nm Assay before standardization to 1 mg/ml. Proteins were then digested with trypsin followed by mass spectrometric analysis for the identification of proteins secreted by *S. aureus* during the different growth phases.

The ten most abundant secreted proteins identified by MudPIT analysis from SH1000 during post-exponential phase are included in Table 6. A total of 38 secreted proteins were identified; the complete list can be found in the Appendix. Though some cytoplasmic proteins such as enolase, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate dehydrogenase can still be identified in the secreted protein fraction of *S. aureus*, filter sterilization of the supernatant excludes whole cells of *S. aureus* minimizing contamination by cytoplasmic proteins. For the most part, secreted proteins of SH1000 identified in the post-exponential phase were surface-associated proteins. These proteins included immunodominant antigen A, bifunctional autolysin, staphylococcal secretory

antigen SsaA2, putative surface proteins, probable transglycosylase SceD, glycerol phosphate lipoteichoic acid synthase, and lipase 1. Because *agr* activity increases during post-exponential phase, toxin production has just begun and has not yet accumulated in the supernatant, accounting for the low number of proteins identified in Table 6.

Identified Proteins (38)	Accession Number	Sample 1	Sample 2
Probable transglycosylase isaA	sp|Q5HCY1|ISAA_STAAC	234	186
Bifunctional autolysin	sp|Q5HH31|ATL_STAAC	42	31
Staphylococcal secretory antigen ssaA2	sp|Q5HDQ9|SSAA2_STAAC	46	39
Staphopain A	sp|Q5HEL3|SSPP_STAAC	10	6
Enolase	sp|Q5HHP1|ENO_STAAC	8	7
Surface protein, putative	tr|Q5HDZ9|Q5HDZ9_STAAC	8	2
Probable transglycosylase sceD	sp|Q5HEA4|SCED_STAAC	5	3
Glycerol phosphate lipoteichoic acid synthase	sp|Q5HHV4|LTAS_STAAC	5	6
Glyceraldehyde-3-phosphate dehydrogenase 1	sp|Q5HHP5|G3P1_STAAC	7	5
Lipase 1	sp|Q5HCM7|LIP1_STAAC	5	0

Table 6. The ten most abundant secreted proteins identified from MudPIT analysis of SH1000 during post-exponential phase

The ten most abundant secreted proteins identified by MudPIT analysis from SH1000 during stationary phase are included in Table 7, with a total of 346 secreted proteins identified. Overall, the number of secreted proteins identified was higher in the stationary phase compared to the post-exponential phase. Most importantly, the production of secreted toxins and exoenzymes increases immensely in stationary phase. Those identified in SH1000 during stationary phase include alpha, delta, and gamma hemolysins, serine proteases SplB and SplC, phenol soluble modulins, leukotoxin LukD, leukocidin-like protein 1, and staphopains A and B.

Identified Proteins (346)	Accession Number	Sample 1	Sample 2
Lipase 1	sp|Q5HCM7|LIP1_STAAC	608	361
Bifunctional autolysin	sp|Q5HH31|ATL_STAAC	269	229
Probable transglycosylase isaA	sp|Q5HCY1|ISAA_STAAC	527	143
Surface protein, putative	tr|Q5HDZ9|Q5HDZ9_STAAC	148	114
Putative uncharacterized protein	tr|Q5HI54|Q5HI54_STAAC	174	119
Enolase	sp|Q5HHP1|ENO_STAAC	70	99
Alpha-hemolysin	tr|Q5HGS1|Q5HGS1_STAAC	85	82
Lipase 2	sp|Q5HJ48|LIP2_STAAC	105	85
Glyceraldehyde-3-phosphate dehydrogenase 1	sp|Q5HHP5|G3P1_STAAC	93	72
Glycerol phosphate lipoteichoic acid synthase	sp|Q5HHV4|LTAS_STAAC	74	46

Table 7. The ten most abundant secreted proteins identified from MudPIT analysis of SH1000 during stationary phase

The application of proteomic methodologies for quantitative analysis of secreted toxins from a variety of *S. aureus* clinical isolates. Having refined effective methods for the extraction and mass spectrometric analysis of *S. aureus* proteomes, we proceeded to apply these methods to a relevant biological question. Clinically significant strains of *S. aureus* currently affecting individuals in healthcare facilities and in the community have thus far only been studied at the genomic and transcriptomic levels. Therefore, we decided to study these strains at the proteomic level, as this analysis has to date not been completed. Understanding the differential expression of secreted proteins could give insight into phenotypic switching mechanisms of *S. aureus* and strain dependent variations in virulence.

S. aureus infections have commonly been confined to health care facilities [41] largely affecting immunocompromised, young, or old individuals. These hospital-acquired methicillin resistant *S. aureus* (HA-MRSA) strains are highly-resistant to antibiotics, making HA-MRSA infections very difficult to treat. The leading HA-MRSA strain in the United States is USA100 with HA-MRSA strain USA200 a close second [127]. Recently, community-acquired methicillin resistant *S. aureus* (CA-MRSA) infections

have been reported in individuals with no ties to health care facilities [1, 94, 138]. In the United States, the most common CA-MRSA strains are known as USA300 and USA400 [110, 177]. These CA-MRSA strains appear to be far more virulent than HA-MRSA and are especially significant because they cause infections in young, healthy individuals with no predisposing factors [24, 54]. Despite their increased virulence, CA-MRSA strains currently have reduced antibiotic resistance compared to HA-MRSA; however it has recently been reported that CA-MRSA are beginning to replace HA-MRSA strain in healthcare facilities [195].

Therefore, synchronous cultures of hospital-associated methicillin resistant *S. aureus* (HA-MRSA) strains (USA100 and USA200) and community-associated methicillin resistant *S. aureus* (CA-MRSA) strains (USA300 and USA400) were grown to the post-exponential (5 hours) and stationary phases of growth (15 hours). After extraction of secreted proteins from the supernatants of these cultures, the concentrations of these proteins were standardized to 100 µg before overnight trypsin digestion. The resulting peptides were labeled using iTRAQ reagents, before mass spectrometric analysis by an LTQ Orbitrap. The reagents (having masses of 114, 115, 116, and 117) were used to label USA100, USA200, USA300, and USA400 respectively. Each of these analyses was performed separately a total of 3 times for each strain. After database searching of the spectral data using Mascot Daemon, Scaffold 3 was used to determine the secretomes of each clinical strain (USA100, USA200, USA300, USA400). Each strain was analyzed using a database derived from the relevant strains genome sequence. Using Scaffold 3, a t-test analysis was performed on the derived data to determine any statistically significant changes in production of major secreted toxins between the various strains, and between

the two different phases of growth (post-exponential and stationary). A p-value ≤ 0.05 at a 95% level of confidence was considered to be statistically significant.

Relative standard deviation and standard error for the three biological replicates. For statistical analyses, each biological sample was replicated a total of three times. To determine variability from sample preparation, relative standard deviation and standard error were calculated for each biological replicate from the comparison of one clinical strain to another. The ten most abundant proteins and one low abundance protein were chosen to determine the variability from one biological replicate to another. The relative standard deviation of all the proteins in a given biological replicate was then averaged. The relative standard deviation value is in the form of a percent and a percent ≤ 20 determines sample variability due to sample preparation is low, meaning the technical replicates were statistically sound (See Appendix).

Analysis of variations in secretomes of HA-MRSA and CA-MRSA strains. Separate databases were created for each strain: N315 for USA100, MRSA252 for USA200, FPR3757 for USA300, and MW2 for USA400. When database searching of the spectral data from each iTRAQ sample, each clinical strain was analyzed by its own database, which can affect the number of proteins identified in Scaffold 3. In the post-exponential phase, 109 proteins were identified using the USA100 database, 89 proteins were identified using the USA200 database, 114 proteins were found using the USA300 database, and 119 proteins were found using the USA400 database. In stationary phase, 246 proteins were discovered using the USA100 database, 230 proteins were identified for USA200, 224 proteins were found for USA300, and 240 proteins were identified using the USA400 database. There was an overall increase in the number of proteins

identified in the transition from post-exponential phase to stationary phase, as one might expect, resulting from toxin accumulation and/or cellular lysis.

Gene Ontology Annotations of HA-MRSA and CA-MRSA clinical strains. Using the complete proteome set for each clinical strain found at Uniprot.org in conjunction with JCVI CMR, a website containing complete genome sets of prokaryotic organisms, the role of each protein identified from each strain was annotated. Gene ontology of HA-MRSA strain USA100 during post-exponential phase is outlined in Figure 5. Proteins abundant during this phase include those involved in protein synthesis, energy metabolism such as glycolysis, gluconeogenesis, tricarboxylic acid cycle, pentose phosphate pathway, fermentation etc., and proteins involved in the biosynthesis and degradation of the cell envelope.

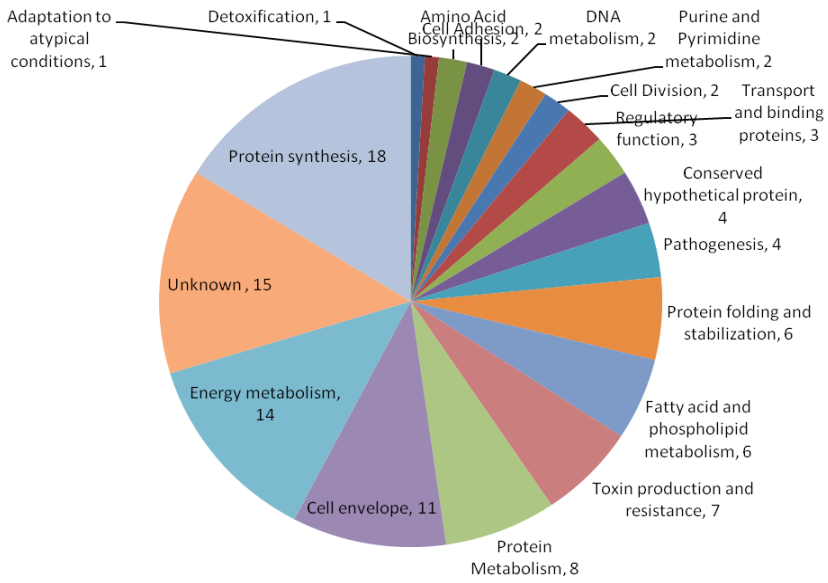

Figure 5. Gene ontology of HA-MRSA USA100 during post-exponential phase

Figure 6 displays gene ontology of USA100 during stationary phase growth. The majority of the proteins involved found during stationary phase are involved in energy

metabolism, protein synthesis and metabolism, purine and pyrimidine metabolism, cell envelope, as well as proteins of unknown function and those that are conserved hypothetical proteins.

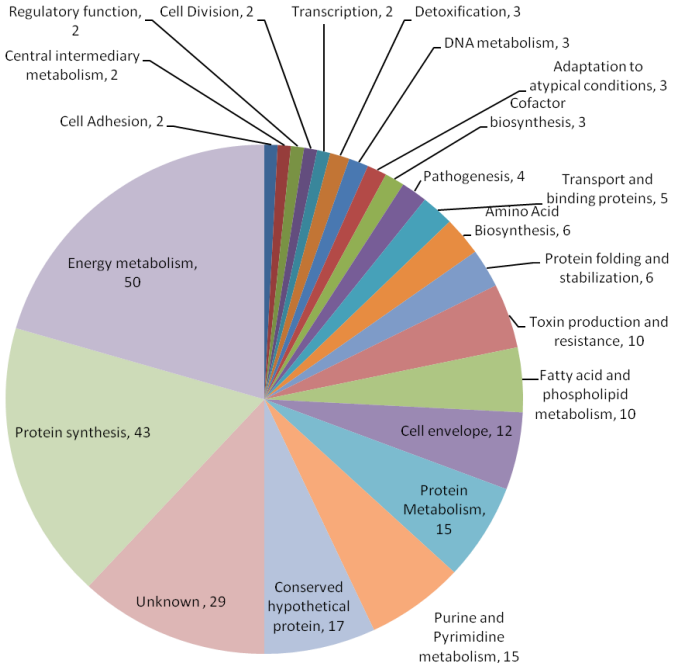

Figure 6. Gene ontology of HA-MRSA USA100 during stationary phase

The gene ontology of the other HA-MRSA known as USA200 during post-exponential phase is demonstrated in Figure 7. Most of the proteins expressed at this growth phase are involved in protein synthesis and energy metabolism.

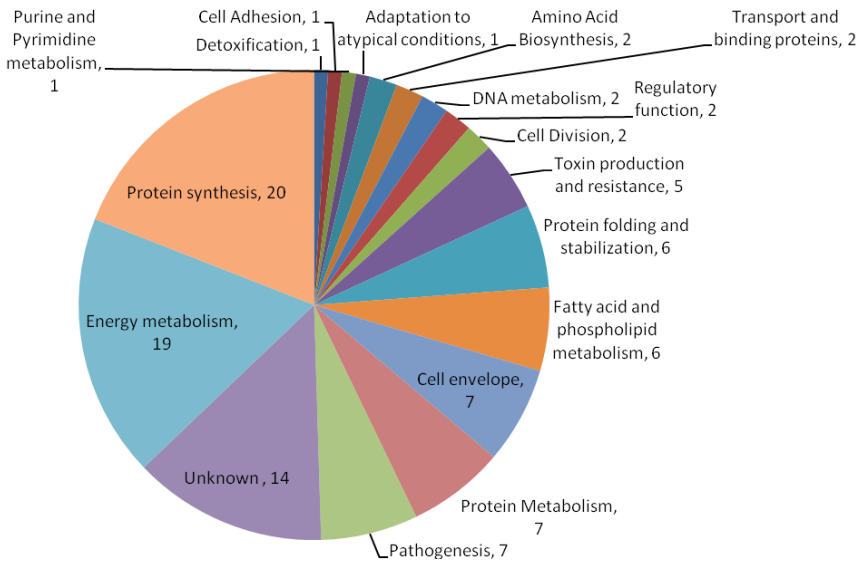

Figure 7. Gene ontology of HA-MRSA USA200 during post-exponential phase

The gene ontology of USA200 during stationary phase is demonstrated in Figure 8. It seems USA200 upregulates the production of proteins involved in purine and pyrimidine metabolism, toxin production, protein metabolism, and detoxification. These proteins would be expected during stationary phase as nutrients become scarce and *S. aureus* upregulates the production of proteins involved in creating a nutrient source.

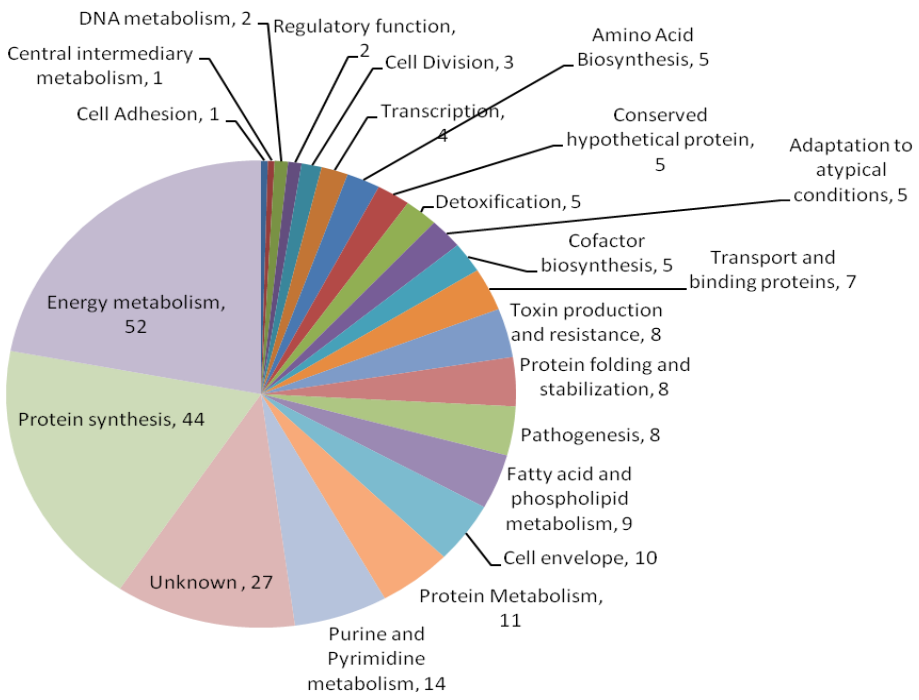

Figure 8. Gene ontology of HA-MRSA USA200 during stationary phase

Figure 9 outlines the gene ontology of the leading CA-MRSA strain known as USA300. The proteins identified are from post-exponential phase of growth. USA300 mainly expresses proteins involved in protein synthesis, energy metabolism, cell envelope, protein metabolism, protein folding, and other uncharacterized proteins, as well as those that are hypothetical conserved proteins.

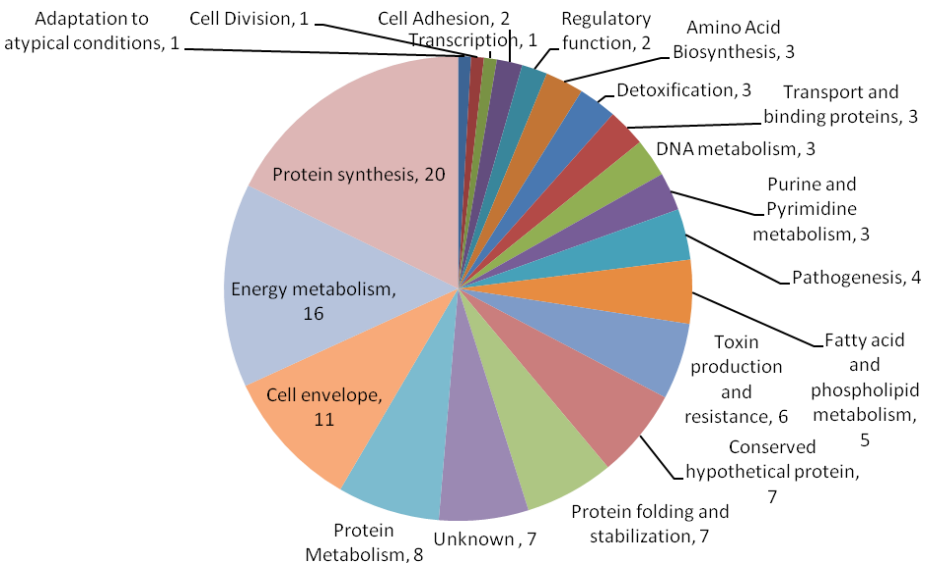

Figure 9. Gene ontology of CA-MRSA USA300 during post-exponential phase

During stationary phase, USA300 generally produces more proteins involved in energy metabolism and protein synthesis. Interestingly, USA300 significantly upregulates the production of certain conserved hypothetical proteins. Proteins involved in purine and pyrimidine metabolism, toxin production, and pathogenesis are generally upregulated during stationary phase (Figure 10).

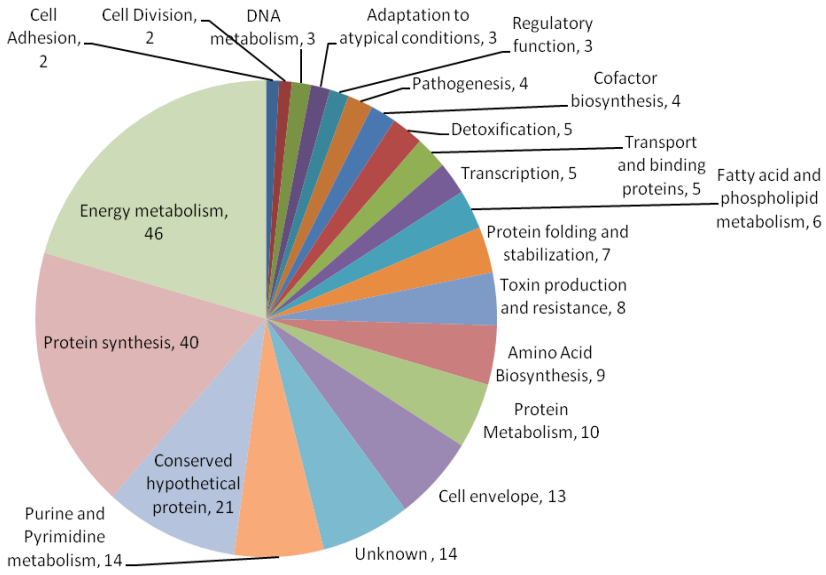

Figure 10. Gene ontology of CA-MRSA USA300 during stationary phase

The other CA-MRSA strain, USA400 seems to produce proteins involved in protein synthesis, energy metabolism, toxin production and resistance, protein metabolism, and in the cell envelope. There also seems to be a significant presence of proteins with unknown functions.

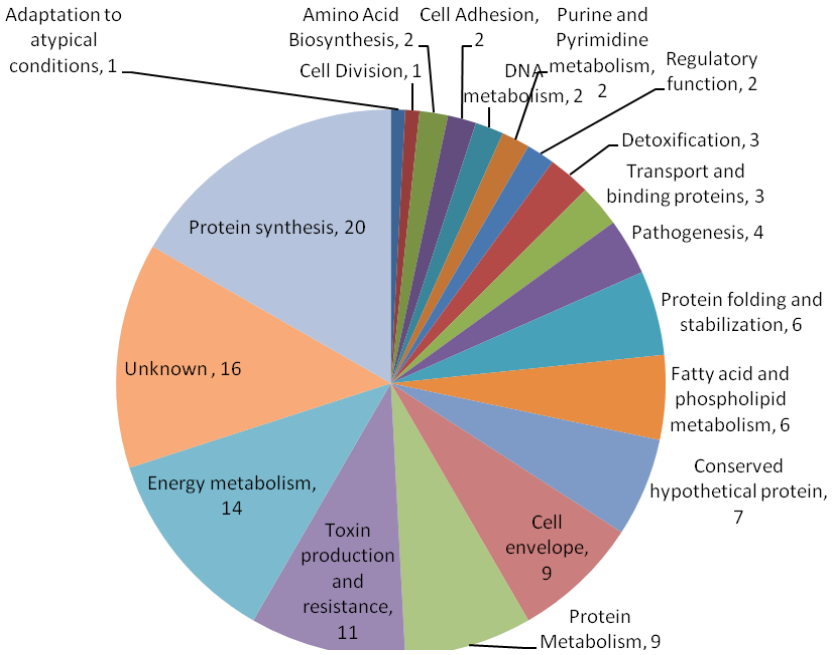

Figure 11. Gene ontology of CA-MRSA USA400 during post-exponential phase

After transitioning from post-exponential phase to stationary phase, USA400 upregulates the expression of even more proteins involved in protein synthesis, energy metabolism, and those with unknown functions. Also, proteins involved in toxin production and resistance, fatty acid and phospholipid metabolism, and purine and pyrimidine metabolism are also upregulated in the later phase of growth.

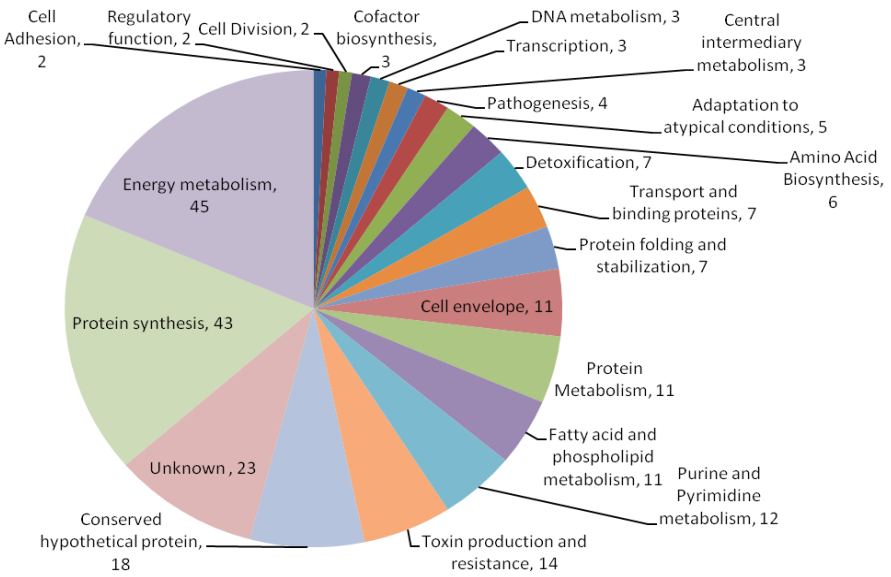

Figure 12. Gene ontology of CA-MRSA USA400 during stationary phase

Changes in the expression of major secreted proteins between two HA-MRSA strains during post-exponential growth. Analysis of the secreted proteins of HA-MRSA USA200 compared to the leading HA-MRSA USA100 strain at this time point revealed there were limited variations in protein levels. Seemingly, USA200 produces 6.8-fold more penicillin binding protein 2' from the *mecA* gene, at this time point when compared to USA100. Further to this, the foldase protein PrsA, which is involved in controlling the rate of protein folding [192], is expressed at levels 3.2-fold higher in USA200 when compared to USA100. The production of putative surface protein SA2285, however, is 20.34 times greater in USA100 than in USA200. This putative surface protein SA2285 is large (1370 aa) and contains domains involved in adhesion, and for the cleavage of human IgA, suggesting a role in pathogenesis [187]. Because toxin production begins during the post-exponential growth, not much variation would be expected at this time point.

Identified Proteins	Accession Number	P-Value	USA100	USA200
Putative surface protein SA2285	P61598|PLS_STAAN	0.0000073	1	0.049164184
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	0.000000000074	1	6.806270155
Foldase protein prsA	P60748|PRSA_STAAN	0.000035	1	3.236942617

Table 8. A comparison of the secretomes of USA100 and USA200 during the post-exponential phase of growth

Changes in the expression of major secreted proteins between two CA-MRSA strains during post-exponential growth. Table 9 depicts the significant changes in secreted protein production between the two CA-MRSA strains, USA300 and USA400. There is a downregulation (0.58-fold) of putative lipoprotein Q2FIT2 in USA300 when compared to USA400. Putative lipoprotein Q2FIT2 contains a potential signal peptide sequence and a conserved DM13 domain that possibly functions as a sugar kinase in bacterial two component systems [90]. On the other hand, the leading CA-MRSA strain USA300 upregulates the production of alpha hemolysin (1.8-fold), phenol soluble modulin (PSMα1) (3.4-fold), a putative uncharacterized protein SAUSA300_pUSA010004 (10.9-fold) that has no known homologs, and an uncharacterized protein Q2FFS8 that is homologous to a beta-lactamase protein (10.8-fold). The presence of thermonuclease, a heat stable DNase, did not change significantly in either strain.

Identified Proteins	Accession Number	P-Value	USA300	USA400
Alpha-hemolysin	Q2FHS2|Q2FHS2_STAA3	0.00018	1	0.550173608
Thermonuclease	Q2FIK2|Q2FIK2_STAA3	0.0016	1	1.219681925
Putative uncharacterized protein SAUSA300_pUSA010004	Q2FDE2|Q2FDE2_STAA3	0.025	1	0.091960838
Putative uncharacterized protein	Q2FFS8|Q2FFS8_STAA3	0.019	1	0.092562355
Putative lipoprotein	Q2FIT2|Q2FIT2_STAA3	0.042	1	1.712581503
Phenol-soluble modulin alpha 1 peptide	P0C7Y0|PSMA1_STAA3	0.046	1	0.306315112

Table 9. Secreted proteins of USA400 demonstrating significant changes in expression compared to USA300 during post-exponential phase of growth

Changes in the expression of major secreted proteins between HA-MRSA and CA-MRSA strains during post-exponential phase. The secreted proteins of the leading CA-MRA strain (USA300) are compared to HA-MRSA USA100 during post-exponential phase of growth, in Table 10. Overall, USA100 produces almost 52 times more putative surface protein SA2285 and 1.55 times more foldase. Immunodominant antigen A and SasD are both upregulated about 13-fold more in USA100 compared to USA300. The immunodominant antigen A, a probable transglycosylase (IsaA), contains a potential signal peptide for secretion and is likely involved in peptidoglycan turnover [170]. The Serine-aspartate repeat-containing protein D, also known as SasD is likely to have a signal peptide sequence as well as conserved domains involved in fibrinogen binding. Interestingly, the data provided in Table 10 correlates well with the literature concerning HA-MRSA and CA-MRSA. Surface proteins are expected to be downregulated in CA-MRSA due to hyperactivity of *agr* that negatively affects production of these proteins as it upregulates production of secreted toxins and proteases. It is unsurprising, therefore, that CA-MRSA strains such as USA300 do not produce surface-associated proteins as much as HA-MRSA strains such as USA100.

Identified Proteins	Accession Number	P-Value	USA100	USA300
Putative surface protein SA2285	P61598|PLS_STAAN	0.0000019	1	0.019251877
Probable transglycosylase isaA	P99160|ISAA_STAAN	0.030	1	0.075564941
Serine-aspartate repeat-containing protein D	Q7A780|SDRD_STAAN	0.0086	1	0.0732664
Foldase protein prsA	P60748|PRSA_STAAN	0.049	1	0.643330071

Table 10. Secreted proteins of USA300 demonstrating significant changes in expression compared to USA100 during post-exponential phase of growth

When compared to USA100 during post-exponential growth, USA400 significantly upregulates the expression of staphopain A (4.1-fold). Interestingly, this correlates with the observation that CA-MRSA strains have higher *agr* activity and therefore produce more secreted toxins and proteases than HA-MRSA [195]. USA400 also seems to produce more penicillin binding protein 2' (1.7-fold) when compared to USA100, but there is a marked downregulation of putative surface protein SA2285 in USA400 (0.04-fold).

Identified Proteins	Accession Number	P-Value	USA100	USA400
Putative surface protein SA2285	P61598|PLS_STAAN	0.00014	1	0.040001113
Staphopain A	P65826|SSPP_STAAN	0.0046	1	4.10146935
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	0.025	1	1.711120053

Table 11. Secreted proteins of USA400 demonstrating significant changes in expression compared to USA100 during post-exponential phase of growth

Production of penicillin binding protein 2' seems to be markedly decreased (0.35-fold) in the leading CA-MRSA strain USA300, when compared to the HA-MRSA USA200. This observation correlates with the finding that HA-MRSA strains seem to be more resistant to antibiotic treatment than CA-MRSA strains.

Identified Proteins	Accession Number	P-Value	USA200	USA300
Penicillin binding protein 2'	Q6GKQ7|Q6GKQ7_STAAR	0.047	1	0.353094018

Table 12. Secreted proteins of USA300 demonstrating significant changes in expression compared to USA200 during post-exponential phase of growth

CA-MRSA USA400 may produce more penicillin binding protein 2' than HA-MRSA USA100 (Table 12), but HA-MRSA USA200 produces 2.8-fold more of this protein than USA300. Because HA-MRSA strains are known to be more antibiotic resistant [127], it is expected to find a HA-MRSA strain such as USA200 producing significantly more penicillin binding protein 2' than a CA-MRSA strain such as USA300. The same relationship between HA-MRSA and CA-MRSA can be seen in Table 13 as USA200 produces 2.2-fold more penicillin binding protein 2' than USA400.

Identified Proteins	Accession Number	P-Value	USA200	USA400
Penicillin-binding protein 2'	Q6GKQ7|Q6GKQ7_STAAR	0.035	1	0.45315937

Table 13. Secreted proteins of USA400 demonstrating significant changes in expression compared to USA200 during post-exponential phase of growth

Changes in expression of major secreted proteins between two HA-MRSA strains during stationary phase. When compared to USA100, USA200 seems to produce significantly more zinc metalloprotease (5.52-fold), less lipase 1 (0.2-fold), and foldase (0.66-fold). Compared to the post-exponential phase of growth, there seems to be a decreased presence of foldase protein PrsA in USA200 during stationary phase.

Identified Proteins	Accession Number	P-Value	USA100	USA200
Zinc metalloproteinase aureolysin	Q7A378|Q7A378_STAAN	0.012	1	5.521879552
Lipase 1	P65289|LIP1_STAAN	0.040	1	4.922412009
Foldase protein prsA	P60748|PRSA_STAAN	0.045	1	1.512870373

Table 14. Secreted proteins of USA200 demonstrating significant changes in expression compared to USA100 during stationary phase of growth

Changes in expression of major secreted proteins between two CA-MRSA strains during stationary phase. There is a marked upregulation of phenol soluble modulin PSMα1 (16.1-fold), PSMα4 (2.5-fold), antibacterial protein SAUSA300_1067 (2.0-fold), known as PSMβ2, antibacterial protein SAUSA300_1068 (10.6-fold), known as PSMβ1, elastin-binding protein EbpS (4.8-fold), a putative cell wall surface anchor family protein (1.4-fold), and a CHAP domain family protein (5.1-fold) in USA400 when compared to USA300. It is predicted to have a signal peptide sequence and it also has a conserved domain involved in cell wall degradation. On the other hand, there is an upregulation of alpha hemolysin (4.7-fold), an ABC transporter substrate-binding protein (1.8-fold), and putative uncharacterized protein SAUSA300_pUSA010004 (7.8-fold) in USA300. Interestingly, PSMα1 is higher in USA300 in the post-exponential phase of growth than in USA400, however the reverse is true during post-exponential growth.

Identified Proteins	**Accession Number**	**P-Value**	**USA300**	**USA400**
Alpha-hemolysin	Q2FHS2|Q2FHS2_STAA3	0.0000000026	1	0.210747
Phenol-soluble modulin alpha 1 peptide	P0C7Y0|PSMA1_STAA3	0.0000018	1	16.10036
Antibacterial protein SAUSA300_1067	Q2FHR4|Q2FHR4_STAA3	0.0012	1	2.022183
CHAP domain family	Q2FIX4|Q2FIX4_STAA3	0.0016	1	5.090964
Elastin-binding protein ebpS	Q2FGW1|EBPS_STAA3	0.0033	1	4.839727
Putative uncharacterized protein SAUSA300_pUSA010004	|Q2FDE2_STAA3	0.0054	1	0.12851
Putative cell wall surface anchor family protein	Q2FE08|Q2FE08_STAA3	0.0074	1	1.362511
Antibacterial protein SAUSA300_1068	Q2FHR3|Q2FHR3_STAA3	0.017	1	10.55861
Phenol-soluble modulin alpha 4 peptide	P0C817|PSMA4_STAA3	0.022	1	2.464562
ABC transporter, substrate-binding protein	Q2FJ07|Q2FJ07_STAA3	0.029	1	0.552823

Table 15. Secreted proteins of USA400 demonstrating significant changes in expression compared to USA300 during stationary phase of growth

Changes in expression of major secreted proteins between HA-MRSA and CA-MRSA strains during stationary phase. Table 16 demonstrates changes in secreted protein production of CA-MRSA USA300 compared to HA-MRSA USA100. Parallel to transcriptomic studies comparing HA-MRSA and CA-MRSA, there is a significant upregulation of gamma (2.3-fold) and alpha hemolysins (12.7-fold) in USA300 compared to USA100. These observations are associated with a hyperactivity of *agr* in CA-MRSA strains compared to HA-MRSA strains. On the other hand, production of the putative surface protein SA2285 is 33.8 times greater in USA100.

Identified Proteins	**Accession Number**	**P-Value**	**USA100**	**USA300**
Putative surface protein SA2285	P61598|PLS_STAAN	0.00069	1	0.02960504
Gamma-hemolysin component C	Q7A3S2|HLGC_STAAN	0.030	1	2.30802049
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	0.032	1	12.6955356

Table 16. Secreted proteins of USA300 demonstrating significant changes in expression compared to USA100 during stationary phase of growth

There is an enormous upregulation of secreted toxins such as lipase 1 (10-fold), lipase 2 (4.6-fold), enterotoxin C (21.9-fold), the zinc metalloprotease aureolysin (1.8-fold), and elastin-binding protein EbpS (18.1-fold) in CA-MRSA USA400 when compared to HA-MRSA USA100. There is also a significant upregulation the SA2006 (8-fold) and SA0841 (5.1-fold) proteins in USA400. These two proteins contain conserved MAP domains involved in adherence.

Identified Proteins	Accession Number	P-Value	USA100	USA400
Lipase 2	Q7A7P2|LIP2_STAAN	0.0022	1	4.611289
Enterotoxin type C	P0A0L4|ENTC3_STAAN	0.0033	1	21.88768
Elastin-binding protein ebpS	Q7A5I6|EBPS_STAAN	0.0033	1	18.1368
Lipase 1	P65289|LIP1_STAAN	0.0047	1	10.02552
SA2006 protein	Q7A483|Q7A483_STAAN	0.020	1	8.034026
Zinc metalloproteinase aureolysin	Q7A378|Q7A378_STAAN	0.031	1	1.837579
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	0.040	1	5.091233

Table 17. Secreted proteins of USA400 demonstrating significant changes in expression compared to USA100 during stationary phase of growth

When comparing the production of secreted proteins between HA-MRSA USA200 and CA-MRSA USA300, it is evident USA200 produces significantly more zinc metalloprotease (3.6-fold) and a putative exported protein Q6GI28 (2.5-fold) than USA300. Putative exported protein Q6GI28 contains a predicted signal peptide sequence, and is homologous to a LytR transcriptional regulator. It also has a conserved domain putatively involved in transcriptional attenuation [77]. Production of gamma hemolysin component C, on the other hand, does not significantly change between USA200 and USA300.

Identified Proteins	Accession Number	P-Value	USA200	USA300
Zinc metalloproteinase aureolysin	Q6GDG5|Q6GDG5_STAAR	0.00038	1	0.279331
Putative exported protein	Q6GI28|Q6GI28_STAAR	0.014	1	0.394861
Gamma-hemolysin component C	Q6GE13|HLGC_STAAR	0.047	1	1.379588

Table 18. Secreted proteins of USA300 demonstrating significant changes in expression compared to USA200 during stationary phase of growth

Side by side, CA-MRSA USA400 produces significantly more elastin-binding protein EbpS (14.8-fold) and an uncharacterized protein SAR1965 (5.2-fold) than USA200. A signal peptide sequence is not been predicted for uncharacterized protein SAR1965, and

appears to be homologous to intracellular proteases. This finding is odd because cytoplasmic proteins are not expected to be identified in the supernatant with secreted proteins. HA-MRSA USA200, however, produces 14.45 times more anti-sigma factor B antagonist protein, also known as anti-anti sigma factor B, than USA400. The high presence of anti-sigma factor B antagonist in HA-MRSA USA100 would lead to higher SigmaB activity in this strain. Because SigmaB has been shown to have a repressive effect on *agr* activity [15] it is of no surprise CA-MRSA strain USA400, due to its hyperactive *agr* would produce SigmaB at significantly reduced levels. SigmaB, being a cytoplasmic protein, is not expected to be identified in the supernatant.

Identified Proteins	**Accession Number**	**P-Value**	**USA200**	**USA400**
Elastin-binding protein ebpS	Q6GGT1|EBPS_STAAR	0.00040	1	14.76076
Anti-sigma-B factor antagonist	Q6GF07|RSBV_STAAR	0.026	1	0.069202
Uncharacterized protein SAR1965	Q6GFI2|Y1965_STAAR	0.042	1	5.188532

Table 19. Secreted proteins of USA400 demonstrating significant changes in expression compared to USA200 during stationary phase of growth

Changes in expression of major secreted proteins of HA-MRSA strains from post-exponential phase to stationary phase. In the transition from post-exponential phase to stationary phase, it is apparent HA-MRSA USA100 upregulates the production of penicillin binding protein 2' (2.4-fold), putative surface protein SA2285 (9.2-fold), immunodominant antigen A (5.7-fold), and enterotoxin type C-3 (1.4-fold). On the other hand, proteins downregulated in USA100 from post-exponential phase include foldase (0.26-fold), lipase 1 (0.14-fold) and 2 (0.12-fold), alpha-hemolysin (0.03-fold), SA0841 protein (0.24-fold), and SA2006 protein (0.07-fold).

Identified Proteins	Accession Number	P-Value	USA100 5hr	USA100 15hr
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	0.0000014	1	2.427176057
Putative surface protein SA2285	P61598|PLS_STAAN	0.000055	1	9.192129976
Foldase protein prsA	P60748|PRSA_STAAN	0.0048	1	0.264551095
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	0.016	1	0.238025085
Lipase 1	P65289|LIP1_STAAN	0.021	1	0.140118214
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	0.02	1	0.028642902
Probable transglycosylase isaA	P99160|ISAA_STAAN	0.033	1	5.734707283
Lipase 2	Q7A7P2|LIP2_STAAN	0.0011	1	0.12382117
Enterotoxin type C-3	P0A0L4|ENTC3_STAAN	0.0012	1	1.40410766
SA2006 protein	Q7A483|Q7A483_STAAN	0.015	1	0.070417081

Table 20. Secreted proteins of USA100 demonstrating significant changes in expression from post-exponential to stationary phase

When switching from post-exponential phase to stationary phase, HA-MRSA USA200 clearly upregulates the production of penicillin binding protein 2' (11-fold). Lipase 1 and a putative exported protein (Q6GIA6), on the other hand, are downregulated in the stationary phase 0.4-fold and 0.6-fold, respectively. Putative exported protein Q6GIA6 is predicted to have a signal peptide sequence, and contains a MAP conserved domain putatively involved in cell adherence. Downregulation of this putative exporter protein can perhaps be associated with the fact that *S. aureus* cells transition from adhesion in the earlier phases of growth to toxin production in the stationary phase.

Identified Proteins	Accession Number	P-Value	USA200 5hr	USA200 15hr
Penicillin-binding protein 2'	Q6GKQ7|Q6GKQ7_STAAR	0.01135	1	10.90122846
Putative exported protein	Q6GIA6|Q6GIA6_STAAR	0.025	1	0.588479043
Lipase 1	Q6GDD3|LIP1_STAAR	0.042	1	0.399557859

Table 21. Secreted proteins of USA200 demonstrating significant changes in expression from post-exponential to stationary phase

Changes in expression of major secreted proteins of CA-MRSA strains from post-exponential phase to stationary phase. Numerous proteins have been found to be significantly changed in CA-MRSA USA300 during the transition from post-exponential phase to stationary phase. A few proteins including, 50S ribosomal protein L11 (1.8-fold), penicillin binding protein 2' (29.6-fold), immunodominant antigen A (9.5-fold), antibacterial protein Q2FHR3 (2.1-fold), and a putative cell wall surface anchor family protein (1.6-fold), were prevalent during stationary growth of USA300. Proteins that were significantly downregulated in stationary phase include: adenylate kinase (0.09-fold), alpha hemolysin (0.2-fold), antibacterial protein Q2FHR4 (0.5-fold), fructose bisphosphate aldolase (0.04-fold), Panton-Valentine leukocidin LukS (0.05-fold), peptidoglycan hydrolase (0.04-fold), phenol soluble modulin PSMα1 (0.02-fold), putative lipoprotein (0.05-fold), putative uncharacterized protein SAUSA300_pUSA010004 (0.2-fold), putative uncharacterized protein SAUSA300_1759 (0.03-fold), putative uncharacterized protein SAUSA300_2164 (0.2-fold), serine protease SplB (0.4-fold), and triacylglycerol lipase (0.3-fold). There was no significant change in thermonuclease production between the two phases. The presence of 50S ribosomal protein L11, adenylate kinase, and fructose bisphosphate is unexpected because these are cytoplasmic proteins and should not be identified in the supernatant as secreted proteins.

Identified Proteins	**Accession Number**	**P-Value**	**USA300 5hr**	**USA300 15hr**
50S ribosomal protein L11	Q2FJA3|RL11_STAA3	0.026	1	1.797912631
Adenylate kinase	Q2FER0|Q2FER0_STAA3	0.0138	1	0.086472995
Alpha-hemolysin	Q2FHS2|Q2FHS2_STAA3	1.07517E-05	1	0.154783038
Antibacterial protein	Q2FHR4|Q2FHR4_STAA3	0.009	1	0.543067428
Antibacterial protein	Q2FHR3|Q2FHR3_STAA3	0.033	1	2.0973797
Fructose bisphosphate aldolase	Q2FF03|Q2FF03_STAA3	0.01485	1	0.039119603
Panton-Valentine leukocidin, LukS	Q2FGU9|Q2FGU9_STAA3	0.024	1	0.049059483
Penicillin-binding protein 2'	Q2FKM6|Q2FKM6_STAA3	1.4E-10	1	29.55802797
Peptidoglycan hydrolase	Q2FJZ4|Q2FJZ4_STAA3	0.039	1	0.039707336
Phenol-soluble modulin alpha 1 peptide	P0C7Y0|PSMA1_STAA3	0.014	1	0.018357338
Probable transglycosylase isaA	Q2FDT8|ISAA_STAA3	0.016	1	9.526965248
Putative cell wall surface anchor family protein	Q2FE08|Q2FE08_STAA3	1.1E-14	1	1.56173773
Putative lipoprotein	Q2FIT2|Q2FIT2_STAA3	0.015	1	0.048020221
Putative uncharacterized protein SAUSA300_1759	Q2FFS8|Q2FFS8_STAA3	0.019	1	0.026126756
Putative uncharacterized protein SAUSA300_2164	Q2FES8|Q2FES8_STAA3	0.031	1	0.209015255
Putative uncharacterized protein SAUSA300_pUSA010004	Q2FDE2|Q2FDE2_STAA3	0.0048	1	0.115393064
Serine protease splB	Q2FFT0|SPLB_STAA3	0.00435	1	0.413303495
Thermonuclease	Q2FIK2|Q2FIK2_STAA3	0.000636	1	0.848936408
Triacylglycerol lipase	Q2FDJ1|Q2FDJ1_STAA3	0.0046	1	0.334270278

Table 22. Secreted proteins of USA300 demonstrating significant changes in expression from post-exponential to stationary phase

When switching from post-exponential growth to stationary growth, CA-MRSA USA400 upregulates the production of surface protein MW2416 (1.6-fold) which is putatively

involved in cleaving human IgA and in adhesion, enterotoxin type C (5.8-fold), and penicillin binding protein 2'(49.5-fold). Proteins downregulated by USA400 during the stationary phase include: lipase 1 (0.5-fold) and 2 (0.2-fold), MW1056 protein (0.1-fold), MW0863 protein (0.5-fold), MW2130 protein (0.4-fold), and alpha hemolysin (0.002-fold). MW0863 and MW2130 proteins contain conserved MAP domains and are homologous to cell adherence proteins.

Identified Proteins	**Accession Number**	**P-Value**	**USA400 5hr**	**USA400 15hr**
Lipase 2	Q8NYC2|LIP2_STAAW	0.00044	1	0.230035026
Lipase 1	Q8NUI5|LIP1_STAAW	0.0043	1	0.453603588
ENTEROTOXIN TYPE C	Q8NXJ6|Q8NXJ6_STAAW	0.020833	1	5.7943177
MW1056 protein	Q8NX40|Q8NX40_STAAW	0.017	1	0.132835969
MW0863 protein	Q8NXE3|Q8NXE3_STAAW	0.019	1	0.518113878
MW2130 protein	Q7A090|Q7A090_STAAW	0.024	1	0.368062689
Putative surface protein MW2416	Q8NUV0|PLS_STAAW	0.032	1	1.554528324
Penicillin binding protein 2'	Q7A209|Q7A209_STAAW	0.019	1	49.50848166
Alpha-Hemolysin	Q8NX49|Q8NX49_STAAW	0.00089	1	0.001530173

Table 23. Secreted proteins of USA400 demonstrating significant changes in expression from post-exponential to stationary phase

Discussion

Proteomics, the identification of entire protein sets present in biological samples, is a useful complementary experimental approach to genomics and transcriptomics. Proteomics has become an extremely useful tool for the study of differential protein expression, gaining significance due to highly dynamic protein expression profiles. Understanding changes in a given proteome from a particular organism can provide much insight into its behavior, physiology and interaction with its environment. Most of the proteomic studies published thus far involve 2D gel electrophoresis with very little emphasis on proteomic analysis of complex protein mixtures. For a proteomic study, it is necessary to maximize protein concentration in order to facilitate the identification of proteins after mass spectrometric analysis. It is also necessary to devise a method that can be easily employed and provide reproducible results. Several published proteomic studies of *S. aureus* involved methods that have not been optimized to provide comprehensive coverage of the proteome. Previous studies have been fraught with problems, including the use of a urea concentration that is too low for the solubilization of insoluble proteins; a lack of filter sterilization for supernatants to remove bacterial cells and ensure purity of secreted proteins; and secreted proteins centrifuged during precipitation at a speed too low to ensure maximal protein recovery [176, 206, 208]. Appropriate method refinement is therefore important for obtaining reproducible data across several biological replicates of a sample. For the extraction of cytoplasmic proteins, we chose to compare various cell lysis techniques and solubilization buffers.

