DEVELOPMENT OF A DIAGNOSTIC METHOD TO ALLOW STRAIN-LEVEL IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS* BASED ON THE NUCLEOTIDE SEQUENCE OF THE ENTEROTOXIN GENE AND THE AMINO ACID SEQUENCE OF ITS ENTEROTOXIN

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DEVELOPMENT OF A DIAGNOSTIC METHOD TO ALLOW STRAIN-LEVEL IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS* BASED ON THE NUCLEOTIDE SEQUENCES OF THE ENTEROTOXIN GENE AND THE AMINO ACID SEQUENCE OF ITS ENTEROTOXIN

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CHAPTER I

INTRODUCTION

Of the 32 bacterial species of staphylococci, *Staphylococcus aureus* is the most important as it is the most common member to be associated with staphylococcal infections and intoxication (Bergdoll 1989). Since the first observance of *S. aureus* in the early 1800's, the pathogen has been implicated in a variety of illnesses including nosocomial infections and foodborne intoxication. *Staphylococcus aureus* is an especially pathogenic microorganism because it possesses a variety of virulence factors that allow it not only to attach to host cells, but invade tissues and evade the host's immune system (Freeman-Cook and Freeman-Cook 2006).

Mammals, including humans, are the primary reservoir of the organism allowing for easy transfer of the microbe leading to nosocomial infections as well as the contamination of foods (Freeman-Cook and Freeman Cook 2006; Bergdoll 1989). The most important feature of some strains of *S. aureus* is the ability to produce potent, heat stable enterotoxins that not only display emetic properties but also act as super antigens, thus increasing the virulence of the organism. Enterotoxigenic strains of *S. aureus* are capable of producing up to 9 serologically different enterotoxins as well as a variety of enterotoxin-like

substances that are capable of causing severe illness in humans (Thomas and others 2007). Illness due to staphylococcal intoxication is usually acquired through ingestion of contaminated foods that contain preformed toxins and is the third most common cause of food poisoning in the world (Atanassova and others 2001). Because of the unique characteristics of the staphylococcal enterotoxins, mainly their ability to resist heat inactivation, they are extremely difficult to eliminate from foods in which they have all ready formed (Schantz and others 1965; Fung and others 1973). When this characteristic is paired with the fact that enterotoxin is rarely detected in foods until a large number of consumers become ill, it is clear that *S. aureus* poses a credible threat to the world-wide population.

CHAPTER II

REVIEW OF LITERATURE

Staphylococcus aureus

History

Alexander Ogston first observed the staphylococci while performing experiments to prove his theory that cocci were pathogenic microorganisms and played a fundamental role in the formation of pus-filled infections, mainly abscesses (Bergdoll 1989). Ogston was able to stain the cocci using methyl-aniline which allowed him to study the different types of cocci in reference to their growth forms. He described the cocci, which he named *Staphylococcus*, as being “individual spheres in masses [that] looked like bunches of grapes”. Ogston was further able to demonstrate a causal relationship between the micrococci and the formation of pus when he fulfilled Koch’s postulates by injecting mice with pus from abscesses and was able to recreate the original infection (Ogston 1984). In 1804, Rosenbach isolated Ogston’s cocci from pus and is credited with describing the bacterial genus *Staphylococcus* and naming the organism *Staphylococcus aureus* for the yellow pigmentation it produced (Bergdoll 1989).

The staphylococci were not originally accepted as an individual genus by many microbiologists, and were instead classified in the genus *Micrococcus*. However, *Staphylococcus* was eventually recognized as an individual genus in the 7th edition of Bergey's Manual of Determinative Bacteriology based on such factors as its ability to grow anaerobically, its cell wall structure and its fatty acid profile (Bergdoll 1989).

Characteristics

Staphylococcus aureus is a Gram positive coccus-shaped microorganism that generally occurs in grape-like clusters, but can also be found in singles and pairs (Tortora and others 2002; Ryan 2004). Cells are non-motile, lack flagella and do not form spores, though they are able to survive in a dormant state for years under unfavorable conditions (Ryan 2004; Freeman-Cook and Freeman-Cook 2006). *Staphylococcus aureus* grows best under aerobic conditions but is able to employ a fermentative metabolism, making it a facultative anaerobe. The organism is able to utilize several different carbohydrates during respiration. However, under anaerobic conditions, *S. aureus* will typically ferment glucose resulting in the production of lactic acid (Minor and Marth 1976).

Members of the species grow in the presence of salt, under high osmotic pressure, low moisture and at wide pH and temperature ranges (Tortora and others 2002; Freeman-Cook and Freeman-Cook 2006). *Staphylococcus aureus* has been shown to grow readily in media containing 5% NaCl and many strains have demonstrated the ability to grow in the presence of 10 to 15% salt (Minor and Marth 1976). Research conducted by Potter and Leistner found that *S.*

aureus can grow at a water activity as low as 0.864 though enterotoxin is usually not produced below 0.90 (1978). *Staphylococcus aureus* is able to grow in a pH range from 4.2 to 9.3 and in a temperature range from 6.5 to 50º C with an optimum growth temperature of 30 to 40º C (Baird-Parker 1965).