We also focused on concentration, precipitation, and washing of secreted proteins for a high yield of protein and subsequent mass spectrometric analysis. In addition, apart from traditional 1D or 2D gel electrophoresis, we performed in-solution trypsin digestion of extracted proteins followed by mass spectrometry.

After our refinement of the methods for extracting cytoplasmic and secreted proteins, we used these newly developed tools to catalogue the intracellular proteome and secretome of a commonly used and well studied lab strain of *S. aureus* known as SH1000 [86]. We focused on proteome coverage at two different phases of growth: post-exponential phase and stationary phase. We did this in order to provide an insight into the physiology of *S. aureus* and how it adapts to its changing environment over time by utilizing differential protein synthesis. From our study we found that there is a clear prevalence of ribosomal proteins, involved in protein synthesis, during post-exponential growth when compared to SH1000 during stationary phase; likely due to the scarcity of nutrients in the later growth phase, resulting in a reduction of translation occurring within the cell. In a study by Becher et al., several subproteomic fractions of a *S. aureus* strain known as COL were analyzed by mass spectrometry [10]. When studying the change of cytoplasmic proteins of COL in the transition from post-exponential to stationary phase, Becher et al. also found ribosomal proteins were no longer being synthesized, and were potentially degraded in stationary phase [10].

Moreover, in our study, the glycolytic enzymes glyceraldehyde 3-phosphate, fructose bisphosphate aldolase, phosphoglycerate kinase, phosphoglycerate mutase, and enolase are generally found to be downregulated in stationary phase when compared to post-exponential phase. This would perhaps be expected as the availability of carbon sources

becomes limited during stationary growth, and as such, enzymes involved in the catabolism of glucose would no longer be required in the absence of primary carbon sources. On the other hand, proteins involved in the tricarboxylic acid (TCA) cycle, such as aconitate dehydrogenase, isocitrate dehydrogenase, components of the 2-oxoglutarate dehydrogenase complex, components of succinate dehydrogenase, fumarate hydratase, and malate dehydrogenase are upregulated during stationary phase growth of *S. aureus*. This could result from the catabolism of any remaining pyruvate molecules generated by glycolysis during post-exponential phase. Also, phosphoenolpyruvate carboxykinase (PckA), a protein involved in gluconeogenesis, is upregulated in the stationary phase of SH1000 and was not detected in post-exponential growth phases. This observation concurs with the 2009 study by Becher et al., where it was suggested that the presence of PckA could be indicative of carbon source starvation, as expected in stationary phase, when nutrients become scarce [10].

In addition to proteins involved in carbon utilization, the production of Clp protease subunits is also higher in stationary phase than in the post-exponential phase. These proteases, which respond to heat, osmotic, and oxidative stresses, enable *S. aureus* to survive in these situations and they tend to accumulate in stationary phase [26, 27,62]. Furthermore, according to our data, anti-sigma factor B antagonist protein is upregulated in the stationary phase whilst the anti-sigma factor of Sigma B known as RsbW is downregulated in the stationary phase. This correlates with reports that Sigma B activity is upregulated in stationary phase [62, 112]. Specifically, the anti-sigma factor B antagonist, also known as anti-anti-sigma factor RsbV, functions to liberate Sigma B

from its anti-sigma factor, RsbW [134], to regulate the transcription of certain genes in stationary phase [111].

When analyzing the secretome of SH1000 in the transition from post-exponential to stationary phase, there is a clear upregulation of toxins and secreted proteases in the later phase of growth. These proteins include alpha hemolysin [146], gamma hemolysin [49], staphopain A and B [25, 99, 203], delta hemolysin [146], serine proteases SplB and SplC [161], PSMα1, PSMβ1, and PSMβ2 [159]. Interestingly, some of these secreted proteins are not detected at all during the post-exponential phase of growth. Upregulation of these secreted proteins is expected in stationary phase of growth due to increased *agr* activity as a direct result of higher cell density [139, 152].

Agr is a quorum sensing, two-component regulator that is expressed in the post-exponential phase of *S. aureus* growth. During the post-exponential phase, *agr* represses surface and attachment proteins and induces the transcription of secreted toxins and proteases [202]. As the population density of *S. aureus* increases, it secretes an auto-inducing peptide that accumulates in the extracellular environment. When *S. aureus* senses the concentration of the AIP has reached a threshold level it induces the transcription of virulence determinants, by the use of an effector molecule, as a response to stress such as nutrient limitation and high population density during stationary growth [139,152]. The *agr* effector molecule is a small regulatory RNA, known as RNAIII, which acts as a regulator of target genes [91, 145] by binding to target mRNA molecules and either stabilizing them or targeting them for destruction [135, 113, 186]. RNAIII facilitates the upregulation of secreted virulence factors [18, 66], and negatively regulates the synthesis of surface proteins, such as protein A and the fibronectin-binding proteins,

which are used for adhesion [91, 143, 171]. Therefore, the upregulation in production of secreted toxins and proteases such as alpha hemolysin, delta hemolysin, gamma hemolysin, staphopains A and B, delta hemolysin, serine proteases SplB and SplC, the phenol-soluble modulins (PSMα1, PSMβ1, and PSMβ2) is indicative of *agr* activity, which is expected in stationary phase. We also observed accumulation of lipase 1 and 2 with the onset of stationary growth. Though it is not clear if the transcription of these enzymes is regulated by *agr*, they are found to be downregulated in *agr* mutants of *S. aureus* [95]. From this observation, it is possible to speculate the potentially positive effect of *agr* activity on the production of lipases 1 and 2.

Though the production of *agr*-regulated proteins is increased in stationary phase; overall *agr* activity would ultimately be expected to be reduced in the strain SH1000. SH1000 is a strain that descended from another *S. aureus* strain known as 8325-4, whose Sigma B activity is diminished by a natural deletion in one of its positive effectors (*rsbU*). SH1000 was derived from 8325-4 via a full restoration of this deletion, and thus Sigma B activity [86]. Because Sigma B is an antagonist of *agr*, *agr*-regulated secreted toxins are downregulated in SH1000 as opposed to 8325-4 and other strains of *S. aureus* [15, 68, 86]. In accordance with this observation, production of the *agr*-regulated protein V8 protease appears to be diminished in SH1000. In our findings, this protein was not detected in the post-exponential phase and was only detected at very low levels in stationary growth, when *agr* activity is at its highest. Furthermore, staphyloxanthin, an orange carotenoid pigment of *S. aureus* [111], is highly pronounced in strain SH1000 [86] due to Sigma B activity [111]. Our data indicate that staphyloxanthin biosynthesis protein is greatly upregulated from post-exponential phase to stationary phase. This

finding concurs with the observation that cultures of SH1000, and therefore the resulting cell pellets, acquire a darker orange color later in growth when Sigma B activity is upregulated [86].

Having determined the reproducibility of our protein extraction protocols, we next set out to characterize the secretomes of clinically relevant strains of *S. aureus*. Specifically, two HA-MRSA (USA100 and USA200) and two CA-MRSA (USA300 and USA400) strains were analyzed via quantitative methods at post-exponential and stationary phases of growth. We focused on these growth phases in particular because toxins are produced beginning in the post-exponential phase and accumulate throughout stationary phase. We undertook a complete proteomic analysis of differential protein production in the secretomes of these clinically significant strains using the popular quantification technique iTRAQ [47]. After extraction of the secretomes, proteins were digested with the protease trypsin to produce peptides. The resulting peptides were isobarically labeled using iTRAQ reagents for mass spectrometric analysis of three biological replicates for subsequent identification and relative quantification of the proteins secreted by these four strains. With this approach we sought to identify and also quantify the production of secreted proteins that enable this pathogen to swiftly infect and cause disease in patients.

When comparing HA-MRSA USA100 to CA-MRSA USA300 during post-exponential phase of growth, it is evident USA100 produces more surface-associated proteins than USA300. Putative surface protein SA2285, serine-aspartate repeat-containing protein D, and immunodominant antigen A, are both significantly upregulated in the HA-MRSA strain USA100. Immunodominant antigen A, involved in peptidoglycan hydrolysis [48], has been identified as a secreted and cell wall associated protein [170]. A study by

Dubrac et al. in 2007 found that transcription of the *isaA* gene, encoding the immunodominant antigen A protein, is directly activated by the essential two-component system known as WalKR (also known as YycFG) [48]. Interestingly, the activity of WalKR, is upregulated during colonization of human nares in carriers of *S. aureus.* Seemingly, colonizing *S. aureus* preferentially expresses proteins involved in adherence to tissues, while at the same time downregulating the production of secreted toxins [21, 22]. Interestingly, *agr* does not appear to be active during nasal colonization, therefore *S. aureus* would be expected to increase production of surface proteins and decrease production of secreted toxins and proteases that are regulated by *agr*, concurring with the observation of increased WalKR activity during nasal colonization. Therefore, it is unsurprising to find a surface protein such as immunodominant antigen A to be significantly upregulated in a HA-MRSA strain such as USA100 where *agr* is not as active as it is in a CA-MRSA strain such as USA300.

On another note, many surface proteins covalently linked to the cell wall require a peptide sorting signal, containing an LPXTG motif located at the C-terminus of the protein [175]. Proteins containing the LPXTG motif are often attached to the cell wall by a protease known as sortase [126, 140]. Instead of the traditional LPXTG motif contained by many surface proteins of *S. aureus*, putative surface protein SA2285 and serine-aspartate repeating protein D, known as SdrD, both contain YSIRK signal peptides [38]. YSIRK is a variation of the peptide sequence that marks a protein destined for the cell wall to be secreted in a ring-like manner near the site of cell division [38]. Proteins containing the YSIRK motif have been associated with adhesion to bones during infection [187]. A study by Trad et al. found a correlation between the prevalence of the

sdrD gene and strains of *S. aureus* that commonly cause bone infections [187]. In fact, a study by Sabat et al. found that a mutation in *sdrD* and *sdrE*, a gene tandemly encoded with *sdrD* in the *sdr* locus, resulted in significantly decreased potential for *S. aureus* to cause bone infections such as osteomyelitis [169]. Interestingly, HA-MRSA strains are known to commonly cause chronic infections such as osteomyelitis [108, 119] whereas CA-MRSA strains are known to cause acute infections such as skin and soft tissue diseases [24, 94, 103]. It is no surprise then that a HA-MRSA strain such as USA100 upregulates the production of surface associated proteins such as immunodominant A, SdrD, and protein SA2285 that could enable the pathogen to infect the host and lead to a chronic disease. Though it is not known whether these surface associated proteins are *agr*-regulated, this observation concurs with the fact that CA-MRSA strains such as USA300, having higher *agr* activity, would therefore express less surface associated proteins than HA-MRSA strains such as USA100 [116, 119].

When comparing HA-MRSA USA100 to CA-MRSA USA400 in the post-exponential phase of growth we observed that USA100 produced 25 times more putative surface protein SA2285 than USA400. As stated earlier, because *agr* activity is expected to be decreased in HA-MRSA than in CA-MRSA, the expression of surface-associated proteins would be upregulated in HA-MRSA as *agr* is a negative effector of surface proteins during the later stages of bacterial growth [116, 119]. On the same note, *agr* is a positive effector on the presence of proteases such as staphopain A [117], which is produced in elevated quantities in CA-MRSA USA400 compared to HA-MRSA USA100 in our study. In a 2007 study by Vincents et al., the extracellular proteases staphopain A and staphopain B were found to have a role in the downregulation of human cystatin

activity [191]. Cystatins are a family of cysteine protease inhibitors that potentially protect against proteases secreted by invading pathogenic microorganisms. Though body fluids may have high concentrations of these protease inhibitors, they are still vulnerable to attack by other proteases [188]. These proteases, such as staphopain A, may have a role in evasion of the host immune system allowing *S. aureus* to survive and cause disease in the individual. Additionally, it was shown by Imamura et al. in a 2005 study that staphopain A can instigate vascular leakage consequently leading to septic shock in affected individuals [88]. Because CA-MRSA strains are not only known for causing cutaneous infections but also sepsis [63, 110, 177], our observation of elevated Staphopain A production in these strains appears to fit with previous studies on this enzyme.

All strains of *S. aureus*, regardless of antibiotic resistance, have penicillin binding proteins known as PBP1, PBP2, and PBP3. These penicillin binding proteins, having high affinity for β-lactam antibiotics, are bound by these antimicrobial agents, in turn compromising the integrity of the cell wall by preventing cross-linking across the layers of peptidoglycan [122]. PBP2A, encoded by the *mecA* gene, has a low affinity for β-lactams, therefore it is not targeted by these antibiotics; consequently preventing weakening of the cell wall. Resistance to β-lactam antibiotics in CA-MRSA, however, is conferred by PBP4 [131], a β-lactamase [141]. On the other hand, loss of PBP2A, also known as penicillin binding protein 2', in HA-MRSA reduces antibiotic resistance whereas loss of PBP4 in HA-MRSA strains has little effect on antibiotic resistance [101]. Therefore, it would be expected to detect penicillin binding protein 2' in much higher quantities in HA-MRSA strains of *S. aureus* than in CA-MRSA. Our data suggests that

this is true as there is a clear upregulation of penicillin binding protein 2' in HA-MRSA USA100 compared to CA-MRSA strains USA300 and USA400.

Overall, toxin production in *S. aureus* is dramatically upregulated during stationary phase [18, 66]. When compared side by side, the production of gamma hemolysin component C and alpha hemolysin are drastically upregulated in CA-MRSA USA300 as opposed to HA-MRSA USA100. As stated earlier, *agr* activity in CA-MRSA strains is expected to be higher than in HA-MRSA strains. This increase in *agr* activity leads to elevated expression of major secreted virulence factors, such as gamma and alpha hemolysins [18, 66, 75, 133]. In a 2010 study by Pang et al., *agr* activity of a USA300 strain was measured by RT-PCR after it had been phagocytosed by polymorphonuclear neutrophils (PMN). PMNs are white blood cells used by the host immune system to ingest and degrade invading pathogenic microorganisms. A USA300 strain containing an *agr* mutation lost significant viability within the PMN as opposed to the USA300 wild type strain containing an intact and fully functional *agr* [147]. It was found that once inside the PMN, *agr* activity by USA300 increased significantly, leading to an increased production of α-hemolysin. This upregulation of α-hemolysin by USA300 in part was responsible for the lysis of PMNs, allowing for evasion of the host immune system, as opposed to a USA300 *agr* mutant strain [147]. Our data concurs with the findings by Pang et al. at the proteomic level, by showing CA-MRSA USA300 upregulates the production of alpha-hemolysin and other toxins due to a higher *agr* activity compared to a HA-MRSA strain such as USA100, where *agr* activity is diminished, and therefore the production of alpha hemolysin and other secreted virulence factors is reduced.

S. aureus produces superantigens such as enterotoxins, which are known to result in gastroenteritis caused by food poisoning [7, 115]. As reported by Baba et al., staphylococcal enterotoxin C is encoded on a pathogenicity island [6], and is not typically found to be expressed in HA-MRSA strains [58]. Unsurprisingly, as shown by our data, CA-MRSA USA400 produced 21.88 times more enterotoxin C than HA-MRSA USA100. In a case report from 2002, an outbreak of gastroenteritis occurred within a family that was caused by a CA-MRSA strain producing enterotoxin C. This report became the first case of CA-MRSA as the sole culprit of a gastroenteritis outbreak [96]. In addition, CA-MRSA USA400 has been found to express the collagen adhesion gene (*cna*), which is associated with increased binding of *S. aureus* to host membrane proteins for the pathogenesis of necrotizing pneumonia [6, 43, 83]. Interestingly, when compared to HA-MRSA USA100, the production of surface proteins such as elastin binding protein, SA2006 protein, and SA0841 involved in adhesion is dramatically upregulated in USA400. Elastin binding protein is also drastically upregulated in USA400 when compared to HA-MRSA USA200, as shown by our data. Not surprisingly, USA400 is known to be a strong former of biofilms [97]. It is possible to speculate that these adhesion proteins may have a strong role in biofilm formation, and perhaps explain the elevated levels of this aggregation phenotype in USA400 strains [95].

Additionally, when compared to USA100 during stationary phase, USA400 also significantly upregulates lipase 1 and lipase 2. In a recent study [95] both lipase 1 and lipase 2, which facilitate tissue invasion [167], were found in low quantities in an *agr* mutant of *S. aureus* [95]. Likewise, a study of HA-MRSA and CA-MRSA strains from Brazil showed that toxins such as enterotoxin C and PVL were rarely detected in HA-

MRSA isolates [181]. According to our study, the production of lipase 1 and lipase 2 is remarkably higher in CA-MRSA strains than HA-MRSA strains. Further to this, in a study by Abdelnour et al., an *agr* mutation in *S. aureus* led to a marked reduction of toxin and enzyme production, including the production of lipases [4]. In a mouse model of septic arthritis infection, an *agr* mutant of *S. aureus* was significantly less virulent than the wild-type counterpart [4]. Also, lipase was found to be important in preventing the phagocytic killing of *S. aureus* after engulfment by granulocytes, also known as polymorphonuclear neutrophils [165]. This observation indicates that lipases can be considered virulence factors, which may be responsible for the pathogenesis of *S. aureus*. As mentioned earlier, having higher *agr* activity, CA-MRSA strains express *agr*-regulated proteins such as lipases 1 and 2 significantly more than HA-MRSA, potentially contributing to its hypervirulence.

Conversely, the high presence of anti-sigma factor B antagonist, also known as anti-anti-sigma factor B, in HA-MRSA USA100 would lead to higher SigmaB activity in this strain. Because SigmaB has been shown to have a repressive effect on *agr* activity [15], it is of no surprise CA-MRSA strain USA400, due to its hyperactive *agr* would produce SigmaB at reduced levels. Though the presence of an intracellular protein such as anti-sigma factor B antagonist in the secretome of *S.aureus* may be puzzling, the resulting quantitation of this protein is exactly what is expected when comparing HA-MRSA and CA-MRSA strains. As indicated by our data, there is an astonishing 14.5 increase of anti-sigma factor B antagonist production in the HA-MRSA USA200 compared to CA-MRSA USA400. Anti-sigma factor B antagonist is a positive effector of Sigma B activity. The positive effect of Sigma B on surface proteins contributes to adhesion of the

cell to host tissue, whilst at the same time negatively impacting the production of secreted virulence factors when *S. aureus* enters stationary phase [206, 208]. As expected, Sigma B activity is reduced in CA-MRSA, as these strains of *S. aureus* are known for the increased secretion of virulence factors, such as toxins and proteases.

The secretion of the zinc metalloprotease aureolysin was significantly upregulated in the CA-MRSA strain USA400 when compared to the HA-MRSA strain USA100. Because this protease is positively regulated by *agr* [30, 49, 183], it is expected to be highly expressed in strains with a hyperactive *agr* regulon, such as a CA-MRSA isolates. Indeed, we have conducted assays in our laboratory to determine the presence of secreted proteases such as aureolysin in HA-MRSA and CA-MRSA strains. A total of 24 USA100 strains and 158 USA300 strains were studied, and it was determined that the CA-MRSA USA300 isolates produced far more secreted proteases than HA-MRSA USA100 isolates (Rivera and Shaw, unpublished observation). This observation correlates with the fact that CA-MRSA strains of *S. aureus*, having higher *agr* activity, secrete significantly more proteases than HA-MRSA strains.

Because most of the scientific studies involving HA-MRSA and CA-MRSA strains are at the genomic and transcriptomic level, our study aimed to compare clinically significant strains of *S. aureus* currently afflicting individuals in healthcare facilities and in the community at the proteomic level. The reproducibility of protein extraction and iTRAQ-labeling followed by mass spectrometric analysis can be used to corroborate present literature and to speculate and answer relevant biological questions. With our study, we found many differences in protein secretion amongst the four clinical strains that support studies of these proteins at the genomic and transcriptomic levels. Overall, we identified

many secreted toxins and proteases that were upregulated in hypervirulent CA-MRSA strains such as USA300 and USA400, typical of increased *agr* activity. On the same token, we found surface-associated proteins to be upregulated in HA-MRSA such as USA100 and USA200, concurring with the finding that *agr* activity in these particular strains is diminished in comparison to CA-MRSA strains.

Consideration of the presence of cytoplasmic proteins in secreted fractions. Even after having refined methods for the extraction of secreted proteins from supernatants of *S. aureus* cultures, contamination by cytoplasmic proteins is a common problem. From our study in particular, we have identified several classes of cytoplasmic proteins in the supernatants of *S. aureus,* such as those involved in central carbon metabolism and protein synthesis. Though the identification of cytoplasmic proteins in the extracellular milieu of *S. aureus* would be unexpected, a few cytoplasmic proteins have been discovered existing as secreted proteins by "non-classical" protein secretion methods. In a study by Pasztor et al., a wild-type strain of *S. aureus* known as strain 22 secreted cytoplasmic proteins into the supernatant, whereas secretion of cytoplasmic proteins in an *atl* (coding for major autolysin) mutant was attenuated [151]. In addition, the presence of cytoplasmic proteins, clearly missing a signal peptide sequence for secretion, has been observed in the culture supernatants of *Bacillus subtilis* and *S. aureus* [179, 184, 206]. Because the most abundant cytoplasmic proteins are not necessarily seen in supernatants, it is possible the cell secretes these proteins by as yet unknown mechanisms, instead of their being present in supernatants as a result of cell lysis. The study by Pasztor et al., found the protein major autolysin to be responsible for the secretion of a common cytoplasmic protein, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [1451]. As

another example, enolase was found to have a role in *S. aureus* adhesion by binding to human laminin and plasminogen [73]. It is thus possible for cytoplasmic proteins, consistently identified as secreted proteins, to have extracellular functions. Because surface-associated proteins mediate host-bacterial interactions, these newly discovered "secreted proteins" could serve as novel antigens, and lead to development of new antimicrobial treatment that could perhaps alleviate the rampant spread of the extremely adaptive and pathogenic *S. aureus*.

Future Directions

With our currently refined method for the extraction of secreted proteins, it is possible we are excluding certain proteins with low molecular weight. The Centricon columns used for the concentration of secreted proteins have a cutoff of 5 kDa. Any protein with a molecular weight less than 5.0 kDa may not be retained by the concentration column, and could thus be omitted from subsequent mass spectrometric analysis. The molecular weights of phenol soluble modulins produced by *S. aureus,* for example, range from 2.17 kDa to 4.5 kDa. Phenol soluble modulins, being important virulence factors of *S. aureus,* which are highly expressed in clinical strains, could potentially be omitted from a proteomic study if Centricon columns are used for the concentration of secreted proteins. The method could be further refined by excluding the concentration step altogether and precipitating secreted proteins from the supernatant with TCA immediately after filter sterilization. Though, with this revision, it is possible to sacrifice overall protein concentration, it could provide full coverage of all the proteins secreted by *S. aureus* at a particular growth phase, including proteins with low molecular weights.

Another important modification of the refined method for extraction of secreted proteins involves fractionation of the sample prior to MS analysis. Our first MudPIT analysis of SH1000 cytoplasmic proteins produced the highest yield of identified proteins. This first MudPIT analysis involved the fractionation of the complex sample using the HPLC. Due to the inconsistent results from HPLC fractionation, we resorted to gas fractionation of the samples. Gas fractionation, however, yielded low protein identifications compared to

HPLC fractionation. Ideally, we would like to fractionate samples using a new HPLC for future proteomic studies.

The next step, after having analyzed the secretomes of HA-MRSA and CA-MRSA strains, would be comparing and analyzing the surface-associated proteomes, known as the "surfome", as well as the membrane-associated proteins, of these same clinical strains. To date, proteomic studies of surface-exposed proteins have been completed using only CA-MRSA strains of *S. aureus*. A proteomic analysis of surface-associated proteins produced by CA-MRSA compared to HA-MRSA could lead to a better understanding of any difference in the pathogenesis amongst these strains. Apart from adhesion to the host tissue, surface-associated proteins can also mediate evasion of the host immune system and invasion of host cells [60, 142]. Though surface-associated proteins have consistently been identified in the secretome, a proteomic method targeted to the surface of the cell could lead to the identification of even more surface-associated proteins that could potentially be specific to a given lineage. Understanding any differences in surface proteins associated with adhesion of the cell to tissue or in-dwelling devices amongst clinical strains could lead to a better understanding of the host-pathogen physiology, and potentially provide novel vaccine candidates.

Also, many conserved hypothetical proteins were found upregulated in the HA-MRSA and CA-MRSA strains. These proteins, not known to be controlled by the central global regulator *agr*, are currently uncharacterized. I would suggest studying and characterizing these proteins in an attempt to determine if they are unique to any particular clinical strain that could result in differential pathogeneses of these strains.

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Appendices

Appendix 1. Cytoplasmic proteins identified from overnight cultures of *S. aureus* SH1000 separated by 1D SDS-PAGE

Identified Proteins (380)	Accession Number	Spectral Counts
bifunctional autolysin (atl)	SACOL1062	67
ATP-dependent Clp protease, putative	SACOL2563	56
sdrD protein (sdrD)	SACOL0609	43
N-acetylmuramoyl-L-alanine amidase domain protein	SACOL2666	36
DNA-directed RNA polymerase, beta' subunit (rpoC)	SACOL0589	35
translation elongation factor G (fusA)	SACOL0593	34
chaperonin, 60 kDa (groEL)	SACOL2016	32
ATP synthase F1, beta subunit (atpD)	SACOL2095	32
dnaK protein (dnaK)	SACOL1637	31
transketolase (tkt)	SACOL1377	30
DNA-directed RNA polymerase, beta subunit (rpoB)	SACOL0588	29
lipase (geh)	SACOL2694	28
enolase (eno)	SACOL0842	25
ribosomal protein S1	SACOL1516	24
fructose-bisphosphate aldolase, class I (fdaB)	SACOL2622	23
Aerolysin-Leukocidin family protein	SACOL2006	23
glutamyl-tRNA(Gln) amidotransferase, B subunit (gatB)	SACOL1960	23
phosphoenolpyruvate-protein phosphotransferase (ptsI)	SACOL1092	23
FeS assembly ATPase SufC	SACOL0914	22
pyruvate kinase (pyk) [2.7.1.40]	SACOL1745	22
cell wall surface anchor family protein (sasG)	SACOL2505	22
translation elongation factor Tu (tuf)	SACOL0594	21
glyceraldehyde 3-phosphate dehydrogenase (gapA1)	SACOL0838	19
lipoprotein, putative	SACOL0444	19
FeS assembly protein SufD (sufD)	SACOL0915	19
sdrC protein (sdrC)	SACOL0608	19
polyribonucleotide nucleotidyltransferase (pnp)	SACOL1293	19
succinyl-CoA synthase, beta subunit (sucC)	SACOL1262	18
alkyl hydroperoxide reductase, subunit F (ahpF)	SACOL0451	18
aldehyde dehydrogenase	SACOL2114	18

Appendix 1. (Continued)

sulfatase family protein	SACOL0778	18
pyruvate dehydrogenase complex E2 component, dihydrolipoamide acetyltransferase (pdhC)	SACOL1104	17
clumping factor A (clfA)	SACOL0856	17
cell division protein FtsZ (ftsZ)	SACOL1199	17
pyruvate dehydrogenase complex E1 component, beta subunit (pdhB)	SACOL1103	16
fumarylacetoacetate hydrolase family protein	SACOL0973	16
acetolactate synthase, catabolic (budB)	SACOL2199	16
fibronectin-binding protein A (fnbA)	SACOL2511	16
cell division protein FtsA (ftsA)	SACOL1198	15
protein export protein PrsA, putative	SACOL1897	15
peptide chain release factor 1 (prfA)	SACOL2110	15
conserved hypothetical protein	SACOL1767	15
amino acid ABC transporter, amino acid-binding protein	SACOL2412	14
DNA polymerase III, beta subunit (dnaN)	SACOL0002	14
phosphoglucosamine mutase GlmM (glmM)	SACOL2151	14
pyruvate dehydrogenase complex E3 component, lipoamide dehydrogenase (pdhD)	SACOL1105	13
cysteine synthase (cysK)	SACOL0557	13
trigger factor (tig)	SACOL1722	13
seryl-tRNA synthetase (serS)	SACOL0009	13
map protein, programmed frameshift (map)	SACOL2002	13
conserved hypothetical protein TIGR00092	SACOL0435	13
alkaline shock protein 23	SACOL2173	12
triosephosphate isomerase (tpiA)	SACOL0840	12
DNA-directed RNA polymerase, alpha subunit (rpoA)	SACOL2213	12
phosphate acetyltransferase (pta)	SACOL0634	12
ATP synthase F1, alpha subunit (atpA)	SACOL2097	12
pyruvate carboxylase (pyc)	SACOL1123	12
phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent (pgm)	SACOL0841	12
fibronectin binding protein B (fnbB)	SACOL2509	12
clumping factor B (clfB)	SACOL2652	12
3-oxoacyl-(acyl-carrier-protein) synthase II (fabF)	SACOL0988	11
pyridoxine biosynthesis protein	SACOL0564	11
hydroxymethylglutaryl-CoA synthase	SACOL2561	11
glucosamine-6-phosphate isomerase, putative	SACOL1912	11
penicillin-binding protein 2 (pbp2)	SACOL1490	11
formate acetyltransferase (pflB)	SACOL0204	10
3-oxoacyl-(acyl-carrier-protein) reductase (fabG1)	SACOL1245	10
rod shape-determining protein MreC (mreC)	SACOL1704	10
conserved hypothetical protein	SACOL2136	10

Appendix 1. (Continued)

lipoprotein, putative	SACOL1101	10
GMP synthase (guaA)	SACOL0461	10
LPXTG cell wall surface anchor family protein (sasF)	SACOL2668	10
phosphoribosylformylglycinamidine synthase II (purL)	SACOL1078	10
phenylalanyl-tRNA synthetase, beta subunit (pheT)	SACOL1149	10
alkyl hydroperoxide reductase, C subunit (ahpC)	SACOL0452	9
staphyloxanthin biosynthesis protein	SACOL2291	9
glucosamine--fructose-6-phosphate aminotransferase (isomerizing) (glmS)	SACOL2145	9
pyruvate dehydrogenase complex E1 component, alpha subunit (pdhA)	SACOL1102	9
ribosomal protein L4 (rplD)	SACOL2238	9
phosphoglycerate kinase (pgk)	SACOL0839	9
oxidoreductase, short chain dehydrogenase-reductase family	SACOL2321	9
2-oxoglutarate dehydrogenase, E2 component, dihydroipoamide succinyltransferase (sucB)	SACOL1448	9
manganese-dependent inorganic pyrophosphatase (ppaC)	SACOL1982	9
branched-chain amino acid aminotransferase (ilvE)	SACOL0600	9
D-alanine aminotransferase (dat)	SACOL1800	9
LysM domain protein	SACOL0507	9
hypoxanthine phosphoribosyltransferase (hpt)	SACOL0554	9
N utilization substance protein A, putative	SACOL1285	9
conserved hypothetical protein	SACOL1792	9
S-adenosylmethionine synthetase (metK)	SACOL1837	9
translation elongation factor Ts (tsf)	SACOL1276	8
aconitate hydratase (acnA)	SACOL1385	8
transaldolase (tal)	SACOL1831	8
ferritins family protein	SACOL1952	8
conserved hypothetical protein	SACOL0597	8
L-lactate dehydrogenase (ldh1)	SACOL0222	8
acid phosphatase5'-nucleotidase, lipoprotein e(P4) family	SACOL0303	8
aerobic glycerol-3-phosphate dehydrogenase (glpD)	SACOL1321	8
glutamyl-tRNA(Gln) amidotransferase, A subunit (gatA)	SACOL1961	8
dihydroorotase (pyrC)	SACOL1213	8
surface protein, putative	SACOL0479	8
leukocidin subunit precursor, putative	SACOL2004	8
catalase (kataA)	SACOL1368	7
glucose-6-phosphate isomerase (pgi)	SACOL0966	7
conserved hypothetical protein	SACOL1902	7
universal stress protein family	SACOL1759	7
acyl carrier protein (acpP)	SACOL1247	7
ribosomal protein L10 (rplJ)	SACOL0585	7

Appendix 1. (Continued)

ribosomal Protein L25 (rplY)	SACOL0545	7
ribosomal protein L1 (rplA)	SACOL0584	7
aminotransferase, class II	SACOL0596	7
conserved hypothetical protein	SACOL1985	7
malonyl CoA-acyl carrier protein transacylase (fabD)	SACOL1244	7
ATP synthase F1, gamma subunit (atpG)	SACOL2096	7
DAK2 domain protein	SACOL1240	7
acetyl-CoA acetyltransferase	SACOL0426	7
conserved hypothetical protein	SACOL0455	7
alpha-hemolysin precursor (hlY)	SACOL1173	7
DNA-binding protein HU (hup)	SACOL1513	6
immunodominant antigen A (isaA)	SACOL2584	6
inosine-5'-monophosphate dehydrogenase (guaB)	SACOL0460	6
fructose-bisphosphate aldolase, class II (fbaA)	SACOL2117	6
ribosomal protein L21 (rplU)	SACOL1702	6
FeS assembly protein SufB (sufB)	SACOL0918	6
threonyl-tRNA synthetase (thrS)	SACOL1729	6
ribosomal protein L6 (rplF)	SACOL2224	6
ribosomal protein S3 (rpsC)	SACOL2233	6
ribosomal protein S4 (rpsD)	SACOL1769	6
ribosomal protein L3 (rplC)	SACOL2239	6
deoxyribose-phosphate aldolase (deoC2)	SACOL2129	6
3-oxoacyl-(acyl-carrier-protein) synthase III (fabH)	SACOL0987	6
6-phosphofructokinase (pfkA)	SACOL1746	6
conserved hypothetical protein	SACOL0669	6
conserved hypothetical protein	SACOL1447	6
sasB protein (sasB)	SACOL2150	6
conserved hypothetical protein	SACOL0912	5
ornithine aminotransferase (rocD2)	SACOL0960	5
ribosomal protein L2 (rplB)	SACOL2236	5
glycyl-tRNA synthetase (glyS)	SACOL1622	5
malate:quinone oxidoreductase (mqo2)	SACOL2623	5
formate dehydrogenase, alpha subunit, putative	SACOL2301	5
ribosomal protein L20 (rplT)	SACOL1725	5
NADP-dependent malic enzyme, putative	SACOL1749	5
conserved hypothetical protein TIGR01033	SACOL0727	5
L-lactate dehydrogenase (ldh2)	SACOL2618	5
anti-sigma B factor (rsbW)	SACOL2055	5
immunodominant antigen B (isaB)	SACOL2660	5

Appendix 1. (Continued)

acetoin reductase	SACOL0111	5
serine hydroxymethyltransferase (glyA)	SACOL2105	5
glutamyl aminopeptidase, putative	SACOL1402	5
DNA-directed RNA polymerase, delta subunit (rpoE)	SACOL2120	5
NAD(P)H dehydrogenase (quinone), putative	SACOL0190	5
oxidoreductase, short-chain dehydrogenase-reductase family	SACOL2488	5
Staphylococcus aureus sex pheromone (camS)	SACOL1964	5
dephospho-CoA kinase (coaE)	SACOL1735	5
lipoprotein, putative	SACOL0449	5
IgG-binding protein SBI	SACOL2418	5
cysteine protease precursor SspB (sspB2)	SACOL1970	5
ribosomal protein L15 (rplO)	SACOL2220	4
hydrolase, alpha-beta hydrolase fold family	SACOL2597	4
succinyl-CoA synthase, alpha subunit (sucD)	SACOL1263	4
valyl-tRNA synthetase (valS)	SACOL1710	4
superoxide dismutase (sodA2)	SACOL1610	4
peptidase, M20-M25-M40 family	SACOL1801	4
conserved hypothetical protein	SACOL1020	4
conserved hypothetical protein	SACOL1789	4
pyrimidine-nucleoside phosphorylase (pdp)	SACOL2128	4
conserved hypothetical protein	SACOL1788	4
naphthoate synthase (menB)	SACOL1054	4
arginyl-tRNA synthetase (argS)	SACOL0663	4
iron compound ABC transporter, iron compound-binding protein	SACOL2277	4
translation elongation factor P (efp)	SACOL1587	4
staphylococcus tandem lipoprotein	SACOL0486	4
2-oxoisovalerate dehydrogenase, E1 component, beta subunit	SACOL1561	4
single-stranded DNA-binding protein (ssb2)	SACOL0438	4
hydrolase, haloacid dehalogenase-like family	SACOL0602	4
transcriptional regulator, putative	SACOL1065	4
penicillin-binding protein 3 (pbp3)	SACOL1609	4
antibacterial protein (phenol soluble modulin)	SACOL1186	3
ThiJ-PfpI family protein	SACOL1933	3
antibacterial protein (phenol soluble modulin)	SACOL1187	3
hexulose-6-phosphate synthase, putative	SACOL0617	3
2-oxoglutarate dehydrogenase, E1 component (sucA)	SACOL1449	3
purine nucleoside phosphorylase (deoD2)	SACOL2130	3
NADH dehydrogenase, putative	SACOL0944	3
ribosomal protein S2 (rpsB)	SACOL1274	3

Appendix 1. (Continued)

acetate kinase (ackA)	SACOL1760	3
adenylosuccinate synthetase (purA)	SACOL0018	3
ribosomal protein S7 (rpsG)	SACOL0592	3
glucose-6-phosphate 1-dehydrogenase (zwf)	SACOL1549	3
ribosomal protein S5 (rpsE)	SACOL2222	3
lysyl-tRNA synthetase (lysS)	SACOL0562	3
uracil phosphoribosyltransferase (upp)	SACOL2104	3
ribosomal protein L5 (rplE)	SACOL2227	3
peptidyl-prolyl cis-trans isomerase, cyclophilin-type	SACOL0957	3
cytochrome aa3 quinol oxidase, subunit II (qoxA)	SACOL1070	3
CTP synthase (pyrG)	SACOL2119	3
4-diphosphocytidyl-2C-methyl-D-erythritol synthase, putative	SACOL0240	3
ribosomal protein L17 (rplQ)	SACOL2212	3
NAD(P)H-flavin oxidoreductase (frp)	SACOL2534	3
cysteine desulfurase, SufS subfamily	SACOL0916	3
ribosomal protein L13 (rplM)	SACOL2207	3
ribosomal protein S12 (rpsL)	SACOL0591	3
elastin binding protein, putative	SACOL1522	3
carbamoyl-phosphate synthase, large subunit (carB)	SACOL1215	3
ribosomal protein L22 (rplV)	SACOL2234	3
D-alanine-activating enzyme-D-alanine-D-alanyl carrier protein ligase (dltA)	SACOL0935	3
phosphomethylpyrimidine kinase (thiD1)	SACOL0626	3
methionine aminopeptidase, type I	SACOL1946	3
ATP-dependent Clp protease, ATP-binding subunit ClpX (clpX)	SACOL1721	3
hydrolase, haloacid dehalogenase-like family	SACOL0931	3
UTP-glucose-1-phosphate uridylyltransferase family protein	SACOL2161	3
1-phosphofructokinase (fruK)	SACOL0758	3
LysM domain protein	SACOL0723	3
ATP-dependent Clp protease, proteolytic subunit ClpP (clpP)	SACOL0833	3
xanthine phosphoribosyltransferase (xpt)	SACOL0458	3
peptide ABC transporter, peptide-binding protein	SACOL2476	3
conserved hypothetical protein	SACOL1464	3
penicillin-binding protein 1 (pbp1)	SACOL1194	3
phage infection protein, putative	SACOL2665	3
protein phosphatase 2C domain protein	SACOL1231	3
secretory extracellular matrix and plasma binding protein (empbp)	SACOL0858	3
thymidylate kinase (tmk)	SACOL0524	3
phospholipase C (hlb)	SACOL2003	3
6,7-dimethyl-8-ribityllumazine synthase (ribH)	SACOL1817	2

Appendix 1. (Continued)

6-phosphogluconate dehydrogenase, decarboxylating (gnd)	SACOL1554	2
delta-1-pyrroline-5-carboxylate dehydrogenase, putative	SACOL2569	2
D-isomer specific 2-hydroxyacid dehydrogenase family protein	SACOL2296	2
alcohol dehydrogenase, zinc-containing	SACOL0660	2
adenylosuccinate lyase (purB)	SACOL1969	2
cold shock protein, CSD family	SACOL1437	2
ribosomal subunit interface protein	SACOL0815	2
phosphocarrier protein HPr (ptsH)	SACOL1091	2
asparaginyl-tRNA synthetase (asnS)	SACOL1494	2
conserved hypothetical protein	SACOL1992	2
fumarate hydratase, class II (fumC)	SACOL1908	2
mannitol-1-phosphate 5-dehydrogenase (mtlD)	SACOL2149	2
immunoglobulin G binding protein A precursor (spa)	SACOL0095	2
conserved hypothetical protein	SACOL2379	2
adenylate kinase (adk)	SACOL2218	2
aspartyl-tRNA synthetase (aspS)	SACOL1685	2
DNA gyrase, A subunit (gyrA)	SACOL0006	2
oxidoreductase, aldo-keto reductase family	SACOL0763	2
lipoprotein, putative	SACOL2365	2
ribosomal protein L11 (rplK)	SACOL0583	2
isocitrate dehydrogenase, NADP-dependent (icd)	SACOL1741	2
cell division protein FtsH, putative	SACOL0555	2
translation initiation factor IF-3 (infC)	SACOL1727	2
molybdenum ABC transporter, molybdenum-binding protein ModA (modA)	SACOL2272	2
tyrosyl-tRNA synthetase (tyrS)	SACOL1778	2
ribose-phosphate pyrophosphokinase (prsA)	SACOL0544	2
DNA repair exonuclease family protein	SACOL1900	2
epimerase-dehydratase, putative	SACOL2446	2
UDP-N-acetylglucosamine 1-carboxyvinyltransferase 2 (murAB)	SACOL2116	2
DHH subfamily 1 protein	SACOL1751	2
copper ion binding protein	SACOL2573	2
pyrroline-5-carboxylate reductase (proC)	SACOL1546	2
ferrochelatase (hemH)	SACOL1888	2
conserved hypothetical protein	SACOL1426	2
thiamine-phosphate pyrophosphorylase (thiE)	SACOL2083	2
cell-division initiation protein, putative	SACOL1205	2
phenylalanyl-tRNA synthetase, alpha subunit (pheS)	SACOL1148	2
decarboxylase family protein	SACOL0740	2
hydrolase, haloacid dehalogenase-like family	SACOL0606	2

Appendix 1. (Continued)

conserved hypothetical protein	SACOL2020	2
hydrolase, haloacid dehalogenase-like family	SACOL1365	2
ABC transporter, substrate-binding protein	SACOL0217	2
transcriptional regulator, putative	SACOL1398	2
acetyl-CoA carboxylase, biotin carboxyl carrier protein (accB)	SACOL1572	2
chaperonin, 33 kDa	SACOL0556	2
ferredoxin (fer)	SACOL1525	2
lipoprotein, putative	SACOL1589	2
serine protease SplB (splB)	SACOL1868	2
formate--tetrahydrofolate ligase (fhs)	SACOL1782	1
conserved hypothetical protein	SACOL2163	1
PTS system, IIA component	SACOL1457	1
thiol peroxidase, putative	SACOL1762	1
thioredoxin (trxA)	SACOL1155	1
translation initiation factor IF-2 (infB)	SACOL1288	1
Dps family protein	SACOL2131	1
ABC transporter, substrate-binding protein	SACOL0688	1
ribosomal protein L16 (rplP)	SACOL2232	1
catabolite control protein A (ccpA)	SACOL1786	1
conserved hypothetical protein	SACOL1630	1
N-acetylglucosamine-6-phosphate deacetylase (nagA)	SACOL0761	1
ATP-dependent Clp protease, ATP-binding subunit ClpB (clpB)	SACOL0979	1
ribosomal protein S11 (rpsK)	SACOL2214	1
pyruvate oxidase	SACOL2553	1
phosphoribosylamine--glycine ligase (purD)	SACOL1083	1
acetyltransferase, GNAT family	SACOL1189	1
quinol oxidase, subunit I (qoxA)	SACOL1069	1
conserved hypothetical protein	SACOL1802	1
oxidoreductase, aldo-keto reductase family	SACOL1835	1
thioredoxin, putative	SACOL0875	1
glutamate-1-semialdehyde-2,1-aminomutase (hemL1)	SACOL1714	1
hydroxyethylthiazole kinase (thiM)	SACOL2084	1
prolyl-tRNA synthetase (proS)	SACOL1282	1
alanyl-tRNA synthetase (alaS)	SACOL1673	1
imidazolonepropionase (hutI)	SACOL2323	1
ribonucleoside-diphosphate reductase, alpha subunit	SACOL0792	1
ribosomal protein L23 (rplW)	SACOL2237	1
heat shock protein GrpE (grpE)	SACOL1638	1
conserved hypothetical protein	SACOL1670	1

Appendix 1. (Continued)

4-oxalocrotonate tautomerase (dmpI)	SACOL1399	1
ABC transporter, ATP-binding protein	SACOL1427	1
heat shock protein HslVU, ATPase subunit HslU (hslU)	SACOL1271	1
DNA polymerase I (polA)	SACOL1737	1
conserved hypothetical protein	SACOL2143	1
serine protease HtrA, putative	SACOL1777	1
aminotransferase, putative	SACOL2000	1
D-alanine--D-alanine ligase	SACOL2074	1
chaperonin, 10 kDa (groES)	SACOL2017	1
glucokinase (glk)	SACOL1604	1
polypeptide deformylase (def1)	SACOL1100	1
glutamate-1-semialdehyde-2,1-aminomutase (hemL2)	SACOL1922	1
ribosome-binding factor A (rbfA)	SACOL1289	1
pyridine nucleotide-disulfide oxidoreductase	SACOL1520	1
ribonucleoside-diphosphate reductase 2, beta subunit (nrdF)	SACOL0793	1
enoyl-(acyl-carrier-protein) reductase (fabI)	SACOL1016	1
phosphoribosylformylglycinamidine synthase I (purQ)	SACOL1077	1
fatty acid-phospholipid synthesis protein PlsX (plsX)	SACOL1243	1
ATP synthase F0, B subunit (atpF)	SACOL2099	1
signal peptidase IB (spsB)	SACOL0969	1
RNA methyltransferase, TrmH family	SACOL0578	1
NifU domain protein	SACOL0917	1
3,4-dihydroxy-2-butanone-4-phosphate synthase-GTP cyclohydrolase II (ribBA)	SACOL1818	1
phosphoribosylformylglycinamidine cyclo-ligase (purM)	SACOL1080	1
glyoxalase family protein	SACOL2533	1
DNA-binding response regulator SrrA (srrA)	SACOL1535	1
phosphoribosylglycinamide formyltransferase (purN)	SACOL1081	1
UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase (murF)	SACOL2073	1
tellurite resistance protein, putative	SACOL1441	1
degV family protein	SACOL1460	1
conserved hypothetical protein	SACOL0742	1
deoxyribose-phosphate aldolase (deoC1)	SACOL0123	1
femB protein	SACOL1411	1
glycerol-3-phosphate dehydrogenase, NAD-dependent (gpsA)	SACOL1514	1
GTP-binding protein, GTP1-OBG family	SACOL1699	1
urease accessory protein UreG (ureG)	SACOL2285	1
DAK2 domain protein	SACOL0708	1
HAM1 protein	SACOL1162	1
conserved hypothetical protein	SACOL1558	1

Appendix 1. (Continued)