As with all Gram-positive bacteria, the cell wall contains peptidoglycan; however, it is also contains ribitol-teichoic acid molecules that are specific to *S. aureus* and act as antigens (Ryan 2004). In addition to the presence of ribitol-teichoic acid in the cell wall of *S. aureus*, some strains may also exhibit the presence of protein A, that can comprise up to 7% of the cell wall and may coat the outside of the cell (Gao and Stewart 2004). Both ribitol-teichoic acid and protein A work to increase the virulence of the microorganism (Ryan 2004). The cell wall of *S. aureus* is also very thick in comparison with other Gram-positive bacteria. This increased thickness provides the organism with a very high internal pressure making it nearly impossible for many antimicrobial drugs to enter the cell (Freeman-Cook and Freeman-Cook 2006).

Staphylococcus aureus can be easily distinguished from pathogenic streptococci by its production of catalase. However, it is relatively similar to the other less virulent staphylococci. Due to this similarity *S. aureus* must be distinguished via diagnostic tests. Most strains of *S. aureus* will exhibit β-hemolysis when grown on blood agar which can be a distinguishing characteristic. However, the production of the enzyme coagulase is the most common distinguishing factor for *S. aureus*. Coagulase binds to prothrombin in the blood causing fibrin to be polymerized, resulting in the formation of a clot

(Ryan 2004). The coagulase produced by *S. aureus* is considered a determinant of the pathogenicity of the organism (Halpin-Dohnalek and Marth 1989).

Virulence Factors

Staphylococcus aureus possesses several virulence factors that can be placed into three broad categories: attachment, evasion of the host immune response and invasion of host tissue. No one factor can be held solely responsible for an infection, but rather it is the sum of all of the organism's virulence factors that make *S. aureus* such a successful pathogen (Freeman-Cook and Freeman-Cook 2006).

Adherence to host cells is the first hurdle that *S. aureus* must overcome because the pathogen must be able to attach to a host surface in order for colonization or infection to occur (Freeman-Cook and Freeman-Cook 2006). *Staphylococcus aureus* usually accomplishes this adhesion by utilizing surface protein binding mechanisms such as fibronectin-binding and collagen-binding (Foster and Höök 1998). Fibronectin-binding occurs when one of two fibronectin-binding proteins, FnBPA and FnBPB, produced by *S. aureus* negotiates the attachment of the bacterial cells to fibronectin in plasma clots and foreign materials, such as heart valves, within the body (Jönsson and others 1991; Foster and Höök 1998). Collagen-binding works in much the same way as fibronectin-binding where the staphylococcal binding protein, Cna, allows the bacterial cells to bind to collagen-containing tissues such as cartilage (Switalski and others 1989). However, collagen-binding via Cna is less common because not all strains of *S. aureus* produce the protein (Foster and Höök 1998). In order

to ease the attachment process, many strains will seek out blood clots or wounded tissue (Freeman-Cook and Freeman-Cook 2006).

Once the pathogen has attached to the host cells, it must find a way to escape the resulting immune response. *Staphylococcus aureus* does this through the production of specific enzymes, proteins, extracellular structures and superantigens (Freeman-Cook and Freeman-Cook 2006). As a defense against infection, host cells will often release lipids capable of disrupting bacterial membranes. In order to circumvent this mechanism, *S. aureus* produces the enzyme lipase to break down the lipids before they can attack the cell membrane (Freeman-Cook and Freeman-Cook 2006). This action gives the pathogen time to solidify its adherence and begin multiplying, thus aiding in the spread of the organism (Rooijakkers and others 2005; Foster 2005). The hallmark of the human immune system is the production of antibodies that mark infecting bacteria for destruction by white blood cells. To counteract this action *S. aureus* cells excrete proteases: enzymes that are able to inactivate antibodies by digesting them into smaller pieces (Freeman-Cook and Freeman-Cook 2006; Foster 2005).

Staphylococcus aureus also produces protein A, which interacts with antibodies to aid in the avoidance of phagocytosis (Freeman-Cook and Freeman-Cook 2006; Dossett and others 1969). Protein A is anchored into the bacterial cell wall and is also excreted by the cell into the surrounding environment. Protein A anchored in the cell wall binds to the Fc region of IgG causing the antibodies to adhere to one another and bind to their complement, resulting in

their inability to bind to the bacterial cell and mark it for phagocytosis (Foster 2005; Freeman-Cook and Freeman-Cook 2006). Additionally, the freely excreted protein A binds to the antibodies adhered to the surface of the bacteria and acts as a sort of smoke screen to hide the cell from new antibodies (Freeman-Cook and Freeman-Cook 2006). A study conducted by Dossett and others found that there is an association between the concentration of protein A produced by the cell and its resistance to phagocytosis; thus, those strains that produce higher amounts of the protein are more likely to survive within the host (1969). Furthermore, it has been shown that protein A is only successful against IgG antibodies and can actually quicken the phagocytic process when other immunoglobulins are involved (Peterson and others 1977).

Lastly, *S. aureus* can avoid phagocytosis by producing a cell wall capsule, or by aggregating into a biofilm. Production of a thin polysaccharide capsule effectively hides the cell because antibodies are not able to bind to the cell wall nor are white blood cells able to recognize it as being pathogenic (Foster 2005; Freeman-Cook and Freeman-Cook 2006). Though researchers know that *S. aureus* is able to produce a capsule and that it plays a role in pathogenicity, it is still unclear how the organism produces the capsule and under what precise conditions (Honeyman and others 2001). Alternatively, when bacteria join to form a biofilm on an artificial surface within the body, they are sheltered by a layer of polysaccharides that prevents antibodies and white blood cells from reaching them (Freeman-Cook and Freeman-Cook 2006).

The last method by which *S. aureus* is able to evade the immune system is through the production of superantigens. Superantigens are exotoxins produced by the cell that are able to activate large numbers of T cells, by binding to the T cells β-chain, resulting in an over-release of the cytokine interleukin-2 (Holtfreter and Bröker 2005; Honeyman and others 2001; Freeman-Cook and Freeman-Cook 2006). An increased level of interleukin-2 in the body generally leads to fever and vomiting, but in some cases the immune response may become severe enough to result in multiple organ failure (Freeman-Cook and Freeman-Cook 2006). Once the over-activation of T cells has occurred, the immune system of the host becomes suppressed and is unable to produce antibodies against the toxins or the bacterial cells leading to the further spread of the microorganism (Foster 2005). *Staphylococcus aureus* is capable of producing 19 different superantigens, including the staphylococcal enterotoxins, which affect T cells in different ways. Because more than one superantigen can be produced by a cell at the same time, it can have a devastating effect on the immune system of the host (Holtfreter and Bröker 2005; Choi and others 1989).

The last category of virulence factors exhibited by *S. aureus* includes those that aid the organism in invading host tissues. In order for a staphylococcal infection to spread, the pathogen must break down surrounding tissues that act as a physical barrier. In the case of *S. aureus* this process is carried out primarily by the protein α-toxin, also known as alpha-hemolysin (Honeyman and others 2001). Alpha-hemolysin works by infiltrating the lipid bilayer of the host tissue cells leading to apoptosis and thus promoting the

spread of the infection (Freer and others 1968). Alpha-toxin is also capable of killing platelets, red blood cells and immune cells resulting in a decreased immune response (Bhakdi and Tranum-Jensen 1991; Freeman-Cook and Freeman-Cook 2006).

Carriage

Staphylococcus aureus is fairly ubiquitous in nature and can be found in water, raw milk, air, decaying vegetation and sewage, though the mucous membranes and skin of mammals, including humans, are its primary reservoir (Freeman-Cook and Freeman-Cook 2006; Bergdoll 1989). The skin of most healthy humans is colonized by *S. aureus*, though they don't usually become infected because the epidermis serves as a barrier through which the bacteria can not pass into the body. However, if the bacteria are given an entry point via an injury or surgical site, it can lead to an infection (Freeman-Cook and Freeman-Cook 2006). Of the possible skin colonization sites, rectal carriage seems to be the most significant because it may lead to colonization of the G.I. tract. Aditionally, research has shown that people who carry *S. aureus* in both the nasal and rectal regions are more likely to develop an infection than those who only exhibit nasal carriage (Squier and others 2002). It is important to note that while all skin carriers are not necessarily nasal carriers; nasal carriers are more likely to carry *S. aureus* at other body sites such as the hands and the perineum (Polakoff and others 1967).

While *S. aureus* lives on the skin of many humans, colonization of the mucous membranes is usually of greater concern. The nasal membranes of

humans provide an ideal colonization site for *S. aureus* because they are damp and in the optimum temperature range of the pathogen (Freeman-Cook and Freeman-Cook 2006). It is important to note that though the nasal membranes are the most common carriage site of *S. aureus* for humans, reported carriage frequencies may vary depending on which part of the nasal mucosa is tested (Williams 1963).

By the early 1960's researchers had identified three general nasal carriage patterns among humans. Nearly 60% of the population displays a sporadic carriage of *S. aureus* where the individual strain may vary over time; these people are referred to as intermittent carriers. Roughly another 20% of the population are constantly carrying a single strain of the pathogen and are commonly referred to as persistent carriers. Lastly, the remaining 20% of the population very rarely carry *S. aureus* and are thus referred to as non-carriers, though some members may carry the organism occasionally (Kluytmans and others 1997; Williams 1963).

These carriage patterns can be further broken down by population demographics including gender, ethnicity, age and overall health. A data study carried out at the Medical University of South Carolina found that men are more likely than women to harbor *S. aureus*; however, there are no gender-based carriage differences in infants (Mainous III and others 2006; Peacock and others 2003). Furthermore, the study indicated that people of Hispanic origin are more likely to be *S. aureus* carriers than non-Hispanic whites or non-Hispanic blacks (Mainous III and others 2006). While still not proven, some researchers believe

that this carriage pattern may be due to some sort of genetic influence (Peacock and others 2001).

Early research into the carriage rates of young children found that in one hospital maternity ward nearly 100% of the newborn infants were nasally positive for *S. aureus* within one week of birth. However, after six months of age the carriage rates declined steadily until six years of age where typical population rates were observed (Cunliffe 1949; Williams 1963). Furthermore, carriage rates begin to increase again during adulthood (Williams 1963; Mainous III and others 2006). It has also been shown that the elderly, adults over 65, are less likely to carry the microorganism; though when they do, it is likely to be a more pathogenic strain (Mainous III and others 2006). Miller and his associates theorized that the carriage rate increases after adolescence due to increased contact with children and is then reduced with older age because of a decrease in that contact (1962).