FtsK-SpoIIIE family protein	SACOL1791	1
conserved hypothetical protein	SACOL2605	1
peptide chain release factor 2, programmed frameshift (prfB)	SACOL0818	1
cytidylate kinase (cmk)	SACOL1518	1
Gid protein (gid)	SACOL1268	1
conserved hypothetical protein	SACOL1120	1
general stress protein 13	SACOL0552	1
conserved domain protein	SACOL2557	1
hypothetical protein	SACOL0272	1
conserved hypothetical protein	SACOL1885	1
uroporphyrinogen decarboxylase (hemE)	SACOL1889	1
transcriptional regulator, putative	SACOL2302	1
YlmF protein (ylmF)	SACOL1202	1
dnaJ protein (dnaJ)	SACOL1636	1
degV family protein	SACOL0812	1
PTS system, mannitol-specific IIBC components	SACOL2146	1
orotidine 5'-phosphate decarboxylase (pyrF)	SACOL1216	1
lipoprotein, putative	SACOL0851	1
cobyric acid synthase, putative	SACOL1950	1
glycerophosphoryl diester phosphodiesterase GlpQ, putative	SACOL0962	1
thioredoxin, putative	SACOL0881	1
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase (metE)	SACOL0428	1
response regulator-related protein	SACOL2360	1
hydrolase, haloacid dehalogenase-like family	SACOL0619	1
conserved hypothetical protein	SACOL0579	1
staphyloxanthin biosynthesis protein, putative	SACOL2295	1
LPXTG cell wall surface anchor protein (sasE)	SACOL1140	1
16S rRNA processing protein RimM (rimM)	SACOL1255	1
conserved hypothetical protein	SACOL1373	1
conserved hypothetical protein	SACOL1375	1
oxygen-independent coproporphyrinogen III oxidase, putative	SACOL1640	1
iron compound ABC transporter, iron compound-binding protein	SACOL2010	1
acetolactate synthase, large subunit, biosynthetic type (ilvB)	SACOL2043	1
monooxygenase family protein	SACOL2297	1
staphylococcus tandem lipoprotein	SACOL2497	1
membrane protein, putative	SACOL2554	1

Appendix 2. Cytoplasmic proteins identified from overnight cultures of *S. aureus* SH1000 from MudPit analysis

Identified Proteins (747)	Accession Number	Spectral Counts
alkaline shock protein 23	SACOL2173	1129
enolase (eno)	SACOL0842	886
translation elongation factor Tu (tuf)	SACOL0594	873
glyceraldehyde 3-phosphate dehydrogenase (gapA1)	SACOL0838	873
formate acetyltransferase (pflB)	SACOL0204	577
aldehyde dehydrogenase (aldA1)	SACOL0154	372
ATP-dependent Clp protease, putative	SACOL2563	371
DNA-binding protein HU (hup)	SACOL1513	356
pyruvate dehydrogenase complex E3 component, lipoamide dehydrogenase (pdhD)	SACOL1105	341
dnaK protein (dnaK)	SACOL1637	298
translation elongation factor Ts (tsf)	SACOL1276	294
antibacterial protein (phenol soluble modulin)	SACOL1186	283
translation elongation factor G (fusA)	SACOL0593	281
fructose-bisphosphate aldolase, class I (fdaB)	SACOL2622	281
alkyl hydroperoxide reductase, C subunit (ahpC)	SACOL0452	265
ThiJ-PfpI family protein	SACOL1933	251
conserved hypothetical protein	SACOL0912	240
phosphoglycerate mutase (gpm)	SACOL2415	219
formate--tetrahydrofolate ligase (fhs)	SACOL1782	208
conserved hypothetical protein	SACOL2163	196
pyruvate dehydrogenase complex E2 component, dihydrolipoamide acetyltransferase (pdhC)	SACOL1104	192
cysteine synthase (cysK)	SACOL0557	192
catalase (kataA)	SACOL1368	187
ornithine aminotransferase (rocD2)	SACOL0960	182
DNA-directed RNA polymerase, beta' subunit (rpoC)	SACOL0589	175
DNA-directed RNA polymerase, beta subunit (rpoB)	SACOL0588	172
acyl-CoA dehydrogenase family protein	SACOL0213	171
ribosomal protein S1 (rpsA)	SACOL1516	169
ribosomal protein L2 (rplB)	SACOL2236	166
phosphoenolpyruvate carboxykinase (ATP) (pckA)	SACOL1838	162
pyruvate dehydrogenase complex E1 component, beta subunit (pdhB)	SACOL1103	156
chaperonin, 60 kDa (groEL)	SACOL2016	155
glucosamine--fructose-6-phosphate aminotransferase (isomerizing) (glmS)	SACOL2145	153
transketolase (tkt)	SACOL1377	146
thioredoxin-disulfide reductase (trxB)	SACOL0829	146
pyruvate kinase (pyk)	SACOL1745	143
antibacterial protein (phenol soluble modulin)	SACOL1187	143
6,7-dimethyl-8-ribityllumazine synthase (ribH)	SACOL1817	143
succinyl-CoA synthase, beta subunit (sucC)	SACOL1262	139
glucose-6-phosphate isomerase (pgi)	SACOL0966	138
glycyl-tRNA synthetase (glyS)	SACOL1622	130
6-phosphogluconate dehydrogenase, decarboxylating (gnd)	SACOL1554	130
hydrolase, alpha-beta hydrolase fold family	SACOL2597	128
aconitate hydratase (acnA)	SACOL1385	124

Appendix 2. (Continued)

inosine-5'-monophosphate dehydrogenase (guaB)	SACOL0460	124
succinyl-CoA synthase, alpha subunit (sucD)	SACOL1263	124
transaldolase (tal)	SACOL1831	123
hexulose-6-phosphate synthase, putative	SACOL0617	123
ribosomal protein L15 (rplO)	SACOL2220	120
cell division protein FtsZ (ftsZ)	SACOL1199	116
ATP synthase F1, beta subunit (atpD)	SACOL2095	115
pyruvate dehydrogenase complex E1 component, alpha subunit (pdhA)	SACOL1102	114
thioredoxin (trxA)	SACOL1155	111
ribosomal protein S8 (rpsH)	SACOL2225	111
glutamine synthetase FemC (femC)	SACOL1329	111
triosephosphate isomerase (tpiA)	SACOL0840	110
delta-1-pyrroline-5-carboxylate dehydrogenase, putative	SACOL2569	107
phosphate acetyltransferase (pta)	SACOL0634	104
ribosomal protein S6 (rpsF)	SACOL0437	103
malate:quinone oxidoreductase (mqo2)	SACOL2623	101
seryl-tRNA synthetase (serS)	SACOL0009	97
formate dehydrogenase, alpha subunit, putative	SACOL2301	97
PTS system, IIA component	SACOL1457	95
3-oxoacyl-(acyl-carrier-protein) reductase (fabG1)	SACOL1245	94
conserved hypothetical protein	SACOL0597	92
alkyl hydroperoxide reductase, subunit F (ahpF)	SACOL0451	91
phosphoglycerate kinase (pgk)	SACOL0839	89
ferritins family protein	SACOL1952	87
alcohol dehydrogenase, zinc-containing	SACOL0660	84
D-isomer specific 2-hydroxyacid dehydrogenase family protein	SACOL2296	84
3-hydroxyacyl-CoA dehydrogenase protein	SACOL0212	84
ornithine carbamoyltransferase (arcB2)	SACOL2656	84
thiol peroxidase, putative	SACOL1762	83
universal stress protein family	SACOL1759	81
staphylococcal accessory regulator A (sarA)	SACOL0672	81
aldehyde dehydrogenase	SACOL2114	80
conserved hypothetical protein	SACOL1484	80
trigger factor (tig)	SACOL1722	79
3-oxoacyl-(acyl-carrier-protein) synthase II (fabF)	SACOL0988	78
2-oxoglutarate dehydrogenase, E1 component (sucA)	SACOL1449	77
NADH dehydrogenase, putative	SACOL0944	77
peptidase, M20-M25-M40 family	SACOL1801	75
fructose-bisphosphate aldolase, class II (fbaA)	SACOL2117	74
glutamate dehydrogenase, NAD-specific (gluD)	SACOL0961	74
bifunctional autolysin (atl)	SACOL1062	71
hydroxymethylglutaryl-CoA synthase	SACOL2561	70
L-lactate dehydrogenase (ldh1)	SACOL0222	70
purine nucleoside phosphorylase (deoD2)	SACOL2130	69
NADP-dependent malic enzyme, putative	SACOL1749	69
glycine cleavage system H protein (gcvH)	SACOL0877	67
isoleucyl-tRNA synthetase (ileS)	SACOL1206	66
methylenetetrahydrofolate dehydrogenase-methenyltetrahydrofolate cyclohydrolase (folD)	SACOL1072	65
ATP synthase F1, alpha subunit (atpA)	SACOL2097	64
adenylosuccinate lyase (purB)	SACOL1969	64
ribosomal protein L1 (rplA)	SACOL0584	63
pyrimidine-nucleoside phosphorylase (pdp)	SACOL2128	63
conserved hypothetical protein	SACOL1902	62
pyruvate carboxylase (pyc)	SACOL1123	62
fumarylacetoacetate hydrolase family protein	SACOL0973	61

Appendix 2. (Continued)

pyridoxine biosynthesis protein	SACOL0564	61
ribosomal protein L7-L12 (rplL)	SACOL0586	61
conserved hypothetical protein	SACOL1680	61
superoxide dismutase (sodA2)	SACOL1610	60
translation initiation factor IF-2 (infB)	SACOL1288	59
arginine deiminase (arcA)	SACOL2657	59
DNA-directed RNA polymerase, alpha subunit (rpoA)	SACOL2213	58
ribosomal protein S2 (rpsB)	SACOL1274	58
aminotransferase, class II	SACOL0596	58
aerobic glycerol-3-phosphate dehydrogenase (glpD)	SACOL1321	58
Dps family protein	SACOL2131	58
alkylhydroperoxidase, AhpD family	SACOL2484	58
lipoprotein, putative	SACOL0444	57
glutamyl-tRNA(Gln) amidotransferase, B subunit (gatB)	SACOL1960	57
2-oxoglutarate dehydrogenase, E2 component, dihydroipoamide succinyltransferase (sucB)	SACOL1448	57
ribosomal subunit interface protein	SACOL0815	56
glutamyl-tRNA synthetase (gltX)	SACOL0574	56
ribosomal protein S13-S18 (rpsM)	SACOL2215	56
ribosomal protein L20 (rplT)	SACOL1725	55
adenylosuccinate synthetase (purA)	SACOL0018	55
oligoendopeptidase F (pepF)	SACOL1005	54
flavohemoprotein, putative	SACOL0220	54
urocanate hydratase (hutU)	SACOL2324	54
FeS assembly protein SufB (sufB)	SACOL0918	53
spoVG protein (spoVG)	SACOL0541	52
phosphoenolpyruvate-protein phosphotransferase (ptsI)	SACOL1092	51
ribosomal protein L21 (rplU)	SACOL1702	51
acetate kinase (ackA)	SACOL1760	51
glucosamine-6-phosphate isomerase, putative	SACOL1912	50
conserved hypothetical protein	SACOL1789	50
conserved hypothetical protein	SACOL1020	50
valyl-tRNA synthetase (valS)	SACOL1710	50
metallo-beta-lactamase family protein	SACOL1098	50
conserved hypothetical protein	SACOL0457	49
ribosomal Protein L25 (rplY)	SACOL0545	48
threonyl-tRNA synthetase (thrS)	SACOL1729	48
succinate dehydrogenase, flavoprotein subunit (sdhA)	SACOL1159	48
conserved hypothetical protein	SACOL2136	47
conserved hypothetical protein	SACOL1992	47
glucose-6-phosphate 1-dehydrogenase (zwf)	SACOL1549	46
fumarate hydratase, class II (fumC)	SACOL1908	46
glutamyl-tRNA(Gln) amidotransferase, A subunit (gatA)	SACOL1961	45
uracil phosphoribosyltransferase (upp)	SACOL2104	45
aldehyde dehydrogenase (aldA2)	SACOL1984	45
D-isomer specific 2-hydroxyacid dehydrogenase family protein	SACOL2535	44
conserved hypothetical protein	SACOL2711	44
alcohol dehydrogenase, zinc-containing	SACOL2178	44
acyl carrier protein (acpP)	SACOL1247	43
ribosomal protein L16 (rplP)	SACOL2232	43
mannitol-1-phosphate 5-dehydrogenase (mtlD)	SACOL2149	43
ribosomal protein S19 (rpsS)	SACOL2235	43
methionyl-tRNA synthetase (metS)	SACOL0533	43
ribosomal protein S5 (rpsE)	SACOL2222	42
ABC transporter, substrate-binding protein	SACOL0688	42
L-lactate dehydrogenase (ldh2)	SACOL2618	42

Appendix 2. (Continued)

asparaginyl-tRNA synthetase (asnS)	SACOL1494	42
conserved hypothetical protein	SACOL0633	42
FeS assembly protein SufD (sufD)	SACOL0915	41
phosphoglycerate mutase, 2,3-bisphosphoglycerate-independent (pgm)	SACOL0841	41
proline dipeptidase	SACOL1588	41
cell division protein FtsA (ftsA)	SACOL1198	40
deoxyribose-phosphate aldolase (deoC1)	SACOL0123	40
catabolite control protein A (ccpA)	SACOL1786	40
conserved hypothetical protein	SACOL1630	40
PTS system, mannitol-specific IIA component	SACOL2148	40
ribosomal protein L4 (rplD)	SACOL2238	39
polyribonucleotide nucleotidyltransferase (pnp)	SACOL1293	39
manganese-dependent inorganic pyrophosphatase (ppaC)	SACOL1982	39
conserved hypothetical protein	SACOL1985	39
lysyl-tRNA synthetase (lysS)	SACOL0562	39
phosphocarrier protein HPr (ptsH)	SACOL1091	39
N-acetylglucosamine-6-phosphate deacetylase (nagA)	SACOL0761	39
deoxyribose-phosphate aldolase (deoC2)	SACOL2129	38
branched-chain amino acid aminotransferase (ilvE)	SACOL0600	38
glycine cleavage system P protein, subunit 2	SACOL1593	38
glycine cleavage system P protein, subunit 1	SACOL1594	38
D-alanine aminotransferase (dat)	SACOL1800	37
ribosomal protein S7 (rpsG)	SACOL0592	37
conserved hypothetical protein	SACOL1788	37
serine hydroxymethyltransferase (glyA)	SACOL2105	37
adenylate kinase (adk)	SACOL2218	37
naphthoate synthase (menB)	SACOL1054	36
peptidyl-prolyl cis-trans isomerase, cyclophilin-type	SACOL0957	36
FeS assembly ATPase SufC	SACOL0914	35
ribosomal protein L10 (rplJ)	SACOL0585	35
protein export protein PrsA, putative	SACOL1897	35
ribosomal protein S4 (rpsD)	SACOL1769	35
ribosomal protein L5 (rplE)	SACOL2227	35
oxidoreductase, aldo-keto reductase family	SACOL1835	35
fructose-1,6-bisphosphatase, putative	SACOL2527	35
oxidoreductase, short chain dehydrogenase-reductase family	SACOL2321	34
ribosomal protein L17 (rplQ)	SACOL2212	34
acetyl-CoA synthetase (acs)	SACOL1783	34
conserved hypothetical protein	SACOL2175	34
alanine dehydrogenase (ald2)	SACOL1758	34
acetolactate synthase, catabolic (budB)	SACOL2199	33
ribosomal protein L22 (rplV)	SACOL2234	33
CTP synthase (pyrG)	SACOL2119	33
acetyltransferase, GNAT family	SACOL1189	33
phosphoribosylaminoimidazolecarboxamide formyltransferase-IMP cyclohydrolase (purH)	SACOL1082	33
glutamyl aminopeptidase, putative	SACOL1402	32
cytochrome aa3 quinol oxidase, subunit II (qoxA)	SACOL1070	32
pyruvate oxidase	SACOL2553	32
thioredoxin, putative	SACOL0875	32
coenzyme A disulfide reductase	SACOL0975	32
phosphoribosylamine--glycine ligase (purD)	SACOL1083	31
acetyl-CoA synthetase, putative	SACOL2624	31
universal stress protein family	SACOL1753	31
phosphoglucosamine mutase GlmM (glmM)	SACOL2151	30
conserved hypothetical protein TIGR01033	SACOL0727	30

Appendix 2. (Continued)

anti-sigma B factor (rsbW)	SACOL2055	30
3-oxoacyl-(acyl-carrier-protein) synthase III (fabH)	SACOL0987	30
acetoin reductase	SACOL0111	30
arginyl-tRNA synthetase (argS)	SACOL0663	30
transcriptional regulator CodY (codY)	SACOL1272	30
argininosuccinate synthase (argG)	SACOL0964	30
clumping factor A (clfA)	SACOL0856	29
DNA polymerase III, beta subunit (dnaN)	SACOL0002	29
NAD(P)H-flavin oxidoreductase (frp)	SACOL2534	29
conserved hypothetical protein	SACOL1802	29
indole-3-pyruvate decarboxylase (ipdC)	SACOL0173	29
ribosomal protein S11 (rpsK)	SACOL2214	28
6-phosphofructokinase (pfkA)	SACOL1746	28
ribosomal protein L11 (rplK)	SACOL0583	28
ATP-dependent Clp protease, ATP-binding subunit ClpB (clpB)	SACOL0979	28
metallo-beta-lactamase family protein	SACOL1294	28
GMP synthase (guaA)	SACOL0461	27
ribosomal protein S3 (rpsC)	SACOL2233	27
immunoglobulin G binding protein A precursor (spa)	SACOL0095	27
conserved hypothetical protein	SACOL2379	27
aminopeptidase PepS (pepS)	SACOL1937	27
cysteine desulfurase, SufS subfamily	SACOL0916	26
4-diphosphocytidyl-2C-methyl-D-erythritol synthase, putative	SACOL0240	26
glutamate-1-semialdehyde-2,1-aminomutase (hemL1)	SACOL1714	26
hydroxyethylthiazole kinase (thiM)	SACOL2084	26
bacterioferritin comigratory protein (bcp)	SACOL1921	26
conserved hypothetical protein	SACOL2596	26
long-chain-fatty-acid--CoA ligase, putative	SACOL0214	26
conserved hypothetical protein	SACOL1975	26
ribosome recycling factor (frr)	SACOL1278	26
RNAIII-activating protein TRAP	SACOL1891	25
phosphopentomutase (deoB)	SACOL0124	25
acetyl-CoA acetyltransferase	SACOL0211	25
ribosomal protein L6 (rplF)	SACOL2224	24
N utilization substance protein A, putative	SACOL1285	24
leucyl-tRNA synthetase (leuS)	SACOL1808	24
anti-anti-sigma factor RsbV (rsbV)	SACOL2056	24
glutamyl aminopeptidase (pepA1)	SACOL1795	24
ribosomal protein L27 (rpmA)	SACOL1700	24
ribosomal protein S16 (rpsP)	SACOL1254	24
conserved hypothetical protein	SACOL1115	23
thioredoxin, putative	SACOL1794	23
HPr kinase-phosphatase (hprK)	SACOL0825	23
phenylalanyl-tRNA synthetase, beta subunit (pheT)	SACOL1149	22
oxidoreductase, aldo-keto reductase family	SACOL0763	22
DNA gyrase, A subunit (gyrA)	SACOL0006	22
imidazolonepropionase (hutI)	SACOL2323	22
conserved hypothetical protein TIGR00103	SACOL0521	22
molybdopterin biosynthesis MoeA protein, putative	SACOL2266	22
glutamyl-aminopeptidase (pepA2)	SACOL2463	22
ribosomal protein S20 (rpsT)	SACOL1642	22
3-methyl-2-oxobutanoate hydroxymethyltransferase (panB)	SACOL2615	22
autoinducer-2 production protein LuxS (luxS)	SACOL2126	22
preprotein translocase, SecA subunit (secA)	SACOL0816	22
arsenate reductase, putative	SACOL0876	22
lipase (geh)	SACOL2694	21

Appendix 2. (Continued)

conserved hypothetical protein	SACOL1792	21
phosphoribosylformylglycinamidine synthase II (purL)	SACOL1078	21
DNA-directed RNA polymerase, delta subunit (rpoE)	SACOL2120	21
heat shock protein GrpE (grpE)	SACOL1638	21
delta-aminolevulinic acid dehydratase (hemB)	SACOL1715	21
phosphoribosylformylglycinamidine synthase, PurS protein (purS)	SACOL1076	21
ornithine carbamoyltransferase (arcB1)	SACOL1181	21
cold shock protein, CSD family	SACOL1437	20
hypoxanthine phosphoribosyltransferase (hpt)	SACOL0554	20
translation elongation factor P (efp)	SACOL1587	20
carbamate kinase (arcC2)	SACOL2654	20
NAD-NADP octopine-nopaline dehydrogenase family protein	SACOL2293	20
formiminoglutamase (hutG)	SACOL2327	20
ribosomal protein L29 (rpmC)	SACOL2231	20
ribosomal protein L3 (rplC)	SACOL2239	19
ribosomal protein L13 (rplM)	SACOL2207	19
acetyl-CoA acetyltransferase	SACOL0426	19
aspartyl-tRNA synthetase (aspS)	SACOL1685	19
ribosomal protein S18 (rpsR)	SACOL0439	19
ribosomal protein S9 (rpsI)	SACOL2206	19
peptidase, M20-M25-M40 family	SACOL0085	19
conserved hypothetical protein	SACOL2609	19
N-acetylmuramoyl-L-alanine amidase domain protein	SACOL2666	18
acid phosphatase5'-nucleotidase, lipoprotein e(P4) family	SACOL0303	18
DAK2 domain protein	SACOL1240	18
dihydroorotase (pyrC)	SACOL1213	18
oxidoreductase, short-chain dehydrogenase-reductase family	SACOL2488	18
tyrosyl-tRNA synthetase (tyrS)	SACOL1778	18
prolyl-tRNA synthetase (proS)	SACOL1282	18
alanyl-tRNA synthetase (alaS)	SACOL1673	18
ribosomal protein L30p-L7e (rpmD)	SACOL2221	18
cysteinyl-tRNA synthetase (cysS)	SACOL0576	18
proline dipeptidase (pepQ)	SACOL1756	18
ribosomal protein L31 (rpmE)	SACOL2112	18
HIT family protein	SACOL1894	18
cytosol aminopeptidase	SACOL0945	18
isocitrate dehydrogenase, NADP-dependent (icd)	SACOL1741	17
glyoxalase family protein	SACOL2533	17
NH(3)-dependent NAD+ synthetase (nadE)	SACOL1974	17
UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1 (murAA)	SACOL2092	17
phosphoribosylaminoimidazole-succinocarboxamide synthase (purC)	SACOL1075	17
phosphoribosylaminoimidazole carboxylase, catalytic subunit (purE)	SACOL1073	17
conserved hypothetical protein	SACOL1987	17
elastin binding protein, putative	SACOL1522	16
methionine aminopeptidase, type I	SACOL1946	16
D-alanine-activating enzyme-D-alanine-D-alanyl carrier protein ligase (dltA)	SACOL0935	16
UDP-N-acetylglucosamine 1-carboxyvinyltransferase 2 (murAB)	SACOL2116	16
glutamate-1-semialdehyde-2,1-aminomutase (hemL2)	SACOL1922	16
ribosomal protein L18 (rplR)	SACOL2223	16
conserved hypothetical protein	SACOL0738	16
sigma factor B regulator protein (rsbU)	SACOL2057	16
conserved hypothetical protein	SACOL1350	16
translation initiation factor IF-3 (infC)	SACOL1727	15
quinol oxidase, subunit I (qoxA)	SACOL1069	15
polypeptide deformylase (def1)	SACOL1100	15
transcriptional regulator, MarR family (norR)	SACOL0746	15

Appendix 2. (Continued)

oligoendopeptidase F, putative	SACOL1419	15
betaine aldehyde dehydrogenase (betB)	SACOL2628	15
nucleoside diphosphate kinase	SACOL1509	15
DNA-binding response regulator YycF (yycF)	SACOL0019	15
conserved hypothetical protein	SACOL1306	15
heat shock protein HslVU, ATPase subunit HslV (hslV)	SACOL1270	15
glyceraldehyde 3-phosphate dehydrogenase (gapA2)	SACOL1734	15
ribosomal protein S12 (rpsL)	SACOL0591	14
phosphomethylpyrimidine kinase (thiD1)	SACOL0626	14
ribonucleoside-diphosphate reductase, alpha subunit	SACOL0792	14
conserved hypothetical protein	SACOL1670	14
DNA polymerase I (polA)	SACOL1737	14
peptidase, M20-M25-M40 family	SACOL1555	14
recA protein	SACOL1304	14
conserved hypothetical protein	SACOL2174	14
GTP-binding protein TypA (typA)	SACOL1118	14
oxidoreductase, putative	SACOL0399	14
succinate dehydrogenase, iron-sulfur protein (sdhB)	SACOL1160	14
alanine dehydrogenase (ald1)	SACOL1478	14
PTS system, IIABC components	SACOL0175	14
ScdA protein (scdA)	SACOL0244	14
amino acid ABC transporter, amino acid-binding protein	SACOL2412	13
conserved hypothetical protein TIGR00092	SACOL0435	13
malonyl CoA-acyl carrier protein transacylase (fabD)	SACOL1244	13
conserved hypothetical protein	SACOL1447	13
lipoprotein, putative	SACOL2365	13
carbamoyl-phosphate synthase, large subunit (carB)	SACOL1215	13
RNA polymerase sigma factor RpoD (rpoD)	SACOL1618	13
ABC transporter, ATP-binding protein	SACOL1427	13
conserved hypothetical protein	SACOL2143	13
glycosyl transferase, group 2 family protein	SACOL0243	13
ribosomal protein L36 (rpmJ)	SACOL2216	13
NADH-dependent flavin oxidoreductase, Oye family	SACOL0959	13
ribosomal protein L35 (rpmI)	SACOL1726	13
conserved hypothetical protein	SACOL1672	13
pyrroline-5-carboxylate reductase (proC)	SACOL1546	12
ribosomal protein L23 (rplW)	SACOL2237	12
cell division protein FtsH, putative	SACOL0555	12
epimerase-dehydratase, putative	SACOL2446	12
fatty acid-phospholipid synthesis protein PlsX (plsX)	SACOL1243	12
aminotransferase, putative	SACOL2000	12
enoyl-(acyl-carrier-protein) reductase (fabI)	SACOL1016	12
transcription elongation factor GreA (greA)	SACOL1665	12
threonine dehydratase, catabolic (ilvA1)	SACOL1477	12
transcriptional regulator, TenA family	SACOL2086	12
alcohol dehydrogenase, zinc-containing	SACOL0241	12
glycerol kinase (glpK)	SACOL1320	12
dihydroxyacetone kinase family protein	SACOL0707	12
conserved hypothetical protein	SACOL0455	11
molybdenum ABC transporter, molybdenum-binding protein ModA (modA)	SACOL2272	11
serine protease HtrA, putative	SACOL1777	11
glucokinase (glk)	SACOL1604	11
glutathione peroxidase (gpxA1)	SACOL1325	11
excinuclease ABC, A subunit (uvrA)	SACOL0824	11
DNA-directed RNA polymerase, omega subunit (rpoZ)	SACOL1222	11
OsmC-Ohr family protein	SACOL0872	11

Appendix 2. (Continued)

SIS domain protein	SACOL0618	11
conserved hypothetical protein	SACOL2456	11
conserved hypothetical protein	SACOL2300	11
conserved hypothetical protein	SACOL1767	10
NAD(P)H dehydrogenase (quinone), putative	SACOL0190	10
hydrolase, haloacid dehalogenase-like family	SACOL0931	10
staphylococcus tandem lipoprotein	SACOL0486	10
ribose-phosphate pyrophosphokinase (prsA)	SACOL0544	10
DNA repair exonuclease family protein	SACOL1900	10
heat shock protein HslVU, ATPase subunit HslU (hslU)	SACOL1271	10
D-alanine--D-alanine ligase	SACOL2074	10
ribonucleoside-diphosphate reductase 2, beta subunit (nrdF)	SACOL0793	10
phosphoribosylformylglycinamidine synthase I (purQ)	SACOL1077	10
femX protein (femX)	SACOL2253	10
malate:quinone oxidoreductase (mqo1)	SACOL2362	10
peptidase T (pepT)	SACOL0806	10
para-nitrobenzyl esterase (pnbA)	SACOL2459	10
transcription antitermination protein NusG (nusG)	SACOL0582	10
conserved hypothetical protein TIGR00294	SACOL0613	10
superoxide dismutase (sodA1)	SACOL0118	10
adenine phosphoribosyltransferase (apt)	SACOL1690	10
ribosomal protein L24 (rplX)	SACOL2228	10
sdrD protein (sdrD)	SACOL0609	9
penicillin-binding protein 2 (pbp2)	SACOL1490	9
conserved hypothetical protein	SACOL0669	9
ATP-dependent Clp protease, proteolytic subunit ClpP (clpP)	SACOL0833	9
ATP synthase F0, B subunit (atpF)	SACOL2099	9
chaperonin, 10 kDa (groES)	SACOL2017	9
pyridine nucleotide-disulfide oxidoreductase	SACOL1520	9
3,4-dihydroxy-2-butanone-4-phosphate synthase-GTP cyclohydrolase II (ribBA)	SACOL1818	9
phosphoribosylformylglycinamidine cyclo-ligase (purM)	SACOL1080	9
exoribonuclease, VacB-RNase II family	SACOL0846	9
chorismate mutase-phospho-2-dehydro-3-deoxyheptonate aldolase	SACOL1787	9
glycine cleavage system T protein (gcvT)	SACOL1595	9
conserved hypothetical protein	SACOL1099	9
dihydrofolate reductase (folA)	SACOL1461	9
ribosomal protein S10 (rpsJ)	SACOL2240	9
accessory gene regulator protein A (agrA)	SACOL2026	9
phosphoribosylaminoimidazole carboxylase, ATPase subunit (purK)	SACOL1074	9
alanine racemase (alr)	SACOL2060	9
D-alanyl carrier protein (dltC)	SACOL0937	9
conserved hypothetical protein	SACOL2288	9
staphylococcal accessory regulator S (sarS)	SACOL0096	9
ATP-dependent RNA helicase, DEAD-DEAH box family	SACOL2072	9
acetyltransferase, GNAT family	SACOL2532	9
urease accessory protein UreE (ureE)	SACOL2283	9
lipoate-protein ligase A family protein	SACOL1591	9
map protein, programmed frameshift (map)	SACOL2002	8
ATP-dependent Clp protease, ATP-binding subunit ClpX (clpX)	SACOL1721	8
1-phosphofructokinase (fruK)	SACOL0758	8
single-stranded DNA-binding protein (ssb2)	SACOL0438	8
UTP-glucose-1-phosphate uridylyltransferase family protein	SACOL2161	8
cell-division initiation protein, putative	SACOL1205	8
RNA methyltransferase, TrmH family	SACOL0578	8
NifU domain protein	SACOL0917	8
amidophosphoribosyltransferase (purF)	SACOL1079	8

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DNA gyrase, B subunit (gyrB)	SACOL0005	8
epidermin immunity protein F (epiF)	SACOL1873	8
cell division protein FtsY, putative	SACOL1251	8
arginase (rocF)	SACOL2154	8
GTP-binding protein, Era-TrmE family	SACOL1515	8
thymidylate synthase (thyA)	SACOL1462	8
repressor of toxins (rot)	SACOL1812	8
ATP-binding protein, Mrp-Nbp35 family	SACOL2156	8
ATP synthase F1, delta subunit (atpH)	SACOL2098	8
ABC transporter, ATP-binding protein	SACOL1994	8
UDP-N-acetylmuramoylalanine--D-glutamate ligase (murD)	SACOL1196	8
beta-hydroxyacyl-(acyl-carrier-protein) dehydratase FabZ (fabZ)	SACOL2091	8
GMP reductase (guaC)	SACOL1371	8
phosphoglucomutase-phosphomannomutase family protein	SACOL2501	8
drP35 protein (drp35)	SACOL2712	8
conserved hypothetical protein	SACOL0525	8
conserved hypothetical protein TIGR00253	SACOL1651	8
ribosomal protein L32 (rpmF)	SACOL1137	8
azoreductase	SACOL0607	8
DNA-binding response regulator VraR (vraR)	SACOL1942	8
peptide chain release factor 1 (prfA)	SACOL2110	7
S-adenosylmethionine synthetase (metK)	SACOL1837	7
4-oxalocrotonate tautomerase (dmpI)	SACOL1399	7
2-oxoisovalerate dehydrogenase, E1 component, beta subunit	SACOL1561	7
DHH subfamily 1 protein	SACOL1751	7
DNA-binding response regulator SrrA (srrA)	SACOL1535	7
degV family protein	SACOL1460	7
conserved hypothetical protein	SACOL0742	7
HAM1 protein	SACOL1162	7
conserved hypothetical protein	SACOL1558	7
UDP-N-acetylglucosamine pyrophosphorylase (glmU)	SACOL0543	7
conserved hypothetical protein	SACOL1483	7
tRNA pseudouridine 55 synthase (truB)	SACOL1290	7
alcohol dehydrogenase, zinc-containing	SACOL2177	7
staphylococcal accessory regulator R (sarR)	SACOL2287	7
conserved hypothetical protein	SACOL0467	7
conserved hypothetical protein	SACOL0409	7
conserved hypothetical protein	SACOL0565	7
glyoxalase family protein	SACOL1553	7
riboflavin biosynthesis protein RibD (ribD)	SACOL1820	7
conserved hypothetical protein	SACOL1836	7
uridylate kinase (pyrH)	SACOL1277	7
femA protein (femA)	SACOL1410	7
5'-methylthioadenosine-S-adenosylhomocysteine nucleosidase (mtn)	SACOL1655	7
molybdenum cofactor biosynthesis protein B (moaB)	SACOL2268	7
delta-hemolysin (hld)	SACOL2022	7
UDP-N-acetylmuramate--alanine ligase (murC)	SACOL1790	7
conserved hypothetical protein	SACOL1310	7
conserved hypothetical protein	SACOL1895	7
esterase, putative	SACOL2549	7
staphyloxanthin biosynthesis protein	SACOL2291	6
ATP synthase F1, gamma subunit (atpG)	SACOL2096	6
dephospho-CoA kinase (coaE)	SACOL1735	6
copper ion binding protein	SACOL2573	6
thiamine-phosphate pyrophosphorylase (thiE)	SACOL2083	6
ferrochelatase (hemH)	SACOL1888	6

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phenylalanyl-tRNA synthetase, alpha subunit (pheS)	SACOL1148	6
femB protein	SACOL1411	6
general stress protein 13	SACOL0552	6
urease accessory protein UreG (ureG)	SACOL2285	6
DAK2 domain protein	SACOL0708	6
ribosomal protein L19 (rplS)	SACOL1257	6
translation initiation factor IF-1 (infA)	SACOL2217	6
NifU domain protein	SACOL0939	6
glucose inhibited division protein A (gidA)	SACOL2737	6
conserved hypothetical protein TIGR01777	SACOL0834	6
phosphomethylpyrimidine kinase (thiD2)	SACOL2085	6
sucrose-6-phosphate hydrolase (cscA)	SACOL2029	6
cmp-binding-factor 1 (cbf1)	SACOL1898	6
conserved hypothetical protein	SACOL0157	6
teichoic acid biosynthesis protein, putative	SACOL0242	6
D-isomer specific 2-hydroxyacid dehydrogenase family protein	SACOL0932	6
isochorismatase family protein	SACOL2667	6
iron compound ABC transporter, iron compound-binding protein	SACOL2167	6
histidine ammonia-lyase (hutH)	SACOL0008	6
lipoate-protein ligase A family protein	SACOL1034	6
queuine tRNA-ribosyltransferase (tgt)	SACOL1694	6
conserved hypothetical protein	SACOL1980	6
phytoene dehydrogenase	SACOL2579	6
FMN reductase-related protein	SACOL0410	6
Rrf2 family protein	SACOL1681	6
glutamyl-tRNA(Gln) amidotransferase, C subunit (gatC)	SACOL1962	6
conserved hypothetical protein	SACOL1387	6
phosphosugar-binding transcriptional regulator, RpiR family	SACOL2308	6
alpha-acetolactate decarboxylase (budA1)	SACOL2198	6
DNA topoisomerase I (topA)	SACOL1267	6
proline dehydrogenase (putA)	SACOL1816	6
conserved hypothetical protein	SACOL1090	6
conserved hypothetical protein TIGR00043	SACOL1627	6
Aerolysin-Leukocidin family protein	SACOL2006	5
ribosome-binding factor A (rbfA)	SACOL1289	5
conserved hypothetical protein	SACOL1426	5
hydrolase, haloacid dehalogenase-like family	SACOL0606	5
UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase (murF)	SACOL2073	5
tellurite resistance protein, putative	SACOL1441	5
GTP-binding protein, GTP1-OBG family	SACOL1699	5
conserved hypothetical protein	SACOL2605	5
peptide chain release factor 2, programmed frameshift (prfB)	SACOL0818	5
conserved hypothetical protein	SACOL1120	5
2-oxoisovalerate dehydrogenase, E1 component, alpha subunit	SACOL1562	5
conserved hypothetical protein	SACOL1940	5
S1 RNA binding domain protein	SACOL2053	5
tryptophanyl-tRNA synthetase (trpS)	SACOL1001	5
HD-HDIG-KH domain protein	SACOL1305	5
histidyl-tRNA synthetase (hisS)	SACOL1686	5
conserved hypothetical protein	SACOL1620	5
2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylic acid synthase-2-oxoglutarate decarboxylase (menD)	SACOL1052	5
conserved hypothetical protein	SACOL2381	5
ribosomal protein L14 (rplN)	SACOL2229	5
citrate synthase (gltA)	SACOL1742	5
excinuclease ABC, B subunit (uvrB)	SACOL0823	5

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NAD(P)H-flavin oxidoreductase, putative	SACOL0453	5
peptide methionine sulfoxide reductase (msrA)	SACOL1397	5
alpha-glucosidase (malA)	SACOL1551	5
conserved hypothetical protein	SACOL2035	5
signal recognition particle protein (ffh)	SACOL1253	5
tRNA (5-methylaminomethyl-2-thiouridylate)-methyltransferase (trmU)	SACOL1676	5
GTP-binding protein Era (era)	SACOL1624	5
2-oxoisovalerate dehydrogenase, E2 component, dihydrolipoamide acetyltransferase	SACOL1560	5
conserved hypothetical protein	SACOL2132	5
conserved hypothetical protein	SACOL1358	5
MutT-nudix family protein	SACOL1542	5
YihY family protein (yihY)	SACOL1941	5
aspartate carbamoyltransferase (pyrB)	SACOL1212	5
conserved hypothetical protein	SACOL0152	5
MutT-nudix family protein	SACOL1724	5
conserved hypothetical protein	SACOL1002	5
arginine repressor (argR)	SACOL1565	5
lipoprotein, putative	SACOL1101	4
iron compound ABC transporter, iron compound-binding protein	SACOL2277	4
hydrolase, haloacid dehalogenase-like family	SACOL0602	4
conserved hypothetical protein	SACOL1464	4
phosphoribosylglycinamide formyltransferase (purN)	SACOL1081	4
xanthine phosphoribosyltransferase (xpt)	SACOL0458	4
hydrolase, haloacid dehalogenase-like family	SACOL1365	4
acetyl-CoA carboxylase, biotin carboxyl carrier protein (accB)	SACOL1572	4
dnaJ protein (dnaJ)	SACOL1636	4
DltD protein (dltD)	SACOL0938	4
GTP pyrophosphokinase (relA2)	SACOL1689	4
primosomal protein N` (priA)	SACOL1224	4
dihydroorotate dehydrogenase (pyrD)	SACOL2606	4
porphobilinogen deaminase (hemC)	SACOL1717	4
exonuclease RexA (rexA)	SACOL0971	4
glycerophosphoryl diester phosphodiesterase, putative	SACOL1130	4
DNA topoisomerase IV, A subunit (parC)	SACOL1390	4
sulfite reductase (NADPH) flavoprotein alpha-component (cysJ)	SACOL2639	4
argininosuccinate lyase (argH)	SACOL0963	4
dehydrosqualene desaturase (crtN)	SACOL2576	4
heat shock protein, Hsp20 family	SACOL2385	4
oxidoreductase, aldo-keto reductase family	SACOL1543	4
glycosyl transferase, group 1 family protein	SACOL1043	4
DNA-dependent DNA polymerase family X	SACOL1153	4
serine acetyltransferase (cysE)	SACOL0575	4
S-adenosyl-methyltransferase MraW	SACOL1192	4
N-acetyltransferase family protein	SACOL2722	4
phosphotyrosine protein phosphatase	SACOL1939	4
exodeoxyribonuclease VII, large subunit (xseA)	SACOL1568	4
deoxyribonuclease, TatD family	SACOL0534	4
pur operon repressor (purR)	SACOL0539	4
glycerate kinase family protein	SACOL0805	4
carbamoyl-phosphate synthase, small subunit (carA)	SACOL1214	4
GTP-binding protein LepA (lepA)	SACOL1641	4
oxidoreductase, aldo-keto reductase family	SACOL2192	4
conserved hypothetical protein	SACOL2318	4
conserved hypothetical protein TIGR00282	SACOL1307	4
conserved hypothetical protein	SACOL0271	4

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conserved hypothetical protein	SACOL1284	4
conserved hypothetical protein	SACOL1899	4
conserved hypothetical protein	SACOL0615	4
inositol monophosphatase family protein	SACOL1116	4
conserved hypothetical protein TIGR00147	SACOL1958	4
trans-sulfuration enzyme family protein	SACOL0503	4
GTP pyrophosphokinase (relA1)	SACOL1010	4
UTP-glucose-1-phosphate uridylyltransferase (galU)	SACOL2508	4
transcriptional regulator, Fur family	SACOL1541	4
sulfatase family protein	SACOL0778	3
immunodominant antigen B (isaB)	SACOL2660	3
FtsK-SpoIIIE family protein	SACOL1791	3
cytidylate kinase (cmk)	SACOL1518	3
Gid protein (gid)	SACOL1268	3
conserved hypothetical protein	SACOL1885	3
uroporphyrinogen decarboxylase (hemE)	SACOL1889	3
transcriptional regulator, putative	SACOL2302	3
YlmF protein (ylmF)	SACOL1202	3
orotidine 5'-phosphate decarboxylase (pyrF)	SACOL1216	3
UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase (murG)	SACOL1453	3
mevalonate kinase (mvk)	SACOL0636	3
pyrimidine operon regulatory protein (pyrR)	SACOL1210	3
lipoate synthase (lipA)	SACOL0927	3
rhodanese-like domain protein	SACOL1807	3
DNA ligase, NAD-dependent (ligA)	SACOL1965	3
transferrin receptor	SACOL0799	3
pyridine nucleotide-disulfide oxidoreductase	SACOL0640	3
conserved hypothetical protein	SACOL0614	3
peptide chain release factor 3 (prfC)	SACOL1025	3
conserved hypothetical protein	SACOL2436	3
DNA topoisomerase IV, B subunit (parE)	SACOL1389	3
N utilization substance protein B (nusB)	SACOL1569	3
rhodanese-like domain protein	SACOL1592	3
ribosomal protein S21 (rpsU)	SACOL1632	3
DNA-3-methyladenine glycosylase	SACOL1711	3
conserved hypothetical protein	SACOL0198	3
conserved hypothetical protein	SACOL1297	3
phosphomevalonate kinase	SACOL0638	3
conserved hypothetical protein	SACOL1286	3
capsular polysaccharide biosynthesis protein Cap5O (cap5O)	SACOL0150	3
RNA polymerase sigma-37 factor (rpoF)	SACOL2054	3
glycosyl transferase, group 1 family protein	SACOL0612	3
conserved hypothetical protein TIGR01741	SACOL0282	3
dihydrodipicolinate reductase (dapB)	SACOL1431	3
conserved hypothetical protein	SACOL0599	3
formate dehydrogenase, NAD-dependent	SACOL0162	3
PTS system, IIBC components	SACOL0516	3
conserved hypothetical protein	SACOL0401	3
phosphopantothenoylcysteine decarboxylase-phosphopantothenate--cysteine ligase (coaBC)	SACOL1223	3
mevalonate diphosphate decarboxylase (mvaD)	SACOL0637	3
conserved hypothetical protein	SACOL0804	3
HD domain protein	SACOL0821	3
conserved hypothetical protein	SACOL1011	3
hypothetical protein	SACOL1042	3

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aminotransferase, class V	SACOL1677	3
protein-export membrane protein SecDF	SACOL1692	3
amino acid ABC transporter, ATP-binding protein	SACOL2453	3
glutamate racemase (murI)	SACOL1161	3
HD domain protein	SACOL0658	3
staphylococcal accessory protein X (sarX)	SACOL0726	3
6-pyruvoyl tetrahydrobiopterin synthase, putative	SACOL0771	3
diaminopimelate decarboxylase (lysA)	SACOL1435	3
PhoH family protein	SACOL1628	3
hypothetical protein	SACOL0156	3
conserved hypothetical protein	SACOL1239	3
DNA repair protein RecN (recN)	SACOL1564	3
acetyl-CoA carboxylase, carboxyl transferase, beta subunit (accD)	SACOL1748	3
sdrC protein (sdrC)	SACOL0608	2
Staphylococcus aureus sex pheromone (camS)	SACOL1964	2
signal peptidase IB (spsB)	SACOL0969	2
conserved hypothetical protein	SACOL2020	2
decarboxylase family protein	SACOL0740	2
ferredoxin (fer)	SACOL1525	2
ABC transporter, substrate-binding protein	SACOL2403	2
conserved domain protein	SACOL1466	2
type I restriction-modification system, M subunit (hsdM1)	SACOL0476 (+1)	2
exonuclease RexB (rexB)	SACOL0970	2
adenosylmethionine--8-amino-7-oxononanoate aminotransferase (bioA)	SACOL2427	2
oxidoreductase, Gfo-Idh-MocA family	SACOL0196	2
conserved hypothetical protein	SACOL2489	2
riboflavin biosynthesis protein RibF (ribF)	SACOL1291	2
UDP-N-acetylglucosamine 2-epimerase Cap5G (cap5G)	SACOL0142	2
conserved domain protein	SACOL0445	2
conserved hypothetical protein	SACOL0830	2
transcriptional regulator, Fur family	SACOL1611	2
protoporphyrinogen oxidase (hemG)	SACOL1887	2
hydrolase, haloacid dehalogenase-like family	SACOL0976	2
hypothetical protein	SACOL0182	2
iron-dependent repressor (sirR)	SACOL0691	2
alkaline phosphatase synthesis transcriptional regulatory protein PhoP (phoP)	SACOL1740	2
peptidase, M16 family	SACOL1298	2
UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase (murE)	SACOL1023	2
hydrolase, alpha-beta hydrolase fold family	SACOL0668	2
thymidine kinase (tdk)	SACOL2111	2
transcriptional regulator, putative	SACOL2650	2
conserved hypothetical protein	SACOL0058	2
PTS system, IIBC components	SACOL0178	2
phosphoglycerate mutase family protein	SACOL0447	2
conserved hypothetical protein	SACOL0753	2
conserved hypothetical protein	SACOL1112	2
fibronectin-fibrinogen binding-related protein	SACOL1220	2
ribosomal protein L28 (rpmB)	SACOL1238	2
conserved hypothetical protein	SACOL1376	2
transcriptional antiterminator LicT, putative	SACOL1393	2
aspartate-semialdehyde dehydrogenase (asd)	SACOL1429	2
dihydrodipicolinate synthase (dapA)	SACOL1430	2
conserved hypothetical protein TIGR00256	SACOL1688	2
cytosolic long-chain acyl-CoA thioester hydrolase family protein	SACOL1936	2
conserved hypothetical protein	SACOL1993	2

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isopentenyl diphosphate isomerase (fni)	SACOL2341	2
staphylococcal accessory protein Z (sarZ)	SACOL2384	2
ABC transporter, ATP-binding protein	SACOL2462	2
gluconokinase (gntK)	SACOL2515	2
galactoside O-acetyltransferase	SACOL2570	2
hydrolase, CocE-NonD family	SACOL2612	2
tributyrin esterase EstA, putative	SACOL2651	2
rod shape-determining protein MreC (mreC)	SACOL1704	1
LysM domain protein	SACOL0507	1
LPXTG cell wall surface anchor family protein (sasF)	SACOL2668	1
lipoprotein, putative	SACOL0449	1
penicillin-binding protein 1 (pbp1)	SACOL1194	1
protein phosphatase 2C domain protein	SACOL1231	1
secretory extracellular matrix and plasma binding protein (empbp)	SACOL0858	1
chaperonin, 33 kDa	SACOL0556	1
immunodominant antigen A (isaA)	SACOL2584	0
cell wall surface anchor family protein (sasG)	SACOL2505	0
fibronectin binding protein B (fnbB)	SACOL2509	0
fibronectin-binding protein A (fnbA)	SACOL2511	0
clumping factor B (clfB)	SACOL2652	0
LysM domain protein	SACOL0723	0
leukocidin subunit precursor, putative	SACOL2004	0
alpha-hemolysin precursor (hlY)	SACOL1173	0
surface protein, putative	SACOL0479	0
IgG-binding protein SBI	SACOL2418	0
cysteine protease precursor SspB (sspB2)	SACOL1970	0
sasB protein (sasB)	SACOL2150	0
transcriptional regulator, putative	SACOL1065	0
phage infection protein, putative	SACOL2665	0
transcriptional regulator, putative	SACOL1398	0
penicillin-binding protein 3 (pbp3)	SACOL1609	0
phospholipase C (hlb)	SACOL2003	0
lipoprotein, putative	SACOL1589	0
peptide ABC transporter, peptide-binding protein	SACOL2476	0
ABC transporter, substrate-binding protein	SACOL0217	0
thymidylate kinase (tmk)	SACOL0524	0
glucose inhibited division protein A (gidA)	SACOL2737-R	0
fibronectin binding protein B (fnbB)	SACOL2509-R	0
serine protease SplB (splB)	SACOL1868	0

Appendix 3. Cytoplasmic proteins identified after MudPit analysis of SH1000 during post-exponential phase from 2 biological replicates

Identified Proteins (346)	Accession Number	Sample 1	Sample 2
Elongation factor Tu	Q5HIC7|EFTU	239	119
Probable transglycosylase isaA	Q5HCY1|ISAA	81	111
Elongation factor G	Q5HIC8|EFG	89	65
Enolase	Q5HHP1|ENO	64	39
Pyruvate kinase	Q5HF76|KPYK	54	27
Dihydrolipoyl dehydrogenase	Q5HGY8|DLDH	39	36
Pyruvate dehydrogenase E1 component subunit beta	Q5HGZ0|ODPB	35	38
Bifunctional autolysin	Q5HH31|ATL	45	24
50S ribosomal protein L30	Q5HDX6|RL30	28	25
50S ribosomal protein L15	Q5HDX7|RL15	15	45
Pyruvate dehydrogenase E1 component subunit alpha	Q5HGZ1|ODPA	34	25
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	Q5HGY9|ODP2	30	23
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	Q5HDD9|GPMA	34	13
Phosphate acetyltransferase	Q5HI88|PTA	29	21
Alkyl hydroperoxide reductase subunit C	Q5HIR5|AHPC	27	16
Transketolase	Q5HG77|TKT	26	21
30S ribosomal protein S8	Q5HDX2|RS8	15	17
50S ribosomal protein L13	Q5HDZ0|RL13	25	14
Glycyl-tRNA synthetase	Q5HFJ5|SYG	29	11
50S ribosomal protein L1	Q5HID7|RL1	22	15
Alkaline shock protein 23	Q5HE23|ASP23	24	11
ATP synthase subunit beta	Q5HE97|ATPB	26	16
Pyridoxal biosynthesis lyase pdxS	Q5HIF5|PDXS	21	16
Staphylococcal secretory antigen ssaA2	Q5HDQ9|SSAA2	22	17
L-lactate dehydrogenase 1	Q5HJD7|LDH1	26	13
50S ribosomal protein L27	Q5HFB8|RL27	23	13
ATP synthase subunit alpha	Q5HE95|ATPA	25	14
Cell division protein ftsZ	Q5HGP5|FTSZ	14	18
3-oxoacyl-[acyl-carrier-protein] reductase	Q5HGK2|FABG	18	17
Probable malate:quinone oxidoreductase 2	Q5HCU5|MQO2	18	9
Succinyl-CoA ligase [ADP-forming] subunit beta	Q5HGI7|SUCC	20	14
GMP synthase [glutamine-hydrolyzing]	Q5HIQ6|GUAA	20	10
50S ribosomal protein L6	Q5HDX3|RL6	19	11
Glutamine synthetase	Q5HGC3|GLNA	21	7
Uracil phosphoribosyltransferase	Q5HE88|UPP	17	13
Glyceraldehyde-3-phosphate dehydrogenase 1	Q5HHP5|G3P1	23	7
DNA-binding protein HU	Q5HFV0|DBH	20	11
Antibacterial protein (Phenol soluble modulin)	Q5HGQ7|Q5HGQ7	18	6
30S ribosomal protein S6	Q5HIS9|RS6	22	10
60 kDa chaperonin	Q5HEH2|CH60	16	9
UPF0365 protein	Q5HFI7|Y1630	19	11
DNA-directed RNA polymerase subunit omega	Q5HGM2|RPOZ	13	12
DNA-directed RNA polymerase subunit beta'	Q5HID2|RPOC	20	8
Fructose-bisphosphate aldolase class 1	Q5HCU6|ALF1	16	10

Appendix 3. (Continued)

DNA-directed RNA polymerase subunit beta	Q5HID3|RPOB	17	9
Glucosamine-6-phosphate isomerase, putative	Q5HES0|Q5HES0	17	12
6-phosphogluconate dehydrogenase, decarboxylating	Q5HFR2|6PGD	17	9
50S ribosomal protein L5	Q5HDX0|RL5	22	8
Putative universal stress protein	Q5HF64|Y1759	15	12
ATP-dependent Clp protease ATP-binding subunit clpL	Q5HD02|CLPL	16	7
Antibacterial protein (Phenol soluble modulin)	Q5HGQ8|Q5HGQ8	16	5
Acetate kinase	Q5HF63|ACKA	16	7
50S ribosomal protein L11	Q5HID8|RL11	11	9
Ribosome-recycling factor	Q5HGH2|RRF	11	13
30S ribosomal protein S19	Q5HDW2|RS19	14	11
Succinyl-CoA ligase [ADP-forming] subunit alpha	Q5HGI6|SUCD	10	9
Putative uncharacterized protein	Q5HIT1|Q5HIT1	10	13
Bifunctional purine biosynthesis protein purH	Q5HH11|PUR9	18	6
Carbamoyl-phosphate synthase large chain	Q5HGM9|CARB	12	12
Probable thiol peroxidase	Q5HF61|TPX	14	7
Chaperone protein hchA	Q5HIC4|HCHA	15	11
Conserved domain protein	Q5HH57|Q5HH57	15	9
Fructose-bisphosphate aldolase	Q5HE75|ALF2	12	6
Pyruvate carboxylase	Q5HGX0	11	5
Ornithine aminotransferase 2	Q5HHC8|OAT2	12	6
Ribose-phosphate pyrophosphokinase	Q5HIH5|KPRS	15	8
Chaperone protein dnaK	Q5HFI0|DNAK	13	8
Elongation factor Ts	Q5HGH4|EFTS	16	5
UPF0342 protein	Q5HET0|Y1902	13	10
Putative 2-hydroxyacid dehydrogenase	Q5HDQ4|Y2296	11	10
Oxidoreductase, putative	Q5HIW6|Q5HIW6	10	4
Formate acetyltransferase	Q5HJF4|PFLB	14	1
N utilization substance protein A, putative	Q5HGG5|Q5HGG5	13	6
30S ribosomal protein S11	Q5HDY3|RS11	14	4
50S ribosomal protein L32	Q5HGV6|RL32	12	6
Naphthoate synthase	Q5HH38|MENB	14	6
D-alanine--D-alanine ligase	Q5HEB7|DDL	14	8
Cell cycle protein gpsB	Q5HFX8|GPSB	13	7
Uncharacterized protein	Q5HGK7|Y1240	10	3
30S ribosomal protein S5	Q5HDX5|RS5	10	9
ATP-dependent Clp protease ATP-binding subunit clpC	P0C281|CLPC	12	6
50S ribosomal protein L10	Q5HID6|RL10	12	4
Alcohol dehydrogenase	Q5HI63|ADH	13	4
Bifunctional protein folD	Q5HH21|FOLD	12	2
Universal stress protein family	Q5HF68|Q5HF68	9	6
Phosphoenolpyruvate-protein phosphotransferase	Q5HH01|PT1	10	6
30S ribosomal protein S2	Q5HGH6|RS2	10	4
NADH dehydrogenase-like protein	Q5HHE4|Y944	11	7
Ribonucleoside-diphosphate reductase	Q5HHU0|Q5HHU0	11	7
Hydroxymethylglutaryl-CoA synthase	Q5HD04|Q5HD04	9	7
Arginyl-tRNA synthetase	Q5HI60|SYR	9	7
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	Q5HG07|ODO2	9	4
30S ribosomal protein S7	Q5HIC9|RS7	11	4
30S ribosomal protein S16	Q5HGJ4|RS16	12	4
Thioredoxin	Q5HGT9|THIO	11	5
Phosphoribosylformylglycinamidine synthase, PurS protein	Q5HH17|Q5HH17	10	5
30S ribosomal protein S1	Q5HFU7|RS1	11	5
UPF0051 protein	Q5HHG8|Y918	10	3
50S ribosomal protein L2	Q5HDW1|RL2	7	5

Appendix 3. (Continued)

NADP-dependent malic enzyme, putative	Q5HF72|Q5HF72	7	7
Translation initiation factor IF-2	Q5HGG2|IF2	7	2
Putative uncharacterized protein	Q5HJK1|Q5HJK1	7	5
3-oxoacyl-[acyl-carrier-protein] synthase 2	Q5HHA1|FABF	6	5
Cell division protein FtsH, putative	Q5HIG4|Q5HIG4	9	5
Asparaginyl-tRNA synthetase	Q5HFW9|SYN	9	4
50S ribosomal protein L21	Q5HFB6|RL21	8	6
Phenol-soluble modulin alpha 1 peptide	P0C7Y4|PSMA1	7	3
30S ribosomal protein S10	Q5HDV7|RS10	7	7
N-acetylmuramoyl-L-alanine amidase domain protein	Q5HCQ3|Q5HCQ3	6	5
Uncharacterized protein	Q5HHB6|Y973	8	5
Putative uncharacterized protein	Q5HHZ0|Q5HHZ0	7	5
Prolyl-tRNA synthetase	Q5HGG8|SYP	7	0
50S ribosomal protein L17	Q5HDY5|RL17	11	1
Imidazolonepropionase	Q5HDM7|HUTI	8	5
50S ribosomal protein L7/L12	Q5HID5|RL7	6	5
Glutamyl-tRNA(Gln) amidotransferase subunit A	Q5HEM2|GATA	6	3
Queuine tRNA-ribosyltransferase	Q5HFC4|TGT	7	5
DNA polymerase III subunit beta	Q5HJZ4|DPO3B	8	2
ATP-dependent protease ATPase subunit HslU	Q5HGH8|HSLU	8	2
1-pyrroline-5-carboxylate dehydrogenase	Q5HCZ6|ROCA	8	2
UvrABC system protein A	UVRA	3	1
Serine hydroxymethyltransferase	Q5HE87|GLYA	9	4
Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B	Q5HEM3|GATB	5	3
Uncharacterized protein	Q5HEP9|Y1933	6	4
Threonyl-tRNA synthetase	Q5HF90|SYT	8	2
Thioredoxin, putative	Q5HF30|Q5HF30	6	4
50S ribosomal protein L18	Q5HDX4|RL18	7	3
GTP-sensing transcriptional pleiotropic repressor codY	Q5HGH7|CODY	6	4
Protein grpE	Q5HFH9|GRPE	7	4
Seryl-tRNA synthetase	Q5HJY7|SYS	6	2
Oxidoreductase, short chain dehydrogenase/reductase family	Q5HDM9|Q5HDM9	7	5
UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1	Q5HEA0|MURA1	6	3
Ferritin	Q5HEN0|FTN	7	4
50S ribosomal protein L29	Q5HDW6|RL29	5	3
Probable catabolite control protein A	Q5HF38|CCPA	7	5
50S ribosomal protein L33 1	Q5HFK9|RL331 (+1)	7	4
50S ribosomal protein L36	Q5HDY1|RL36	3	2
Thioredoxin reductase	Q5HHQ4|TRXB	10	0
Phosphoglucosamine mutase	Q5HE43|GLMM	6	2
Transaldolase	Q5HEZ4|Q5HEZ4	8	3
6-phosphofructokinase	Q5HF75|K6PF	5	4
50S ribosomal protein L23	Q5HDW0|RL23	4	4
UPF0082 protein	Q5HHZ9|Y727	7	3
Formate--tetrahydrofolate ligase	Q5HF42|FTHS	8	1
ABC transporter, ATP-binding protein	Q5HG28|Q5HG28	9	2
NifU domain protein	Q5HHG9|Q5HHG9	7	3
Valyl-tRNA synthetase	Q5HFA8|SYV	5	3
GTP-binding protein engA	Q5HFU8|ENGA	5	3
Deoxyribose-phosphate aldolase 1	Q5HJN0|DEOC1	5	2
Chorismate mutase/phospho-2-dehydro-3-deoxyheptonate aldolase	Q5HF37|Q5HF37	5	4
Putative aldehyde dehydrogenase AldA	Q5HJK3|ALDA	7	3
HTH-type transcriptional regulator sarR	Q5HDR3|SARR	5	3
Orotidine 5'-phosphate decarboxylase	Q5HGM8|PYRF	6	4

Appendix 3. (Continued)

UPF0355 protein	Q5HIR0|UP355	4	4
Adenylosuccinate synthetase	Q5HJX8|PURA	6	2
Proline dipeptidase	Q5HFM9|Q5HFM9	7	1
Putative uncharacterized protein	Q5HET6	5	2
Probable manganese-dependent inorganic pyrophosphatase	Q5HEK1|PPAC	7	3
UPF0435 protein	Q5HEP4|Y1938	6	4
Putative NAD(P)H nitroreductase	Q5HD30|Y2534	3	5
Probable acetyl-CoA acyltransferase	Q5HIU0|THLA	6	1
Isoleucyl-tRNA synthetase	Q5HGN8|SYI	5	2
Alcohol dehydrogenase, zinc-containing	Q5HE18|Q5HE18	3	3
Hydroxymethylglutaryl-CoA reductase, degradative	Q5HD05|Q5HD05	3	2
Putative dipeptidase	Q5HF23|PEPVL	4	3
3-hexulose-6-phosphate synthase	Q5HIA5|HPS	5	1
Dephospho-CoA kinase	Q5HF85|COAE	5	1
Aminoacyltransferase femA	Q5HG45|FEMA	4	3
FeS assembly ATPase SufC	Q5HHH2|Q5HHH2	7	1
NifU domain protein	Q5HHE8|Q5HHE8	4	4
Delta-hemolysin	Q5HEG6|HLD	5	4
Hypoxanthine-guanine phosphoribosyltransferase	Q5HIG5|HPRT	3	4
Inositol monophosphatase family protein	Q5HGX7|Q5HGX7	5	3
Phosphoglycerate kinase	Q5HHP4|PGK	5	3
30S ribosomal protein S4	Q5HF54|RS4	6	0
Protein translocase subunit secA 1	Q5HHR7|SECA1	4	0
CTP synthase	Q5HE73|PYRG	3	2
Serine-protein kinase rsbW	Q5HED6|RSBW	3	2
Anti-sigma-B factor antagonist	P60071|RSBV	5	2
50S ribosomal protein L14	RL14	3	1
S1 RNA binding domain protein	Q5HED8|Q5HED8	2	4
3-oxoacyl-[acyl-carrier-protein] synthase 3	Q5HHA2|FABH	5	1
tRNA uridine 5-carboxymethylaminomethyl modification enzyme mnmG	Q5HCI4|MNMG	2	2
Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta	Q5HF73|ACCD	4	2
Polyribonucleotide nucleotidyltransferase	Q5HGF7|PNP	2	1
N-acetylmuramoyl-L-alanine amidase sle1	Q5HIL2|SLE1	3	2
D-alanine aminotransferase	Q5HF24|DAAA	3	2
ATP synthase subunit delta	Q5HE94|ATPD	6	1
2-oxoglutarate dehydrogenase E1 component	Q5HG06|ODO1	4	3
30S ribosomal protein S20	Q5HFH5|RS20	4	0
Succinate dehydrogenase, iron-sulfur protein	Q5HGT4|Q5HGT4	4	3
Rrf2 family protein	Q5HFD6|Q5HFD6	3	2
Probable DEAD-box ATP-dependent RNA helicase	Q5HEB9|Y2072	3	3
Putative uncharacterized protein	Q5HEF7|Q5HEF7	3	3
Phosphoribosylglycinamide formyltransferase	Q5HH12|PUR3	2	3
50S ribosomal protein L25	Q5HIH4|RL25	3	2
Tyrosyl-tRNA synthetase	Q5HF45|SYY	2	2
Deoxyribonuclease, TatD family	Q5HII5|Q5HII5	2	1
Aconitate hydratase	Q5HG69|ACON	3	1
Low molecular weight protein-tyrosine-phosphatase ptpA	Q5HEP3|PTPA	1	4
Peptide chain release factor 1	Q5HE82|RF1	2	3
Peroxide-responsive repressor perR	Q5HER3|PERR	1	2
Mannitol-specific phosphotransferase enzyme IIA component	PTMA	3	1
PhoH family protein	Q5HFI9|Q5HFI9	1	2
Mannitol-1-phosphate 5-dehydrogenase	MTLD	4	0
Lysyl-tRNA synthetase	Q5HIF7|SYK	3	1
UPF0297 protein	Q5HFE5|Y1672	6	1
6,7-dimethyl-8-ribityllumazine synthase	Q5HF08|RISB	4	2

Appendix 3. (Continued)

Putative uncharacterized protein	Q5HEL5|Q5HEL5	4	2
Probable transglycosylase sceD	Q5HEA4|SCED	2	2
Guanylate kinase	Q5HGM3|KGUA	4	2
2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase 2	Q5HJC1|ISPD2	3	3
Oxidoreductase, aldo/keto reductase family	Q5HHW7|Q5HHW7	3	0
50S ribosomal protein L9	Q5HJY1|RL9	4	0
Ribonuclease J 1	Q5HGZ5|RNJ1	5	0
33 kDa chaperonin	Q5HIG3|HSLO	5	0
Putative uncharacterized protein	Q5HGE0|Q5HGE0	3	3
Phosphoribosylamine--glycine ligase	Q5HH10|PUR2	3	1
ATP synthase gamma chain	Q5HE96|ATPG	2	1
Trigger factor	Q5HF97|TIG	3	0
Nitrite reductase [NAD(P)H], large subunit	Q5HDF6|Q5HDF6	2	2
Penicillin-binding protein 2	Q5HFX3|Q5HFX3	4	0
Riboflavin biosynthesis protein RibF	Q5HGF9|Q5HGF9	2	0
UPF0122 protein	Q5HGJ6|Y1252	3	0
Uridylate kinase	Q5HGH3|PYRH	3	2
Phosphoribosylformylglycinamidine synthase 2	Q5HH15|PURL	1	1
Catalase	Q5HG86|CATA	3	1
Phosphate acyltransferase	Q5HGK4|PLSX	3	2
Phosphoribosylformylglycinamidine synthase 1	Q5HH16|PURQ	4	2
Probable cysteine desulfurase	Q5HHH0|CSD	5	1
30S ribosomal protein S18	Q5HIS7|RS18	3	1
Molybdopterin molybdenumtransferase	Q5HDT4|MOEA	3	2
MutT/nudix family protein	Q5HF95|Q5HF95	3	0
5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase	Q5HIT8|METE	2	0
Putative uncharacterized protein	Q5HIN0|Q5HIN0	3	0
Malonyl CoA-acyl carrier protein transacylase	Q5HGK3|FABD	6	0
Uncharacterized protein	Q5HG10|Y1445	2	1
Alcohol dehydrogenase, zinc-containing	Q5HJC0|Q5HJC0	1	3
50S ribosomal protein L3	Q5HDV8|RL3	2	1
ComE operon protein 2	Q5HFH2|RANDOM_Q5HFH2-R	1	1
Xanthine phosphoribosyltransferase	Q5HIQ9|XPT	2	1
Chaperone protein clpB	Q5HHB0|CLPB	4	1
Fumarate hydratase class II	Q5HES4|FUMC	3	1
Copper chaperone copZ	Q5HCZ2|COPZ	1	2
Probable glycine dehydrogenase [decarboxylating] subunit 1	Q5HFM3|GCSPA	3	2
Glucose-specific phosphotransferase enzyme IIA component	Q5HFZ9|PTGA	4	1
UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase	Q5HG02|MURG	3	1
Methionyl-tRNA formyltransferase	Q5HGL6|FMT	4	1
Putative trmH family tRNA/rRNA methyltransferase	Q5HIE3|TRMHL	3	1
Translation initiation factor IF-3	Q5HF92|IF3	2	2
30S ribosomal protein S3	Q5HDW4|RS3	2	1
Alcohol dehydrogenase, zinc-containing	Q5HDI5|Q5HDI5	2	2
Histidyl-tRNA synthetase	Q5HFD2|SYH	2	2
Aspartate carbamoyltransferase	Q5HGN2|PYRB	2	2
Glutamate-1-semialdehyde 2,1-aminomutase 1	Q5HFA5|GSA1	2	2
Putative uncharacterized protein	Q5HGK8	2	2
Pyrimidine-nucleoside phosphorylase	Q5HE64|PDP	2	0
PTS system, IIBC components	Q5HJD5|Q5HJD5	1	0
50S ribosomal protein L4	RANDOM_RL4-R	1	0
Elastin-binding protein ebpS	Q5HFU2|EBPS	2	0

Appendix 3. (Continued)

Extracellular matrix-binding protein ebh	Q5HFY8|EBH	1	0
Septation ring formation regulator ezrA	Q5HF56|EZRA	4	0
Phosphopentomutase	Q5HJM9|DEOB	5	0
Phosphoribosylaminoimidazole carboxylase ATPase subunit	Q5HH19|PURK	3	0
Aspartate-semialdehyde dehydrogenase	Q5HG26|Q5HG26	2	0
Bacterioferritin comigratory protein	Q5HER1|Q5HER1	1	0
50S ribosomal protein L24	Q5HDW9|RL24	2	0
Dps family protein	Q5HE61|Q5HE61	3	0
Uroporphyrinogen decarboxylase	Q5HEU2|DCUP	4	0
DNA polymerase I	Q5HF83|Q5HF83	4	0
UPF0133 protein	Q5HIJ8|Y521	2	1
Putative hemin transport system permease protein hrtB	RANDOM_HRTB-R	1	0
Glutamate-1-semialdehyde 2,1-aminomutase 2	Q5HER0|GSA2	2	2
Phosphoribosylformylglycinamidine cyclo-ligase	Q5HH13|PUR5	3	1
30S ribosomal protein S12	Q5HID0|RS12	2	1
Putative septation protein spoVG	Q5HIH8|SP5G	3	1
Inosine-5'-monophosphate dehydrogenase	Q5HIQ7|IMDH	1	3
Putative uncharacterized protein	Q5HFG6|Q5HFG6	2	2
Pyruvate carboxylase	Q5HGX0|Q5HGX0	12	5
Putative uncharacterized protein	Q5HFX9|Q5HFX9	1	1
S-adenosylmethionine synthase	Q5HEY9|METK	2	1
Peptidase, M20/M25/M40 family	Q5HJR7|Q5HJR7	2	1
Iron compound ABC transporter, iron compound-binding protein	Q5HDS3|Q5HDS3	2	1
S-ribosylhomocysteine lyase	Q5HE66|LUXS	1	1
Putative uncharacterized protein	Q5HI16|Q5HI16	1	0
DNA topoisomerase 4 subunit B	Q5HG65|PARE	2	0
30S ribosomal protein S21	Q5HFI5|RS21	0	1
Peptide deformylase	Q5HGZ3|DEF	0	2
Acetyl-coenzyme A carboxylase carboxyl transferase subunit alpha	Q5HF74|ACCA	3	0
UDP-N-acetylmuramoylalanine--D-glutamate ligase	Q5HGP8|MURD	1	0
Trans-sulfuration enzyme family protein	Q5HIL6|Q5HIL6	2	0
Dehydrosqualene synthase	Q5HCY8|CRTM	1	0
Serine-aspartate repeat-containing protein C	Q5HIB4|SDRC	4	0
Transcription antitermination protein nusG	Q5HID9|NUSG	3	0
Bifunctional protein glmU	Q5HIH6|GLMU	3	0
GTP-binding protein TypA	Q5HGX5|Q5HGX5	3	0
Sun protein	Q5HGL5	2	0
Aspartyl-tRNA synthetase	Q5HFD3|SYD	2	0
Lipoate-protein ligase A family protein	Q5HH58|Q5HH58	1	0
Coenzyme A disulfide reductase	Q5HHB4|CDR	3	0
Cysteine synthase	Q5HIG2|CYSK	0	3
Acetyl-CoA carboxylase, biotin carboxylase	Q5HFP5|Q5HFP5	2	0
N-acetylglucosamine-6-phosphate deacetylase	Q5HHW9|Q5HHW9	4	0
Probable branched-chain-amino-acid aminotransferase	Q5HIC1|ILVE	2	0
Probable malate:quinone oxidoreductase 1	Q5HDJ0|MQO1	1	2
LexA repressor	Q9L4P1|LEXA	2	1
FtsK/SpoIIIE family protein	Q5HF33|Q5HF33	1	1
Menaquinone biosynthesis methyltransferase ubiE	Q5HFV2|UBIE	1	1
Adenylate kinase	Q5HDX9|KAD	1	0
UPF0403 protein	Q5HFZ5|Y1464	1	0
Conserved virulence factor B	Q5HG29|CVFB	1	0
UTP--glucose-1-phosphate uridylyltransferase	Q5HD54|GTAB	2	0
Urease accessory protein ureE	Q5HDR7|UREE	2	0
UDP-N-acetylenolpyruvoylglucosamine reductase	Q5HHT2|MURB	1	0

Appendix 3. (Continued)

Ribonuclease J 2	Q5HGF6|RNJ2	1	0
Aerobic glycerol-3-phosphate dehydrogenase	Q5HGD1|GLPD	1	0
Glutamate synthase, small subunit	Q5HIK4|Q5HIK4	1	0
Alanyl-tRNA synthetase	Q5HFE4|SYA	3	0
Aminotransferase, putative	Q5HEI3|Q5HEI3	3	0
Putative uncharacterized protein	Q5HEU6|Q5HEU6	3	0
Hydrolase, haloacid dehalogenase-like family	Q5HHB3|Q5HHB3	3	0
Acid phosphatase5'-nucleotidase, lipoprotein e(P4) family	Q5HJ61|Q5HJ61	3	0
DNA ligase	Q5HEL8|DNLJ	2	0
Amino acid ABC transporter, ATP-binding protein	Q5HDA5|Q5HDA5	1	0
Ribonucleoside-diphosphate reductase 2, beta subunit	Q5HHT9|Q5HHT9	2	0
Probable ctpA-like serine protease	Q5HG01|CTPAL	1	0
Capsular polysaccharide biosynthesis protein Cap5F	Q5HJL6|Q5HJL6	2	0
Aminomethyltransferase	Q5HFM2|GCST	1	0
Lipase 1	Q5HCM7|LIP1	2	0
Methionine aminopeptidase	Q5HEN6|AMPM	2	0
Thymidylate synthase	Q5HFZ6|TYSY	2	0
Serine protease HtrA, putative	Q5HF46|Q5HF46	2	0
Putative uncharacterized protein	Q5HIE4|Q5HIE4	2	0
Putative uncharacterized protein	Q5HI54|Q5HI54	1	0
Peptidase, M20/M25/M40 family	Q5HFR1|Q5HFR1	1	0
Leucyl-tRNA synthetase	Q5HF16|SYL	1	0
UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase	Q5HEB8|Q5HEB8	1	0
Putative uncharacterized protein	Q5HE21|Q5HE21	0	1
Glutamyl-tRNA synthetase	Q5HIE7|SYE	0	1
Cobyric acid synthase, putative	Q5HEN2|Q5HEN2	0	1
Putative phosphotransferase	Q5HFJ7|Y1620	1	0
ATP-dependent protease subunit HslV	Q5HGH9|HSLV	1	0
Glycerol kinase	Q5HGD2|GLPK	1	0
77 kDa membrane protein	Q5HEI2|RANDOM_OMP7-R	1	0
Protein esaA	Q5HJ90|RANDOM_ESAA-R	1	0
ATP-dependent Clp protease proteolytic subunit	Q5HHQ0|CLPP	1	0

Appendix 4. Cytoplasmic proteins identified after MudPit analysis of SH1000 during stationary phase from 2 biological replicates

Identified Proteins (366)	Accession Number	Sample 1	Sample 2
Elongation factor Tu	Q5HIC7|EFTU	246	521
Antibacterial protein (Phenol soluble modulin)	Q5HGQ7|Q5HGQ7	115	215
Probable transglycosylase isaA	Q5HCY1|ISAA	140	124
Pyruvate kinase	Q5HF76|KPYK	115	58
Uracil phosphoribosyltransferase	Q5HE88|UPP	66	66
Elongation factor G	Q5HIC8|EFG	81	67
Dihydrolipoyl dehydrogenase	Q5HGY8|DLDH	78	48
Bifunctional autolysin	Q5HH31|ATL	92	31
Cell division protein ftsZ	Q5HGP5|FTSZ	68	43
Delta-hemolysin	Q5HEG6|HLD	98	18
Pyruvate dehydrogenase E1 component subunit beta	Q5HGZ0|ODPB	36	59
Antibacterial protein (Phenol soluble modulin)	Q5HGQ8|Q5HGQ8	40	31
50S ribosomal protein L1	Q5HID7|RL1	59	30
50S ribosomal protein L30	Q5HDX6|RL30	45	31
Cysteine synthase	Q5HIG2|CYSK	43	39
Inosine-5'-monophosphate dehydrogenase	Q5HIQ7|IMDH	47	28
Pyruvate dehydrogenase E1 component subunit alpha	Q5HGZ1|ODPA	44	26
50S ribosomal protein L6	Q5HDX3|RL6	42	24
Pyridoxal biosynthesis lyase pdxS	Q5HIF5|PDXS	36	31
60 kDa chaperonin	Q5HEH2|CH60	46	15
DNA-binding protein HU	Q5HFV0|DBH	37	17
Bifunctional purine biosynthesis protein purH	Q5HH11|PUR9	35	21
Staphylococcal secretory antigen ssaA2	Q5HDQ9|SSAA2	32	26
30S ribosomal protein S5	Q5HDX5|RS5	32	23
Bifunctional protein folD	Q5HH21|FOLD	40	9
Enolase	Q5HHP1|ENO	36	15
Ornithine aminotransferase 2	Q5HHC8|OAT2	25	26
Phenol-soluble modulin alpha 1 peptide	P0C7Y4|PSMA1	22	11
Putative universal stress protein	Q5HF64|Y1759	17	24
50S ribosomal protein L27	Q5HFB8|RL27	27	12
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	Q5HGY9|ODP2	19	22
Chaperone protein hchA	Q5HIC4|HCHA	25	18
Alkaline shock protein 23	Q5HE23|ASP23	29	12
Alkyl hydroperoxide reductase subunit C	Q5HIR5|AHPC	23	18
30S ribosomal protein S1	Q5HFU7|RS1	34	9
50S ribosomal protein L5	Q5HDX0|RL5	25	17
Carbamoyl-phosphate synthase large chain	Q5HGM9|CARB	19	21
30S ribosomal protein S8	Q5HDX2|RS8	7	17
Phosphoribosylformylglycinamidine synthase 2	Q5HH15|PURL	24	14
Phosphate acetyltransferase	Q5HI88|PTA	20	20
Fructose-bisphosphate aldolase class 1	Q5HCU6|ALF1	23	9
Ribose-phosphate pyrophosphokinase	Q5HIH5|KPRS	20	16
Probable malate:quinone oxidoreductase 2	Q5HCU5|MQO2	20	18
UPF0365 protein	Q5HFI7|Y1630	17	13

Appendix 4. (Continued)

N-acetylmuramoyl-L-alanine amidase domain protein	Q5HCQ3|Q5HCQ3	14	18
3-oxoacyl-[acyl-carrier-protein] reductase	Q5HGK2|FABG	23	14
50S ribosomal protein L13	Q5HDZ0|RL13	20	16
50S ribosomal protein L21	Q5HFB6|RL21	23	7
Glycyl-tRNA synthetase	Q5HFJ5|SYG	19	11
D-alanine--D-alanine ligase	Q5HEB7|DDL	20	10
Chaperone protein dnaK	Q5HFI0|DNAK	23	6
ATP-dependent Clp protease ATP-binding subunit clpL	Q5HD02|CLPL	15	12
Acetate kinase	Q5HF63|ACKA	17	13
sp|Q5HG77|TKT_STAAC	Q5HG77|TKT	20	11
Oxidoreductase, short chain dehydrogenase/reductase family	Q5HDM9|Q5HDM9	14	15
Translation initiation factor IF-2	Q5HGG2|IF2	17	11
Ribonucleoside-diphosphate reductase	Q5HHU0|Q5HHU0	17	8
ATP synthase subunit beta	Q5HE97|ATPB	13	11
DNA-directed RNA polymerase subunit beta	Q5HID3|RPOB	12	13
UDP-N-acetylglucosamine 1-carboxyvinyltransferase 1	Q5HEA0|MURA1	18	7
Succinyl-CoA ligase [ADP-forming] subunit beta	Q5HGI7|SUCC	9	15
ATP synthase subunit alpha	Q5HE95|ATPA	12	17
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	Q5HG07|ODO2	14	14
Oxidoreductase, putative	Q5HIW6|Q5HIW6	18	8
DNA-directed RNA polymerase subunit beta'	Q5HID2|RPOC	13	7
1-pyrroline-5-carboxylate dehydrogenase	Q5HCZ6|ROCA	8	14
Imidazolonepropionase	Q5HDM7|HUTI	14	8
Cell cycle protein gpsB	Q5HFX8|GPSB	19	6
N utilization substance protein A, putative	Q5HGG5|Q5HGG5	13	10
UvrABC system protein A	UVRA	5	2
Putative 2-hydroxyacid dehydrogenase	Q5HDQ4|Y2296	16	8
Uncharacterized protein	Q5HHB6|Y973	13	11
GMP synthase [glutamine-hydrolyzing]	GUAA	14	2
Succinyl-CoA ligase [ADP-forming] subunit alpha	Q5HGI6|SUCD	14	7
NADH dehydrogenase-like protein	Q5HHE4|Y944	15	9
Uncharacterized protein	Q5HGK7|Y1240	13	10
Pyruvate carboxylase	Q5HGX0	9	2
Pyrimidine-nucleoside phosphorylase	Q5HE64|PDP	9	9
Uncharacterized protein	Q5HEP9|Y1933	10	7
Probable catabolite control protein A	Q5HF38|CCPA	14	8
GTPase obg	Q5HFB9|OBG	8	6
Putative uncharacterized protein	Q5HIT1|Q5HIT1	10	11
ATP-dependent Clp protease ATP-binding subunit clpC	P0C281|CLPC	8	12
3-oxoacyl-[acyl-carrier-protein] synthase 2	Q5HHA1|FABF	13	8
Alanine dehydrogenase 2	Q5HF65|DHA2	14	4
Bifunctional protein glmU	Q5HIH6|GLMU	15	4
30S ribosomal protein S10	Q5HDV7|RS10	8	13
50S ribosomal protein L15	Q5HDX7|RL15	5	12
Elongation factor Ts	Q5HGH4|EFTS	12	7
30S ribosomal protein S11	Q5HDY3|RS11	12	4
Glucosamine-6-phosphate isomerase, putative	Q5HES0|Q5HES0	12	5
Catalase	Q5HG86|CATA	13	6
FeS assembly ATPase SufC	Q5HHH2|Q5HHH2	9	10
30S ribosomal protein S2	Q5HGH6|RS2	9	8
NADP-dependent malic enzyme, putative	Q5HF72|Q5HF72	7	10
DNA-directed RNA polymerase subunit omega	Q5HGM2|RPOZ	7	10
Naphthoate synthase	Q5HH38|MENB	7	8
Phosphoglucosamine mutase	Q5HE43|GLMM	10	5
Aspartate-semialdehyde dehydrogenase	Q5HG26|Q5HG26	9	4

Appendix 4. (Continued)

Ribosome-recycling factor	Q5HGH2|RRF	6	10
6-phosphogluconate dehydrogenase, decarboxylating	Q5HFR2|6PGD	11	2
UPF0342 protein	Q5HET0|Y1902	13	5
Dephospho-CoA kinase	Q5HF85|COAE	8	5
L-lactate dehydrogenase 1	Q5HJD7|LDH1	5	8
Probable manganese-dependent inorganic pyrophosphatase	Q5HEK1|PPAC	7	5
30S ribosomal protein S7	Q5HIC9|RS7	4	9
30S ribosomal protein S19	Q5HDW2|RS19	10	6
ATP-dependent protease ATPase subunit HslU	Q5HGH8|HSLU	6	9
30S ribosomal protein S4	Q5HF54|RS4	10	4
Glutamyl-tRNA(Gln) amidotransferase subunit A	Q5HEM2|GATA	7	5
Protein recA	Q5HGE6|RECA	10	3
Conserved domain protein	Q5HH57|Q5HH57	9	5
Fructose-bisphosphate aldolase	Q5HE75|ALF2	12	2
Transcriptional regulator sarA	Q5HI51|SARA	10	5
Putative uncharacterized protein	Q5HG68|RANDOM_Q5HG68-R	2	0
Urocanate hydratase	Q5HDM6|HUTU	8	3
Hydroxymethylglutaryl-CoA reductase, degradative	Q5HD05|Q5HD05	6	5
Aconitate hydratase	Q5HG69|ACON	8	3
Probable thiol peroxidase	Q5HF61|TPX	5	7
S-adenosylmethionine synthase	Q5HEY9|METK	7	5
Phenol-soluble modulin alpha 4 peptide	P0C821|PSMA4	8	5
Universal stress protein family	Q5HF68|Q5HF68	6	2
50S ribosomal protein L14	Q5HDW8|RL14	4	4
Transaldolase	Q5HEZ4|Q5HEZ4	6	3
Valyl-tRNA synthetase	Q5HFA8|SYV	10	3
GTP-binding protein engA	Q5HFU8|ENGA	6	4
Prolyl-tRNA synthetase	Q5HGG8|SYP	6	3
2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase 2	Q5HJC1|ISPD2	7	4
DNA polymerase III subunit beta	Q5HJZ4|DPO3B	5	7
50S ribosomal protein L16	Q5HDW5|RL16	9	3
50S ribosomal protein L33 2	Q5HG85|RL332 (+1)	8	3
PTS system, IIBC components	Q5HJD5|Q5HJD5	1	0
Alcohol dehydrogenase, zinc-containing	Q5HE18|Q5HE18	8	3
Asparaginyl-tRNA synthetase	Q5HFW9|SYN	4	2
6-phosphofructokinase	Q5HF75|K6PF	5	5
Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B	Q5HEM3|GATB	7	4
Putative aldehyde dehydrogenase	Q5HE78|ALD1	4	4
Acid phosphatase5'-nucleotidase, lipoprotein e(P4) family	Q5HJ61|Q5HJ61	7	2
UPF0082 protein	Q5HHZ9|Y727	7	2
Aminotransferase, putative	Q5HEI3|Q5HEI3	7	3
Glutamine synthetase	Q5HGC3|GLNA	6	0
Capsular polysaccharide biosynthesis protein Cap5B	Q5HJM0|Q5HJM0	6	6
HTH-type transcriptional regulator sarR	Q5HDR3|SARR	9	1
Glutamate-1-semialdehyde 2,1-aminomutase 2	Q5HER0|GSA2	3	6
Polyribonucleotide nucleotidyltransferase	Q5HGF7|PNP	8	3
Inositol monophosphatase family protein	Q5HGX7|Q5HGX7	8	4
sp|Q5HGP8|MURD_STAAC	Q5HGP8|MURD	3	2
UPF0051 protein	Q5HHG8|Y918	3	5
HTH-type transcriptional regulator mgrA	Q5HHY2|MGRA	6	5
50S ribosomal protein L10	Q5HID6|RL10	2	6
Putative aldehyde dehydrogenase AldA	Q5HJK3|ALDA	5	2
50S ribosomal protein L2	Q5HDW1|RL2	6	3
Queuine tRNA-ribosyltransferase	Q5HFC4|TGT	4	6

Appendix 4. (Continued)

Putative uncharacterized protein	Q5HEL5|Q5HEL5	7	3
S1 RNA binding domain protein	Q5HED8|Q5HED8	4	5
Putative dipeptidase	Q5HF23|PEPVL	6	4
Putative uncharacterized protein	Q5HI16|Q5HI16	6	4
Hypoxanthine-guanine phosphoribosyltransferase	Q5HIG5|HPRT	3	5
Penicillin-binding protein 2'	Q5HJW3|RANDOM_Q5HJW3-R	2	0
Deoxyribose-phosphate aldolase 2	DEOC2	0	1
Putative uncharacterized protein	Q5HF32|Q5HF32	8	0
Threonyl-tRNA synthetase	Q5HF90|SYT	8	2
ABC transporter, ATP-binding protein	Q5HG28|Q5HG28	4	5
30S ribosomal protein S12	RS12	4	2
50S ribosomal protein L3	Q5HDV8|RL3	4	2
Succinate dehydrogenase, iron-sulfur protein	Q5HGT4|Q5HGT4	2	6
Extracellular matrix-binding protein ebh	Q5HFY8|RANDOM_EBH-R	1	0
Serine hydroxymethyltransferase	Q5HE87|GLYA	4	3
Glyceraldehyde-3-phosphate dehydrogenase 1	Q5HHP5|G3P1	4	3
FtsK/SpoIIIE family protein	Q5HF33|Q5HF33	5	3
Rrf2 family protein	Q5HFD6|Q5HFD6	4	5
Putative uncharacterized protein	Q5HHZ0|Q5HHZ0	4	6
Pyruvate oxidase	Q5HD12|Q5HD12	5	2
Uncharacterized protein	Q5HG10|Y1445	2	4
50S ribosomal protein L23	Q5HDW0|RL23	4	5
50S ribosomal protein L17	Q5HDY5|RL17	5	4
Formate acetyltransferase	Q5HJF4|PFLB	3	7
Phosphoribosylformylglycinamidine synthase, PurS protein	Q5HH17|Q5HH17	4	6
Molybdopterin molybdenumtransferase	Q5HDT4|MOEA	5	3
Isocitrate dehydrogenase [NADP]	Q5HF79|IDH	1	7
ATP synthase subunit delta	Q5HE94|ATPD	3	4
Formate--tetrahydrofolate ligase	Q5HF42|FTHS	5	1
Xanthine phosphoribosyltransferase	Q5HIQ9|XPT	8	1
50S ribosomal protein L18	Q5HDX4|RL18	4	2
Hydroxymethylglutaryl-CoA synthase	Q5HD04|Q5HD04	4	0
ATP-dependent Clp protease ATP-binding subunit clpX	Q5HF98|CLPX	2	5
50S ribosomal protein L4	Q5HDV9|RL4	2	2
Iron compound ABC transporter, iron compound-binding protein	Q5HE28|Q5HE28	3	3
30S ribosomal protein S16	Q5HGJ4|RS16	3	3
Cell division protein FtsH, putative	Q5HIG4|Q5HIG4	6	3
ABC transporter, ATP-binding/permease protein	Q5HI25	1	0
Teichoic acids export ATP-binding protein TagH	TAGH	0	2
Tetrapyrrole methylase family protein	Q5HII8|Q5HII8	2	2
Putative uncharacterized protein	Q5HGX3|Q5HGX3	7	1
Elastin-binding protein ebpS	Q5HFU2|EBPS	6	1
Methylenetetrahydrofolate--tRNA-(uracil-5-)-methyltransferase trmFO	Q5HGI1|TRMFO	3	4
50S ribosomal protein L9	Q5HJY1|RL9	6	2
Alcohol dehydrogenase, zinc-containing	Q5HJC4|Q5HJC4	2	5
sp|Q5HHP4|PGK_STAAC	Q5HHP4|PGK	5	2
Transcription termination factor Rho	Q5HE79|Q5HE79	2	4
Uridylate kinase	Q5HGH3|PYRH	4	4
CTP synthase	Q5HE73|PYRG	3	2
Aerobic glycerol-3-phosphate dehydrogenase	Q5HGD1|GLPD	3	2
Dihydroorotate dehydrogenase	Q5HCW1|Q5HCW1	4	3
50S ribosomal protein L32	Q5HGV6|RL32	3	4
Putative pyridoxine kinase	Q5HI96|PDXK	4	3

Appendix 4. (Continued)

Serine protease HtrA, putative	Q5HF46|Q5HF46	3	4
2-oxoglutarate dehydrogenase E1 component	Q5HG06|ODO1	4	2
Probable DEAD-box ATP-dependent RNA helicase	Q5HEB9|Y2072	1	5
Extracellular matrix-binding protein ebh	Q5HFY8|EBH	0	1
N-acetylglucosamine-6-phosphate deacetylase	Q5HHW9|Q5HHW9	3	1
Seryl-tRNA synthetase	Q5HJY7|SYS	5	2
Mannitol-1-phosphate 5-dehydrogenase	MTLD	3	0
30S ribosomal protein S13	Q5HDY2|RS13	5	1
Putative NAD(P)H nitroreductase	Q5HD30|Y2534	4	3
Low molecular weight protein-tyrosine-phosphatase ptpA	Q5HEP3|PTPA	5	2
Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta	Q5HF73|ACCD	4	4
GTP-sensing transcriptional pleiotropic repressor codY	Q5HGH7|CODY	5	2
Phosphoribosylaminoimidazole-succinocarboxamide synthase	Q5HH18|PUR7	3	2
Probable endonuclease 4	Q5HFK3|END4	5	0
GTP-binding protein TypA	Q5HGX5|Q5HGX5	6	0
Lysyl-tRNA synthetase	Q5HIF7|SYK	6	0
Chaperone protein clpB	Q5HHB0|CLPB	1	4
Orotidine 5'-phosphate decarboxylase	Q5HGM8|PYRF	4	3
Alcohol dehydrogenase, iron-containing	Q5HJM2|Q5HJM2	0	1
Putative uncharacterized protein	Q5HJK1|Q5HJK1	4	1
UDP-N-acetylenolpyruvoylglucosamine reductase	Q5HHT2|MURB	4	2
Succinate dehydrogenase, flavoprotein subunit	Q5HGT5|Q5HGT5	4	1
Putative uncharacterized protein	Q5HET6	1	3
Phosphopantothenoylcysteine decarboxylase/phosphopantothenate--cysteine ligase	Q5HGM1|Q5HGM1	0	2
Primosomal protein DnaI	Q5HF89	3	0
UPF0297 protein	Q5HFE5|Y1672	6	1
Alcohol dehydrogenase	Q5HI63|ADH	4	3
Lipoate-protein ligase A family protein	Q5HH58|Q5HH58	3	3
Membrane protein, putative	Q5HDB3|RANDOM_Q5HDB3-R	1	1
Phosphoribosylaminoimidazole carboxylase ATPase subunit	Q5HH19|PURK	5	0
Dihydropteroate synthase	Q5HIG1|DHPS	3	0
Probable transglycosylase sceD	Q5HEA4|SCED	3	0
3-hydroxyacyl-CoA dehydrogenase protein	Q5HJE6|Q5HJE6	3	0
sp|Q5HGG0|TRUB_STAAC	Q5HGG0|TRUB	5	0
Adenylosuccinate synthetase	Q5HJX8|PURA	5	0
Guanylate kinase	Q5HGM3|KGUA	7	0
Putative septation protein spoVG	Q5HIH8|SP5G	6	0
Pseudouridine synthase	Q5HGN5|Q5HGN5	2	2
Translation initiation factor IF-3	Q5HF92|IF3	3	3
Protein nagD homolog	Q5HHF6|NAGD	3	3
Ferritin	Q5HEN0|FTN	2	3
Signal recognition particle protein	Q5HGJ5|Q5HGJ5	2	2
Acetyltransferase, GNAT family	Q5HD32|Q5HD32	2	2
Phosphoribosylglycinamide formyltransferase	Q5HH12|PUR3	0	4
Aspartyl-tRNA synthetase	Q5HFD3|SYD	2	1
UPF0355 protein	Q5HIR0|UP355	4	2
Putative uncharacterized protein	Q5HE56|Q5HE56	2	1
Uroporphyrinogen decarboxylase	Q5HEU2|DCUP	3	3
Probable cysteine desulfurase	Q5HHH0|CSD	2	4
N-acetylmuramoyl-L-alanine amidase sle1	Q5HIL2|SLE1	3	2
NifU domain protein	Q5HHG9|Q5HHG9	3	3
Transferrin receptor	Q5HHT4|Q5HHT	2	2

Appendix 4. (Continued)