Lastly, carriage rates of *Staphylococcus aureus* can be affected by the overall health of the population. Research has shown that cigarette smoking, exposure to cigarette smoke and use of antibiotics do not increase the risk of carriage. However, people who suffer from asthma have significantly higher carriage rates than those who do not, though the reason is still unclear (Mainous III and others 2006). Similarly, HIV-positive people or those suffering from chronic diseases such as AIDS have increased carriage rates, probably due to decreased immune function though the reason is still unclear because of the

extensive use of antibiotics in these demographic groups (Kluytmans and others 1997).

Transmission

In addition to being responsible for severe infections such as pneumonia, meningitis, septic arthritis and blood infections, *S. aureus* is also the leading cause of surgical wound infections in the United States (Freeman-Cook and Freeman-Cook 2006). It has been estimated that nearly 20% of all surgical patients will acquire an infection during recovery; thus making *S. aureus* an important cause of illness and death in hospital patients (Wertheim and others 2005; Ahmed and others 1998). Most researchers believed that post-operative staphylococcal infections were due to cross-colonization from other hospital patients, but early work carried out by Kluytmans and his associates identified nasal carriage of the pathogen as a risk factor in the development of infections and attributed many of the infections to bacteria all ready colonizing the patient (1995).

When comparing the strains of *S. aureus* found in the blood of hospital patients suffering from bacteremia to those found in the patient's nasal cavities, researchers found that nasal isolates were responsible for the blood infections in nearly 80% of the cases, establishing that the majority of *S. aureus* infections are of host origin (von Eiff and others 2001). Further research carried out by Wertheim and his colleagues found that nasal carriers of *S. aureus* displayed an increased risk of developing nosocomial staphylococcal bacteremia, but that non-carriers were more likely to die from the disease. They attributed this finding to

an immunological adaptation by the carriers to their endogenous strains (2004). Wenzel and Perl theorize that the pathogen is carried from the nose to other body areas where infection is likely to occur such as surgical sites and skin wounds via the hands; if this theory is correct, it may also account for the presence of *S. aureus* on the hands of nasal carriers as described by Polkaloff and her associates (1995; 1967).

Not only does the presence of *S. aureus* in the nasal cavities of a patient increase the likelihood of a post-operative infection, but the amount being carried is a factor as well. A study carried out at the University of Louisville found that patients harboring large quantities of the pathogen, referred to as heavy carriers, had an increased chance of developing a post-operative infection when compared to non-carriers or people carrying lower numbers of the bacteria (White 1963).

In order to prevent infection from endogenous strains of *S. aureus*, researchers have suggested the elimination of nasal carriage prior to hospitalization or surgery (Kluytmans and Wertheim 2004). Most of the work conducted in this area has focused on the use of mupirocin to eradicate the organism from colonized individuals. A study conducted by the University Hospitals of Geneva compared the efficacy of intranasal treatment of colonized individuals with mupirocin to that of individuals treated with placebo and found that, while nasal carriage rates were lowered in some patients, there was no associated reduction in the carriage rates of other body areas (Harbarth and others 1999).

A later study investigated the effects of preoperative administration of mupirocin on the rates of operation site infections after surgery. Patients were treated with mupirocin intranasally for 5 days before surgery and were monitored for the presence of any *S. aureus* infections for one month postoperatively. Researchers were able to eradicate nasal carriage of the pathogen in 83.4% of the patients receiving the antibiotic; however, they observed no appreciable difference in infection rate between the treatment and placebo groups though the treatment was able to lower the number of nosocomial infections among the colonized patients (Perl and others 2002). Thus, the prophylactic use of mupirocin may be beneficial in the elimination of nosocomial infections in post-operative hospital wards (Kluytmans and Wertheim 2004).

While nosocomial or hospital acquired infections involving *S. aureus* can come directly from other patients, a large number of patients acquire the infection from hospital workers who carry their own endogenous bacteria on their hands or transfer the pathogen from other colonized patients in the hospital (Lowy 1998). This method of transfer is evidenced by the fact that not all patients who acquire a staphylococcal infection are carriers of the bacteria. A study conducted in the Netherlands looked at the *S. aureus* carriage rates of healthcare workers and found that 35% are colonized with the pathogen (Ballemans and others 1999). In order to control the number of nosocomial infections due to transient carriage of the organism by healthcare workers, many researchers have recommended that hospitals institute cleanliness measures that require employees to wear masks and follow rigorous hand hygiene protocols (Lacey and others 2001; Pittet 2001).

A hospital in the Netherlands has even gone as far as to prohibit colonized employees from going to work until they can be treated and cleared of the organism (Blok and others 2003).

Another method of *S. aureus* transfer that is becoming increasingly more common is the transfer from pets to people and vice versa. One of the first cases of this phenomenon occurred at Wrexham Maelor Hospital where screening of staff for the pathogen revealed a nurse to be colonized with the organism. After treatment, the nurse continued to test positive for *S. aureus* leading him to suspect his dog with which he shared a home. Nasal swabs from the dog tested positive for the same strain carried by the nurse, indicating that the dog was the reservoir of the pathogen in the home (Cefai and others 1994). A 2005 study looking at the staphylococcal carriage rates of veterinary personnel and dogs in a small animal clinic found that 9% of dogs may carry methicillin-resistant *S. aureus*, demonstrating the potential for cross-colonization between pets and people (Loeffler and others 2005). Lastly, Weese and his associates reviewed several cases where staphylococci were transmitted between pets and their owners and found that in some cases, the pets actually acquire the bacteria from their owners who typically work in hospital environments (2006).

Staphylococcal Enterotoxins

The staphylococcal enterotoxins are a group of globular proteins, produced by the bacterial cell during growth, with molecular weights ranging from 28,000 to 35,000 daltons (Halpin-Dohnalek and Marth1989). The molecules are single-chained, charged and incorporate large amounts of aspartic acid,

threonine and serine which account for roughly 30% of their amino acid content (Johnson and others 1991). The enterotoxins are heat stable, water and saline soluble; they exhibit emetic properties and are resistant to digestion by proteolytic enzymes (Thomas and others 2007, Honeyman and others 2001).

Heat resistance of the enterotoxins is an especially important food safety consideration (Balaban and Rasooly 2000). Early research showed that staphylococcal enterotoxins are able to survive the milk pasteurization process even at low initial concentrations (Tatini 1976). Similarly, a study conducted by Schantz and his colleagues found that enterotoxin B is able to retain activity after being heated to 60º C for up to 16 hours (1965). Even if enterotoxins are inactivated by a severe thermal process the result may only be temporary as research has shown that many of the enterotoxins possess the ability to reactivate making it nearly impossible to eliminate them once they're been formed (Fung and others 1973).

Extracellular Factors

There are a variety of outside factors such as pH, temperature, atmospheric makeup, and salt concentration that can affect the production of enterotoxins by *S. aureus* (Halpin-Dohnalek and Marth 1989). As stated earlier, *S. aureus* is able to grow at a wide pH range; however, enterotoxin production requires a more specific range. The staphylococcal enterotoxins are produced optimally at a pH range of 6.44 to 7.20, though research has shown some serotypes are produced at a pH as low as 4.5 and as high as 9.02 under certain conditions (Halpin-Dohnalek and Marth 1989; Scheusner and others 1973; Tatini

and others 1971). Temperature is also correlated with pH as optimal growth temperatures can counteract the effects of an undesirable pH (Halpin-Dohnalek and Marth 1989). The ideal temperature for growth of *S. aureus* is 37º C, though enterotoxins can be produced over a temperature range of 10 to 46º C with maximum production occurring between 40 and 45º C (Halpin-Dohnalek and Marth 1989; Tatini 1973).

Because *S. aureus* is a facultative anaerobe, it is able to grow in the absence of oxygen; however, enterotoxin production is quite limited under anaerobic conditions (Baird-Parker 1971). Jarvis and his associates observed the production of staphylococcal enterotoxins by *S. aureus* in baffled and non-baffled flasks where baffling was said to create greater aeration in the culture medium. They found that more enterotoxin was generally produced in the aerated flasks; furthermore, some strains produced nearly twice as much enterotoxin in the oxygen-rich environment (1973). However, research conducted by Marland demonstrated that enterotoxin production could actually be reduced in environments where oxygen levels were too high, though *S. aureus* was still able to grow (1967).

As stated earlier, *S. aureus* is able to grow readily in media containing 5% salt while some strains are able to survive in the presence of 10 to 15% salt; however, the salt concentration at which enterotoxins can be produced is dependent upon the pH (Minor and Marth 1976; Genigeorgis and Sadler 1966). In their study, enterotoxin was produced by *S. aureus* in media containing 8% NaCl at a pH of 5.5 but was only able to be produced up to a salt concentration

of 4% at a pH of 5.1. Similarly, enterotoxin was produced in media containing 10% salt at a pH of 6.9; thus, they concluded that enterotoxin production in the presence of NaCl is optimized at a less acidic pH (Genigeorgis and Sadler 1966). Thus, the production of enterotoxin in foods can be somewhat controlled by salt concentration and pH of the product.

Classification

The staphylococcal enterotoxins are strain specific; however, each strain has the ability to produce more than one serotype of the toxin (Halpin-Dohnalek and Marth 1989). There are several enterotoxins, currently designated as staphylococcal enterotoxins A through E and G through J along with a number of staphylococcal enterotoxin-like compounds that are closely related but lack emetic properties (Honeyman and others 2001; Thomas and others 2007). Staphylococcal enterotoxin C is further divided into 3 subtypes: C_1, C_2 and C_3 (Bennet 2001). The enterotoxins produced by *S. aureus* exhibit a high degree of homology in their amino acid sequences which has led researchers to suggest that the different enterotoxins are the result of gene duplication from an ancestral toxin-like compound (Iandolo 1989). The homology can range between 30 and 86% with all serotypes displaying similar biological roles (Betley and Mekalanos 1988; Johnson and others 1991). This homology has also enabled the categorization of the enterotoxins into three groups based on their genetic relationships. Group 1 contains staphylococcal enterotoxins A, D, E, H and J; while group 2 contains enterotoxins B, C and G. Staphylococcal enterotoxin I is

the only true enterotoxin in group 3 which also contains several enterotoxin-like substances (Thomas and others 2007).