Acetyl-CoA carboxylase, biotin carboxylase	Q5HFP5|Q5HFP5	4	0
Spermidine/putrescine import ATP-binding protein PotA	Q5HGY5|RANDOM_POTA-R	0	1
NH(3)-dependent NAD(+) synthetase	Q5HEK9|NADE	5	0
Ribonuclease J 1	Q5HGZ5|RNJ1	5	0
33 kDa chaperonin	Q5HIG3|HSLO	2	0
tRNA uridine 5-carboxymethylaminomethyl modification enzyme mnmG	Q5HCI4|MNMG	5	0
D-alanine aminotransferase	Q5HF24|DAAA	0	4
Penicillin-binding protein 2	Q5HFX3|Q5HFX3	3	0
Thioredoxin reductase	Q5HHQ4|TRXB	4	0
Fumarate hydratase class II	Q5HES4|FUMC	3	1
UvrABC system protein A	Q5HHQ9|UVRA	4	2
Phosphoenolpyruvate-protein phosphotransferase	Q5HH01|PT1	2	1
Putative uncharacterized protein	Q5HFY2|Q5HFY2	1	1
Probable branched-chain-amino-acid aminotransferase	Q5HIC1|ILVE	3	1
Pyruvate carboxylase	Q5HGX0|Q5HGX0	9	3
Peptide deformylase	Q5HGZ3|DEF	1	1
50S ribosomal protein L29	Q5HDW6|RL29	2	3
Serine protease htrA-like	Q5HH63|HTRAL	3	1
Arginyl-tRNA synthetase	Q5HI60|SYR	4	1
NADH-dependent flavin oxidoreductase, Oye family	Q5HHC9|Q5HHC9	4	1
Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]	Q5HE49|GLMS	3	1
Putative uncharacterized protein	Q5HET6|Q5HET6	2	3
Phosphoribosylamine--glycine ligase	Q5HH10|PUR2	2	1
Acetyl-CoA synthetase, putative	Q5HCU4|Q5HCU4	2	1
Putative uncharacterized protein	Q5HGK8	2	2
Phosphoglycerate mutase family protein	Q5HIS0|Q5HIS0	2	0
50S ribosomal protein L36	Q5HDY1|RL36	0	2
10 kDa chaperonin	Q5HEH1|CH10	4	0
Glucokinase	Q5HFL3|Q5HFL3	2	0
Prophage L54a, major capsid protein, putative	Q5HIZ5|RANDOM_Q5HIZ5-R	1	0
UPF0637 protein	Q5HGX8|Y1115	2	0
FeS assembly protein SufD	Q5HHH1|Q5HHH1	3	0
Uncharacterized oxidoreductase	Q5HD73|Y2488	0	4
Triosephosphate isomerase	Q5HHP3|TPIS	2	0
NAD-specific glutamate dehydrogenase	Q5HHC7|DHE2	3	0
Acetoin(diacetyl) reductase	Q5HJP2|BUTA	5	0
Enoyl-(Acyl-carrier-protein) reductase	Q5HH75|Q5HH75	1	2
Isoleucyl-tRNA synthetase	Q5HGN8|SYI	2	1
Putative uncharacterized protein	Q5HIN0|Q5HIN0	2	1
Putative phosphotransferase	Q5HFJ7|Y1620	3	1
Protein translocase subunit secA 1	Q5HHR7|SECA1	2	1
Putative uncharacterized protein	Q5HE21|Q5HE21	2	1
Putative uncharacterized protein	Q5HGR9|Q5HGR9	1	3
50S ribosomal protein L25	Q5HIH4|RL25	1	1
Probable tautomerase	Q5HG56|Y1399	2	1
Protoporphyrinogen oxidase	Q5HEU4|Q5HEU4	1	2
Thioredoxin, putative	Q5HHL0|Q5HHL0	1	2
UPF0403 protein	Q5HFZ5|Y1464	2	0
Phage infection protein, putative	Q5HCQ4|Q5HCQ4	2	0
tr|Q5HEB8|Q5HEB8_STAAC	Q5HEB8|Q5HEB8	2	0
sp|Q5HFM2|GCST_STAAC	Q5HFM2|GCST	0	2
ATP-dependent DNA helicase RecQ	Q5HFU1|Q5HFU1	2	0
DNA translocase ftsK	Q5HGF5|FTSK	2	0

Appendix 4. (Continued)

Threonine dehydratase catabolic	Q5HFY5|THD2	1	0
Transcription-repair-coupling factor	Q5HIH2|RANDOM_MFD-R	1	0
Ribonuclease J 2	Q5HGF6|RNJ2	2	0
DNA ligase	Q5HEL8|DNLJ	2	0
Glycerol phosphate lipoteichoic acid synthase	Q5HHV4|LTAS	4	0
Proline dipeptidase	Q5HFM9|Q5HFM9	3	0
UDP-N-acetylmuramate--L-alanine ligase	Q5HF34|MURC	4	0
Amino acid ABC transporter, ATP-binding protein	Q5HDA5|Q5HDA5	0	4
NAD/NADP octopine/nopaline dehydrogenase family protein	Q5HDQ7|Q5HDQ7	4	0
50S ribosomal protein L19	Q5HGJ1|RL19	1	1
30S ribosomal protein S3	Q5HDW4|RS3	1	1
Uncharacterized hydrolase	Q5HCW9|Y2597	1	1
S1 RNA binding domain protein	Q5HED8|RANDOM_Q5HED8-R	1	1
Signal peptidase IB	Q5HHB9|LEP	1	1
Dehydrosqualene desaturase	Q5HCY9|CRTN	0	1
Sortase	Q5HD25|Q5HD25	1	0
Glucose-specific phosphotransferase enzyme IIA component	Q5HFZ9|PTGA	2	0
Cobyric acid synthase, putative	Q5HEN2|Q5HEN2	0	2
6,7-dimethyl-8-ribityllumazine synthase	Q5HF08|RISB	2	0
Putative uncharacterized protein	Q5HIQ0|Q5HIQ0	1	0
Exodeoxyribonuclease 7 large subunit	Q5HFP8|RANDOM_EX7L-R	0	1
Anti-sigma-B factor antagonist	P60071|RSBV	3	0
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	Q5HDD9|GPMA	3	0
Delta-aminolevulinic acid dehydratase	Q5HFA4|HEM2	2	0
Uncharacterized protein	Q5HG67|Y1387	2	0
Dihydroorotase	Q5HGN1|PYRC	3	0
Thioredoxin	Q5HGT9|THIO	3	0
Transcription antitermination protein nusG	Q5HID9|NUSG	3	0
2-dehydropantoate 2-reductase	Q5HCV2|Q5HCV2	3	0
Acetolactate synthase, catabolic	Q5HDZ7|Q5HDZ7	0	2
Chorismate mutase/phospho-2-dehydro-3-deoxyheptonate aldolase	Q5HF37|Q5HF37	0	3
OsmC/Ohr family protein	Q5HF52|Q5HF52	2	0
Phosphomevalonate kinase	Q5HI84|Q5HI84	1	0
Lipoate-protein ligase A family protein	Q5HFM6|Q5HFM6	2	0
L-serine dehydratase, iron-sulfur-dependent, beta subunit	Q5HD20|Q5HD20	0	1
Putative uncharacterized protein	Q5HIY4|RANDOM_Q5HIY4-R	0	1
Acyl-CoA dehydrogenase family protein	Q5HJE5|Q5HJE5	1	0
Dehydrosqualene synthase	Q5HCY8|CRTM	2	0
Elongation factor P	Q5HFN0|EFP	2	0
50S ribosomal protein L11	Q5HID8|RL11	2	0
Oxidoreductase, aldo/keto reductase family	Q5HFS2|Q5HFS2	1	0
PhoH family protein	Q5HFI9|Q5HFI9	1	0
2-oxoisovalerate dehydrogenase, E2 component, dihydrolipoamide acetyltransferase	Q5HFQ6|Q5HFQ6	1	0
Transcriptional regulator, DeoR family	Q5HHX1|Q5HHX1	0	1
Protein-export membrane protein SecDF	Q5HFC6|Q5HFC6	1	0
IS1272, transposase	Q5HJW0|RANDOM_Q5HJW0-R	1	0
ABC transporter, substrate-binding protein	Q5HJE1|Q5HJE1	0	1
Formate dehydrogenase, NAD-dependent	Q5HJJ6|Q5HJJ6	1	0
Lysostaphin resistance protein A	Q5HDM2|LYRA	1	0

Appendix 4. (Continued)

Peptidase, M20/M25/M40 family	Q5HFR1|Q5HFR1	1	0
Putative 8-amino-7-oxononanoate synthase/2-amino-3-ketobutyrate coenzyme A ligase	Q5HIC5|BIKB	0	1
Aminoacyltransferase femA	FEMA	0	1
ABC transporter, ATP-binding protein	Q5HI35|Q5HI35	1	0
Pur operon repressor	Q5HII0|Q5HII0	1	0
Ornithine cyclodeaminase, putative	Q5HJQ2|Q5HJQ2	1	0

Appendix 5. Secreted proteins identified after MudPit analysis of SH1000 during post-exponential phase from 2 biological replicates

Identified Proteins (38)	Accession Number	Sample 1	Sample 2
Probable transglycosylase isaA	Q5HCY1|ISAA	234	186
Bifunctional autolysin	Q5HH31|ATL	42	31
Staphylococcal secretory antigen ssaA2	Q5HDQ9|SSAA2	46	39
Staphopain A	Q5HEL3|SSPP	10	6
Enolase	Q5HHP1|ENO	8	7
Surface protein, putative	Q5HDZ9|Q5HDZ9	8	2
Probable transglycosylase sceD	Q5HEA4|SCED	5	3
Glycerol phosphate lipoteichoic acid synthase	Q5HHV4|LTAS	5	6
Glyceraldehyde-3-phosphate dehydrogenase 1	Q5HHP5|G3P1	7	5
Lipase 1	Q5HCM7|LIP1	5	0
N-acetylmuramoyl-L-alanine amidase domain protein	Q5HCQ3|Q5HCQ3	4	2
Lipase 2	Q5HJ48|LIP2	4	4
50S ribosomal protein L17	Q5HDY5|RL17	3	2
Hyaluronate lyase	Q5HE02|Q5HE02	0	2
Putative uncharacterized protein	Q5HI54|Q5HI54	3	0
30S ribosomal protein S18	Q5HIS7|RS18	3	4
50S ribosomal protein L28	Q5HGK9|RL28	5	5
SdrH protein, putative	Q5HEG9|RANDOM_Q5HEG9-R	0	1
6-phosphogluconate dehydrogenase, decarboxylating	Q5HFR2|6PGD	3	2
Penicillin-binding protein 3	Q5HFK8|Q5HFK8	5	3
Elastin-binding protein ebpS	Q5HFU2|EBPS	0	1
UvrABC system protein A	Q5HHQ9|RANDOM_UVRA-R	1	0
Gamma-hemolysin component B	Q5HDD3|HLGB	1	0
50S ribosomal protein L22	Q5HDW3|RL22	3	3
Helicase, putative	Q5HD63|RANDOM_Q5HD63-R	1	0
Penicillin-binding protein 1	Q5HGQ0|RANDOM_Q5HGQ0-R	0	1
tRNA modification GTPase mnmE	Q5HCI3|RANDOM_MNME-R	0	1
Type-1 restriction enzyme R protein	Q5HJH8|RANDOM_HSDR-R	1	0
Pyruvate dehydrogenase E1 component subunit beta	Q5HGZ0|ODPB	2	0
N-acetylmuramoyl-L-alanine amidase sle1	Q5HIL2|SLE1	2	1
Putative uncharacterized protein	Q5HI78|Q5HI78	0	1
Leukotoxin LukE	Q5HEU9|RANDOM_Q5HEU9-R	1	0
Siderophore biosynthesis protein, IucC family	Q5HJQ1|RANDOM_Q5HJQ1-R	0	2
Phosphonate ABC transporter, phosphonate-binding protein	Q5HJM5|RANDOM_Q5HJM5-R	0	1
Cell division protein sepF	Q5HGP2|SEPF	1	1
Bifunctional protein folD	Q5HH21|FOLD	2	0
Staphyloxanthin biosynthesis protein, putative	Q5HDQ5|Q5HDQ5	0	1

Appendix 6. Secreted proteins identified after MudPit analysis of SH1000 during stationary phase from 2 biological replicates

Identified Proteins (346)	Accession Number	Sample 1	Sample 2
Lipase 1	Q5HCM7|LIP1	608	361
Bifunctional autolysin	Q5HH31|ATL	269	229
Probable transglycosylase isaA	Q5HCY1|ISAA	527	143
Surface protein, putative	Q5HDZ9|Q5HDZ9	148	114
Putative uncharacterized protein	Q5HI54|Q5HI54	174	119
Enolase	Q5HHP1|ENO	70	99
Alpha-hemolysin	Q5HGS1|Q5HGS1	85	82
Lipase 2	Q5HJ48|LIP2	105	85
Glyceraldehyde-3-phosphate dehydrogenase 1	Q5HHP5|G3P1	93	72
Glycerol phosphate lipoteichoic acid synthase	Q5HHV4|LTAS	74	46
Glutamine synthetase	Q5HGC3|GLNA	12	58
LysM domain protein	Q5HI03|Q5HI03	56	41
N-acetylmuramoyl-L-alanine amidase domain protein	Q5HCQ3|Q5HCQ3	36	40
Inosine-5'-monophosphate dehydrogenase	Q5HIQ7|IMDH	33	35
Dihydrolipoyl dehydrogenase	Q5HGY8|DLDH	30	25
Phospholipase C	Q5HEI1|PHLC	7	29
Formate--tetrahydrofolate ligase	Q5HF42|FTHS	28	23
Staphopain A	Q5HEL3|SSPP	42	15
N-acetylmuramoyl-L-alanine amidase sle1	Q5HIL2|SLE1	23	31
Gamma-hemolysin component B	Q5HDD3|HLGB	33	10
Fructose-bisphosphate aldolase	Q5HE75|ALF2	16	18
Alkyl hydroperoxide reductase subunit C	Q5HIR5|AHPC	15	26
Staphylococcal secretory antigen ssaA2	Q5HDQ9|SSAA2	47	12
Phosphate acetyltransferase	Q5HI88|PTA	16	32
6-phosphogluconate dehydrogenase, decarboxylating	Q5HFR2|6PGD	32	16
Probable transglycosylase sceD	Q5HEA4|SCED	19	31
Staphopain B	Q5HH36|SSPB	27	12
Catalase	Q5HG86|CATA	30	15
Clumping factor A	Q5HHM8|CLFA	33	8
Glutamyl-tRNA synthetase	Q5HIE7|SYE	19	16
Delta-hemolysin	Q5HEG6|HLD	6	24
Succinyl-CoA ligase [ADP-forming] subunit alpha	Q5HGI6|SUCD	4	26
Transcriptional regulator, putative	Q5HH28|Q5HH28	5	4
Penicillin-binding protein 3	Q5HFK8|Q5HFK8	30	4
Alkaline shock protein 23	Q5HE23|ASP23	13	30
Succinyl-CoA ligase [ADP-forming] subunit beta	Q5HGI7|SUCC	1	12
DNA-binding protein HU	Q5HFV0|DBH	4	25
ABC transporter, substrate-binding protein	Q5HI37|Q5HI37	3	24
2,3-bisphosphoglycerate-independent phosphoglycerate mutase	Q5HHP2|GPMI	28	10
Pyruvate carboxylase	Q5HGX0	1	23
3-oxoacyl-[acyl-carrier-protein] synthase 2	Q5HHA1|FABF	24	13
50S ribosomal protein L9	Q5HJY1|RL9	2	5
ATP synthase subunit beta	Q5HE97|ATPB	3	29
GTP-sensing transcriptional pleiotropic repressor codY	Q5HGH7|CODY	9	15
Foldase protein prsA	Q5HET4|PRSA	0	20

Appendix 6. (Continued)

ATP synthase subunit alpha	Q5HE95|ATPA	8	17
Seryl-tRNA synthetase	Q5HJY7|SYS	11	14
Antibacterial protein (Phenol soluble modulin)	Q5HGQ7|Q5HGQ7	7	10
1-pyrroline-5-carboxylate dehydrogenase	Q5HCZ6|ROCA	1	29
Elastin-binding protein ebpS	Q5HFU2|EBPS	12	3
Pyruvate kinase	Q5HF76|KPYK	2	19
Staphyloxanthin biosynthesis protein, putative	Q5HDQ5|Q5HDQ5	15	15
Phosphoenolpyruvate-protein phosphotransferase	Q5HH01|PT1	13	10
Glycerophosphoryl diester phosphodiesterase GlpQ, putative	Q5HHC6|Q5HHC6	3	16
3-oxoacyl-[acyl-carrier-protein] reductase	Q5HGK2|FABG	13	6
DNA-directed RNA polymerase subunit beta	Q5HID3|RPOB	3	7
Glycyl-glycine endopeptidase lytM	Q5HJ99|LYTM	12	12
Putative uncharacterized protein	Q5HIS3|Q5HIS3	7	15
Serine-aspartate repeat-containing protein C	Q5HIB4|SDRC	14	8
Elongation factor Ts	Q5HGH4|EFTS	4	11
GMP synthase [glutamine-hydrolyzing]	GUAA	9	9
Putative uncharacterized protein	Q5HHW2|Q5HHW2	5	2
Transketolase	Q5HG77|TKT	4	14
Purine nucleoside phosphorylase	Q5HE62|Q5HE62	10	2
Primosomal protein N`	Q5HGM0|RANDOM_Q5HGM0-R	2	0
Chaperone protein dnaK	Q5HFI0|DNAK	2	11
Triosephosphate isomerase	Q5HHP3|TPIS	8	5
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	Q5HDD9|GPMA	1	10
Gamma-hemolysin component C	Q5HDD4|HLGC	2	8
50S ribosomal protein L7/L12	Q5HID5|RL7	0	1
Cell wall surface anchor family protein	Q5HJN4|Q5HJN4	9	13
SdrH protein, putative	Q5HEG9|Q5HEG9	19	0
Glucose-6-phosphate isomerase	Q5HHC2|G6PI	8	13
L-lactate dehydrogenase 1	Q5HJD7|LDH1	6	11
Phosphoribosylaminoimidazole carboxylase, catalytic subunit	Q5HH20|Q5HH20	12	6
Surface protein, putative	Q5HHA4|Q5HHA4	8	5
Elongation factor Tu	Q5HIC7|EFTU	6	11
Chaperone protein hchA	Q5HIC4|HCHA	10	10
Urocanate hydratase	Q5HDM6|HUTU	16	0
Phosphoglycerate kinase	Q5HHP4|PGK	0	20
Gamma-hemolysin component A	Q5HDD6|HLGA	8	8
Thioredoxin reductase	Q5HHQ4|TRXB	4	9
Hydroxymethylglutaryl-CoA synthase	Q5HD04|Q5HD04	6	3
Transaldolase	Q5HEZ4|Q5HEZ4	3	9
DNA-directed RNA polymerase subunit beta'	Q5HID2|RPOC	5	11
30S ribosomal protein S12	RS12	14	2
Fructose-bisphosphate aldolase class 1	Q5HCU6|ALF1	6	10
Uncharacterized lipoprotein	Q5HDI7|Y2365	3	9
50S ribosomal protein L27	Q5HFB8|RL27	7	5
Trigger factor	Q5HF97|TIG	5	0
Deoxyribose-phosphate aldolase 2	DEOC2	0	3
50S ribosomal protein L11	Q5HID8|RL11	5	2
Acetoin(diacetyl) reductase	Q5HJP2|BUTA	5	6
6-phosphofructokinase	Q5HF75|K6PF	3	8
30S ribosomal protein S16	Q5HGJ4|RS16	1	9
50S ribosomal protein L21	Q5HFB6|RL21	10	8
Leucyl-tRNA synthetase	Q5HF16|SYL	4	4
3-oxoacyl-[acyl-carrier-protein] synthase 3	Q5HHA2|FABH	1	6
3-hydroxyacyl-CoA dehydrogenase protein	Q5HJE6|Q5HJE6	3	1

Appendix 6. (Continued)

Putative aldehyde dehydrogenase AldA	Q5HJK3|ALDA	1	8
Acetate kinase	Q5HF63|ACKA	2	14
NA polymerase III subunit beta	Q5HJZ4|DPO3B	13	1
Phenylalanyl-tRNA synthetase beta chain	Q5HGU5|SYFB	4	4
50S ribosomal protein L30	Q5HDX6|RL30	1	8
50S ribosomal protein L3	Q5HDV8|RL3	0	7
LPXTG cell wall surface anchor family protein	Q5HCQ1|Q5HCQ1	2	9
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	Q5HGY9|ODP2	0	9
Methionyl-tRNA synthetase	Q5HII6|SYM	0	9
Elongation factor G	Q5HIC8|EFG	2	5
Arginase	P60086|ARGI	14	0
33 kDa chaperonin	Q5HIG3|HSLO	7	1
Amino acid ABC transporter, amino acid-binding protein	Q5HDE2|Q5HDE2	13	2
Serine hydroxymethyltransferase	Q5HE87|GLYA	1	10
Phosphate acyltransferase	Q5HGK4|PLSX	9	2
Phosphoenolpyruvate carboxykinase [ATP]	Q5HEY8|PCKA	0	14
50S ribosomal protein L17	Q5HDY5|RL17	3	2
Antibacterial protein (Phenol soluble modulin)	Q5HGQ8|Q5HGQ8	2	7
50S ribosomal protein L14	RL14	7	0
Uncharacterized hydrolase	Q5HCW9|Y2597	8	6
Putative uncharacterized protein	Q5HIY1	3	0
50S ribosomal protein L22	Q5HDW3|RL22	5	3
Phenol-soluble modulin alpha 1 peptide	P0C7Y4|PSMA1	3	7
S-adenosylmethionine synthase	Q5HEY9|METK	9	0
Pyruvate dehydrogenase E1 component subunit alpha	Q5HGZ1|ODPA	0	13
Cell wall surface anchor family protein	Q5HD57|Q5HD57	10	5
NAD-specific glutamate dehydrogenase	Q5HHC7|DHE2	3	8
Superoxide dismutase [Mn/Fe] 1	Q5HFK7|SODM1	4	10
Alanine dehydrogenase 2	Q5HF65|DHA2	1	9
Alcohol dehydrogenase	Q5HI63|ADH	1	7
50S ribosomal protein L25	Q5HIH4|RL25	1	3
Glucose-specific phosphotransferase enzyme IIA component	Q5HFZ9|PTGA	6	8
30S ribosomal protein S2	Q5HGH6|RS2	4	4
Mannitol-1-phosphate 5-dehydrogenase	MTLD	0	2
Probable thiol peroxidase	Q5HF61|TPX	3	8
Putative septation protein spoVG	Q5HIH8|SP5G	1	6
Uncharacterized protein	Q5HEP9|Y1933	6	3
Glutamyl endopeptidase	Q5HH35|SSPA	0	8
Succinate dehydrogenase, iron-sulfur protein	Q5HGT4|Q5HGT4	6	6
Diaminopimelate decarboxylase	Q5HG20|Q5HG20	1	0
Isoleucyl-tRNA synthetase	Q5HGN8|SYI	0	4
Immunoglobulin G binding protein A	Q5HJQ8|Q5HJQ8	1	10
Fumarate hydratase class II	Q5HES4|FUMC	4	1
Adenylate kinase	Q5HDX9|KAD	3	6
Naphthoate synthase	Q5HH38|MENB	4	2
50S ribosomal protein L15	Q5HDX7|RL15	2	7
30S ribosomal protein S8	Q5HDX2|RS8	3	4
Glucosamine-6-phosphate isomerase, putative	Q5HES0|Q5HES0	1	2
N-acetylglucosamine-6-phosphate deacetylase	Q5HHW9|Q5HHW9	5	2
60 kDa chaperonin	Q5HEH2|CH60	0	7
Polyribonucleotide nucleotidyltransferase	Q5HGF7|PNP	0	10
Putative 2-hydroxyacid dehydrogenase	Q5HDQ4|Y2296	2	3
2-oxoglutarate dehydrogenase E1 component	Q5HG06|ODO1	2	2
Formate acetyltransferase	Q5HJF4|PFLB	3	2

Appendix 6. (Continued)

Putative uncharacterized protein	Q5HEP7	1	0
Putative uncharacterized protein	Q5HF32|Q5HF32	2	1
Chitinase-related protein	Q5HH22|Q5HH22	3	1
AcrB/AcrD/AcrF family protein	Q5HDU7|RANDOM_Q5HDU7-R	2	0
Dihydroorotase	Q5HGN1|PYRC	7	4
Putative uncharacterized protein	Q5HIA8|Q5HIA8	0	3
Uncharacterized leukocidin-like protein 1	P21224|LUKL1	3	2
50S ribosomal protein L6	Q5HDX3|RL6	2	5
Bifunctional protein folD	Q5HH21|FOLD	2	5
CTP synthase	Q5HE73|PYRG	0	4
Glutathione peroxidase homolog BsaA	Q5HGC7|BSAA	0	7
Alkyl hydroperoxide reductase subunit F	Q5HIR6|AHPF	3	4
Universal stress protein family	Q5HF68|Q5HF68	3	3
50S ribosomal protein L13	Q5HDZ0|RL13	7	1
UPF0312 protein	Q5HCL0|Y2711	5	5
Cell division protein ftsZ	Q5HGP5|FTSZ	7	1
Pyruvate dehydrogenase E1 component subunit beta	Q5HGZ0|ODPB	0	7
Threonyl-tRNA synthetase	Q5HF90|SYT	0	8
Staphylococcus aureus sex pheromone	Q5HEL9|Q5HEL9	1	3
Ribonuclease J 1	Q5HGZ5|RNJ1	1	6
Valyl-tRNA synthetase	Q5HFA8|SYV	3	5
Adenylosuccinate synthetase	Q5HJX8|PURA	1	4
Immunodominant staphylococcal antigen B	ISAB	0	1
Iron compound ABC transporter, iron compound-binding protein	Q5HDS3|Q5HDS3	3	3
Chorismate mutase/phospho-2-dehydro-3-deoxyheptonate aldolase	Q5HF37|Q5HF37	1	4
Phosphopentomutase	Q5HJM9|DEOB	0	6
Fibrinogen-binding protein	Q5HGS6|FIB	5	0
Serine-protein kinase rsbW	Q5HED6|RSBW	2	7
FtsK/SpoIIIE family protein	Q5HF33|Q5HF33	0	1
Penicillin-binding protein 1	Q5HGQ0|Q5HGQ0	0	3
Putative uncharacterized protein	Q5HFP6|Q5HFP6	2	3
Rod shape-determining protein MreC	Q5HFB4|Q5HFB4	5	3
Putative uncharacterized protein	Q5HG81|Q5HG81	2	3
Probable acetyl-CoA acyltransferase	Q5HIU0|THLA	0	1
Cysteine synthase	Q5HIG2|CYSK	2	5
S-ribosylhomocysteine lyase	Q5HE66|LUXS	2	2
Competence protein ComEC/Rec2, putative	Q5HFH3|Q5HFH3	1	0
Phosphoribosylformylglycinamidine cyclo-ligase	Q5HH13|PUR5	0	5
Putative uncharacterized protein	Q5HGE4|Q5HGE4	5	0
10 kDa chaperonin	Q5HEH1|CH10	3	1
Putative dipeptidase	Q5HF23|PEPVL	1	4
Ornithine aminotransferase 2	Q5HHC8|OAT2	0	6
Putative uncharacterized protein	Q5HET6	0	3
2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase 2	Q5HJC1|ISPD2	5	2
Thioredoxin, putative	Q5HHK5|Q5HHK5	0	4
UPF0133 protein	Y521	0	2
Putative uncharacterized protein	Q5HDH4|Q5HDH4	0	5
Elongation factor P	Q5HFN0|EFP	2	7
Probable branched-chain-amino-acid aminotransferase	Q5HIC1|ILVE	5	2
HIT family protein	Q5HET7|Q5HET7	1	1
Aminopeptidase PepS	Q5HEP5|Q5HEP5	0	5
Respiratory nitrate reductase, alpha subunit	Q5HDF9|Q5HDF9	1	0
Adenylosuccinate lyase	Q5HEL4|PUR8	0	5
Uracil phosphoribosyltransferase	Q5HE88|UPP	0	5
Alcohol dehydrogenase, zinc-containing	Q5HE18|Q5HE18	2	2

Appendix 6. (Continued)

Ribosomal large subunit pseudouridine synthase, RluD subfamily	Q5HES5|Q5HES5	0	1
Cell division protein FtsY, putative	Q5HGJ7|Q5HGJ7	0	3
30S ribosomal protein S1	Q5HFU7|RS1	0	4
Putative uncharacterized protein	Q5HCV8|Q5HCV8	1	5
Ribosome-recycling factor	Q5HGH2|RRF	3	2
Phosphoglucomutase	Q5HD61|PGCA	4	1
ATP-dependent Clp protease ATP-binding subunit clpL	Q5HD02|CLPL	0	1
Putative acetyl-CoA C-acetyltransferase vraB	Q5HIA0|VRAB	1	0
Thioredoxin, putative	Q5HHL0|Q5HHL0	0	6
Protein essC	Q5HJ86|ESSC	0	1
Uncharacterized protein	Q5HHB6|Y973	2	3
30S ribosomal protein S9	Q5HDZ1|RS9	1	2
Putative uncharacterized protein	Q5HFN8|RANDOM_Q5HFN8-R	0	1
Xanthine permease	Q5HIQ8	0	1
5' nucleotidase family protein	RANDOM_Q5HH61-R	0	1
Phage infection protein, putative	Q5HCQ4|Q5HCQ4	1	3
50S ribosomal protein L24	Q5HDW9|RL24	2	4
Putative uncharacterized protein	Q5HGA4|Q5HGA4	1	1
Transcriptional regulator, putative	Q5HDP8|Q5HDP8	0	2
1-phosphatidylinositol phosphodiesterase	Q5HJS4|Q5HJS4	0	4
Iron-regulated surface determinant protein A	Q5HGV4|ISDA	0	1
Flavohemoprotein, putative	Q5HJD8|Q5HJD8	0	5
Coenzyme A disulfide reductase	Q5HHB4|CDR	1	1
Putative uncharacterized protein	Q5HGZ2|Q5HGZ2	2	0
Glutamyl aminopeptidase, putative	Q5HG53|Q5HG53	0	2
5'-nucleotidase family protein	Q5HJX2|Q5HJX2	0	2
50S ribosomal protein L23	Q5HDW0|RL23	1	1
50S ribosomal protein L18	Q5HDX4|RL18	1	4
Serine protease splB	Q5HEW1|SPLB	2	1
Indole-3-pyruvate decarboxylase	Q5HJI5|Q5HJI5	4	1
Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]	Q5HE49|GLMS	4	1
50S ribosomal protein L35	Q5HF93|RL35	1	3
Sensor protein srrB	Q5HFT1|SRRB	1	0
Delta-aminolevulinic acid dehydratase	Q5HFA4|HEM2	0	5
Iron compound ABC transporter, iron compound-binding protein	Q5HE28|Q5HE28	0	1
Arginyl-tRNA synthetase	Q5HI60|SYR	0	4
ATP-dependent Clp protease ATP-binding subunit clpC	P0C281|CLPC	0	2
50S ribosomal protein L14	Q5HDW8|RL14	9	0
Putative peptidyl-prolyl cis-trans isomerase	Q5HHD1|PPI1	0	4
30S ribosomal protein S7	Q5HIC9|RS7	0	2
Inosine-uridine preferring nucleoside hydrolase	Q5HJD4|RANDOM_Q5HJD4-R	1	0
Formimidoylglutamase	Q5HDM3|HUTG	0	1
3-methyl-2-oxobutanoate hydroxymethyltransferase	Q5HCV3|PANB	0	4
50S ribosomal protein L19	Q5HGJ1|RANDOM_RL19-R	2	0
Glucose-6-phosphate 1-dehydrogenase	Q5HFR7|Q5HFR7	0	4
3-phosphoshikimate 1-carboxyvinyltransferase	Q5HFV9|AROA	1	0
Uncharacterized protein	Q5HHR8|Y815	0	4
2-oxoisovalerate dehydrogenase, E1 component, alpha subunit	Q5HFQ4|Q5HFQ4	2	0
Uncharacterized N-acetyltransferase	Q5HGQ5|Y1189	0	4
Probable manganese-dependent inorganic pyrophosphatase	Q5HEK1|PPAC	1	1
Serine-aspartate repeat-containing protein D	Q5HIB3|SDRD	0	1
Probable ctpA-like serine protease	Q5HG01|CTPAL	0	1

Appendix 6. (Continued)

Pathogenicity island protein, integrase	Q5HHK1|Q5HHK1	0	1
Aminoacyltransferase femA	FEMA	0	1
Serine protease splC	Q5HEW2|SPLC	2	2
D-lactate dehydrogenase	Q5HD29|LDHD	1	2
ATP-dependent Clp protease proteolytic subunit	Q5HHQ0|CLPP	1	2
Single-stranded-DNA-specific exonuclease RecJ	Q5HFC7|RANDOM_Q5HFC7-R	1	0
Putative uncharacterized protein	Q5HE42|Q5HE42	0	2
Ribose-phosphate pyrophosphokinase	Q5HIH5|KPRS	1	0
ABC transporter, ATP-binding protein, putative	Q5HEQ3|RANDOM_Q5HEQ3-R	1	0
Histidine ammonia-lyase	Q5HJY8|HUTH	0	2
Probable DNA-directed RNA polymerase subunit delta	Q5HE72|RPOE	0	1
Putative uncharacterized protein isdD	Q5HGV2|Q5HGV2	0	1
DNA-dependent DNA polymerase family X	Q5HGU1|Q5HGU1	1	0
Porphobilinogen deaminase	Q5HFA2|HEM3	0	2
Signal transduction protein TRAP	Q5HEU0|TRAP	0	3
Putative NAD(P)H nitroreductase	Q5HD30|Y2534	0	2
Putative uncharacterized protein	Q5HF22|Q5HF22	0	3
Pseudouridine synthase	Q5HFS9|Q5HFS9	0	3
sp|Q5HEL0|Y1973_STAAC	Q5HEL0|Y1973	1	0
sp|Q5HE32|Y2163_STAAC	Q5HE32|Y2163	0	2
Oligoendopeptidase F	Q5HH84|Q5HH84	0	1
Lipoprotein, putative	RANDOM_Q5HHT0-R	0	2
DNA-directed RNA polymerase subunit omega	Q5HGM2|RPOZ	0	4
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	Q5HG07|ODO2	0	3
UPF0477 protein	Q5HH71|Y1020	0	1
Putative uncharacterized protein	Q5HE56|Q5HE56	0	3
Putative uncharacterized protein	Q5HIR8	0	1
sp|Q5HHF6|NAGD_STAAC	Q5HHF6|NAGD	1	2
Malonyl CoA-acyl carrier protein transacylase	Q5HGK3|FABD	1	2
Deoxyribose-phosphate aldolase 1	Q5HJN0|DEOC1	0	3
Anthranilate synthase component I	Q5HG52|RANDOM_Q5HG52-R	0	1
Oxidoreductase, short chain dehydrogenase/reductase family	Q5HDM9|Q5HDM9	0	2
50S ribosomal protein L33 2	Q5HG85|RL332	0	1
Metallo-beta-lactamase family protein	Q5HJT8|Q5HJT8	0	1
Peptide chain release factor 1	Q5HE82|RF1	0	1
Glutamyl aminopeptidase	Q5HF29|Q5HF29	0	1
High-affinity nickel-transport protein	Q5HCK0|RANDOM_Q5HCK0-R	0	2
Hypoxanthine-guanine phosphoribosyltransferase	Q5HIG5|HPRT	1	0
Methionine import ATP-binding protein MetN 2	Q5HHK4|METN2	0	1
D-alanine--poly(phosphoribitol) ligase subunit 1	Q5HHF2|DLTA	2	0
Glycerol-3-phosphate acyltransferase	Q5HG66|RANDOM_PLSY-R	1	0
Extracellular matrix protein-binding protein emp	Q5HHM6|EMP	0	4
Pyruvate carboxylase	Q5HGX0|Q5HGX0	0	24
Urease subunit alpha	Q5HDR8|URE1	0	3
Uncharacterized lipoprotein	Q5HIN1|Y486	0	3
Probable malate:quinone oxidoreductase 1	Q5HDJ0|MQO1	2	0
Putative uncharacterized protein	Q5HF36|RANDOM_Q5HF36-R	1	0

Appendix 6. (Continued)

5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase	Q5HFG2|MTNN	0	4
Dps family protein	Q5HE61|Q5HE61	0	3
NADP-dependent malic enzyme, putative	Q5HF72|Q5HF72	0	4
tr|Q5HH17|Q5HH17_STAAC	Q5HH17|Q5HH17	0	3
Ribonucleoside-diphosphate reductase 2, beta subunit	Q5HHT9|Q5HHT9	0	3
Putative formate dehydrogenase	Q5HDP9|FDHL	0	1
Putative uncharacterized protein	Q5HFT3|RANDOM_Q5HFT3-R	0	1
Putative uncharacterized protein	Q5HJ95|RANDOM_Q5HJ95-R	0	1
Leukotoxin LukD	Q5HEV0|Q5HEV0	22	0
tr|Q5HDI9|Q5HDI9_STAAC	Q5HDI9|Q5HDI9	1	1
3-hexulose-6-phosphate synthase	Q5HIA5|HPS	0	1
Putative uncharacterized protein	Q5HF36|Q5HF36	0	1
sp|Q5HIE5|SYC_STAAC	Q5HIE5|SYC	0	2
Organic hydroperoxide resistance protein-like	Q5HHL3|OHRL	1	0
Transcription-repair-coupling factor	Q5HIH2|MFD	0	1
GMP synthase [glutamine-hydrolyzing]	Q5HIQ6|GUAA	9	9
Glycerol phosphate lipoteichoic acid synthase	Q5HHV4|RANDOM_LTAS-R	0	1
Dihydrolipoyl dehydrogenase	Q5HFQ3|Q5HFQ3	0	2
Peptidase, M20/M25/M40 family	Q5HFR1|Q5HFR1	0	3
Putative uncharacterized protein	Q5HJT9|RANDOM_Q5HJT9-R	1	0
Clumping factor B	Q5HCR7|CLFB	0	2
Probable glycine dehydrogenase [decarboxylating] subunit 1	Q5HFM3|GCSPA	0	1
Arsenate reductase, putative	Q5HHK9|Q5HHK9	0	2
6,7-dimethyl-8-ribityllumazine synthase	Q5HF08|RISB	0	1
Chaperone protein dnaJ	Q5HFI1|DNAJ	0	2
Amidophosphoribosyltransferase	Q5HH14|PUR1	0	2
Copper-exporting P-type ATPase A	Q5HCZ3|COPA	0	1
UvrABC system protein A	Q5HHQ9|UVRA	1	0
Mannitol-specific phosphotransferase enzyme IIA component	Q5HE46|PTMA	0	2
Pyrroline-5-carboxylate reductase	Q5HFR9|P5CR	0	1
Pyridoxal biosynthesis lyase pdxS	Q5HIF5|PDXS	0	2
Thioredoxin	Q5HGT9|THIO	0	1
NADH-dependent flavin oxidoreductase, Oye family	Q5HHC9|Q5HHC9	0	2
sp|Q5HDW6|RL29_STAAC	Q5HDW6|RL29	0	1
Transcription-repair-coupling factor	Q5HIH2|RANDOM_MFD-R	0	1
Mevalonate kinase	Q5HI86|RANDOM_Q5HI86-R	0	1
Putative 8-amino-7-oxononanoate synthase/2-amino-3-ketobutyrate coenzyme A ligase	Q5HIC5|BIKB	0	1
Phosphate import ATP-binding protein pstB	P69881|RANDOM_PSTB-R	1	0
Putative uncharacterized protein	Q5HGB8|RANDOM_Q5HGB8-R	0	1

Appendix 7. Changes in secreted proteins of HA-MRSA USA200 compared to HA-MRSA USA100 during post-exponential phase from 3 biological replicates

Identified Proteins (109)	Accession Number	USA100 #1	USA100 #2	USA100 #3	USA200 #1	USA200 #2	USA200 #3
Lipase 1	P65289|LIP1	Ref	-2.8	-2.6	1.6	-2.6	0.1
Putative surface protein SA2285	P61598|PLS	Ref	3.9	-0.4	-4	-0.5	-4.3
Bifunctional autolysin	Q99V41|ATL	Ref	-0.5	-1.6	0.6	-0.3	-1
Lipase 2	Q7A7P2|LIP2	Ref	0.6	0.8	1.3	1.8	3
SA0841 protein	Q7A6G0|Q7A6G0	Ref	-2.3	-1	2.5	-0.3	1.5
Penicillin binding protein 2 prime	Q7A8C6|Q7A8C6	Ref	-0.9	-3.5	3	1.2	-0.4
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS	Ref	1.2	-0.2	1	1.3	1.4
Staphopain B	Q7A6A7|SSPB	Ref	-5	-2.5	2.3	-4.8	0.4
Thermonuclease	Q7A6P2|NUC	Ref	3.8	2.7	1.6	4.7	4.6
Alpha-Hemolysin	Q7A632|Q7A632	Ref	-2.5	2.5	1.5	-2	3.5
Probable transglycosylase isaA	P99160|ISAA	Ref	2.7	-1	-0.6	2.2	-1
SA2437 protein	Q7A371|Q7A371	Ref	1.2	0.7	3	2	3.3
SA0620 protein	Q7A6Y9|Q7A6Y9	Ref	-2.8	-3.1	1.3	-1.1	-2.6
Glutamyl endopeptidase	Q7A6A6|SSPA	Ref	-4.5	-3	3	-1.8	0.8
Probable beta-lactamase	Q7A4X8|Q7A4X8	Ref	No Values	-6.9	Reference Missing	Reference Missing	-2.8
Immunoglobulin G-binding protein A	P99134|SPA	Ref	5.9	4.9	-2.6	3	1.9
Enterotoxin type C-3	P0A0L4|ENTC3	Ref	0.5	-0.8	0.1	0.3	-0.9
Putative uncharacterized protein SA0359	Q7A7J8|Q7A7J8	Ref	2.3	-2.1	0.9	2.2	-1.6
SA2006 protein	Q7A483|Q7A483	Ref	-2.5	-1.3	1.4	-2	-0.4
Staphopain A	P65826|SSPP	Ref	1.9	1.9	-0.8	2.8	1.9
Alkyl hydroperoxide reductase subunit C	P99074|AHPC	Ref	-2	-4.3	-0.5	-2.8	-4.9
Putative uncharacterized protein SA0570	Q7A735|Q7A735	Ref	9.8	4.3	0.6	10.9	5.4
Elongation factor Tu	P99152|EFTU	Ref	1.7	0.6	-0.5	2.1	2.3

Appendix 7. (Continued)

Glycyl-glycine endopeptidase lytM	Q7A7T0|LYTM	Ref	5.4	1.7	0.7	6.6	3.7
Immunoglobulin-binding protein sbi	Q99RL2|SBI	Ref	2.4	-0.3	0.1	1.4	0.1
Truncated beta-hemplysin	Q99QR7|Q99QR7	Ref	No Values	3.2	Reference Missing	No Values	7.2
Uncharacterized leukocidin-like protein 1	Q7A4L0|LUKL1	Ref	4.6	1.4	0.5	4.6	2.6
Enolase	P99088|ENO	Ref	1.5	0.2	-0.6	1.6	-0.3
Fructose-bisphosphate aldolase	P99075|ALF2	Ref	4.2	1.6	-2.4	4	1
Probable transglycosylase sceD	Q7A4F2|SCED	Ref	3.9	-1.6	1.7	6.3	-1.3
Serine-aspartate repeat-containing protein D	Q7A780|SDRD	Ref	-0.8	-3.1	-4.4	-5	-3.8
SA0587 protein	Q7A719|Q7A719	Ref	0	0	No Values	-0.3	0.1
SA0914 protein	Q99V35|Q99V35	Ref	5	3.6	0.1	4.8	4.5
60 kDa chaperonin	P99083|CH60	Ref	-4	-0.2	-0.5	-3.2	0.8
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1	Ref	No Values	2.1	-0.6	No Values	2.6
Zinc metalloproteinase aureolysin	Q7A378|Q7A378	Ref	0	0	No Values	1.6	2.4
Virulence factor esxA	Q7A7S4|ESXA	Ref	0	0	No Values	-0.5	0.6
Serine protease splB	Q7A4Y1|SPLB	Ref	0.1	-1.3	No Values	0.1	-0.8
Foldase protein prsA	P60748|PRSA	Ref	-1.3	-2.4	1.4	0.9	-0.6
50S ribosomal protein L7/L12	P99154|RL7	Ref	No Values	4	-0.7	No Values	2.8
Iron-regulated surface determinant protein A	Q7A655|ISDA	Ref	0.8	-0.3	0.9	1.1	0
DNA-binding protein HU	Q7A5J1|DBH	Ref	8.2	3.5	-1.4	9.6	5.3
Staphylokinase	Q99SU7|SAK	Ref	1.2	-0.4	-2.3	2.7	-0.5
Pyruvate dehydrogenase E1 component subunit alpha	Q820A6|ODPA	Ref	0	0	No Values	-2.6	-0.4
Putative uncharacterized protein SA0663	Q7A6V1|Q7A6V1	Ref	-3.1	2.7	-0.6	-2.8	3.3
Chaperone protein dnaK	P99110|DNAK	Ref	No Values	0.3	2.4	No Values	-0.2
Adenylate kinase	P99062|KAD	Ref	6.5	1.2	-1.1	5.6	1
Putative uncharacterized protein SA0908	Q7A6A3|Q7A6A3	Ref	-0.3	-1.2	0.8	-1.7	-1

Appendix 7. (Continued)

50S ribosomal protein L30	P0A0G0|RL30	Ref	0	0	No Values	1.2	-1.1
Uncharacterized leukocidin-like protein 2	Q99SN7|LUKL2	Ref	-3.8	1.8	1.3	-4.1	2.7
Dihydrolipoyl dehydrogenase	P99084|DLDH	Ref	No Values	3.8	-0.3	No Values	5.2
Phenol-soluble modulin alpha 1 peptide	P0C7Y7|PSMA1	Ref	1.3	2.2	0.2	3.2	3.3
Serine protease splF	Q7A4Y4|SPLF	Ref	0	No Values	No Values	1	No Values
Pyruvate dehydrogenase E1 component subunit beta	P99063|ODPB	Ref	7.3	8.3	-2.5	7.8	10.6
50S ribosomal protein L17	Q7A469|RL17	Ref	2.7	0.2	-1.2	3.3	2.2
Glutamine synthetase	P99095|GLNA	Ref	No Values	0.4	-0.7	No Values	0.5
N-acetylmuramoyl-L-alanine amidase sle1	Q7A7E0|SLE1	Ref	No Values	4.5	0.5	No Values	4.6
Alkaline shock protein 23	P99157|ASP23	Ref	1.7	0.4	-0.5	1.3	0.3
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	P65636|ODP2	Ref	-1.8	-2.5	1.6	-1.7	Value Missing
SA2097 protein	Q7A418|Q7A418	Ref	0	0	No Values	1.4	0.9
Ribosome-recycling factor	P99130|RRF	Ref	No Values	0.7	-0.4	No Values	-0.3
Elongation factor G	P68789|EFG	Ref	No Values	0.7	-0.6	No Values	0.8
Serine protease splC	Q7A4Y2|SPLC	Ref	0	No Values	No Values	0.2	No Values
50S ribosomal protein L11	P0A0F2|RL11	Ref	2.6	0.5	-2	2.9	-0.4
Thioredoxin	P99122|THIO	Ref	No Values	No Values	-1.7	No Values	No Values
Trigger factor	P99080|TIG	Ref	No Values	2.4	-0.6	No Values	1
30S ribosomal protein S9	P66646|RS9	Ref	No Values	-1	-2.2	No Values	0.2
50S ribosomal protein L13	Q7A473|RL13	Ref	No Values	1.8	-0.4	No Values	1
Phosphate acetyltransferase	P99092|PTA	Ref	3.4	1.4	-1.7	2.8	1.2
Acyl carrier protein	P0A002|ACP	Ref	No Values	2.7	-1.9	No Values	1.5
Serine-aspartate repeat-containing protein E	Q99W46|SDRE	Ref	No Values	2	-0.5	No Values	2.9