Of the staphylococcal enterotoxins, enterotoxin A is considered to be the most toxic and is most often responsible for staphylococcal food poisoning (Huang and others 1987; Holmberg and Blake 1984). Researchers suggest that enterotoxin A is more prevalent in staphylococcal food poisoning because it is the most common type carried by humans (Casman 1965). Enterotoxin D is also commonly incriminated in outbreaks of staphylococcal foodborne illness, followed by enterotoxin B which can be implicated in 10% of cases (Jarvis and others 1973; Casman 1965). Similarly, strains of *S. aureus* that produce staphylococcal enterotoxin B are most often associated with nosocomial infections, indicating that it may be more successful in enabling tissue invasion than the other enterotoxins (Halpin-Dohnalek and Marth 1989).

Foodborne Intoxication

Staphylococcal food poisoning is one of the most common types of foodborne illness based on the number of outbreaks and cases that are reported annually; coming in third behind food borne illnesses due to *Salmonella* and *Clostridium perfringens* (Atanassova and others 2001). However, most isolated cases of staphylococcal intoxication go unreported, indicating that the disease may be more prevalent than statistics imply (Genigeorgis 1989; Tranter 1990). The primary cause of staphylococcal food poisoning is contamination of foods by carriers of enterotoxigenic *S. aureus* which then grow in the food and produce enterotoxin (Bania and others 2006). The bacteria can be introduced into food

when it comes into contact with the hands of infected workers or when nasal carriers cough or sneeze on the food (Bennet 2001). Once the bacteria has incubated in the food for anywhere from 30 minutes to 8 hours, depending on temperature, it will have produced enough toxin to make a consumer ill (Holmburg and Blake 1984). A person can experience symptoms after ingesting only 1 microgram of the preformed toxin: an amount that is produced once the bacterial population surpasses 10^5 per gram of contaminated food (International Medical Publishing 2004). In very sensitive people, symptoms may be induced by as little as 100 to 200 ng of toxin (Bennett 2001).

Once the food containing preformed enterotoxin has been ingested, the consumer will usually begin to experience symptoms within 2 to 6 hours (Balaban and Rasooly 2000). The exact mechanism by which staphylococcal enterotoxins induce illness is still unknown, but researchers theorize that they bind to “emesis receptors” in the GI tract to induce vomiting, thus freeing the bacteria from the acidic digestive system (Freeman-Cook and Freeman-Cook 2006). Symptoms of intoxication include nausea, vomiting, stomach cramps, diarrhea and fatigue (Freeman-Cook and Freeman-Cook 2006; International Medical Publishing 2004). People affected by the illness will usually recover within 1 to 3 days without medical intervention and the intoxication is rarely fatal; though the elderly have displayed increased rates of illness and death (Tranter 1990; Balaban and Rasooly 2000).

Any food or food product that has the appropriate pH and salt concentration and is held at the right temperature can serve as a medium for

enterotoxigenic *S. aureus* to grow and produce enterotoxin (Halpin-Dohnalek and Marth 1989). Foods that have been implicated in staphylococcal intoxication outbreaks include milk products, meat and poultry, eggs products, salads, sandwiches and sandwich fillings, cream-filled bakery products such as pastries, pies and éclairs; as well as foods that require hands-on preparation and those that are held above refrigeration temperatures after preparation (Bennett 2001). The foods that are implicated in outbreaks differ depending on the country in which the outbreak occurs. For example staphylococcal intoxication outbreaks in the UK are typically due to meat products such as ham; outbreaks in France are most often due to milk products; in the United States the culprit is usually red meat (Le Loir and others 2003).

Staphylococcus aureus is quite ubiquitous in nature and so cannot be eliminated from the people who prepare food. However, researchers have made suggestions to aid consumers and food service establishments in avoiding outbreaks of staphylococcal intoxication: foods should be held at or below 10º C, or above 45º C to prevent excessive growth of the pathogen. They also suggest that refrigerated foods be packaged into small portions to ease quick cooling of the food product (Bennett 2001).

Genetic Control of Enterotoxin Production

Each staphylococcal enterotoxin is encoded by an individual gene within the bacterial DNA; however, strains of *S. aureus* typically carry more than one of these enterotoxin genes and are thus able to produce multiple serotypes of the toxin (Halpin-Dohnalek and Marth 1989).

The staphylococcal enterotoxin A gene, *entA*, consists of approximately 770 base pairs and encodes for a 257 amino acid protein precursor which includes a 24 amino acid signaling sequence. This sequence is cleaved after translation resulting in an active protein containing 233 amino acids (Betley and Mekalanos 1988). The *entA* gene differs from the genes encoding the other staphylococcal enterotoxins in a few important ways. The gene encoding enterotoxin A is carried by a bacteriophage that is integrated into the bacterial chromosome as opposed to being naturally present like some other staphylococcal enterotoxin genes (Betley and Mekalanos 1985; Balaban and Rasooly 2000). Another special characteristic of the *entA* gene is that, unlike the other enterotoxin genes, it is not regulated by the accessory gene regulator or *agr*. Researchers believe that this lack of *agr* regulation may be responsible for the prevalence of enterotoxin A in foodborne intoxications involving *S. aureus* (Tremaine and others 1993).