Appendix 7. (Continued)

Beta-lactamase	Q9AC80|Q9AC80	Ref	1.1	1.2	0	1.6	1.2
Cell division protein	Q7A620|Q7A620	Ref	No Values	2.3	-1.7	No Values	0.8
Fructose-bisphosphate aldolase class 1	P99117|ALF1	Ref	No Values	0	No Values	No Values	0.3
50S ribosomal protein L14	Q7A463|RL14	Ref	No Values	0.1	2.9	No Values	0
Phenol-soluble modulin alpha 4 peptide	P0C824|PSMA4	Ref	0	0	No Values	0.9	0.7
Staphylococcal secretory antigen ssaA2	Q7A423|SSAA2	Ref	No Values	2.4	0.8	No Values	2.7
Glycerophosphoryl diester phosphodiesterase	Q7A6H7|Q7A6H7	Ref	0	0	No Values	-1	1.7
SA2202 protein	Q99RL6|Q99RL6	Ref	0	0	No Values	0.2	-1
50S ribosomal protein L22	Q7A460|RL22	Ref	3.6	No Values	-1	2.7	No Values
Elastin-binding protein ebpS	Q7A5I6|EBPS	Ref	0	0	No Values	0.6	0.4
Triosephosphate isomerase	P99133|TPIS	Ref	No Values	0.3	-0.6	No Values	2.5
P99156|GREA_STAAN	P99156|GREA	Ref	No Values	3.4	-1.3	No Values	4.9
Cysteine synthase	P63871|CYSK	Ref	No Values	-0.1	2.7	No Values	Reference Missing
Elongation factor Ts	P99171|EFTS	Ref	No Values	No Values	-1.2	Reference Missing	No Values
30S ribosomal protein S6	P99142|RS6	Ref	No Values	No Values	-2	No Values	No Values
Leukotoxin, LukD	Q99T54|Q99T54	Ref	0	No Values	No Values	-0.5	No Values
Inosine-5'-monophosphate dehydrogenase	P99106|IMDH	Ref	1.8	-1.2	-1.1	1.5	-0.9
Protein grpE	P99086|GRPE	Ref	No Values	0.3	-1	No Values	0.3
50S ribosomal protein L24	P60735|RL24	Ref	No Values	0	No Values	No Values	-1.1
L-lactate dehydrogenase 1	P65256|LDH1	Ref	1.4	No Values	-3	1.1	No Values
Superoxide dismutase [Mn/Fe] 1	P99098|SODM1	Ref	No Values	No Values	2.2	No Values	No Values
Transketolase	P99161|TKT	Ref	No Values	0	No Values	No Values	-0.4
30S ribosomal protein S16	P66440|RS16	Ref	1.8	No Values	-0.4	2.5	No Values
10 kDa chaperonin	P99104|CH10	Ref	No Values	2.9	-1.9	No Values	5.5
SA0295 protein	Q7A7Q2|Q7A7Q2	Ref	No Values	No Values	0.4	No Values	No Values

Appendix 7. (Continued)

Glucose-6-phosphate isomerase	P99078|G6PI	Ref	No Values	No Values	-0.4	No Values	No Values
Protein esaA	Q7A7S3|ESAA	Ref	0.1	No Values	0.4	0.3	No Values
Methicillin resistance mecR1 protein	P0A0B0|RANDOM_MECR-R	Ref	No Values	0	1.2	No Values	1.5
Uncharacterized protein SA1692	P0A0K1|Y1692	Ref	No Values	0	No Values	No Values	1.2
Chaperone protein hchA	P64313|HCHA	Ref	No Values	No Values	0.5	No Values	No Values
Clumping factor A	Q99VJ4|CLFA	Ref	No Values	0	No Values	No Values	1.2
50S ribosomal protein L10	P99155|RL10	Ref	No Values	2.7	0.7	No Values	3
50S ribosomal protein L18	Q7A467|RL18	Ref	No Values	No Values	-2.5	No Values	No Values
Putative peptidyl-prolyl cis-trans isomerase	Q7A6I1|PPI1	Ref	No Values	No Values	-1.2	No Values	No Values
Fibrinogen-binding protein	P68800|FIB	Ref	No Values	No Values	-0.1	No Values	No Values
1-phosphatidylinositol phosphodiesterase precurosr	Q7A888|Q7A888	Ref	0	No Values	No Values	1	No Values
Virulence factor esxA	ESXA	Ref	No Values	No Values	No Values	No Values	No Values
50S ribosomal protein L15	P0A0F6|RL15	Ref	No Values	No Values	0.6	No Values	No Values

Appendix 8. Changes in secreted proteins of CA-MRSA USA300 compared to HA-MRSA USA100 during post-exponential phase 3 biological replicates

Identified Proteins (109)	Accession Number	USA100 #1	USA100 #2	USA100 #3	USA300 #1	USA300 #2	USA300 #3
Lipase 1	P65289|LIP1	Ref	-2.8	-2.6	2.8	-1.4	-1.3
Putative surface protein SA2285	P61598|PLS	Ref	3.9	-0.4	-3.7	-2.3	-4.6
Bifunctional autolysin	Q99V41|ATL	Ref	-0.5	-1.6	0.2	-2.1	-2.8
Lipase 2	Q7A7P2|LIP2	Ref	0.6	0.8	2	2.2	1.5
SA0841 protein	Q7A6G0|Q7A6G0	Ref	-2.3	-1	1.6	-1.4	-0.7
Penicillin binding protein 2 prime	Q7A8C6|Q7A8C6	Ref	-0.9	-3.5	0.8	-1.5	-3.3
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS	Ref	1.2	-0.2	0.2	0.3	-2
Staphopain B	Q7A6A7|SSPB	Ref	-5	-2.5	1.2	-3.4	-2.3
Thermonuclease	Q7A6P2|NUC	Ref	3.8	2.7	1.8	4.3	2.5
Alpha-Hemolysin	Q7A632|Q7A632	Ref	-2.5	2.5	2.8	-0.8	3.2
Probable transglycosylase isaA	P99160|ISAA	Ref	2.7	-1	-1.6	-2.1	-4.6
SA2437 protein	Q7A371|Q7A371	Ref	1.2	0.7	1.7	-0.5	1
SA0620 protein	Q7A6Y9|Q7A6Y9	Ref	-2.8	-3.1	0.4	-4.4	-2.8
Glutamyl endopeptidase	Q7A6A6|SSPA	Ref	-4.5	-3	0.9	-2.9	-2
Probable beta-lactamase	Q7A4X8|Q7A4X8	Ref	No Values	-6.9	Reference Missing	Reference Missing	-6.6
Immunoglobulin G-binding protein A	P99134|SPA	Ref	5.9	4.9	-2.7	1	2.3
Enterotoxin type C-3	P0A0L4|ENTC3	Ref	0.5	-0.8	0.1	-2.5	-1.4
Putative uncharacterized protein SA0359	Q7A7J8|Q7A7J8	Ref	2.3	-2.1	0	3.4	-3.2
SA2006 protein	Q7A483|Q7A483	Ref	-2.5	-1.3	2.8	-1.4	-1.1
Staphopain A	P65826|SSPP	Ref	1.9	1.9	0.8	3.1	0.7
Alkyl hydroperoxide reductase subunit C	P99074|AHPC	Ref	-2	-4.3	-1.5	-5.3	-6.5
Putative uncharacterized protein SA0570	Q7A735|Q7A735	Ref	9.8	4.3	1.2	10.4	3
Elongation factor Tu	P99152|EFTU	Ref	1.7	0.6	0.2	0.2	-0.6
Glycyl-glycine endopeptidase lytM	Q7A7T0|LYTM	Ref	5.4	1.7	-1.2	4.4	0.7

Appendix 8. (Continued)

Immunoglobulin-binding protein sbi	Q99RL2|SBI	Ref	2.4	-0.3	0.3	2.3	-1.1
Truncated beta-hemplysin	Q99QR7|Q99QR7	Ref	No Values	3.2	Reference Missing	No Values	3.3
Uncharacterized leukocidin-like protein 1	Q7A4L0|LUKL1	Ref	4.6	1.4	2.3	3.3	2.4
Enolase	P99088|ENO	Ref	1.5	0.2	-0.6	-0.7	-1.2
Fructose-bisphosphate aldolase	P99075|ALF2	Ref	4.2	1.6	-2.5	5.8	-0.1
Probable transglycosylase sceD	Q7A4F2|SCED	Ref	3.9	-1.6	0.4	3.4	-3.2
Serine-aspartate repeat-containing protein D	Q7A780|SDRD	Ref	-0.8	-3.1	-3.7	-4.8	-6.5
SA0587 protein	Q7A719|Q7A719	Ref	0	0	No Values	-1.7	-2
SA0914 protein	Q99V35|Q99V35	Ref	5	3.6	-0.2	5.4	2.6
60 kDa chaperonin	P99083|CH60	Ref	-4	-0.2	-0.2	-5	-1.1
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1	Ref	No Values	2.1	-1.5	Reference Missing	1.8
Zinc metalloproteinase aureolysin	Q7A378|Q7A378	Ref	0	0	No Values	1.6	1
Virulence factor esxA	Q7A7S4|ESXA	Ref	0	0	No Values	-0.1	-0.1
Serine protease splB	Q7A4Y1|SPLB	Ref	0.1	-1.3	Reference Missing	1.6	-0.3
Foldase protein prsA	P60748|PRSA	Ref	-1.3	-2.4	-0.2	-4.2	-3.3
50S ribosomal protein L7/L12	P99154|RL7	Ref	No Values	4	-1.9	No Values	1.4
Iron-regulated surface determinant protein A	Q7A655|ISDA	Ref	0.8	-0.3	-0.3	-0.9	-1.2
DNA-binding protein HU	Q7A5J1|DBH	Ref	8.2	3.5	-2.2	7.1	1.1
Staphylokinase	Q99SU7|SAK	Ref	1.2	-0.4	1.2	2.9	-1.2
Pyruvate dehydrogenase E1 component subunit alpha	Q820A6|ODPA	Ref	0	0	No Values	-2.4	-3
Putative uncharacterized protein SA0663	Q7A6V1|Q7A6V1	Ref	-3.1	2.7	1	-1.4	2.2
Chaperone protein dnaK	P99110|DNAK	Ref	No Values	0.3	2.7	Reference Missing	-1.9
Adenylate kinase	P99062|KAD	Ref	6.5	1.2	-2.2	4.6	-1.3
Putative uncharacterized protein SA0908	Q7A6A3|Q7A6A3	Ref	-0.3	-1.2	1.7	-2.5	-2.3

Appendix 8. (Continued)

50S ribosomal protein L30	P0A0G0|RL30	Ref	0	0	No Values	-3.7	-2.2
Uncharacterized leukocidin-like protein 2	Q99SN7|LUKL2	Ref	-3.8	1.8	1.4	-2.2	-0.2
Dihydrolipoyl dehydrogenase	P99084|DLDH	Ref	No Values	3.8	-2.4	Reference Missing	3.9
Phenol-soluble modulin alpha 1 peptide	P0C7Y7|PSMA1	Ref	1.3	2.2	2.8	2.9	4.7
Serine protease splF	Q7A4Y4|SPLF	Ref	0	No Values	No Values	1.3	No Values
Pyruvate dehydrogenase E1 component subunit beta	P99063|ODPB	Ref	7.3	8.3	-1.9	7	9.4
50S ribosomal protein L17	Q7A469|RL17	Ref	2.7	0.2	-1	2.9	-1.4
Glutamine synthetase	P99095|GLNA	Ref	No Values	0.4	-1.5	Reference Missing	-2.5
N-acetylmuramoyl-L-alanine amidase sle1	Q7A7E0|SLE1	Ref	No Values	4.5	0.8	Reference Missing	Value Missing
Alkaline shock protein 23	P99157|ASP23	Ref	1.7	0.4	-0.9	2.8	Value Missing
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	P65636|ODP2	Ref	-1.8	-2.5	2.8	-4.3	-2.3
SA2097 protein	Q7A418|Q7A418	Ref	0	0	No Values	-0.5	-0.8
Ribosome-recycling factor	P99130|RRF	Ref	No Values	0.7	-1.2	No Values	-2
Elongation factor G	P68789|EFG	Ref	No Values	0.7	1	No Values	-1.6
Serine protease splC	Q7A4Y2|SPLC	Ref	0	No Values	No Values	1.6	No Values
50S ribosomal protein L11	P0A0F2|RL11	Ref	2.6	0.5	-2	-1.1	-2.9
Thioredoxin	P99122|THIO	Ref	No Values	No Values	0.2	No Values	No Values
Trigger factor	P99080|TIG	Ref	No Values	2.4	-1.5	No Values	-1.9
30S ribosomal protein S9	P66646|RS9	Ref	No Values	-1	-1.6	No Values	-2
50S ribosomal protein L13	Q7A473|RL13	Ref	No Values	1.8	-0.4	No Values	-0.3
Phosphate acetyltransferase	P99092|PTA	Ref	3.4	1.4	-2.9	3.2	0.2
Acyl carrier protein	P0A002|ACP	Ref	No Values	2.7	-2.3	No Values	0.2

Appendix 8. (Continued)

Serine-aspartate repeat-containing protein E	Q99W46|SDRE	Ref	No Values	2	-1.7	No Values	1.4
Beta-lactamase	Q9AC80|Q9AC80	Ref	1.1	1.2	-0.3	1.4	1.3
Cell division protein	Q7A620|Q7A620	Ref	No Values	2.3	-1.3	No Values	-0.5
Fructose-bisphosphate aldolase class 1	P99117|ALF1	Ref	No Values	0	No Values	No Values	-0.6
50S ribosomal protein L14	Q7A463|RL14	Ref	No Values	0.1	1.1	No Values	-0.3
Phenol-soluble modulin alpha 4 peptide	P0C824|PSMA4	Ref	0	0	No Values	1.3	2.2
Staphylococcal secretory antigen ssaA2	Q7A423|SSAA2	Ref	No Values	2.4	0.3	No Values	1
Glycerophosphoryl diester phosphodiesterase	Q7A6H7|Q7A6H7	Ref	0	0	No Values	0.7	-0.5
SA2202 protein	Q99RL6|Q99RL6	Ref	0	0	No Values	-1.6	-1.4
50S ribosomal protein L22	Q7A460|RL22	Ref	3.6	No Values	-1.7	2.6	No Values
Elastin-binding protein ebpS	Q7A5I6|EBPS	Ref	0	0	No Values	-2	-1.2
Triosephosphate isomerase	P99133|TPIS	Ref	No Values	0.3	2.8	No Values	0.3
P99156|GREA_STAAN	P99156|GREA	Ref	No Values	3.4	-2.4	No Values	3.2
Cysteine synthase	P63871|CYSK	Ref	No Values	-0.1	2.8	No Values	3.2
Elongation factor Ts	P99171|EFTS	Ref	No Values	No Values	-2.9	Reference Missing	No Values
30S ribosomal protein S6	P99142|RS6	Ref	No Values	No Values	-0.3	No Values	No Values
Leukotoxin, LukD	Q99T54|Q99T54	Ref	0	No Values	No Values	1.7	No Values
Inosine-5'-monophosphate dehydrogenase	P99106|IMDH	Ref	1.8	-1.2	-1.4	-0.2	-3.3
Protein grpE	P99086|GRPE	Ref	No Values	0.3	-2	No Values	-1
50S ribosomal protein L24	P60735|RL24	Ref	No Values	0	No Values	No Values	-1.6
L-lactate dehydrogenase 1	P65256|LDH1	Ref	1.4	No Values	-1.2	0.6	No Values
Superoxide dismutase [Mn/Fe] 1	P99098|SODM1	Ref	No Values	No Values	0.9	No Values	No Values
Transketolase	P99161|TKT	Ref	No Values	0	No Values	No Values	-2.7
30S ribosomal protein S16	P66440|RS16	Ref	1.8	No Values	-1.3	0.2	No Values
10 kDa chaperonin	P99104|CH10	Ref	No Values	2.9	0.5	No Values	3

Appendix 8. (Continued)

SA0295 protein	Q7A7Q2|Q7A7Q2	Ref	No Values	No Values	0.1	No Values	No Values
Glucose-6-phosphate isomerase	P99078|G6PI	Ref	No Values	No Values	-1.5	No Values	No Values
Protein esaA	Q7A7S3|ESAA	Ref	0.1	No Values	0.2	-1.6	No Values
Methicillin resistance mecR1 protein	P0A0B0|RANDOM_MECR-R	Ref	No Values	0	2.3	No Values	0.4
Uncharacterized protein SA1692	P0A0K1|Y1692	Ref	No Values	0	No Values	No Values	0
Chaperone protein hchA	P64313|HCHA	Ref	No Values	No Values	-0.1	No Values	No Values
Clumping factor A	Q99VJ4|CLFA	Ref	No Values	0	No Values	No Values	-0.4
50S ribosomal protein L10	P99155|RL10	Ref	No Values	2.7	-0.7	No Values	1.8
50S ribosomal protein L18	Q7A467|RL18	Ref	No Values	No Values	-2.2	No Values	No Values
Putative peptidyl-prolyl cis-trans isomerase	Q7A6I1|PPI1	Ref	No Values	No Values	-1.8	No Values	No Values
Fibrinogen-binding protein	P68800|FIB	Ref	No Values	No Values	0.4	No Values	No Values
1-phosphatidylinositol phosphodiesterase precurosr	Q7A888|Q7A888	Ref	0	No Values	No Values	-1.7	No Values
Virulence factor esxA	ESXA	Ref	No Values	No Values	No Values	No Values	No Values
50S ribosomal protein L15	P0A0F6|RL15	Ref	No Values	No Values	-0.9	No Values	No Values

Appendix 9. Changes in secreted proteins of CA-MRSA USA400 compared to HA-MRSA USA100 during post-exponential phase from 3 biological replicates

Identified Proteins (109)	Accession Number	USA100 #1	USA100 #2	USA100 #3	USA400 #1	USA400 #2	USA400 #3
Lipase 1	P65289|LIP1	Ref	-2.8	-2.6	-0.2	0.1	0.5
Putative surface protein SA2285	P61598|PLS	Ref	3.9	-0.4	-2.9	-1.1	-3.9
Bifunctional autolysin	Q99V41|ATL	Ref	-0.5	-1.6	0.8	-1	-1.2
Lipase 2	Q7A7P2|LIP2	Ref	0.6	0.8	2	1.4	2.7
SA0841 protein	Q7A6G0|Q7A6G0	Ref	-2.3	-1	1.2	0	1.2
Penicillin binding protein 2 prime	Q7A8C6|Q7A8C6	Ref	-0.9	-3.5	0.5	0.3	-2.9
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS	Ref	1.2	-0.2	0.3	2.5	0.4
Staphopain B	Q7A6A7|SSPB	Ref	-5	-2.5	1.6	-4.8	-0.6
Thermonuclease	Q7A6P2|NUC	Ref	3.8	2.7	0.8	4.2	3.5
Alpha-Hemolysin	Q7A632|Q7A632	Ref	-2.5	2.5	0.8	-2.9	3.2
Probable transglycosylase isaA	P99160|ISAA	Ref	2.7	-1	-0.8	2.8	-1.3
SA2437 protein	Q7A371|Q7A371	Ref	1.2	0.7	1.7	2.9	2.8
SA0620 protein	Q7A6Y9|Q7A6Y9	Ref	-2.8	-3.1	0.8	-2.6	-1.9
Glutamyl endopeptidase	Q7A6A6|SSPA	Ref	-4.5	-3	1.7	-3.2	0.1
Probable beta-lactamase	Q7A4X8|Q7A4X8	Ref	No Values	-6.9	Reference Missing	Reference Missing	-6
Immunoglobulin G-binding protein A	P99134|SPA	Ref	5.9	4.9	-3.2	1.3	4.6
Enterotoxin type C-3	P0A0L4|ENTC3	Ref	0.5	-0.8	1.1	3.1	1.2
Putative uncharacterized protein SA0359	Q7A7J8|Q7A7J8	Ref	2.3	-2.1	1	3.9	-0.7
SA2006 protein	Q7A483|Q7A483	Ref	-2.5	-1.3	1.1	-2	0.7
Staphopain A	P65826|SSPP	Ref	1.9	1.9	1.4	3.9	4.1
Alkyl hydroperoxide reductase subunit C	P99074|AHPC	Ref	-2	-4.3	-0.6	-4	-4.5
Putative uncharacterized protein SA0570	Q7A735|Q7A735	Ref	9.8	4.3	2.7	12	5.5
Elongation factor Tu	P99152|EFTU	Ref	1.7	0.6	1.4	1.7	1.7

Appendix 9. (Continued)

Glycyl-glycine endopeptidase lytM	Q7A7T0|LYTM	Ref	5.4	1.7	0.2	4.9	2.2
Immunoglobulin-binding protein sbi	Q99RL2|SBI	Ref	2.4	-0.3	0.7	3.5	0.6
Truncated beta-hemplysin	Q99QR7|Q99QR7	Ref	No Values	3.2	Reference Missing	No Values	3.5
Uncharacterized leukocidin-like protein 1	Q7A4L0|LUKL1	Ref	4.6	1.4	2.3	1	2.8
Enolase	P99088|ENO	Ref	1.5	0.2	0.3	-0.3	-0.1
Fructose-bisphosphate aldolase	P99075|ALF2	Ref	4.2	1.6	-1.1	1.2	1
Probable transglycosylase sceD	Q7A4F2|SCED	Ref	3.9	-1.6	0.1	2.2	-1.7
Serine-aspartate repeat-containing protein D	Q7A780|SDRD	Ref	-0.8	-3.1	-2.4	-3.5	-5.5
SA0587 protein	Q7A719|Q7A719	Ref	0	0	No Values	-0.1	0.2
SA0914 protein	Q99V35|Q99V35	Ref	5	3.6	0.2	6	4.1
60 kDa chaperonin	P99083|CH60	Ref	-4	-0.2	0.8	-3.7	1
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1	Ref	No Values	2.1	-0.4	No Values	1.9
Zinc metalloproteinase aureolysin	Q7A378|Q7A378	Ref	0	0	No Values	1.6	1
Virulence factor esxA	Q7A7S4|ESXA	Ref	0	0	No Values	0.4	-0.1
Serine protease splB	Q7A4Y1|SPLB	Ref	0.1	-1.3	Reference Missing	0.3	0.7
Foldase protein prsA	P60748|PRSA	Ref	-1.3	-2.4	-0.4	-1.9	-1.8
50S ribosomal protein L7/L12	P99154|RL7	Ref	No Values	4	-0.6	No Values	3.7
Iron-regulated surface determinant protein A	Q7A655|ISDA	Ref	0.8	-0.3	0.4	0.4	0.1
DNA-binding protein HU	Q7A5J1|DBH	Ref	8.2	3.5	-0.5	9.4	2.6
Staphylokinase	Q99SU7|SAK	Ref	1.2	-0.4	0.1	2.9	0.4
Pyruvate dehydrogenase E1 component subunit alpha	Q820A6|ODPA	Ref	0	0	No Values	-2.2	-0.1
Putative uncharacterized protein SA0663	Q7A6V1|Q7A6V1	Ref	-3.1	2.7	-0.2	-3	3.5
Chaperone protein dnaK	P99110|DNAK	Ref	No Values	0.3	1.3	Reference Missing	0.5
Adenylate kinase	P99062|KAD	Ref	6.5	1.2	0.2	5.1	0.8

Appendix 9. (Continued)

Putative uncharacterized protein SA0908	Q7A6A3|Q7A6A3	Ref	-0.3	-1.2	1.1	-0.7	-1.2
50S ribosomal protein L30	P0A0G0|RL30	Ref	0	0	No Values	-2.2	-0.5
Uncharacterized leukocidin-like protein 2	Q99SN7|LUKL2	Ref	-3.8	1.8	1.7	-2.3	1.9
Dihydrolipoyl dehydrogenase	P99084|DLDH	Ref	No Values	3.8	-0.7	No Values	4
Phenol-soluble modulin alpha 1 peptide	P0C7Y7|PSMA1	Ref	1.3	2.2	0.8	2.3	3.7
Serine protease splF	Q7A4Y4|SPLF	Ref	0	No Values	No Values	0	No Values
Pyruvate dehydrogenase E1 component subunit beta	P99063|ODPB	Ref	7.3	8.3	0.1	7.4	12
50S ribosomal protein L17	Q7A469|RL17	Ref	2.7	0.2	-0.1	2.9	1.6
Glutamine synthetase	P99095|GLNA	Ref	No Values	0.4	-1.1	No Values	0.5
N-acetylmuramoyl-L-alanine amidase sle1	Q7A7E0|SLE1	Ref	No Values	4.5	0.3	No Values	5.9
Alkaline shock protein 23	P99157|ASP23	Ref	1.7	0.4	0.3	2.1	2.6
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	P65636|ODP2	Ref	-1.8	-2.5	Value Missing	-1.6	-1.7
SA2097 protein	Q7A418|Q7A418	Ref	0	0	No Values	0.1	0.1
Ribosome-recycling factor	P99130|RRF	Ref	No Values	0.7	-0.7	No Values	-0.4
Elongation factor G	P68789|EFG	Ref	No Values	0.7	0.4	No Values	1.7
Serine protease splC	Q7A4Y2|SPLC	Ref	0	No Values	No Values	1	No Values
50S ribosomal protein L11	P0A0F2|RL11	Ref	2.6	0.5	-1	-0.3	-0.1
Thioredoxin	P99122|THIO	Ref	No Values	No Values	-0.3	No Values	No Values
Trigger factor	P99080|TIG	Ref	No Values	2.4	-1.2	No Values	1.4
30S ribosomal protein S9	P66646|RS9	Ref	No Values	-1	0.5	No Values	-0.5
50S ribosomal protein L13	Q7A473|RL13	Ref	No Values	1.8	0.9	No Values	2.7
Phosphate acetyltransferase	P99092|PTA	Ref	3.4	1.4	-1	1.8	1
Acyl carrier protein	P0A002|ACP	Ref	No Values	2.7	-1.3	No Values	1.5

Appendix 9. (Continued)

Serine-aspartate repeat-containing protein E	Q99W46|SDRE	Ref	No Values	2	-0.9	No Values	2.6
Beta-lactamase	Q9AC80|Q9AC80	Ref	1.1	1.2	1.2	1	1.7
Cell division protein	Q7A620|Q7A620	Ref	No Values	2.3	-1.3	No Values	2.7
Fructose-bisphosphate aldolase class 1	P99117|ALF1	Ref	No Values	0	No Values	No Values	0.4
50S ribosomal protein L14	Q7A463|RL14	Ref	No Values	0.1	1.1	No Values	0.6
Phenol-soluble modulin alpha 4 peptide	P0C824|PSMA4	Ref	0	0	No Values	3.2	3
Staphylococcal secretory antigen ssaA2	Q7A423|SSAA2	Ref	No Values	2.4	0.7	No Values	2.3
Glycerophosphoryl diester phosphodiesterase	Q7A6H7|Q7A6H7	Ref	0	0	No Values	2.3	-0.3
SA2202 protein	Q99RL6|Q99RL6	Ref	0	0	No Values	-0.1	0
50S ribosomal protein L22	Q7A460|RL22	Ref	3.6	No Values	0.4	2.5	No Values
Elastin-binding protein ebpS	Q7A5I6|EBPS	Ref	0	0	No Values	-0.5	-0.6
Triosephosphate isomerase	P99133|TPIS	Ref	No Values	0.3	1.1	No Values	0.5
P99156|GREA_STAAN	P99156|GREA	Ref	No Values	3.4	-0.3	No Values	3.9
Cysteine synthase	P63871|CYSK	Ref	No Values	-0.1	0.1	No Values	3.1
Elongation factor Ts	P99171|EFTS	Ref	No Values	No Values	-1.5	Reference Missing	No Values
30S ribosomal protein S6	P99142|RS6	Ref	No Values	No Values	-0.9	No Values	No Values
Leukotoxin, LukD	Q99T54|Q99T54	Ref	0	No Values	No Values	-0.5	No Values
Inosine-5'-monophosphate dehydrogenase	P99106|IMDH	Ref	1.8	-1.2	-1.4	0.6	-1.2
Protein grpE	P99086|GRPE	Ref	No Values	0.3	-1.4	No Values	0.2
50S ribosomal protein L24	P60735|RL24	Ref	No Values	0	No Values	No Values	-0.3
L-lactate dehydrogenase 1	P65256|LDH1	Ref	1.4	No Values	-0.4	0.9	No Values
Superoxide dismutase [Mn/Fe] 1	P99098|SODM1	Ref	No Values	No Values	1.3	No Values	No Values
Transketolase	P99161|TKT	Ref	No Values	0	No Values	No Values	-0.8
30S ribosomal protein S16	P66440|RS16	Ref	1.8	No Values	-0.1	1.9	No Values
10 kDa chaperonin	P99104|CH10	Ref	No Values	2.9	-1.5	No Values	3.1

Appendix 9. (Continued)

SA0295 protein	Q7A7Q2|Q7A7Q2	Ref	No Values	No Values	2.2	No Values	No Values
Glucose-6-phosphate isomerase	P99078|G6PI	Ref	No Values	No Values	-0.6	No Values	No Values
Protein esaA	Q7A7S3|ESAA	Ref	0.1	No Values	0.1	Value Missing	No Values
Methicillin resistance mecR1 protein	P0A0B0|RANDOM_MECR-R	Ref	No Values	0	0.8	No Values	1.4
Uncharacterized protein SA1692	P0A0K1|Y1692	Ref	No Values	0	No Values	No Values	3.1
Chaperone protein hchA	P64313|HCHA	Ref	No Values	No Values	1	No Values	No Values
Clumping factor A	Q99VJ4|CLFA	Ref	No Values	0	No Values	No Values	-0.2
50S ribosomal protein L10	P99155|RL10	Ref	No Values	2.7	0.4	No Values	3.4
50S ribosomal protein L18	Q7A467|RL18	Ref	No Values	No Values	-0.1	No Values	No Values
Putative peptidyl-prolyl cis-trans isomerase	Q7A6I1|PPI1	Ref	No Values	No Values	-0.5	No Values	No Values
Fibrinogen-binding protein	P68800|FIB	Ref	No Values	No Values	0.6	No Values	No Values
1-phosphatidylinositol phosphodiesterase precurosr	Q7A888|Q7A888	Ref	0	No Values	No Values	-0.1	No Values
Virulence factor esxA	ESXA	Ref	No Values	No Values	No Values	Reference Missing	No Values
50S ribosomal protein L15	P0A0F6|RL15	Ref	No Values	No Values	-0.6	No Values	No Values

Appendix 10. Changes in secreted proteins of HA-MRSA USA200 compared to HA-MRSA USA100 during stationary phase from 3 biological replicates

Identified Proteins (246)	Accession Number	USA100#1	USA100 #2	USA100 #3	USA200 #1	USA200 #2	USA200 #3
Lipase 2	Q7A7P2|LIP2	Ref	-0.4	1.6	-0.2	0.7	3.8
Lipase 1	P65289|LIP1	Ref	-1	-0.8	0.4	2.6	1.5
Alpha-Hemolysin	Q7A632|Q7A632	Ref	0.6	0.2	-0.2	1	2.7
Elongation factor Tu	P99152|EFTU	Ref	-1.8	-6.7	-0.1	-2.6	-6.7
Enolase	P99088|ENO	Ref	-0.6	-6.6	-0.4	-0.9	-6.6
Putative surface protein SA2285	P61598|PLS	Ref	-4.6	-9	-4.4	-9.8	-9
Bifunctional autolysin	Q99V41|ATL	Ref	0.1	-3.1	-1.3	2.6	-3.1
SA0841 protein	Q7A6G0|Q7A6G0	Ref	-1.4	-3	2.4	2.1	-1
Chaperone protein dnaK	P99110|DNAK	Ref	-3.6	-7	-0.3	-3.9	-7
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1	Ref	-2.5	-5.6	-0.5	-1.5	-5.6
DNA-binding protein HU	Q7A5J1|DBH	Ref	-1.7	-5.1	-0.3	-6	-5.1
Alkyl hydroperoxide reductase subunit C	P99074|AHPC	Ref	-4.7	-7	-0.9	-6.4	-7
Inosine-5'-monophosphate dehydrogenase	P99106|IMDH	Ref	0.2	-5.8	-0.4	0.7	-5.8
Immunoglobulin G-binding protein A	SPA	Ref	No Values	0	No Values	No Values	1.1
Formate--tetrahydrofolate ligase	Q7A535|FTHS	Ref	-1.6	-7.6	-0.8	-3.2	-7.6
Dihydrolipoyl dehydrogenase	P99084|DLDH	Ref	-0.7	-7.2	-0.3	0.3	-7.2
50S ribosomal protein L7/L12	P99154|RL7	Ref	-0.2	-7.4	-1.6	0.1	-7.4
Pyruvate kinase	Q7A559|KPYK	Ref	-4.6	-3.7	-1.3	-4.5	-3.7
SA0587 protein	Q7A719|Q7A719	Ref	-1.3	-5	-0.1	-2.1	-5
Phosphate acetyltransferase	P99092|PTA	Ref	-2	-6.2	-0.6	-1.2	-6.2
Elongation factor G	P68789|EFG	Ref	-1.5	-6.2	0.1	-2.1	-6.2
Glutamine synthetase	P99095|GLNA	Ref	-2.4	-6.7	-0.3	-2.6	-6.7
SA2006 protein	Q7A483|Q7A483	Ref	0.3	0.8	-0.6	2.9	2.4

Appendix 10. (Continued)

Fructose-bisphosphate aldolase	P99075|ALF2	Ref	-3.8	-7.4	Value Missing	-3.5	-7.4
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS	Ref	-1	-0.5	-0.4	0.3	-0.5
Alkaline shock protein 23	P99157|ASP23	Ref	2.4	-3.9	-0.9	2.5	-3.9
Triosephosphate isomerase	P99133|TPIS	Ref	-2	-4	-1.2	-0.8	-4
Enterotoxin type C-3	P0A0L4|ENTC 3	Ref	-5.3	-5.4	-0.3	-4.7	-2.6
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	P65636|ODP2	Ref	-0.6	-5.7	-0.7	-2.5	-5.7
Cysteine synthase	P63871|CYSK	Ref	-0.6	-5.1	0.3	-1.2	-5.1
Elongation factor Ts	P99171|EFTS	Ref	2.5	-5	-0.5	3.4	-5
Pyruvate dehydrogenase E1 component subunit alpha	Q820A6|ODPA	Ref	-5.5	-9.6	-0.6	-6.8	-9.6
Putative uncharacterized protein SA0663	Q7A6V1|Q7A6 V1	Ref	0.1	-0.2	-0.3	2.6	Value Missing
Ornithine aminotransferase 2	P60298|OAT2	Ref	-3.2	-9.4	-0.7	-5	-9.4
Staphopain B	Q7A6A7|SSPB	Ref	-5.1	-3.5	-0.4	-3.1	-2.8
Succinyl-CoA ligase [ADP-forming] subunit beta	P99071|SUCC	Ref	-1.6	-6.2	-0.9	-4.1	-6.2
Phosphocarrier protein HPr	P99143|PTHP	Ref	1.1	-5.6	-0.3	2.5	-5.6
Putative uncharacterized protein SA0359	Q7A7J8|Q7A7J 8	Ref	-5.8	-6.1	Value Missing	-3.4	-6.1
Thermonuclease	Q7A6P2|NUC	Ref	1.6	-0.9	-0.5	4.4	0.2
Trigger factor	P99080|TIG	Ref	-1.3	-4.9	-0.7	-0.6	-4.9
Aconitate hydratase	P99148|ACON	Ref	-0.1	-4.9	-0.1	0.7	-4.9
Adenylate kinase	P99062|KAD	Ref	-3.1	-4.8	-0.4	-2.6	-4.8
Fructose-bisphosphate aldolase class 1	P99117|ALF1	Ref	-2.3	-6.6	-1.1	-2.5	-6.6
50S ribosomal protein L17	Q7A469|RL17	Ref	1.2	-3.6	-0.1	2.4	-3.6
Glutamyl endopeptidase	Q7A6A6|SSPA	Ref	-1.7	-3.3	-0.6	1.5	-1.3
SA0620 protein	Q7A6Y9|Q7A6 Y9	Ref	-0.1	-2.2	1.7	2	3.1
60 kDa chaperonin	P99083|CH60	Ref	-1.3	-5.4	-0.7	-0.3	-5.4
Probable beta-lactamase	Q7A4X8|Q7A4 X8	Ref	0.8	No Values	4.4	4.4	No Values

Appendix 10. (Continued)

Putative uncharacterized protein SA0570	Q7A735|Q7A735	Ref	0.1	-3.9	Value Missing	2.6	-3.9
Alcohol dehydrogenase	Q7A742|ADH	Ref	-0.4	-4.8	0.5	-1.3	-4.8
Catalase	Q7A5T2|CATA	Ref	-3.8	-6.9	0.1	-5.3	-6.9
SA2097 protein	Q7A418|Q7A418	Ref	-1.4	-2.7	0.8	1.7	-1.4
Penicillin binding protein 2 prime	Q7A8C6|Q7A8C6	Ref	-4.4	-6.8	0.2	-1.1	-6.1
Putative uncharacterized protein SAP003	Q9AC87|Q9AC87	Ref	3.5	-6.8	-0.9	5.5	-6.8
Probable thiol peroxidase	P99146|TPX	Ref	0.7	-6	Reference Missing	-0.3	-6
30S ribosomal protein S1	Q7A5J0|RS1	Ref	-0.9	-5.3	Value Missing	-2.3	-5.3
L-lactate dehydrogenase 1	P65256|LDH1	Ref	2.2	-1.1	-0.5	4	-0.8
Phosphoglycerate kinase	P99135|PGK	Ref	-1.6	-5.4	-0.3	-1.2	-5.4
Glucose-6-phosphate isomerase	P99078|G6PI	Ref	-1.5	-5.4	0	-1.3	-5.4
Transketolase	P99161|TKT	Ref	-1.9	-5.5	-3.1	-0.7	-5.5
Pyruvate dehydrogenase E1 component subunit beta	P99063|ODPB	Ref	No Values	-8.1	-1.4	Reference Missing	-8.1
Glycerophosphoryl diester phosphodiesterase	Q7A6H7|Q7A6H7	Ref	-2.9	-4.4	Value Missing	-1	Value Missing
Delta-hemolysin	P0A0M2|HLD	Ref	-2.5	No Values	0.4	1.1	No Values
Phenol-soluble modulin alpha 4 peptide	P0C824|PSMA4	Ref	-2.3	0.1	-0.3	-1.3	3.2
Foldase protein prsA	P60748|PRSA	Ref	-3.3	-5.7	0.3	-1.2	-5.1
Glycyl-glycine endopeptidase lytM	Q7A7T0|LYTM	Ref	-0.3	-0.9	2.3	3.3	5.2
Thioredoxin	P99122|THIO	Ref	0.2	-4.6	0.2	-0.7	-4.6
SA2437 protein	Q7A371|Q7A371	Ref	-10	-8.7	Reference Missing	-9.1	Value Missing
UPF0477 protein SA0873	Q7A6D4|Y873	Ref	-4.7	-5.9	-1.1	-6.9	Value Missing
Citrate synthase II	Q7A561|Q7A561	Ref	5.5	-5.8	-0.7	7.9	-5.8
6-phosphogluconate dehydrogenase, decarboxylating	P63334|6PGD	Ref	-0.9	-4.1	0	-0.1	Value Missing
Superoxide dismutase [Mn/Fe] 1	P99098|SODM1	Ref	-4.7	-7.7	1.3	-3.3	-7.7
Phenol-soluble modulin alpha 1 peptide	P0C7Y7|PSMA1	Ref	-5.3	-4.2	-0.2	-3.8	-0.8

Appendix 10. (Continued)

Phosphoenolpyruvate carboxykinase [ATP]	P99128|PCKA	Ref	-0.6	-8.4	Value Missing	-2.3	-8.4
Probable transglycosylase isaA	P99160|ISAA	Ref	-2.4	-4.1	-0.4	-2.1	-4.1
Glycyl-tRNA synthetase	P99129|SYG	Ref	-0.3	-1.7	Value Missing	-1.4	-1.7
DNA-directed RNA polymerase subunit beta'	P60285|RPOC	Ref	-2.4	-3.7	1.8	-2.4	Value Missing
Glycine cleavage system H protein	P64214|GCSH	Ref	0.9	-6.8	0	2.7	Value Missing
50S ribosomal protein L30	P0A0G0|RL30	Ref	0.8	0.7	-2.1	-0.7	0.7
Cold shock protein cspA	Q7A5P3|CSPA	Ref	No Values	-3.9	No Values	No Values	-3.9
Serine protease splB	Q7A4Y1|SPLB	Ref	-0.2	-4.1	-0.4	-0.2	-2.5
DNA-directed RNA polymerase subunit beta	P60278|RPOB	Ref	No Values	-6.1	-0.1	No Values	-6.1
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	P99153|GPMA	Ref	No Values	-3.4	0.3	Reference Missing	-3.4
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	Q7A5N4|ODO2	Ref	-5.2	-4.6	1.2	-8.3	Value Missing
Seryl-tRNA synthetase	P99178|SYS	Ref	-6.1	-9.3	-0.6	-8.5	-9.3
NAD-specific glutamate dehydrogenase	Q7A6H8|DHE2	Ref	-2.1	-3	-0.5	-3.6	-2.8
Succinyl-CoA ligase [ADP-forming] subunit alpha	P99070|SUCD	Ref	-16.8	-25.2	No Values	-17.8	-25.2
Uncharacterized leukocidin-like protein 1	Q7A4L0|LUKL1	Ref	1.8	1.4	-0.4	1.8	1.9
Acyl carrier protein	P0A002|ACP	Ref	-2.5	-2.9	-0.3	-3.8	Value Missing
50S ribosomal protein L21	Q7A583|RL21	Ref	1.8	-5.2	Reference Missing	2.9	Value Missing
UPF0337 protein SA0772	Q7A6L9|Y772	Ref	-2.6	-3.3	No Values	-4.2	-3.3
3-oxoacyl-[acyl-carrier-protein] synthase 2	Q7A6F8|FABF	Ref	-2.6	-3.1	No Values	-1.8	-3.1
50S ribosomal protein L9	P66318|RL9	Ref	-3.2	-6.4	Value Missing	-3.8	-6.4
Beta-lactamase	Q9AC80|Q9AC80	Ref	0.3	-1.8	-1.6	0.2	-1.8

Appendix 10. (Continued)

Thioredoxin reductase	P99101|TRXB	Ref	No Values	-7.7	Value Missing	No Values	-7.7
Putative dipeptidase SA1572	Q7A522|PEPV L	Ref	-5.8	-9.3	Reference Missing	-6	-9.3
SA0916 protein	Q7A696|Q7A69 6	Ref	-3.9	-4.7	No Values	-6.9	Value Missing
Phosphoenolpyruvat e-protein phosphotransferase	Q99V14|PT1	Ref	0	-5.4	Value Missing	1	-5.2
30S ribosomal protein S9	P66646|RS9	Ref	2.5	-6.4	-1	6.1	Value Missing
Uncharacterized protein SA1692	P0A0K1|Y1692	Ref	-0.3	-1.9	-0.4	-0.9	-1.7
50S ribosomal protein L11	P0A0F2|RL11	Ref	-0.6	-5.9	-0.7	-2.6	Value Missing
Putative universal stress protein SA1532	Q7A551|Y1532	Ref	0.6	-2	-0.5	0.6	Value Missing
1-pyrroline-5-carboxylate dehydrogenase	P99076|ROCA	Ref	-1	-4.6	1	-5.2	-4.6
Gamma-hemolysin component C	Q7A3S2|HLGC	Ref	-5.4	-5.2	-1.1	-5.3	Value Missing
Serine-aspartate repeat-containing protein D	Q7A780|SDRD	Ref	0	0	No Values	-2.7	0
Glutamyl-tRNA synthetase	P99170|SYE	Ref	-2.6	-7.5	-0.9	-2.9	-7.5
2,3-bisphosphoglycerate-independent phosphoglycerate mutase	P64270|GPMI	Ref	1.2	-4.5	-0.8	4.8	Value Missing
D-lactate dehydrogenase	P99116|LDHD	Ref	No Values	0	No Values	No Values	0
50S ribosomal protein L10	P99155|RL10	Ref	2.6	-5	-0.7	3.6	-5.1
SA2202 protein	Q99RL6|Q99R L6	Ref	-0.1	-2.5	-0.4	0.2	-2.4
Gamma-hemolysin component A	P0A072|HLGA	Ref	2.2	No Values	-0.8	3.7	No Values
10 kDa chaperonin	P99104|CH10	Ref	-2.1	-5.9	-0.4	-2	-5.9
Dihydroorotase	P65906|PYRC	Ref	-1.6	-3.8	0.1	-0.7	-3.8
UPF0457 protein SA1975.1	Q99S93|Y197A	Ref	-1.5	-5.6	No Values	-4.8	Value Missing
Ribosome-recycling factor	P99130|RRF	Ref	-2.6	-4.7	Reference Missing	-2.2	-3.9
Elongation factor P	P99066|EFP	Ref	-1	-5.3	-0.3	-2	-5.3
Uncharacterized leukocidin-like protein 2	Q99SN7|LUKL 2	Ref	0.6	No Values	-1.7	0.6	No Values
UPF0355 protein SA0372	Q7A7I6|UP355	Ref	-1.6	-4.6	Value Missing	-1.3	-4.6
Acetate kinase	Q99TF2|ACKA	Ref	2.6	-2.1	-1.5	4.2	-1.6
Elastin-binding protein ebpS	Q7A5I6|EBPS	Ref	-2.6	-5	-0.2	-3.3	-5

Appendix 10. (Continued)

30S ribosomal protein S6	P99142|RS6	Ref	No Values	-2.2	-0.8	No Values	Value Missing
General stress protein 20U	Q7A4C8|Q7A4C8	Ref	-0.9	-1.4	Value Missing	-0.6	Value Missing
50S ribosomal protein L24	P60735|RL24	Ref	-2.7	-7.6	No Values	-3.8	Value Missing
Bifunctional protein folD	Q7A697|FOLD	Ref	No Values	-0.3	0.1	No Values	Value Missing
50S ribosomal protein L22	Q7A460|RL22	Ref	-2	-4.7	0.4	0.7	-3.1
Putative uncharacterized protein SAS040	Q7A5U6|Q7A5U6	Ref	-2.5	-8	-0.8	-1.8	-8
Gamma-hemolysin component B	P0A075|HLGB	Ref	1	No Values	-0.2	2.1	No Values
Putative uncharacterized protein SA0395	Q99WG7|Q99WG7	Ref	No Values	No Values	0.7	Reference Missing	No Values
Putative peptidyl-prolyl cis-trans isomerase	Q7A6I1|PPI1	Ref	2.4	0.1	-2.2	6	4.4
50S ribosomal protein L1	Q99W68|RL1	Ref	No Values	-2.8	0.1	Reference Missing	-2.8
Virulence factor esxA	ESXA (+1)	Ref	-0.5	No Values	-0.8	0.8	No Values
ATP-dependent Clp protease ATP-binding subunit clpC	Q7A797|CLPC	Ref	1.9	-4.7	2.2	4.8	-4.7
SA0914 protein	Q99V35|Q99V35	Ref	2.4	-3.6	0.2	4.8	-3.3
30S ribosomal protein S16	P66440|RS16	Ref	-1.5	-8.5	No Values	-1.2	-8.5
Translation initiation factor IF-1	P65119|IF1	Ref	-5.8	-9.1	Reference Missing	-9.3	-9
DNA-directed RNA polymerase subunit alpha	P66706|RPOA	Ref	-1.1	-5.9	-0.1	-2	-5.9
Truncated beta-hemplysin	Q99QR7|Q99QR7	Ref	-19.6	No Values	No Values	-16	No Values
Deoxyribose-phosphate aldolase 1	P99102|DEOC1 (+1)	Ref	-3	-4.4	-0.1	-3.1	-4.4
Pyruvate carboxylase	Q7A666|Q7A666	Ref	0	0	No Values	-1.5	0
Uncharacterized protein SA0829	Q7A6H3|Y829	Ref	-6.5	No Values	No Values	-6.8	No Values
Probable acetyl-CoA acyltransferase	Q7A7L2|THLA	Ref	-2.7	No Values	Value Missing	-4.1	No Values
Protein grpE	P99086|GRPE	Ref	-3.6	-4.6	-0.4	-4.4	Value Missing
50S ribosomal protein L3	P60449|RL3	Ref	No Values	No Values	0	No Values	No Values
ATP synthase subunit beta	P99112|ATPB	Ref	-1.6	-5.5	-0.5	0	-5.5

Appendix 10. (Continued)

Uncharacterized protein SA0707	Q7A6R6|Y707	Ref	-2.5	-5.2	-0.3	-3.9	-5.2
Glucose-specific phosphotransferase enzyme IIA component	P60857|PTGA	Ref	No Values	0	No Values	Reference Missing	0
Transcription elongation factor greA	P99156|GREA	Ref	-2.1	-3.6	-0.7	-2.6	Value Missing
SA0758 protein	Q7A6M7	Ref	-0.3	-3.9	-2.9	-0.5	-3.9
Chaperone protein hchA	P64313|HCHA	Ref	3	No Values	0	5.5	No Values
50S ribosomal protein L5	Q7A465|RL5	Ref	-0.6	No Values	1.8	2.4	No Values
Serine hydroxymethyltransferase	P99091|GLYA	Ref	-4	-8	-0.9	-3.3	-8
Methionine aminopeptidase	AMPM	Ref	-5.7	-9.4	-0.3	-5.9	-9.4
50S ribosomal protein L15	P0A0F6|RL15	Ref	0.9	-4.1	0.4	3.6	-4.1
ATP-dependent Clp protease ATP-binding subunit clpL	Q7A3F4|CLPL	Ref	-0.4	-7.4	Value Missing	1.8	-6.8
UPF0342 protein SA1663	Q7A4V3|Y1663	Ref	-2.4	-3.6	-1.1	-0.2	Value Missing
SA0759 protein	Q7A6M6|Q7A6M6	Ref	1.8	-3.2	No Values	3.4	Value Missing
Staphylokinase	Q99SU7|SAK	Ref	0.8	-1.5	Value Missing	-0.3	Value Missing
Iron-regulated surface determinant protein A	Q7A655|ISDA	Ref	No Values	-4.7	Value Missing	No Values	-4.2
Serine-aspartate repeat-containing protein E	Q99W46|SDRE	Ref	0	0	No Values	1.8	1.8
Leukotoxin, LukD	Q99T54|Q99T54	Ref	No Values	No Values	No Values	No Values	No Values
Amidophosphoribosyltransferase	P99164|PUR1	Ref	No Values	No Values	-0.3	No Values	No Values
SA0859 protein	Q7A6E5|Q7A6E5	Ref	No Values	-6.3	No Values	No Values	Value Missing
Phosphoribosylformylglycinamidine synthase 1	P99166|PURQ	Ref	No Values	No Values	No Values	No Values	No Values
Staphylococcal complement inhibitor	Q99SU9|SCIN	Ref	No Values	-1.8	No Values	No Values	-1
Uncharacterized N-acetyltransferase SA1019	Q99UT4|Y1019	Ref	-2.5	-4.4	0	-4	-4.4
50S ribosomal protein L27	P66133|RL27	Ref	No Values	-6.7	0	No Values	Value Missing
Adenylosuccinate lyase	Q7A4Q3|PUR8	Ref	0	0	No Values	1	Value Missing

Appendix 10. (Continued)

Naphthoate synthase	Q7A6A9|MENB	Ref	-8.8	No Values	No Values	-9.8	No Values
Putative uncharacterized protein SA0771	Q7A6M0|Q7A6M0	Ref	-1.3	-2.8	Value Missing	-1.6	-2.8
Xaa-Pro dipeptidase	Q99TW4|Q99TW4	Ref	-5.1	-8	No Values	-3.7	-8
1-phosphatidylinositol phosphodiesterase precurosr	Q7A888|Q7A888	Ref	0.7	No Values	Value Missing	2.5	No Values
Probable glycine dehydrogenase [decarboxylating] subunit 1	P64218|GCSPA	Ref	No Values	0	No Values	No Values	0
Putative aldehyde dehydrogenase AldA	Q7A825|ALDA	Ref	No Values	0	No Values	No Values	0
L-lactate dehydrogenase 2	P99119|LDH2	Ref	1.4	-4	Value Missing	3.1	-3.8
Formate acetyltransferase	Q7A7X6|PFLB	Ref	-1.2	-4.1	1.3	-1.8	-3.3
Signal transduction protein TRAP	Q7A4W3|TRAP	Ref	-0.6	0	-0.2	-0.5	0.6
Purine nucleoside phosphorylase	Q7A4C9|Q7A4C9	Ref	No Values	No Values	-1.1	No Values	No Values
Arginyl-tRNA synthetase	Q99W05|SYR	Ref	No Values	-7.3	-0.8	No Values	-7.3
50S ribosomal protein L13	Q7A473|RL13	Ref	-1.3	No Values	1	0	No Values
Probable transglycosylase sceD	Q7A4F2|SCED	Ref	0	No Values	No Values	2	No Values
Organic hydroperoxide resistance protein-like	Q7A6M9|OHRL	Ref	-1.8	No Values	No Values	-0.2	No Values
Serine protease splD	Q7A4Y3|SPLD	Ref	No Values	No Values	Value Missing	No Values	No Values
Putative uncharacterized protein SA1986	Q7A493|Q7A493	Ref	No Values	No Values	0.3	No Values	No Values
D-alanine--poly(phosphoribitol) ligase subunit 2	P0A019|DLTC	Ref	-2.5	-5.9	Value Missing	-1.5	Value Missing
Immunoglobulin-binding protein sbi	Q99RL2|SBI	Ref	-3.3	-6.8	Value Missing	-4.9	Value Missing
Putative uncharacterized protein SA1528	Q7A553|Q7A553	Ref	1.7	No Values	-0.2	1.9	No Values
GMP synthase [glutamine-hydrolyzing]	P99105|GUAA	Ref	No Values	-5.9	Value Missing	No Values	Value Missing
SA1524 protein	Q7A556|Q7A556	Ref	1.6	-3.1	Value Missing	3.1	-3.1

Appendix 10. (Continued)

30S ribosomal protein S11	P66357|RS11	Ref	-2.3	-7.6	No Values	-3.3	Value Missing
Putative uncharacterized protein SA0919	Q7A694|Q7A694	Ref	No Values	-4.8	-0.2	No Values	-3.2
Phenol-soluble modulin alpha 3 peptide	P0C811|PSMA3	Ref	-0.6	No Values	0.4	0.3	No Values
Ferritin	Q7A4R2|FTN	Ref	No Values	0	No Values	No Values	Value Missing
Alkyl hydroperoxide reductase subunit F	P99118|AHPF	Ref	No Values	-6.2	Value Missing	No Values	-6.2
Anti-sigma-B factor antagonist	P66838|RSBV	Ref	No Values	-3.2	-0.6	No Values	-2.3
Nucleoside diphosphate kinase	NDK (+1)	Ref	-3.7	-9.6	Value Missing	-6.2	-9.6
Zinc metalloproteinase aureolysin	Q7A378|Q7A378	Ref	No Values	-2.5	Reference Missing	Reference Missing	1.7
UPF0173 metal-dependent hydrolase SA1529	P99149|Y1529	Ref	-14	-19.1	No Values	-13.9	Value Missing
Pyridoxal biosynthesis lyase pdxS	P60798|PDXS	Ref	No Values	-6.5	0	No Values	-5.9
Alanine dehydrogenase 2	Q99TF4|DHA2	Ref	No Values	-5.4	-0.6	No Values	Value Missing
Urocanate hydratase	P67417|HUTU	Ref	No Values	0	No Values	No Values	Value Missing
Putative uncharacterized protein SA2309	Q7A3I0|Q7A3I0	Ref	4.7	-3.5	Value Missing	Reference Missing	Value Missing
CTP synthase	P99072|PYRG	Ref	0.6	-5.5	Value Missing	1.2	Value Missing
Putative 8-amino-7-oxononanoate synthase/2-amino-3-ketobutyrate coenzyme A ligase	P60120|BIKB	Ref	No Values	-23	No Values	No Values	Value Missing
Threonyl-tRNA synthetase	P67585|SYT	Ref	No Values	No Values	-0.6	No Values	No Values
Probable malate:quinone oxidoreductase 2	P99115|MQO2	Ref	0	0	No Values	-2.8	0
Probable branched-chain-amino-acid aminotransferase	P99138|ILVE	Ref	0	0	No Values	0.1	0.4
Methionyl-tRNA synthetase	P67579|SYM	Ref	No Values	0	No Values	No Values	Value Missing
Tryptophanyl-tRNA synthetase	P67593|SYW	Ref	0	No Values	No Values	-3	No Values
Mannitol-1-phosphate 5-dehydrogenase	P99140|MTLD	Ref	0	No Values	No Values	1.8	No Values
SA1343 protein	Q7A5G2|Q7A5G2	Ref	No Values	0	No Values	No Values	Value Missing

Appendix 10. (Continued)

Staphopain A	P65826|SSPP	Ref	No Values	No Values	No Values	No Values	No Values
UPF0082 protein SA0624	P67182|Y624	Ref	-2.2	-5.3	Value Missing	-1.9	Value Missing
Cell division protein ftsZ	P99108|FTSZ	Ref	2.5	-3.1	-0.3	2.3	Value Missing
Adenylosuccinate synthetase	P99099|PURA	Ref	No Values	-5.1	-0.5	No Values	-4.8
Histidine ammonia-lyase	P64416|HUTH	Ref	No Values	-9.6	No Values	No Values	Value Missing
Imidazolonepropionase	P64418|HUTI	Ref	No Values	0	No Values	No Values	Value Missing
6-phosphofructokinase	P99165|K6PF	Ref	-3.2	No Values	No Values	-1.7	No Values
Lysyl-tRNA synthetase	P67610|SYK	Ref	No Values	-1.3	-0.9	No Values	0.8
SA1599 protein	Q7A501|Q7A501	Ref	No Values	0	No Values	No Values	0.8
SA0231 protein	Q7A7W3|Q7A7W3	Ref	No Values	-3.4	Value Missing	No Values	-3.3
Phenylalanyl-tRNA synthetase beta chain	P67041|SYFB	Ref	No Values	0	No Values	No Values	Value Missing
P60855|Y370_STAAN	P60855|Y370	Ref	No Values	0	No Values	No Values	0
50S ribosomal protein L23	Q7A459|RL23	Ref	No Values	No Values	2.3	No Values	No Values
Putative uncharacterized protein SA0908	Q7A6A3|Q7A6A3	Ref	3	-2.3	-0.2	5.1	Value Missing
SA1475 protein	Q7A581|Q7A581	Ref	0	0	No Values	2.3	0.4
Leucyl-tRNA synthetase	P67513|SYL	Ref	No Values	-6	Value Missing	No Values	-5.8
Clumping factor A	Q99VJ4|CLFA	Ref	0	0	No Values	0.9	Value Missing
Acetate-CoA ligase	Q7A3A2|Q7A3A2	Ref	0	No Values	No Values	3.6	No Values
Lactonase drp35	RANDOM_DRP35-R	Ref	0	No Values	No Values	-0.3	No Values
50S ribosomal protein L2	P60432|RL2	Ref	No Values	0	No Values	No Values	0
Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]	GLMS	Ref	No Values	No Values	No Values	No Values	No Values
Putative uncharacterized protein SA1743	Q7A4N7|Q7A4N7	Ref	No Values	0	No Values	No Values	0
Cell division protein	Q7A620|Q7A620	Ref	No Values	0	No Values	No Values	0
50S ribosomal protein L18	Q7A467|RL18	Ref	No Values	-18.9	No Values	No Values	-18.8
3-hexulose-6-phosphate synthase	Q7A774|HPS	Ref	No Values	No Values	Reference Missing	No Values	No Values
Acetoin(diacetyl) reductase	P99120|BUTA	Ref	No Values	0	No Values	No Values	1.3

Appendix 10. (Continued)

SA0022 protein	Q99XE9|Q99XE9	Ref	0	No Values	No Values	1.9	No Values
3-hydroxy-3-methylglutaryl CoA synthase	Q7A3F6|Q7A3F6	Ref	No Values	No Values	Value Missing	No Values	No Values
30S ribosomal protein S7	P66616|RS7	Ref	-1.5	No Values	-1.5	0.1	No Values
Uncharacterized lipoprotein SA2158	Q7A3W5|Y2158	Ref	No Values	No Values	No Values	No Values	No Values
Ribonuclease J 1	Q7A682|RNJ1	Ref	No Values	No Values	Value Missing	Reference Missing	No Values
Trans-2-enoyl-ACP reductase	Q7A6D8	Ref	0	No Values	No Values	2.7	No Values
2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase 2	Q7A7V0|ISPD2	Ref	No Values	No Values	-0.6	No Values	No Values
30S ribosomal protein S13	P66388|RS13	Ref	No Values	No Values	No Values	No Values	No Values
30S ribosomal protein S2	P66544|RS2	Ref	No Values	0	No Values	No Values	0.2
3-oxoacyl-[acyl-carrier-protein] synthase 3	P99159|FABH	Ref	No Values	No Values	No Values	No Values	No Values

Appendix 11. Changes in secreted proteins of CA-MRSA USA300 compared to HA-MRSA USA100 during stationary phase from 3 biological replicates

Identified Proteins (246)	Accession Number	USA100 #1	USA100 #2	USA100 #3	USA300 #1	USA300 #2	USA300 #3
Lipase 2	Q7A7P2|LIP2	Ref	-0.4	1.6	0.6	3	4.8
Lipase 1	P65289|LIP1	Ref	-1	-0.8	0.6	1.5	2.5
Alpha-Hemolysin	Q7A632|Q7A632	Ref	0.6	0.2	0.6	4.1	4.8
Elongation factor Tu	P99152|EFTU	Ref	-1.8	-6.7	0.6	-1.4	-7
Enolase	P99088|ENO	Ref	-0.6	-6.6	0.6	-0.3	-6.8
Putative surface protein SA2285	P61598|PLS	Ref	-4.6	-9	-5.1	-9.5	-11.5
Bifunctional autolysin	Q99V41|ATL	Ref	0.1	-3.1	-0.7	1	-2.8
SA0841 protein	Q7A6G0|Q7A6G0	Ref	-1.4	-3	0.6	0.5	-0.3
Chaperone protein dnaK	P99110|DNAK	Ref	-3.6	-7	0.6	-4.1	-7.8
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1	Ref	-2.5	-5.6	0.6	-1.8	-6.1
DNA-binding protein HU	Q7A5J1|DBH	Ref	-1.7	-5.1	-0.2	-3	-4.9
Alkyl hydroperoxide reductase subunit C	P99074|AHPC	Ref	-4.7	-7	0.6	-6.2	-6.8
Inosine-5'-monophosphate dehydrogenase	P99106|IMDH	Ref	0.2	-5.8	0.6	1.7	-5.8
Immunoglobulin G-binding protein A	SPA	Ref	No Values	0	No Values	No Values	1.7
Formate--tetrahydrofolate ligase	Q7A535|FTHS	Ref	-1.6	-7.6	0.5	-2.4	-8.9
Dihydrolipoyl dehydrogenase	P99084|DLDH	Ref	-0.7	-7.2	0.6	0.2	-7.5
50S ribosomal protein L7/L12	P99154|RL7	Ref	-0.2	-7.4	0.6	-0.3	-7.4
Pyruvate kinase	Q7A559|KPYK	Ref	-4.6	-3.7	-0.9	-3.8	-3.6
SA0587 protein	Q7A719|Q7A719	Ref	-1.3	-5	0.6	-0.6	-4.2
Phosphate acetyltransferase	P99092|PTA	Ref	-2	-6.2	0	-2	-7.8
Elongation factor G	P68789|EFG	Ref	-1.5	-6.2	0.6	-0.4	-5.3
Glutamine synthetase	P99095|GLNA	Ref	-2.4	-6.7	0.6	-1.9	-6.7

Appendix 11. (Continued)

SA2006 protein	Q7A483|Q7A483	Ref	0.3	0.8	0.6	3.6	2.5
Fructose-bisphosphate aldolase	P99075|ALF2	Ref	-3.8	-7.4	0.6	-3.3	-10
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS	Ref	-1	-0.5	0.6	-0.5	-0.3
Alkaline shock protein 23	P99157|ASP23	Ref	2.4	-3.9	0.6	3.5	-2.5
Triosephosphate isomerase	P99133|TPIS	Ref	-2	-4	0.1	-1.4	-4.4
Enterotoxin type C-3	P0A0L4|ENTC3	Ref	-5.3	-5.4	-2	-4.5	-4.3
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	P65636|ODP2	Ref	-0.6	-5.7	0.4	-1.4	-6.1
Cysteine synthase	P63871|CYSK	Ref	-0.6	-5.1	0.6	-0.4	-4.3
Elongation factor Ts	P99171|EFTS	Ref	2.5	-5	0.6	3.1	-5.2
Pyruvate dehydrogenase E1 component subunit alpha	Q820A6|ODPA	Ref	-5.5	-9.6	0.6	-6.5	-10.4
Putative uncharacterized protein SA0663	Q7A6V1|Q7A6V1	Ref	0.1	-0.2	0.6	3.4	3.6
Ornithine aminotransferase 2	P60298|OAT2	Ref	-3.2	-9.4	0	-4.7	-10.3
Staphopain B	Q7A6A7|SSPB	Ref	-5.1	-3.5	0.6	-3.6	-1.4
Succinyl-CoA ligase [ADP-forming] subunit beta	P99071|SUCC	Ref	-1.6	-6.2	0.1	-1.5	-6.7
Phosphocarrier protein HPr	P99143|PTHP	Ref	1.1	-5.6	-1.2	1.9	-6
Putative uncharacterized protein SA0359	Q7A7J8|Q7A7J8	Ref	-5.8	-6.1	0.6	-4.7	-5.1
Thermonuclease	Q7A6P2|NUC	Ref	1.6	-0.9	0.6	2.8	1.6
Trigger factor	P99080|TIG	Ref	-1.3	-4.9	0.2	-2.1	-4.7
Aconitate hydratase	P99148|ACON	Ref	-0.1	-4.9	0.5	0.5	-4.7
Adenylate kinase	P99062|KAD	Ref	-3.1	-4.8	0.6	-3.7	-5.1
Fructose-bisphosphate aldolase class 1	P99117|ALF1	Ref	-2.3	-6.6	0.2	-2.4	-6.8
50S ribosomal protein L17	Q7A469|RL17	Ref	1.2	-3.6	0.6	1.4	-3.6
Glutamyl endopeptidase	Q7A6A6|SSPA	Ref	-1.7	-3.3	0.6	-0.1	-1.4
SA0620 protein	Q7A6Y9|Q7A6Y9	Ref	-0.1	-2.2	-1.4	1	0.5

Appendix 11. (Continued)

60 kDa chaperonin	P99083|CH60	Ref	-1.3	-5.4	-0.2	-0.9	-5.6
Probable beta-lactamase	Q7A4X8|Q7A4X8	Ref	0.8	No Values	-3.7	0.8	No Values
Putative uncharacterized protein SA0570	Q7A735|Q7A735	Ref	0.1	-3.9	0.6	1.5	-2.9
Alcohol dehydrogenase	Q7A742|ADH	Ref	-0.4	-4.8	0.6	0.6	-4.4
Catalase	Q7A5T2|CATA	Ref	-3.8	-6.9	Reference Missing	-5	-7.9
SA2097 protein	Q7A418|Q7A418	Ref	-1.4	-2.7	-1.1	-0.5	-2.6
Penicillin binding protein 2 prime	Q7A8C6|Q7A8C6	Ref	-4.4	-6.8	0.3	-4.5	-7.5
Putative uncharacterized protein SAP003	Q9AC87|Q9AC87	Ref	3.5	-6.8	0.6	4.8	-7
Probable thiol peroxidase	P99146|TPX	Ref	0.7	-6	0.6	-0.3	-8.5
30S ribosomal protein S1	Q7A5J0|RS1	Ref	-0.9	-5.3	0.6	-1	-5
L-lactate dehydrogenase 1	P65256|LDH1	Ref	2.2	-1.1	0.6	3.8	0.4
Phosphoglycerate kinase	P99135|PGK	Ref	-1.6	-5.4	0.6	-0.5	-5.7
Glucose-6-phosphate isomerase	P99078|G6PI	Ref	-1.5	-5.4	0.6	-1.4	-6
Transketolase	P99161|TKT	Ref	-1.9	-5.5	-2.2	-1	-6
Pyruvate dehydrogenase E1 component subunit beta	P99063|ODPB	Ref	No Values	-8.1	-1.2	Reference Missing	-8.6
Glycerophosphoryl diester phosphodiesterase	Q7A6H7|Q7A6H7	Ref	-2.9	-4.4	0.6	-3.1	-4.3
Delta-hemolysin	P0A0M2|HLD	Ref	-2.5	No Values	0.6	-2	No Values
Phenol-soluble modulin alpha 4 peptide	P0C824|PSMA4	Ref	-2.3	0.1	0.5	0	2.3
Foldase protein prsA	P60748|PRSA	Ref	-3.3	-5.7	0.1	-2.9	-5.5
Glycyl-glycine endopeptidase lytM	Q7A7T0|LYTM	Ref	-0.3	-0.9	-1.7	0.7	0.9
Thioredoxin	P99122|THIO	Ref	0.2	-4.6	0.6	0.5	-5.1
SA2437 protein	Q7A371|Q7A371	Ref	-10	-8.7	Reference Missing	-10	-8.3
UPF0477 protein SA0873	Q7A6D4|Y873	Ref	-4.7	-5.9	-0.5	-4.7	-6.8
Citrate synthase II	Q7A561|Q7A561	Ref	5.5	-5.8	0	8.5	-6.9
6-phosphogluconate dehydrogenase, decarboxylating	P63334|6PGD	Ref	-0.9	-4.1	0.5	-0.5	-4.5
Superoxide dismutase [Mn/Fe] 1	P99098|SODM1	Ref	-4.7	-7.7	0.6	-5	-8.1

Appendix 11. (Continued)

Phenol-soluble modulin alpha 1 peptide	P0C7Y7|PSMA 1	Ref	-5.3	-4.2	-0.9	-4.1	-1.7
Phosphoenolpyruvate carboxykinase [ATP]	P99128|PCKA	Ref	-0.6	-8.4	0.6	-1.4	-9.4
Probable transglycosylase isaA	P99160|ISAA	Ref	-2.4	-4.1	-1	-3.1	-4.8
Glycyl-tRNA synthetase	P99129|SYG	Ref	-0.3	-1.7	0.6	-1.3	-1
DNA-directed RNA polymerase subunit beta'	P60285|RPOC	Ref	-2.4	-3.7	0.6	-1.1	-5.4
Glycine cleavage system H protein	P64214|GCSH	Ref	0.9	-6.8	0.6	-1.5	-5
50S ribosomal protein L30	P0A0G0|RL30	Ref	0.8	0.7	-4	0	1.2
Cold shock protein cspA	Q7A5P3|CSPA	Ref	No Values	-3.9	Reference Missing	No Values	-3.8
Serine protease splB	Q7A4Y1|SPLB	Ref	-0.2	-4.1	0.5	3	-1.5
DNA-directed RNA polymerase subunit beta	P60278|RPOB	Ref	No Values	-6.1	-1	No Values	-5.8
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	P99153|GPMA	Ref	No Values	-3.4	0.6	No Values	-2.8
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	Q7A5N4|ODO2	Ref	-5.2	-4.6	0	-4.9	-5.9
Seryl-tRNA synthetase	P99178|SYS	Ref	-6.1	-9.3	-0.5	-7.6	-9.7
NAD-specific glutamate dehydrogenase	Q7A6H8|DHE2	Ref	-2.1	-3	0.6	-3.2	-3.4
Succinyl-CoA ligase [ADP-forming] subunit alpha	P99070|SUCD	Ref	-16.8	-25.2	No Values	-17.5	-25.3
Uncharacterized leukocidin-like protein 1	Q7A4L0|LUKL 1	Ref	1.8	1.4	0.4	4	3.5
Acyl carrier protein	P0A002|ACP	Ref	-2.5	-2.9	-0.1	-4	-3.2
50S ribosomal protein L21	Q7A583|RL21	Ref	1.8	-5.2	Reference Missing	2.4	-5.9
UPF0337 protein SA0772	Q7A6L9|Y772	Ref	-2.6	-3.3	Reference Missing	-2.9	-3.1
3-oxoacyl-[acyl-carrier-protein] synthase 2	Q7A6F8|FABF	Ref	-2.6	-3.1	Reference Missing	-2.2	-2.5

Appendix 11. (Continued)

50S ribosomal protein L9	P66318|RL9	Ref	-3.2	-6.4	0.6	-4.1	-6.5
Beta-lactamase	Q9AC80|Q9AC80	Ref	0.3	-1.8	0.6	2.4	-1.2
Thioredoxin reductase	P99101|TRXB	Ref	No Values	-7.7	Value Missing	No Values	-8.4
Putative dipeptidase SA1572	Q7A522|PEPVL	Ref	-5.8	-9.3	Reference Missing	-5.7	-9.8
SA0916 protein	Q7A696|Q7A696	Ref	-3.9	-4.7	Reference Missing	Value Missing	Value Missing
Phosphoenolpyruvate-protein phosphotransferase	Q99V14|PT1	Ref	0	-5.4	0.6	1.2	-4.4
30S ribosomal protein S9	P66646|RS9	Ref	2.5	-6.4	0.5	Value Missing	-6.4
Uncharacterized protein SA1692	P0A0K1|Y1692	Ref	-0.3	-1.9	0.3	1	-0.1
50S ribosomal protein L11	P0A0F2|RL11	Ref	-0.6	-5.9	-0.7	-2.2	-5.8
Putative universal stress protein SA1532	Q7A551|Y1532	Ref	0.6	-2	0.5	2.5	-1.6
1-pyrroline-5-carboxylate dehydrogenase	P99076|ROCA	Ref	-1	-4.6	-0.9	-2.2	-6
Gamma-hemolysin component C	Q7A3S2|HLGC	Ref	-5.4	-5.2	0.6	-2	-0.6
Serine-aspartate repeat-containing protein D	Q7A780|SDRD	Ref	0	0	No Values	-2.8	-1.6
Glutamyl-tRNA synthetase	P99170|SYE	Ref	-2.6	-7.5	0.6	-2.9	-7.6
2,3-bisphosphoglycerate-independent phosphoglycerate mutase	P64270|GPMI	Ref	1.2	-4.5	-0.9	1.8	-4.8
D-lactate dehydrogenase	P99116|LDHD	Ref	No Values	0	No Values	No Values	-2.2
50S ribosomal protein L10	P99155|RL10	Ref	2.6	-5	-0.7	3.4	-4.8
SA2202 protein	Q99RL6|Q99RL6	Ref	-0.1	-2.5	0.4	-0.1	-1.5
Gamma-hemolysin component A	P0A072|HLGA	Ref	2.2	No Values	0.6	4.3	No Values
10 kDa chaperonin	P99104|CH10	Ref	-2.1	-5.9	0.2	-2.6	-6.6
Dihydroorotase	P65906|PYRC	Ref	-1.6	-3.8	0.2	-2.2	-2.9
UPF0457 protein SA1975.1	Q99S93|Y197A	Ref	-1.5	-5.6	Reference Missing	-1.7	-5.5
Ribosome-recycling factor	P99130|RRF	Ref	-2.6	-4.7	Reference Missing	-3.6	-3.9
Elongation factor P	P99066|EFP	Ref	-1	-5.3	-0.4	-2.5	-5.7
Uncharacterized leukocidin-like protein 2	Q99SN7|LUKL2	Ref	0.6	No Values	0.5	2.6	No Values

Appendix 11. (Continued)

UPF0355 protein SA0372	Q7A7I6|UP355	Ref	-1.6	-4.6	0.6	-0.7	-4.1
Acetate kinase	Q99TF2|ACKA	Ref	2.6	-2.1	-0.9	4.2	-0.1
Elastin-binding protein ebpS	Q7A5I6|EBPS	Ref	-2.6	-5	-0.4	-1.7	-4.2
30S ribosomal protein S6	P99142|RS6	Ref	No Values	-2.2	0.6	No Values	-1.9
General stress protein 20U	Q7A4C8|Q7A4C8	Ref	-0.9	-1.4	0.6	-1.2	Value Missing
50S ribosomal protein L24	P60735|RL24	Ref	-2.7	-7.6	Reference Missing	-3	-7.6
Bifunctional protein folD	Q7A697|FOLD	Ref	No Values	-0.3	-0.1	No Values	1.4
50S ribosomal protein L22	Q7A460|RL22	Ref	-2	-4.7	0.6	1	-4.1
Putative uncharacterized protein SAS040	Q7A5U6|Q7A5U6	Ref	-2.5	-8	-0.2	-2	-8.5
Gamma-hemolysin component B	P0A075|HLGB	Ref	1	No Values	0.6	2.7	No Values
Putative uncharacterized protein SA0395	Q99WG7|Q99WG7	Ref	No Values	No Values	-0.2	Reference Missing	No Values
Putative peptidyl-prolyl cis-trans isomerase	Q7A6I1|PPI1	Ref	2.4	0.1	0.6	Reference Missing	-0.2
50S ribosomal protein L1	Q99W68|RL1	Ref	No Values	-2.8	-0.3	Reference Missing	-2.6
Virulence factor esxA	ESXA (+1)	Ref	-0.5	No Values	0.6	1.8	No Values
ATP-dependent Clp protease ATP-binding subunit clpC	Q7A797|CLPC	Ref	1.9	-4.7	-2.4	4	-4.3
SA0914 protein	Q99V35|Q99V35	Ref	2.4	-3.6	0.6	3.3	-2.8
30S ribosomal protein S16	P66440|RS16	Ref	-1.5	-8.5	Reference Missing	-1.4	-8.9
Translation initiation factor IF-1	P65119|IF1	Ref	-5.8	-9.1	Reference Missing	-8	-8.7
DNA-directed RNA polymerase subunit alpha	P66706|RPOA	Ref	-1.1	-5.9	0.6	-1.8	-5.4
Truncated beta-hemplysin	Q99QR7|Q99QR7	Ref	-19.6	No Values	No Values	-19.4	No Values
Deoxyribose-phosphate aldolase 1	P99102|DEOC1 (+1)	Ref	-3	-4.4	-0.9	-3.6	-3.9
Pyruvate carboxylase	Q7A666|Q7A666	Ref	0	0	No Values	-0.5	-0.3
Uncharacterized protein SA0829	Q7A6H3|Y829	Ref	-6.5	No Values	Reference Missing	-9.7	No Values
Probable acetyl-CoA acyltransferase	Q7A7L2|THLA	Ref	-2.7	No Values	0.6	-3.2	No Values
Protein grpE	P99086|GRPE	Ref	-3.6	-4.6	-1	-5	-4.6
50S ribosomal protein L3	P60449|RL3	Ref	No Values	No Values	0.5	No Values	No Values
ATP synthase subunit beta	P99112|ATPB	Ref	-1.6	-5.5	0.6	-0.1	-4.9

Appendix 11. (Continued)

Uncharacterized protein SA0707	Q7A6R6|Y707	Ref	-2.5	-5.2	0.6	-1.6	-5.6
Glucose-specific phosphotransferase enzyme IIA component	P60857|PTGA	Ref	No Values	0	No Values	Reference Missing	0.4
Transcription elongation factor greA	P99156|GREA	Ref	-2.1	-3.6	-0.4	-1.7	-6.2
SA0758 protein	Q7A6M7	Ref	-0.3	-3.9	-2.2	-1.2	-4
Chaperone protein hchA	P64313|HCHA	Ref	3	No Values	0.6	5	No Values
50S ribosomal protein L5	Q7A465|RL5	Ref	-0.6	No Values	0.3	2.5	No Values
Serine hydroxymethyltransferase	P99091|GLYA	Ref	-4	-8	-0.7	-4	-8.2
Methionine aminopeptidase	AMPM	Ref	-5.7	-9.4	-2.9	-7	-9.5
50S ribosomal protein L15	P0A0F6|RL15	Ref	0.9	-4.1	0.6	0.9	-3.7
ATP-dependent Clp protease ATP-binding subunit clpL	Q7A3F4|CLPL	Ref	-0.4	-7.4	0.5	0.7	-6.7
UPF0342 protein SA1663	Q7A4V3|Y1663	Ref	-2.4	-3.6	0.6	-2.5	-4.6
SA0759 protein	Q7A6M6|Q7A6M6	Ref	1.8	-3.2	Reference Missing	2.5	-3
Staphylokinase	Q99SU7|SAK	Ref	0.8	-1.5	0	2.5	-0.4
Iron-regulated surface determinant protein A	Q7A655|ISDA	Ref	No Values	-4.7	0.2	No Values	-3.7
Serine-aspartate repeat-containing protein E	Q99W46|SDRE	Ref	0	0	No Values	-1.1	-2.5
Leukotoxin, LukD	Q99T54|Q99T54	Ref	No Values	No Values	Reference Missing	Reference Missing	No Values
Amidophosphoribosyltransferase	P99164|PUR1	Ref	No Values	No Values	0.2	No Values	No Values
SA0859 protein	Q7A6E5|Q7A6E5	Ref	No Values	-6.3	Reference Missing	No Values	-8.9
Phosphoribosylformylglycinamidine synthase 1	P99166|PURQ	Ref	No Values	No Values	Reference Missing	No Values	No Values
Staphylococcal complement inhibitor	Q99SU9|SCIN	Ref	No Values	-1.8	Reference Missing	No Values	-0.2
Uncharacterized N-acetyltransferase SA1019	Q99UT4|Y1019	Ref	-2.5	-4.4	-1	-2.9	-4.1
50S ribosomal protein L27	P66133|RL27	Ref	No Values	-6.7	0.6	No Values	-6.8
Adenylosuccinate lyase	Q7A4Q3|PUR8	Ref	0	0	No Values	-0.2	-0.3

Appendix 11. (Continued)

Naphthoate synthase	Q7A6A9|MENB	Ref	-8.8	No Values	Reference Missing	-9	No Values
Putative uncharacterized protein SA0771	Q7A6M0|Q7A6M0	Ref	-1.3	-2.8	0.6	-1.3	-1.8
Xaa-Pro dipeptidase	Q99TW4|Q99TW4	Ref	-5.1	-8	Reference Missing	-4.2	-8.1
1-phosphatidylinositol phosphodiesterase precurosr	Q7A888|Q7A888	Ref	0.7	No Values	0.4	3.1	No Values
Probable glycine dehydrogenase [decarboxylating] subunit 1	P64218|GCSPA	Ref	No Values	0	No Values	No Values	-0.9
Putative aldehyde dehydrogenase AldA	Q7A825|ALDA	Ref	No Values	0	No Values	No Values	-0.7
L-lactate dehydrogenase 2	P99119|LDH2	Ref	1.4	-4	-1.1	3.3	-2.7
Formate acetyltransferase	Q7A7X6|PFLB	Ref	-1.2	-4.1	-0.5	-0.3	-3.7
Signal transduction protein TRAP	Q7A4W3|TRAP	Ref	-0.6	0	-0.2	-0.5	0.9
Purine nucleoside phosphorylase	Q7A4C9|Q7A4C9	Ref	No Values	No Values	-0.5	No Values	No Values
Arginyl-tRNA synthetase	Q99W05|SYR	Ref	No Values	-7.3	-0.8	No Values	-7.7
50S ribosomal protein L13	Q7A473|RL13	Ref	-1.3	No Values	-0.2	0.3	No Values
Probable transglycosylase sceD	Q7A4F2|SCED	Ref	0	No Values	No Values	-1.3	No Values
Organic hydroperoxide resistance protein-like	Q7A6M9|OHRL	Ref	-1.8	No Values	Reference Missing	-1.6	No Values
Serine protease splD	Q7A4Y3|SPLD	Ref	No Values	No Values	-0.6	No Values	No Values
Putative uncharacterized protein SA1986	Q7A493|Q7A493	Ref	No Values	No Values	-2.4	No Values	No Values
D-alanine--poly(phosphoribitol) ligase subunit 2	P0A019|DLTC	Ref	-2.5	-5.9	0.2	-1.7	-5.3
Immunoglobulin-binding protein sbi	Q99RL2|SBI	Ref	-3.3	-6.8	0.6	-4.9	-7.2
Putative uncharacterized protein SA1528	Q7A553|Q7A553	Ref	1.7	No Values	-0.4	4.2	No Values
GMP synthase [glutamine-hydrolyzing]	P99105|GUAA	Ref	No Values	-5.9	0.6	No Values	-6.3
SA1524 protein	Q7A556|Q7A556	Ref	1.6	-3.1	-0.6	2.7	-4.5

Appendix 11. (Continued)

30S ribosomal protein S11	P66357|RS11	Ref	-2.3	-7.6	Reference Missing	-2.9	-8.6
Putative uncharacterized protein SA0919	Q7A694|Q7A694	Ref	No Values	-4.8	-0.4	No Values	-4.3
Phenol-soluble modulin alpha 3 peptide	P0C811|PSMA3	Ref	-0.6	No Values	0.3	0	No Values
Ferritin	Q7A4R2|FTN	Ref	No Values	0	No Values	No Values	0.3
Alkyl hydroperoxide reductase subunit F	P99118|AHPF	Ref	No Values	-6.2	Value Missing	No Values	-6.1
Anti-sigma-B factor antagonist	P66838|RSBV	Ref	No Values	-3.2	-0.1	No Values	-2.9
Nucleoside diphosphate kinase	NDK (+1)	Ref	-3.7	-9.6	0.2	-5.5	-9.8
Zinc metalloproteinase aureolysin	Q7A378|Q7A378	Ref	No Values	-2.5	Reference Missing	Reference Missing	-0.5
UPF0173 metal-dependent hydrolase SA1529	P99149|Y1529	Ref	-14	-19.1	No Values	-14.2	-19.5
Pyridoxal biosynthesis lyase pdxS	P60798|PDXS	Ref	No Values	-6.5	-2	No Values	-5.9
Alanine dehydrogenase 2	Q99TF4|DHA2	Ref	No Values	-5.4	-1.2	No Values	-6.9
Urocanate hydratase	P67417|HUTU	Ref	No Values	0	No Values	No Values	-0.9
Putative uncharacterized protein SA2309	Q7A3I0|Q7A3I0	Ref	4.7	-3.5	0.5	Reference Missing	-3.4
CTP synthase	P99072|PYRG	Ref	0.6	-5.5	0.3	0.6	-5.1
Putative 8-amino-7-oxononanoate synthase/2-amino-3-ketobutyrate coenzyme A ligase	P60120|BIKB	Ref	No Values	-23	No Values	No Values	-24.1
Threonyl-tRNA synthetase	P67585|SYT	Ref	No Values	No Values	0.2	No Values	No Values
Probable malate:quinone oxidoreductase 2	P99115|MQO2	Ref	0	0	No Values	0.2	0.5
Probable branched-chain-amino-acid aminotransferase	P99138|ILVE	Ref	0	0	No Values	0.3	-0.4
Methionyl-tRNA synthetase	P67579|SYM	Ref	No Values	0	No Values	No Values	0.1
Tryptophanyl-tRNA synthetase	P67593|SYW	Ref	0	No Values	No Values	-3.1	No Values
Mannitol-1-phosphate 5-dehydrogenase	P99140|MTLD	Ref	0	No Values	No Values	0.7	No Values
SA1343 protein	Q7A5G2|Q7A5G2	Ref	No Values	0	No Values	No Values	-1.7

Appendix 11. (Continued)

Staphopain A	P65826|SSPP	Ref	No Values	No Values	No Values	No Values	No Values
UPF0082 protein SA0624	P67182|Y624	Ref	-2.2	-5.3	0.6	-1.8	-5
Cell division protein ftsZ	P99108|FTSZ	Ref	2.5	-3.1	-0.5	3	-3.6
Adenylosuccinate synthetase	P99099|PURA	Ref	No Values	-5.1	-1.9	No Values	-5.2
Histidine ammonia-lyase	P64416|HUTH	Ref	No Values	-9.6	Reference Missing	No Values	-9.6
Imidazolonepropionase	P64418|HUTI	Ref	No Values	0	No Values	No Values	-1.4
6-phosphofructokinase	P99165|K6PF	Ref	-3.2	No Values	Reference Missing	-0.7	No Values
Lysyl-tRNA synthetase	P67610|SYK	Ref	No Values	-1.3	-1.8	No Values	2.3
SA1599 protein	Q7A501|Q7A501	Ref	No Values	0	No Values	No Values	1.3
SA0231 protein	Q7A7W3|Q7A7W3	Ref	No Values	-3.4	-0.2	No Values	-3.9
Phenylalanyl-tRNA synthetase beta chain	P67041|SYFB	Ref	No Values	0	No Values	No Values	-1.5
P60855|Y370_STAAN	P60855|Y370	Ref	No Values	0	No Values	No Values	-0.7
50S ribosomal protein L23	Q7A459|RL23	Ref	No Values	No Values	0.5	No Values	No Values
Putative uncharacterized protein SA0908	Q7A6A3|Q7A6A3	Ref	3	-2.3	-1	3.6	-2.3
SA1475 protein	Q7A581|Q7A581	Ref	0	0	No Values	0.7	0.3
Leucyl-tRNA synthetase	P67513|SYL	Ref	No Values	-6	0.3	No Values	-5.8
Clumping factor A	Q99VJ4|CLFA	Ref	0	0	No Values	0.2	Reference Missing
Acetate-CoA ligase	Q7A3A2|Q7A3A2	Ref	0	No Values	No Values	0.6	No Values
Lactonase drp35	RANDOM_DRP35-R	Ref	0	No Values	No Values	-1.5	No Values
50S ribosomal protein L2	P60432|RL2	Ref	No Values	0	No Values	No Values	-0.2
Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]	GLMS	Ref	No Values	No Values	Reference Missing	No Values	No Values
Putative uncharacterized protein SA1743	Q7A4N7|Q7A4N7	Ref	No Values	0	No Values	No Values	0.6
Cell division protein	Q7A620|Q7A620	Ref	No Values	0	No Values	Reference Missing	0.5
50S ribosomal protein L18	Q7A467|RL18	Ref	No Values	-18.9	No Values	No Values	-18.5
3-hexulose-6-phosphate synthase	Q7A774|HPS	Ref	No Values	No Values	No Values	No Values	No Values
Acetoin(diacetyl) reductase	P99120|BUTA	Ref	No Values	0	No Values	No Values	-0.2

Appendix 11. (Continued)

SA0022 protein	Q99XE9|Q99XE9	Ref	0	No Values	No Values	1.2	No Values
3-hydroxy-3-methylglutaryl CoA synthase	Q7A3F6|Q7A3F6	Ref	No Values	No Values	0.6	No Values	No Values
30S ribosomal protein S7	P66616|RS7	Ref	-1.5	No Values	-0.2	-1.2	No Values
Uncharacterized lipoprotein SA2158	Q7A3W5|Y2158	Ref	No Values	No Values	Reference Missing	No Values	No Values
Ribonuclease J 1	Q7A682|RNJ1	Ref	No Values	No Values	-0.5	No Values	No Values
Trans-2-enoyl-ACP reductase	Q7A6D8	Ref	0	No Values	No Values	0.7	No Values
2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase 2	Q7A7V0|ISPD2	Ref	No Values	No Values	-1.6	No Values	No Values
30S ribosomal protein S13	P66388|RS13	Ref	No Values	No Values	Reference Missing	No Values	No Values
30S ribosomal protein S2	P66544|RS2	Ref	No Values	0	No Values	No Values	0.2
3-oxoacyl-[acyl-carrier-protein] synthase 3	P99159|FABH	Ref	No Values	No Values	No Values	No Values	No Values

Appendix 12. Changes in secreted proteins of CA-MRSA USA400 compared to HA-MRSA USA100 during stationary phase from 3 biological replicates

Identified Proteins (246)	Accession Number	USA100 #1	USA100 #2	USA100 #3	USA400 #1	USA400 #2	USA400 #3
Lipase 2	Q7A7P2|LIP2	Ref	-0.4	1.6	0.7	2.9	3.7
Lipase 1	P65289|LIP1	Ref	-1	-0.8	1.1	2.5	3.7
Alpha-Hemolysin	Q7A632|Q7A632	Ref	0.6	0.2	0.5	1	2.5
Elongation factor Tu	P99152|EFTU	Ref	-1.8	-6.7	0.9	-1.7	-7.3
Enolase	P99088|ENO	Ref	-0.6	-6.6	0.6	-0.8	-6.9
Putative surface protein SA2285	P61598|PLS	Ref	-4.6	-9	-4.2	-9.2	-9.7
Bifunctional autolysin	Q99V41|ATL	Ref	0.1	-3.1	-0.2	1.8	-1.8
SA0841 protein	Q7A6G0|Q7A6G0	Ref	-1.4	-3	1.5	1.5	1
Chaperone protein dnaK	P99110|DNAK	Ref	-3.6	-7	0.4	-3.6	-7.7
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1	Ref	-2.5	-5.6	0.4	-2.4	-6.3
DNA-binding protein HU	Q7A5J1|DBH	Ref	-1.7	-5.1	0	-2.7	-5.8
Alkyl hydroperoxide reductase subunit C	P99074|AHPC	Ref	-4.7	-7	1.2	-6.4	-6
Inosine-5'-monophosphate dehydrogenase	P99106|IMDH	Ref	0.2	-5.8	0.6	0.5	-6.5
Immunoglobulin G-binding protein A	SPA	Ref	No Values	0	No Values	No Values	Value Missing
Formate--tetrahydrofolate ligase	Q7A535|FTHS	Ref	-1.6	-7.6	0.5	-2.8	-8.3
Dihydrolipoyl dehydrogenase	P99084|DLDH	Ref	-0.7	-7.2	0.8	-0.9	-7.1
50S ribosomal protein L7/L12	P99154|RL7	Ref	-0.2	-7.4	0.4	0.1	-7.6
Pyruvate kinase	Q7A559|KPYK	Ref	-4.6	-3.7	0.1	-4.5	-4.2
SA0587 protein	Q7A719|Q7A719	Ref	-1.3	-5	0.9	-0.8	-4.8
Phosphate acetyltransferase	P99092|PTA	Ref	-2	-6.2	1.4	-2.1	-6.9
Elongation factor G	P68789|EFG	Ref	-1.5	-6.2	1.2	-1.3	-6.2
Glutamine synthetase	P99095|GLNA	Ref	-2.4	-6.7	0.4	-2.4	-7.3
SA2006 protein	Q7A483|Q7A483	Ref	0.3	0.8	1.4	4.1	3.6

Appendix 12. (Continued)

Fructose-bisphosphate aldolase	P99075|ALF2	Ref	-3.8	-7.4	1.8	-3.8	-8.1
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS	Ref	-1	-0.5	0.9	-0.1	-1.2
Alkaline shock protein 23	P99157|ASP23	Ref	2.4	-3.9	2.2	4.1	-2.8
Triosephosphate isomerase	P99133|TPIS	Ref	-2	-4	0	-1.7	-4.7
Enterotoxin type C-3	P0A0L4|ENTC 3	Ref	-5.3	-5.4	4.4	-1.2	0.5
Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex	P65636|ODP2	Ref	-0.6	-5.7	-0.7	-2.3	-6.4
Cysteine synthase	P63871|CYSK	Ref	-0.6	-5.1	1.1	-0.3	-5.1
Elongation factor Ts	P99171|EFTS	Ref	2.5	-5	0.7	2.7	-5.7
Pyruvate dehydrogenase E1 component subunit alpha	Q820A6|ODPA	Ref	-5.5	-9.6	0.5	-7.2	-10.3
Putative uncharacterized protein SA0663	Q7A6V1|Q7A6 V1	Ref	0.1	-0.2	0.3	4.2	3.2
Ornithine aminotransferase 2	P60298|OAT2	Ref	-3.2	-9.4	0.9	-4.4	-9.7
Staphopain B	Q7A6A7|SSPB	Ref	-5.1	-3.5	0	-3	-0.5
Succinyl-CoA ligase [ADP-forming] subunit beta	P99071|SUCC	Ref	-1.6	-6.2	0	-2.2	-6.6
Phosphocarrier protein HPr	P99143|PTHP	Ref	1.1	-5.6	0.8	1.9	-6.3
Putative uncharacterized protein SA0359	Q7A7J8|Q7A7J 8	Ref	-5.8	-6.1	0.9	-2.6	-3.5
Thermonuclease	Q7A6P2|NUC	Ref	1.6	-0.9	1.2	2.4	0.7
Trigger factor	P99080|TIG	Ref	-1.3	-4.9	-0.2	-1	-5
Aconitate hydratase	P99148|ACON	Ref	-0.1	-4.9	0.9	0.8	-5.2
Adenylate kinase	P99062|KAD	Ref	-3.1	-4.8	0.4	-3.3	-5.5
Fructose-bisphosphate aldolase class 1	P99117|ALF1	Ref	-2.3	-6.6	0.4	-2.3	-7.2
50S ribosomal protein L17	Q7A469|RL17	Ref	1.2	-3.6	1.4	1.5	-3.7
Glutamyl endopeptidase	Q7A6A6|SSPA	Ref	-1.7	-3.3	2.7	1.3	1
SA0620 protein	Q7A6Y9|Q7A6 Y9	Ref	-0.1	-2.2	2.2	2.1	2.9
60 kDa chaperonin	P99083|CH60	Ref	-1.3	-5.4	0.9	-1.4	-5.8
Probable beta-lactamase	Q7A4X8|Q7A4 X8	Ref	0.8	No Values	2.9	0.4	No Values