The *entB* gene is composed of roughly 900 nucleotides that encode for an enterotoxin B precursor protein consisting of 267 amino acid residues including a 27 residue membrane-bound signaling sequence; thus, the active protein contains 240 amino acids (Johns and Khan 1988; Tweten and Iandolo 1981). Research has shown that *entB* can be chromosomally located in *S. aureus* as well as being carried by a plasmid. Early work conducted by Shalita and others found that enterotoxin B production in *S. aureus* can be encoded by a plasmid that also carries genes for resistance to methicillin and tetracycline (1977). However, further research carried out at Kansas State University found

that the *entB* gene is located on the actual bacterial chromosome in strains of *S. aureus* isolated from foods involved in outbreaks of staphylococcal intoxication (Shafer and Iandolo 1978).

The 3 subtypes of staphylococcal enterotoxin C: SEC_1, SEC_2, and SEC_3 are antigenically different but exhibit considerable cross-reactivity (Balaban and Rasooly 2000; Bergdoll and others 1965). The staphylococcal enterotoxin C_3 gene, *entC3*, consists of about 800 base pairs and encodes for a precursor protein containing 266 amino acids, 27 of which serve as a signaling sequence. The mature SEC_3 protein contains 239 amino acids (Hovde and others 1990). When researchers aligned the nucleotide sequences of the 3 SEC genes, they found that not only did each gene contain 801 base pairs, but they demonstrated substantial homology as well. When mature, SEC_2 and SEC_3 proteins only differ by 4 amino acids; similarly, SEC_1 differs from SEC_2 by 7 amino acids and from SEC_3 by 9 (Hovde and others 1990). This extensive similarity is most likely responsible for the cross-reactivity of the enterotoxin C subtypes.

The gene encoding for staphylococcal enterotoxin D, *entD*, is located on a plasmid that also encodes for production of staphylococcal enterotoxin J and the enzyme penicillinase, which confers the bacterium with resistance to the antibiotic penicillin. The gene consists of 774 nucleotides that encode for a protein containing 258 amino acids, 30 of which make up the signaling peptide (Bayles and Iandolo 1989).

The gene, *entE*, encoding for production of staphylococcal enterotoxin E, is made up of 774 base pairs that encode for a protein precursor with 257 amino

acids; once processed, the active extracellular protein contains 230 amino acids. Staphylococcal enterotoxin E is very closely related to enterotoxin A. When the nucleotide sequences of the *entA* and *entE* genes were compared, an 84% sequences homology was observed. Comparison of the amino acid sequences of the 2 genes also indicated a close genetic relationship (Couch and others 1988).

Staphylococcal enterotoxin G is encoded by the *entG* gene which consists of 774 base pairs that are processed to form a protein precursor made up of 258 amino acids. Once the signaling sequence has been cleaved, the active protein contains 233 amino acids. Nucleotide sequence comparison has shown staphylococcal enterotoxin G to be genetically similar to both SEB and SEC, as well as an enterotoxin produced by some species of *Streptococcus*; streptococcal pyrogenic enterotoxin A or SpeA (Munson and others 1998).

The staphylococcal enterotoxin H gene, *entH*, contains 725 nucleotides that encode for a precursor protein that is 241 amino acid residues long, 24 of which comprise the signaling sequence; thus, the active protein contains 217 amino acids. Comparison of the SEH protein sequence to those of other staphylococcal enterotoxins revealed that enterotoxin H is most closely related to SEA, SED, and SEB where homologies of 37%, 33%, and 27% respectively were observed (Ren and others 1994). Because SEH displays relatively low homology to other staphylococcal enterotoxins, it is theorized that it may be a more distant enterotoxin (Balaban and Rasooly 2000).

Staphylococcal enterotoxin I is encoded by the *entI* gene which consists of 726 base pairs that encode for a precursor protein containing 242 amino acids

including a 24 residue signaling sequence. The active protein contains 218 amino acids (Munson and others 1998; Balaban and Rasooly 2000). When nucleotide and protein sequences are compared, staphylococcal enterotoxin I has the lowest homology to other enterotoxins (Balaban and Rasooly 2000). Research has shown SEI to be structurally different from other enterotoxins in that it is vulnerable to damaging enzymes such as proteases. Similarly, the protein is less stable and must be ingested in higher doses to elicit an emetic reaction (Munson and others 1998).

As stated earlier, the plasmid that carries the *entD* gene also carries the staphylococcal enterotoxin J gene, *entJ*. The entJ gene encodes for a precursor protein containing 269 amino acids that, when aligned, exhibits a 64-66% homology to the SED protein. The 2 genes are separated by 895 nucleotides and are oriented as to be read in opposite directions on the plasmid. Further research utilizing PCR amplification indicates that the staphylococcal J gene is present on all plasmids that encode for the production of SED (Zhang and others 1998).

OBJECTIVE OF PRESENT STUDY

Vegetative cells of *S. aureus* are easily killed by most heat treatments currently being utilized in the food industry, including pasteurization. However, staphylococcal enterotoxins are able to survive these thermal processes and remain in the food where they pose a serious threat of illness to consumers. While the serotype of the staphylococcal enterotoxin present in an intoxicated food can be easily deduced using various laboratory testing kits, at the current time there are no means by which to trace the enterotoxin to the specific strain of *Staphylococcus aureus* by which it was produced.