Appendix 12. (Continued)

Putative uncharacterized protein SA0570	Q7A735|Q7A735	Ref	0.1	-3.9	0.9	2.9	-1.5
Alcohol dehydrogenase	Q7A742|ADH	Ref	-0.4	-4.8	0.5	-0.7	-5.1
Catalase	Q7A5T2|CATA	Ref	-3.8	-6.9	Reference Missing	-5.3	-7.6
SA2097 protein	Q7A418|Q7A418	Ref	-1.4	-2.7	0.3	-0.1	-2.1
Penicillin binding protein 2 prime	Q7A8C6|Q7A8C6	Ref	-4.4	-6.8	1	-3.5	-5.8
Putative uncharacterized protein SAP003	Q9AC87|Q9AC87	Ref	3.5	-6.8	-0.1	5.3	-7.5
Probable thiol peroxidase	P99146|TPX	Ref	0.7	-6	3.1	-1.7	-6.7
30S ribosomal protein S1	Q7A5J0|RS1	Ref	-0.9	-5.3	2	-1.2	-5.7
L-lactate dehydrogenase 1	P65256|LDH1	Ref	2.2	-1.1	1.5	3.5	0.1
Phosphoglycerate kinase	P99135|PGK	Ref	-1.6	-5.4	1.1	-0.5	-5.3
Glucose-6-phosphate isomerase	P99078|G6PI	Ref	-1.5	-5.4	0.9	-2.1	-5.8
Transketolase	P99161|TKT	Ref	-1.9	-5.5	0.6	-2.3	-5.9
Pyruvate dehydrogenase E1 component subunit beta	P99063|ODPB	Ref	No Values	-8.1	0.6	Reference Missing	-8.7
Glycerophosphoryl diester phosphodiesterase	Q7A6H7|Q7A6H7	Ref	-2.9	-4.4	1	-2.6	-4.1
Delta-hemolysin	P0A0M2|HLD	Ref	-2.5	No Values	1.6	-0.3	No Values
Phenol-soluble modulin alpha 4 peptide	P0C824|PSMA4	Ref	-2.3	0.1	2.4	1.9	4
Foldase protein prsA	P60748|PRSA	Ref	-3.3	-5.7	1.7	-1.9	-4.9
Glycyl-glycine endopeptidase lytM	Q7A7T0|LYTM	Ref	-0.3	-0.9	0.8	1.9	2.5
Thioredoxin	P99122|THIO	Ref	0.2	-4.6	-0.2	0.6	-5.4
SA2437 protein	Q7A371|Q7A371	Ref	-10	-8.7	Reference Missing	-8.2	-6.9
UPF0477 protein SA0873	Q7A6D4|Y873	Ref	-4.7	-5.9	1.3	-5.4	-5.5
Citrate synthase II	Q7A561|Q7A561	Ref	5.5	-5.8	0.2	8.2	-6.5
6-phosphogluconate dehydrogenase, decarboxylating	P63334|6PGD	Ref	-0.9	-4.1	0.9	-0.5	-3.9
Superoxide dismutase [Mn/Fe] 1	P99098|SODM1	Ref	-4.7	-7.7	0.9	-3.9	-8
Phenol-soluble modulin alpha 1 peptide	P0C7Y7|PSMA1	Ref	-5.3	-4.2	3.9	-1.1	0.2

Appendix 12. (Continued)

Phosphoenolpyruvate carboxykinase [ATP]	P99128|PCKA	Ref	-0.6	-8.4	1.1	-4.7	-9.1
Probable transglycosylase isaA	P99160|ISAA	Ref	-2.4	-4.1	0.9	-2.9	-4.6
Glycyl-tRNA synthetase	P99129|SYG	Ref	-0.3	-1.7	-0.5	-3.9	-0.8
DNA-directed RNA polymerase subunit beta'	P60285|RPOC	Ref	-2.4	-3.7	0.2	-2.4	-4.5
Glycine cleavage system H protein	P64214|GCSH	Ref	0.9	-6.8	0.1	1.2	-6.8
50S ribosomal protein L30	P0A0G0|RL30	Ref	0.8	0.7	0.2	-0.1	1.4
Cold shock protein cspA	Q7A5P3|CSPA	Ref	No Values	-3.9	No Values	No Values	-4.6
Serine protease splB	Q7A4Y1|SPLB	Ref	-0.2	-4.1	0.6	1.5	-2.2
DNA-directed RNA polymerase subunit beta	P60278|RPOB	Ref	No Values	-6.1	0.5	No Values	-5.8
2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	P99153|GPMA	Ref	No Values	-3.4	1.4	No Values	-3.9
Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex	Q7A5N4|ODO2	Ref	-5.2	-4.6	0.3	-7.1	-4.7
Seryl-tRNA synthetase	P99178|SYS	Ref	-6.1	-9.3	0.6	-7.6	-9.3
NAD-specific glutamate dehydrogenase	Q7A6H8|DHE2	Ref	-2.1	-3	0.5	-2.9	-3.4
Succinyl-CoA ligase [ADP-forming] subunit alpha	P99070|SUCD	Ref	-16.8	-25.2	No Values	-17.9	-25.7
Uncharacterized leukocidin-like protein 1	Q7A4L0|LUKL1	Ref	1.8	1.4	1.9	3.9	3.3
Acyl carrier protein	P0A002|ACP	Ref	-2.5	-2.9	2	-2.5	Value Missing
50S ribosomal protein L21	Q7A583|RL21	Ref	1.8	-5.2	Reference Missing	3	-5.9
UPF0337 protein SA0772	Q7A6L9|Y772	Ref	-2.6	-3.3	No Values	-2.1	-3.9
3-oxoacyl-[acyl-carrier-protein] synthase 2	Q7A6F8|FABF	Ref	-2.6	-3.1	Reference Missing	-2.7	-2
50S ribosomal protein L9	P66318|RL9	Ref	-3.2	-6.4	0.4	-3.5	-6.8
Beta-lactamase	Q9AC80|Q9AC80	Ref	0.3	-1.8	1.1	2.1	-0.6

Appendix 12. (Continued)

Thioredoxin reductase	P99101|TRXB	Ref	No Values	-7.7	Value Missing	No Values	-8.5
Putative dipeptidase SA1572	Q7A522|PEPV L	Ref	-5.8	-9.3	Reference Missing	-5.1	-10
SA0916 protein	Q7A696|Q7A69 6	Ref	-3.9	-4.7	Reference Missing	Value Missing	0.9
Phosphoenolpyruvat e-protein phosphotransferase	Q99V14|PT1	Ref	0	-5.4	0.7	0.1	-4.4
30S ribosomal protein S9	P66646|RS9	Ref	2.5	-6.4	0.9	Value Missing	-6.2
Uncharacterized protein SA1692	P0A0K1|Y1692	Ref	-0.3	-1.9	1.7	1.9	-0.7
50S ribosomal protein L11	P0A0F2|RL11	Ref	-0.6	-5.9	0.5	-2.4	-5.7
Putative universal stress protein SA1532	Q7A551|Y1532	Ref	0.6	-2	1.6	1.4	-1.6
1-pyrroline-5-carboxylate dehydrogenase	P99076|ROCA	Ref	-1	-4.6	-0.3	-3.7	-5.1
Gamma-hemolysin component C	Q7A3S2|HLGC	Ref	-5.4	-5.2	-0.4	-1.7	-1.3
Serine-aspartate repeat-containing protein D	Q7A780|SDRD	Ref	0	0	No Values	-2.8	-0.4
Glutamyl-tRNA synthetase	P99170|SYE	Ref	-2.6	-7.5	2.3	-3.4	-7.8
2,3-bisphosphoglycerate-independent phosphoglycerate mutase	P64270|GPMI	Ref	1.2	-4.5	-0.7	Reference Missing	-5
D-lactate dehydrogenase	P99116|LDHD	Ref	No Values	0	No Values	No Values	-0.7
50S ribosomal protein L10	P99155|RL10	Ref	2.6	-5	1	3.9	-4.4
SA2202 protein	Q99RL6|Q99R L6	Ref	-0.1	-2.5	-0.4	0.6	-1.7
Gamma-hemolysin component A	P0A072|HLGA	Ref	2.2	No Values	0.8	6.3	No Values
10 kDa chaperonin	P99104|CH10	Ref	-2.1	-5.9	0.7	-1.6	-5.9
Dihydroorotase	P65906|PYRC	Ref	-1.6	-3.8	0.4	-0.7	-4.2
UPF0457 protein SA1975.1	Q99S93|Y197A	Ref	-1.5	-5.6	No Values	-1.2	-5
Ribosome-recycling factor	P99130|RRF	Ref	-2.6	-4.7	Reference Missing	-2.2	-3.7
Elongation factor P	P99066|EFP	Ref	-1	-5.3	1.4	-1.6	-5
Uncharacterized leukocidin-like protein 2	Q99SN7|LUKL 2	Ref	0.6	No Values	0.7	3.1	No Values
UPF0355 protein SA0372	Q7A7I6|UP355	Ref	-1.6	-4.6	Reference Missing	-0.2	-4.1
Acetate kinase	Q99TF2|ACKA	Ref	2.6	-2.1	0	2.9	-0.2
Elastin-binding protein ebpS	Q7A5I6|EBPS	Ref	-2.6	-5	4.4	-1.3	-2.5

Appendix 12. (Continued)

30S ribosomal protein S6	P99142|RS6	Ref	No Values	-2.2	2	No Values	Value Missing
General stress protein 20U	Q7A4C8|Q7A4C8	Ref	-0.9	-1.4	Value Missing	-0.6	2.4
50S ribosomal protein L24	P60735|RL24	Ref	-2.7	-7.6	Reference Missing	-3.6	-8
Bifunctional protein folD	Q7A697|FOLD	Ref	No Values	-0.3	0.3	No Values	Value Missing
50S ribosomal protein L22	Q7A460|RL22	Ref	-2	-4.7	1.8	0.8	-3.7
Putative uncharacterized protein SAS040	Q7A5U6|Q7A5U6	Ref	-2.5	-8	0.2	-2.3	-8.5
Gamma-hemolysin component B	P0A075|HLGB	Ref	1	No Values	0.4	4.5	No Values
Putative uncharacterized protein SA0395	Q99WG7|Q99WG7	Ref	No Values	No Values	1.2	Reference Missing	No Values
Putative peptidyl-prolyl cis-trans isomerase	Q7A6I1|PPI1	Ref	2.4	0.1	-1.2	6.5	Value Missing
50S ribosomal protein L1	Q99W68|RL1	Ref	No Values	-2.8	2	Reference Missing	-1.6
Virulence factor esxA	ESXA (+1)	Ref	-0.5	No Values	-0.2	1.2	No Values
ATP-dependent Clp protease ATP-binding subunit clpC	Q7A797|CLPC	Ref	1.9	-4.7	0.1	5.5	-3.7
SA0914 protein	Q99V35|Q99V35	Ref	2.4	-3.6	1.5	4.9	-2.1
30S ribosomal protein S16	P66440|RS16	Ref	-1.5	-8.5	No Values	-1.2	-9.2
Translation initiation factor IF-1	P65119|IF1	Ref	-5.8	-9.1	Reference Missing	-8	-8.2
DNA-directed RNA polymerase subunit alpha	P66706|RPOA	Ref	-1.1	-5.9	0.2	-2.6	-4.9
Truncated beta-hemplysin	Q99QR7|Q99QR7	Ref	-19.6	No Values	No Values	-19.4	No Values
Deoxyribose-phosphate aldolase 1	P99102|DEOC1 (+1)	Ref	-3	-4.4	1	-2.3	-4.1
Pyruvate carboxylase	Q7A666|Q7A666	Ref	0	0	No Values	-1.5	-0.7
Uncharacterized protein SA0829	Q7A6H3|Y829	Ref	-6.5	No Values	Reference Missing	-9	No Values
Probable acetyl-CoA acyltransferase	Q7A7L2|THLA	Ref	-2.7	No Values	Reference Missing	-2.1	No Values
Protein grpE	P99086|GRPE	Ref	-3.6	-4.6	1.3	-4.6	-4.1
50S ribosomal protein L3	P60449|RL3	Ref	No Values	No Values	Value Missing	No Values	No Values
ATP synthase subunit beta	P99112|ATPB	Ref	-1.6	-5.5	1.1	-1.5	-5.4
Uncharacterized protein SA0707	Q7A6R6|Y707	Ref	-2.5	-5.2	Reference Missing	-2.4	-5.9

Appendix 12. (Continued)

Glucose-specific phosphotransferase enzyme IIA component	P60857|PTGA	Ref	No Values	0	No Values	Reference Missing	0.2
Transcription elongation factor greA	P99156|GREA	Ref	-2.1	-3.6	2	-1.7	-4.4
SA0758 protein	Q7A6M7	Ref	-0.3	-3.9	0.3	-2.4	-4.7
Chaperone protein hchA	P64313|HCHA	Ref	3	No Values	0.5	5.6	No Values
50S ribosomal protein L5	Q7A465|RL5	Ref	-0.6	No Values	1.2	2.7	No Values
Serine hydroxymethyltransferase	P99091|GLYA	Ref	-4	-8	-0.2	-4.1	-8.8
Methionine aminopeptidase	AMPM	Ref	-5.7	-9.4	-0.1	-6.5	-9.3
50S ribosomal protein L15	P0A0F6|RL15	Ref	0.9	-4.1	1.5	1.1	-2.8
ATP-dependent Clp protease ATP-binding subunit clpL	Q7A3F4|CLPL	Ref	-0.4	-7.4	1.6	1.4	-5.4
UPF0342 protein SA1663	Q7A4V3|Y1663	Ref	-2.4	-3.6	1.3	-1.2	-4.3
SA0759 protein	Q7A6M6|Q7A6M6	Ref	1.8	-3.2	Reference Missing	3.2	-2.6
Staphylokinase	Q99SU7|SAK	Ref	0.8	-1.5	-0.9	2.9	-1.2
Iron-regulated surface determinant protein A	Q7A655|ISDA	Ref	No Values	-4.7	2	No Values	-2.8
Serine-aspartate repeat-containing protein E	Q99W46|SDRE	Ref	0	0	No Values	0.3	-0.7
Leukotoxin, LukD	Q99T54|Q99T54	Ref	No Values	No Values	No Values	Reference Missing	No Values
Amidophosphoribosyltransferase	P99164|PUR1	Ref	No Values	No Values	0.2	No Values	No Values
SA0859 protein	Q7A6E5|Q7A6E5	Ref	No Values	-6.3	No Values	No Values	-7.1
Phosphoribosylformylglycinamidine synthase 1	P99166|PURQ	Ref	No Values	No Values	No Values	No Values	No Values
Staphylococcal complement inhibitor	Q99SU9|SCIN	Ref	No Values	-1.8	Reference Missing	No Values	0
Uncharacterized N-acetyltransferase SA1019	Q99UT4|Y1019	Ref	-2.5	-4.4	1.8	-2.5	-3.2
50S ribosomal protein L27	P66133|RL27	Ref	No Values	-6.7	2.7	No Values	-6.5
Adenylosuccinate lyase	Q7A4Q3|PUR8	Ref	0	0	No Values	0	0.1

Appendix 12. (Continued)

Naphthoate synthase	Q7A6A9|MENB	Ref	-8.8	No Values	No Values	-11.1	No Values
Putative uncharacterized protein SA0771	Q7A6M0|Q7A6M0	Ref	-1.3	-2.8	0.9	-1.2	-1.9
Xaa-Pro dipeptidase	Q99TW4|Q99TW4	Ref	-5.1	-8	Reference Missing	-3.2	-7
1-phosphatidylinositol phosphodiesterase precurosr	Q7A888|Q7A888	Ref	0.7	No Values	0	2.9	No Values
Probable glycine dehydrogenase [decarboxylating] subunit 1	P64218|GCSPA	Ref	No Values	0	No Values	No Values	-0.4
Putative aldehyde dehydrogenase AldA	Q7A825|ALDA	Ref	No Values	0	No Values	No Values	0.5
L-lactate dehydrogenase 2	P99119|LDH2	Ref	1.4	-4	0.6	2.5	-3.4
Formate acetyltransferase	Q7A7X6|PFLB	Ref	-1.2	-4.1	2.1	-1	-3.4
Signal transduction protein TRAP	Q7A4W3|TRAP	Ref	-0.6	0	2.1	0.3	1.3
Purine nucleoside phosphorylase	Q7A4C9|Q7A4C9	Ref	No Values	No Values	-0.4	No Values	No Values
Arginyl-tRNA synthetase	Q99W05|SYR	Ref	No Values	-7.3	1.3	No Values	-7.8
50S ribosomal protein L13	Q7A473|RL13	Ref	-1.3	No Values	2.4	-0.3	No Values
Probable transglycosylase sceD	Q7A4F2|SCED	Ref	0	No Values	No Values	-0.6	No Values
Organic hydroperoxide resistance protein-like	Q7A6M9|OHRL	Ref	-1.8	No Values	Reference Missing	-0.5	No Values
Serine protease splD	Q7A4Y3|SPLD	Ref	No Values	No Values	-0.2	No Values	No Values
Putative uncharacterized protein SA1986	Q7A493|Q7A493	Ref	No Values	No Values	0.4	No Values	No Values
D-alanine--poly(phosphoribitol) ligase subunit 2	P0A019|DLTC	Ref	-2.5	-5.9	0.6	-1.2	-6.4
Immunoglobulin-binding protein sbi	Q99RL2|SBI	Ref	-3.3	-6.8	0.7	-2.9	-6.7
Putative uncharacterized protein SA1528	Q7A553|Q7A553	Ref	1.7	No Values	1	2.3	No Values
GMP synthase [glutamine-hydrolyzing]	P99105|GUAA	Ref	No Values	-5.9	0.4	No Values	Value Missing
SA1524 protein	Q7A556|Q7A556	Ref	1.6	-3.1	0.1	Value Missing	-3.5

Appendix 12. (Continued)

30S ribosomal protein S11	P66357|RS11	Ref	-2.3	-7.6	Reference Missing	-3	-8.3
Putative uncharacterized protein SA0919	Q7A694|Q7A694	Ref	No Values	-4.8	1.5	No Values	-2.7
Phenol-soluble modulin alpha 3 peptide	P0C811|PSMA3	Ref	-0.6	No Values	3.6	3	No Values
Ferritin	Q7A4R2|FTN	Ref	No Values	0	No Values	No Values	0.2
Alkyl hydroperoxide reductase subunit F	P99118|AHPF	Ref	No Values	-6.2	Value Missing	No Values	-5.6
Anti-sigma-B factor antagonist	P66838|RSBV	Ref	No Values	-3.2	1.4	No Values	-2.1
Nucleoside diphosphate kinase	NDK (+1)	Ref	-3.7	-9.6	1.8	-5.5	-9.4
Zinc metalloproteinase aureolysin	Q7A378|Q7A378	Ref	No Values	-2.5	Reference Missing	Reference Missing	1
UPF0173 metal-dependent hydrolase SA1529	P99149|Y1529	Ref	-14	-19.1	No Values	-13.7	Value Missing
Pyridoxal biosynthesis lyase pdxS	P60798|PDXS	Ref	No Values	-6.5	0.8	No Values	-5.2
Alanine dehydrogenase 2	Q99TF4|DHA2	Ref	No Values	-5.4	0.1	No Values	Value Missing
Urocanate hydratase	P67417|HUTU	Ref	No Values	0	No Values	No Values	0.2
Putative uncharacterized protein SA2309	Q7A3I0|Q7A3I0	Ref	4.7	-3.5	0.5	Reference Missing	Value Missing
CTP synthase	P99072|PYRG	Ref	0.6	-5.5	0.7	0.5	-4.5
Putative 8-amino-7-oxononanoate synthase/2-amino-3-ketobutyrate coenzyme A ligase	P60120|BIKB	Ref	No Values	-23	No Values	No Values	-23.5
Threonyl-tRNA synthetase	P67585|SYT	Ref	No Values	No Values	0.1	No Values	No Values
Probable malate:quinone oxidoreductase 2	P99115|MQO2	Ref	0	0	No Values	0.1	0.7
Probable branched-chain-amino-acid aminotransferase	P99138|ILVE	Ref	0	0	No Values	0	-0.7
Methionyl-tRNA synthetase	P67579|SYM	Ref	No Values	0	No Values	No Values	-0.4
Tryptophanyl-tRNA synthetase	P67593|SYW	Ref	0	No Values	No Values	Value Missing	No Values
Mannitol-1-phosphate 5-dehydrogenase	P99140|MTLD	Ref	0	No Values	No Values	0.8	No Values
SA1343 protein	Q7A5G2|Q7A5G2	Ref	No Values	0	No Values	No Values	-0.7

Appendix 12. (Continued)

Staphopain A	P65826|SSPP	Ref	No Values	No Values	No Values	No Values	No Values
UPF0082 protein SA0624	P67182|Y624	Ref	-2.2	-5.3	1.3	-1.9	-5.4
Cell division protein ftsZ	P99108|FTSZ	Ref	2.5	-3.1	1.9	3.8	Value Missing
Adenylosuccinate synthetase	P99099|PURA	Ref	No Values	-5.1	-0.5	No Values	-4.8
Histidine ammonia-lyase	P64416|HUTH	Ref	No Values	-9.6	Reference Missing	No Values	-9.8
Imidazolonepropionase	P64418|HUTI	Ref	No Values	0	No Values	No Values	-0.7
6-phosphofructokinase	P99165|K6PF	Ref	-3.2	No Values	Reference Missing	-1.2	No Values
Lysyl-tRNA synthetase	P67610|SYK	Ref	No Values	-1.3	-0.4	No Values	3.5
SA1599 protein	Q7A501|Q7A501	Ref	No Values	0	No Values	No Values	1.5
SA0231 protein	Q7A7W3|Q7A7W3	Ref	No Values	-3.4	Value Missing	No Values	-3.8
Phenylalanyl-tRNA synthetase beta chain	P67041|SYFB	Ref	No Values	0	No Values	No Values	-0.7
P60855|Y370_STAAN	P60855|Y370	Ref	No Values	0	No Values	No Values	-0.7
50S ribosomal protein L23	Q7A459|RL23	Ref	No Values	No Values	1.2	No Values	No Values
Putative uncharacterized protein SA0908	Q7A6A3|Q7A6A3	Ref	3	-2.3	1.5	5	-1.1
SA1475 protein	Q7A581|Q7A581	Ref	0	0	No Values	3.1	0.7
Leucyl-tRNA synthetase	P67513|SYL	Ref	No Values	-6	0.9	No Values	-6.1
Clumping factor A	Q99VJ4|CLFA	Ref	0	0	No Values	0.7	Value Missing
Acetate-CoA ligase	Q7A3A2|Q7A3A2	Ref	0	No Values	No Values	-0.2	No Values
Lactonase drp35	RANDOM_DRP35-R	Ref	0	No Values	No Values	Value Missing	No Values
50S ribosomal protein L2	P60432|RL2	Ref	No Values	0	No Values	No Values	0.1
Glucosamine--fructose-6-phosphate aminotransferase [isomerizing]	GLMS	Ref	No Values	No Values	No Values	No Values	No Values
Putative uncharacterized protein SA1743	Q7A4N7|Q7A4N7	Ref	No Values	0	No Values	No Values	2
Cell division protein	Q7A620|Q7A620	Ref	No Values	0	No Values	Reference Missing	0.7
50S ribosomal protein L18	Q7A467|RL18	Ref	No Values	-18.9	No Values	No Values	-19.2
3-hexulose-6-phosphate synthase	Q7A774|HPS	Ref	No Values	No Values	No Values	No Values	No Values
Acetoin(diacetyl) reductase	P99120|BUTA	Ref	No Values	0	No Values	No Values	0.5

Appendix 12. (Continued)

SA0022 protein	Q99XE9|Q99XE9	Ref	0	No Values	No Values	1.4	No Values
3-hydroxy-3-methylglutaryl CoA synthase	Q7A3F6|Q7A3F6	Ref	No Values	No Values	0.5	No Values	No Values
30S ribosomal protein S7	P66616|RS7	Ref	-1.5	No Values	0.8	0.3	No Values
Uncharacterized lipoprotein SA2158	Q7A3W5|Y2158	Ref	No Values	No Values	No Values	No Values	No Values
Ribonuclease J 1	Q7A682|RNJ1	Ref	No Values	No Values	-0.5	Reference Missing	No Values
Trans-2-enoyl-ACP reductase	Q7A6D8	Ref	0	No Values	No Values	1.2	No Values
2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase 2	Q7A7V0|ISPD2	Ref	No Values	No Values	0.7	No Values	No Values
30S ribosomal protein S13	P66388|RS13	Ref	No Values	No Values	Reference Missing	No Values	No Values
30S ribosomal protein S2	P66544|RS2	Ref	No Values	0	No Values	No Values	0.9
3-oxoacyl-[acyl-carrier-protein] synthase 3	P99159|FABH	Ref	No Values	No Values	No Values	No Values	No Values

Appendix 13. Statistical analyses of the first biological replicate comparing HA-MRSA USA100 and HA-MRSA USA200 from post-exponential phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	6.975562	0.483219009	6.927312411	0.2789862
Bifunctional autolysin	Q99V41|ATL_STAAN	5.104569	0.956585247	18.73978299	0.5522847
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS_STAAN	4.947719	0.572118202	11.56327181	0.3303125
Lipase 1	P65289|LIP1_STAAN	5.200267	0.790831535	15.20751659	0.4565868
Lipase 2	Q7A7P2|LIP2_STAAN	6.217630	0.485646794	7.810801323	0.2803883
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	18.71768	4.147290327	22.15706589	2.3944391
Probable transglycosylase isaA	P99160|ISAA_STAAN	1.468505	0.299009925	20.36151418	0.1726334
Putative surface protein SA2285	P61598|PLS_STAAN	0.157861	0.043353443	27.46291637	0.0250301
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	19.05092	3.570102378	18.73978299	2.0611995
Thermonuclease	Q7A6P2|NUC_STAAN	5.176559	0.538252457	10.39787968	0.3107602
Least Abundant Protein					
Enolase	P99088|ENO_STAAN	1.420859	0.164297809	11.56327181	0.0948573
				Relative Standard Deviation Average 15.53919237	

Appendix 14. Statistical analyses of the second biological replicate comparing HA-MRSA USA100 and HA-MRSA USA200 from post-exponential phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	1.551979	0.062808912	4.047019914	0.0362627 4
Bifunctional autolysin	Q99V41|ATL_STAAN	1.073382	0.273251875	25.45708615	0.1577620 4
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS_STAAN	0.816069	0.094364312	11.56327181	0.0544812 6
Lipase 1	P65289|LIP1_STAAN	1.262571	0.098616943	7.810801323	0.0569365 1
Lipase 2	Q7A7P2|LIP2_STAAN	1.705025	0.139465418	8.179669083	0.0805203 9
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	3.498565	0.40454866	11.56327181	0.2335662 7
Probable transglycosylase isaA	P99160|ISAA_STAAN	0.751904	0.163402663	21.73185194	0.0943405 7
Putative surface protein SA2285	P61598|PLS_STAAN	0.090796	0.00975663	10.74561596	0.0056329 9
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	3.326740	0.13463385	4.047019914	0.0777308 8
Thermonuclease	Q7A6P2|NUC_STAAN	2.166928	0.377881349	17.43856716	0.2181698 9
Least Abundant Protein					
Enolase	P99088|ENO_STAAN	1.131773	0.17211471	15.20751659	0.0993704 7
				Relative Standard Deviation Average 12.52651742	

Appendix 15. Statistical analyses of the third biological replicate comparing HA-MRSA USA100 and HA-MRSA USA200 from post-exponential phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	11.3863	1.574696933	13.82975009	0.90915169
Bifunctional autolysin	Q99V41|ATL_STAAN	2.31777	0.703685578	30.36035003	0.40627305
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS_STAAN	3.06652	0.58416065	19.04959703	0.33726530
Lipase 1	P65289|LIP1_STAAN	6.45108	1.345409444	20.85556249	0.77677250
Lipase 2	Q7A7P2|LIP2_STAAN	5.66591	0.392495798	6.927312411	0.22660755
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	8.42533	2.547636686	30.23782481	1.47087872
Probable transglycosylase isaA	P99160|ISAA_STAAN	1.26059	0.051016689	4.047019914	0.02945449
Putative surface protein SA2285	P61598|PLS_STAAN	0.09355	0.02793254	29.85727152	0.01612685
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	6.13305	1.168321299	19.04959703	0.67453061
Thermonuclease	Q7A6P2|NUC_STAAN	4.42601	0.737871913	16.67126423	0.42601054
Least Abundant Protein					
Enolase	P99088|ENO_STAAN	0.9137	0.074737667	8.179669083	0.04314981
				Relative Standard Deviation Average	
				18.09683806	

Appendix 16. Statistical analyses of the first biological replicate comparing HA-MRSA USA100 and CA-MRSA USA300 from post-exponential phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	20.6408	0.816267	3.954618	0.471272
Bifunctional autolysin	Q99V41|ATL_STAAN	3.60024	0.636098	17.66821	0.367252
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS_STAAN	3.48220	0	0	0
Lipase 1	P65289|LIP1_STAAN	12.7267	1.041007	8.179669	0.601025
Lipase 2	Q7A7P2|LIP2_STAAN	8.29239	1.553977	18.73978	0.897189
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	5.92752	0.234411	3.954618	0.135337
Probable transglycosylase isaA	P99160|ISAA_STAAN	0.89696	0.125036	13.93987	0.072189
Putative surface protein SA2285	P61598|PLS_STAAN	0.21798	0.015101	6.927312	0.008718
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	6.13305	1.168321	19.0496	0.674531
Thermonuclease	Q7A6P2|NUC_STAAN	10.4798	2.185624	20.85556	1.261871
Least Abundant Protein					
Enolase	P99088|ENO_STAAN	1.10097	0.118306	10.74562	0.068304
				Relative Standard Deviation Average	
				11.27408	

Appendix 17. Statistical analyses of the second biological replicate comparing HA-MRSA USA100 and CA-MRSA USA300 from post-exponential phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	12.4560	1.338483	10.74562	0.772773
Bifunctional autolysin	Q99V41|ATL_STAAN	1.15527	0.538799	46.63829	0.311076
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS_STAAN	3.41005	0.278931	8.179669	0.161041
Lipase 1	P65289|LIP1_STAAN	14.2620	0.577188	4.04702	0.33324
Lipase 2	Q7A7P2|LIP2_STAAN	15.8009	4.717723	29.85727	2.723778
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	4.40974	1.812254	41.09659	1.046306
Probable transglycosylase isaA	P99160|ISAA_STAAN	0.33369	0.063568	19.0496	0.036701
Putative surface protein SA2285	P61598|PLS_STAAN	0.1252	0.008673	6.927312	0.005007
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	8.46258	1.44561	17.08238	0.834623
Thermonuclease	Q7A6P2|NUC_STAAN	7.36710	1.258477	17.08238	0.726582
Least Abundant Protein					
Enolase	P99088|ENO_STAAN	2.05105	0.160204	7.810801	0.092494
				Relative Standard Deviation Average	
				18.95608	

Appendix 18. Statistical analyses of the third biological replicate comparing HA-MRSA USA100 and CA-MRSA USA300 from post-exponential phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	4.25341	0.924345	21.73185	0.533671
Bifunctional autolysin	Q99V41|ATL_STAAN	0.47842	0.037369	7.810801	0.021575
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS_STAAN	0.41789	0.11444	27.38478	0.066072
Lipase 1	P65289|LIP1_STAAN	2.25452	0.672825	29.84336	0.388456
Lipase 2	Q7A7P2|LIP2_STAAN	1.74389	0.120805	6.927312	0.069747
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	1.10097	0.118306	10.74562	0.068304
Probable transglycosylase isaA	P99160|ISAA_STAAN	0.17882	0.034065	19.0496	0.019667
Putative surface protein SA2285	P61598|PLS_STAAN	0.06506	0.015199	23.35989	0.008775
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	1.29216	0.105695	8.179669	0.061023
Thermonuclease	Q7A6P2|NUC_STAAN	0.87194	0.060402	6.927312	0.034873
Least Abundant Protein					
Enolase	P99088|ENO_STAAN	0.40873	0.056527	13.82975	0.032636
				Relative Standard Deviation Average	
				15.9809	

Appendix 19. Statistical analyses of the first biological replicate comparing HA-MRSA USA100 and CA-MRSA USA400 from post-exponential phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	1.01446	0.20982	20.68284	0.12114
Bifunctional autolysin	Q99V41|ATL_STAAN	2.17455	0.44976	20.68284	0.259669
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS_STAAN	1.13374	0.193671	17.08238	0.111816
Lipase 1	P65289|LIP1_STAAN	1.05598	0.160589	15.20752	0.092716
Lipase 2	Q7A7P2|LIP2_STAAN	3.41005	0.278931	8.179669	0.161041
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	1.09741	0.044413	4.04702	0.025642
Probable transglycosylase isaA	P99160|ISAA_STAAN	0.58809	0.0238	4.04702	0.013741
Putative surface protein SA2285	P61598|PLS_STAAN	0.13222	0.022588	17.08238	0.013041
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	1.48431	0.121412	8.179669	0.070097
Thermonuclease	Q7A6P2|NUC_STAAN	1.67714	0.279601	16.67126	0.161428
Least Abundant Protein					
Enolase	P99088|ENO_STAAN	0.93762	0.116176	12.39047	0.067074
				Relative Standard Deviation Average	
				13.11391	

Appendix 20. Statistical analyses of the second biological replicate comparing HA-MRSA USA100 and CA-MRSA USA400 from post-exponential phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	1.26468	0.135898	10.74562	0.078461
Bifunctional autolysin	Q99V41|ATL_STAAN	0.85670	0.127044	14.82936	0.073349
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS_STAAN	3.11402	0.334621	10.74562	0.193193
Lipase 1	P65289|LIP1_STAAN	10.5729	0.732423	6.927312	0.422865
Lipase 2	Q7A7P2|LIP2_STAAN	3.32674	0.134634	4.04702	0.077731
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	3.28699	1.428436	43.45718	0.824708
Probable transglycosylase isaA	P99160|ISAA_STAAN	1.58824	0.062809	3.954618	0.036263
Putative surface protein SA2285	P61598|PLS_STAAN	0.11620	0.027602	23.75351	0.015936
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	5.79219	0.234411	4.04702	0.135337
Thermonuclease	Q7A6P2|NUC_STAAN	2.04051	0.481623	23.603	0.278065
Least Abundant Protein					
Enolase	P99088|ENO_STAAN	0.56152	0.022206	3.954618	0.012821
				Relative Standard Deviation Average	
				13.64226	

Appendix 21. Statistical analyses of the third biological replicate comparing HA-MRSA USA100 and CA-MRSA USA400 from post-exponential phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	3.31918	0.860895	25.93696	0.497038
Bifunctional autolysin	Q99V41|ATL_STAAN	2.15409	0.266903	12.39047	0.154096
Glycerol phosphate lipoteichoic acid synthase	Q7A6U1|LTAS_STAAN	2.10945	0.312818	14.82936	0.180606
Lipase 1	P65289|LIP1_STAAN	11.4446	2.180165	19.0496	1.258719
Lipase 2	Q7A7P2|LIP2_STAAN	5.16867	0.42278	8.179669	0.244092
Penicillin binding protein 2'	Q7A8C6|Q7A8C6_STAAN	2.00154	0.555139	27.7356	0.32051
Probable transglycosylase isaA	P99160|ISAA_STAAN	1.17618	0.0476	4.04702	0.027482
Putative surface protein SA2285	P61598|PLS_STAAN	0.14702	0.00595	4.04702	0.003435
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	4.82990	0.502207	10.39788	0.28995
Thermonuclease	Q7A6P2|NUC_STAAN	2.83787	0.759821	26.77427	0.438683
Least Abundant Protein					
Enolase	P99088|ENO_STAAN	1.29414	0.134563	10.39788	0.07769
				Relative Standard Deviation Average	
				14.88961	

Appendix 22. Statistical analyses of the first biological replicate comparing HA-MRSA USA100 and HA-MRSA USA200 from stationary phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	2.58828	0.269126	10.39788	0.15538
DNA-binding protein HU	Q7A5J1|DBH_STAAN	0.52736	0.078205	14.82936	0.045151
Elongation factor Tu	P99152|EFTU_STAAN	1.00641	0.139185	13.82975	0.080358
Enolase	P99088|ENO_STAAN	1.38491	0.113281	8.179669	0.065403
Formate--tetrahydrofolate ligase	Q7A535|FTHS_STAAN	0.73816	0.181347	24.5674	0.104701
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1_STAAN	2.85928	0.498617	17.43857	0.287877
Lipase 1	P65289|LIP1_STAAN	9.46840	1.319883	13.93987	0.762035
Lipase 2	Q7A7P2|LIP2_STAAN	3.10395	0.125618	4.04702	0.072525
Putative surface protein SA2285	P61598|PLS_STAAN	0.11164	0.038674	34.64102	0.022329
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	12.1451	0.841333	6.927312	0.485744
Least Abundant Protein					
Serine protease splB	Q7A4Y1|SPLB_STAAN	2.58010	0.102033	3.954618	0.058909
				Relative Standard Deviation Average	
				13.88659	

Appendix 23. Statistical analyses of the second biological replicate comparing HA-MRSA USA100 and HA-MRSA USA200 from stationary phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	1.12305	0.044413	3.954618	0.025642
DNA-binding protein HU	Q7A5J1|DBH_STAAN	1.04784	0.041438	3.954618	0.023924
Elongation factor Tu	P99152|EFTU_STAAN	1.15053	0.079701	6.927312	0.046016
Enolase	P99088|ENO_STAAN	1.15606	0.159881	13.82975	0.092308
Formate--tetrahydrofolate ligase	Q7A535|FTHS_STAAN	1.17999	0.126798	10.74562	0.073207
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1_STAAN	1.20366	0.0476	3.954618	0.027482
Lipase 1	P65289|LIP1_STAAN	1.59106	0.426635	26.81436	0.246318
Lipase 2	Q7A7P2|LIP2_STAAN	1.20563	0.098617	8.179669	0.056937
Putative surface protein SA2285	P61598|PLS_STAAN	0.09647	0.021376	22.15707	0.012342
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	1.55701	0.16731	10.74562	0.096597
Least Abundant Protein					
Serine protease splB	Q7A4Y1|SPLB_STAAN	1.35545	0.145652	10.74562	0.084092
				Relative Standard Deviation Average	
				11.09171	

Appendix 24. Statistical analyses of the third biological replicate comparing HA-MRSA USA100 and HA-MRSA USA200 from stationary phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	0.60183	0.0238	3.954618	0.013741
DNA-binding protein HU	Q7A5J1|DBH_STAAN	0.16219	0.072474	44.68319	0.041843
Elongation factor Tu	P99152|EFTU_STAAN	0.07019	0.002776	3.954618	0.001603
Enolase	P99088|ENO_STAAN	0.08088	0.00841	10.39788	0.004856
Formate--tetrahydrofolate ligase	Q7A535|FTHS_STAAN	0.25040	0.017346	6.927312	0.010015
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1_STAAN	0.05993	0.006437	10.74562	0.003716
Lipase 1	P65289|LIP1_STAAN	0.49038	0.05099	10.39788	0.029439
Lipase 2	Q7A7P2|LIP2_STAAN	0.58901	0.046006	7.810801	0.026562
Putative surface protein SA2285	P61598|PLS_STAAN	0.05076	0	0	0
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	0.33828	0.026424	7.810801	0.015256
Least Abundant Protein					
Serine protease splB	Q7A4Y1|SPLB_STAAN	0.32728	0.071125	21.73185	0.041064
				Relative Standard Deviation Average	
				11.67405	

Appendix 25. Statistical analyses of the first biological replicate comparing HA-MRSA USA100 and CA-MRSA USA300 from stationary phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	15.6923	1.631676	10.39788	0.942049
DNA-binding protein HU	Q7A5J1|DBH_STAAN	0.77599	0.031404	4.04702	0.018131
Elongation factor Tu	P99152|EFTU_STAAN	2.19483	0.088825	4.04702	0.051283
Enolase	P99088|ENO_STAAN	1.92932	0.340877	17.66821	0.196805
Formate--tetrahydrofolate ligase	Q7A535|FTHS_STAAN	1.71544	0.260877	15.20752	0.150617
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1_STAAN	5.61599	1.171247	20.85556	0.67622
Lipase 1	P65289|LIP1_STAAN	5.68343	0.657191	11.56327	0.37943
Lipase 2	Q7A7P2|LIP2_STAAN	7.87275	1.167478	14.82936	0.674044
Putative surface protein SA2285	P61598|PLS_STAAN	0.11324	0.035899	31.69967	0.020726
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	5.16021	0.204067	3.954618	0.117818
Least Abundant Protein					
Serine protease splB	Q7A4Y1|SPLB_STAAN	16.7655	0.663015	3.954618	0.382792
				Relative Standard Deviation Average	
				12.56589	

Appendix 26. Statistical analyses of the second biological replicate comparing HA-MRSA USA100 and CA-MRSA USA300 from stationary phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	14.2620	0.577188	4.04702	0.33324
DNA-binding protein HU	Q7A5J1|DBH_STAAN	10.0847	0.408134	4.04702	0.235636
Elongation factor Tu	P99152|EFTU_STAAN	16.1745	2.820606	17.43857	1.628478
Enolase	P99088|ENO_STAAN	15.0243	2.077825	13.82975	1.199633
Formate--tetrahydrofolate ligase	Q7A535|FTHS_STAAN	9.30818	3.193771	34.31142	1.843925
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1_STAAN	10.9418	2.050485	18.73978	1.183848
Lipase 1	P65289|LIP1_STAAN	18.4655	2.135221	11.56327	1.23277
Lipase 2	Q7A7P2|LIP2_STAAN	19.6983	0	0	0
Putative surface protein SA2285	P61598|PLS_STAAN	0.46480	0.110408	23.75351	0.063744
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	19.6983	0	0	0
Least Abundant Protein					
Serine protease splB	Q7A4Y1|SPLB_STAAN	11.4446	2.180165	19.0496	1.258719
				Relative Standard Deviation Average	
				13.34363	

Appendix 27. Statistical analyses of the third biological replicate comparing HA-MRSA USA100 and CA-MRSA USA300 from stationary phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	7.15414	0.768757	10.74562	0.443842
DNA-binding protein HU	Q7A5J1|DBH_STAAN	0.37107	0.030353	8.179669	0.017524
Elongation factor Tu	P99152|EFTU_STAAN	0.21798	0.015101	6.927312	0.008718
Enolase	P99088|ENO_STAAN	0.30827	0.021355	6.927312	0.01233
Formate--tetrahydrofolate ligase	Q7A535|FTHS_STAAN	0.23440	0.029044	12.39047	0.016769
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1_STAAN	0.17706	0.012265	6.927312	0.007081
Lipase 1	P65289|LIP1_STAAN	4.01386	1.149492	28.63803	0.663659
Lipase 2	Q7A7P2|LIP2_STAAN	6.71829	1.187002	17.66821	0.685316
Putative surface protein SA2285	P61598|PLS_STAAN	0.06129	0.006374	10.39788	0.00368
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	1.95856	0.160204	8.179669	0.092494
Least Abundant Protein					
Serine protease splB	Q7A4Y1|SPLB_STAAN	1.74389	0.120805	6.927312	0.069747
				Relative Standard Deviation Average	
				11.26443	

Appendix 28. Statistical analyses of the first biological replicate comparing HA-MRSA USA100 and CA-MRSA USA400 from stationary phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	1.741101	0	0	0
DNA-binding protein HU	Q7A5J1|DBH_STAAN	0.630299	0.025508	4.04702	0.014727
Elongation factor Tu	P99152|EFTU_STAAN	1.124897	0.092013	8.179669	0.053124
Enolase	P99088|ENO_STAAN	0.961342	0.13401	13.93987	0.077371
Formate--tetrahydrofolate ligase	Q7A535|FTHS_STAAN	0.795422	0.065063	8.179669	0.037564
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1_STAAN	1.387025	0.144221	10.39788	0.083266
Lipase 1	P65289|LIP1_STAAN	6.997131	0.809097	11.56327	0.467133
Lipase 2	Q7A7P2|LIP2_STAAN	5.665917	0.392496	6.927312	0.226608
Putative surface protein SA2285	P61598|PLS_STAAN	0.084859	0.023239	27.38478	0.013417
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	5.53058	0.218713	3.954618	0.126274
Least Abundant Protein					
Serine protease splB	Q7A4Y1|SPLB_STAAN	4.396529	0.343404	7.810801	0.198264
				Relative Standard Deviation Average	
				9.307717	

Appendix 29. Statistical analyses of the second biological replicate comparing HA-MRSA USA100 and CA-MRSA USA400 from stationary phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	1.41647	0.098124	6.927312	0.056652
DNA-binding protein HU	Q7A5J1|DBH_STAAN	1.15059	0.079701	6.927312	0.046016
Elongation factor Tu	P99152|EFTU_STAAN	1.78853	0.192189	10.74562	0.11096
Enolase	P99088|ENO_STAAN	1.76009	0.306935	17.43857	0.177209
Formate--tetrahydrofolate ligase	Q7A535|FTHS_STAAN	1.49338	0.227107	15.20752	0.13112
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1_STAAN	1.66876	0.179319	10.74562	0.10353
Lipase 1	P65289|LIP1_STAAN	2.04784	0.082877	4.04702	0.047849
Lipase 2	Q7A7P2|LIP2_STAAN	1.82740	0.149475	8.179669	0.0863
Putative surface protein SA2285	P61598|PLS_STAAN	0.14046	0.064804	46.13595	0.037415
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	2.58010	0.102033	3.954618	0.058909
Least Abundant Protein					
Serine protease splB	Q7A4Y1|SPLB_STAAN	1.87803	0.259728	13.82975	0.149954
				Relative Standard Deviation Average	
				13.10354	

Appendix 30. Statistical analyses of the third biological replicate comparing HA-MRSA USA100 and CA-MRSA USA400 from stationary phase

Ten Most Abundant Proteins	**Accession Number**	**Average**	**Standard Deviation**	**Relative Standard Deviation**	**Standard Error**
Alpha-Hemolysin	Q7A632|Q7A632_STAAN	0.72515	0.056641	7.810801	0.032701
DNA-binding protein HU	Q7A5J1|DBH_STAAN	0.17286	0.067446	39.01742	0.03894
Elongation factor Tu	P99152|EFTU_STAAN	0.08880	0.010269	11.56327	0.005929
Enolase	P99088|ENO_STAAN	0.12221	0.004833	3.954618	0.00279
Formate--tetrahydrofolate ligase	Q7A535|FTHS_STAAN	0.23961	0.025748	10.74562	0.014866
Glyceraldehyde-3-phosphate dehydrogenase 1	P99136|G3P1_STAAN	0.07031	0.018855	26.81436	0.010886
Lipase 1	P65289|LIP1_STAAN	2.60351	0.817272	31.39105	0.471852
Lipase 2	Q7A7P2|LIP2_STAAN	1.70223	0.067317	3.954618	0.038865
Putative surface protein SA2285	P61598|PLS_STAAN	0.05076	0	0	0
SA0841 protein	Q7A6G0|Q7A6G0_STAAN	1.35107	0.054678	4.04702	0.031569
Least Abundant Protein					
Serine protease splB	Q7A4Y1|SPLB_STAAN	0.45432	0.196599	43.27277	0.113506
				Relative Standard Deviation	
				16.59741	