The purpose of this research study is to examine enterotoxigenic strains of *S. aureus* for variations in the nucleotide sequence of the enterotoxin gene and the amino acid sequence of the enterotoxin using staphylococcal enterotoxin A as a model. Our hope is that any observed differences will allow for a strain-level identification of the strain of *S. aureus* that produced the toxin. This methodology could then be applied to all enterotoxin serotypes produced by strains of *S. aureus* resulting in the development of a database that would facilitate the identification of a specific strain of *Staphylococcus* based on the nucleotide sequence of its enterotoxin.

The development of such a database would not only be beneficial to food manufacturers in locating the possible sources of staphylococcal contamination, but could also be employed in the event that staphylococcal enterotoxin is purposely introduced into the food supply.

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CHAPTER III

DEVELOPMENT OF A DIAGNOSTIC METHOD TO ALLOW STRAIN-LEVEL IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS* BASED ON THE NUCLEOTIDE SEQUENCE OF THE ENTEROTOXIN GENE AND THE AMINO ACID SEQUENCE OF ITS ENTEROTOXIN

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ABSTRACT

Staphylococcal enterotoxin is a common cause of foodborne intoxication and has recently been designated as a select agent. Staphylococcal enterotoxins are tolerant of heat and can often be found in foods that have undergone thermal processes severe enough to kill the vegetative cells of the organism. Currently, there is no method available to trace a staphylococcal enterotoxin to its biological source.

Two sets of primers were developed to amplify the *entA* gene of several isolates of *Staphylococcus aureus* using PCR. The PCR products were then compared to look for variations in the nucleotide sequences among the strains of *S. aureus*. The DNA sequences were also translated into proteins which were compared to see if any variations in their amino acid sequences could be observed.

Variations were observed in both the nucleotide and amino acid sequences of many of the isolates of *S. aureus*. However, a few isolates exhibited identical nucleotide and amino acid sequences. In these cases, the PCR products were of differing lengths; thus, there could have been sequence variations outside of the amplified region of the gene.

This study indicates that it may be possible to identify a strain of *S. aureus* based on the nucleotide or amino acid sequence of its enterotoxin. Further research is required to determine if this method is applicable to serotypes of staphylococcal enterotoxin other than type A.

INTRODUCTION

Staphylococcus aureus is a pathogenic microorganism that is commonly responsible for nosocomial infections and foodborne intoxication in humans (Freeman-Cook and Freeman-Cook 2006). Staphylococcal intoxication is the third most common form of foodborne illness, surpassed only by the occurrence of *Salmonella* and *Clostridium perfringens* infections (Atanassova and others 2001). Food is typically contaminated with the bacterium by food preparers who carry enterotoxigenic *S. aureus* in their nasal passages and transfer it to the food via their hands or sneezing (Bania and others 2006; Bennet 2001). A 2007 CDC report estimates that nearly 32% of Americans carry the microbe (CDC 2007). Foodborne staphylococcal intoxication occurs when food, containing the pre-formed enterotoxins produced by *S. aureus*, is consumed.

The staphylococcal enterotoxins are a group of proteins produced by the organism that induce an emetic response in humans along with other gastrointestinal symptoms (Freeman-Cook and Freeman-Cook 2006). While *S. aureus* is able to produce several enterotoxin serotypes, enterotoxin A is the most common type to be associated with foodborne illness; probably because it is also the most common serotype carried by humans (Holmberg and Blake 1984; Casman 1965).

The presence of staphylococcal enterotoxins is especially important in food safety because they are very resistant to heat (Balaban and Rasooly 2000). The enterotoxins produced by *S. aureus* have been shown to maintain their activity through thermal processes such as milk pasteurization, and enterotoxin B

is able to stay active after being held at 60ºC for several hours (Tatini 1976; Schantz and others 1965). Many of the thermal processes used in foods are severe enough to kill any vegetative cells of *S. aureus*, leaving only the toxin behind.

In addition to food safety concerns, the staphylococcal enterotoxins have recently been added to HHS and USDA Select Agents and Toxins list because of their ability to act as superantigens (CDC 2006; Ulrich and others 2007). While there are several ways to detect staphylococcal enterotoxin in foods, there are not currently any methods by which the enterotoxin can be traced back to the strain of *S. aureus* that produced it.

The objective of the present study was to examine the nucleotide sequence of the enterotoxin gene and the amino acid sequence of the actual enterotoxin produced by various strains of *S. aureus* for variations using enterotoxin A as a model. If successful, this methodology could be utilized in building a database that would allow for the identification of a specific strain of *S. aureus* based on the nucleotide sequence of its enterotoxin. Such a database could not only be used by food manufacturers in establishing the source of staphylococcal contamination, but would also be forensically beneficial to investigators if staphylococcal enterotoxin were ever purposely introduced into the food supply.

MATERIALS AND METHODS

Isolation and Identification of *Staphylococcus aureus*

Nine commercial strains of *Staphylococcus aureus* were purchased from American Type Culture Collection (ATCC). These strains are: *S. aureus* subsp *aureus* ATCC 14458, ATCC 8095, ATCC 51650, ATCC 51651, ATCC 13565, ATCC 13566, ATCC 27664, ATCC 19095, and ATCC 51740. Additional strains were isolated from a variety of biological sources including raw milk, fecal samples from various animal species and human nasal swabs.

Isolation of *S. aureus* from milk was carried out using raw milk samples collected from local dairies. Ten milliliters of milk was added to one-hundred milliliters of sterile Bacto Tryptic Soy Broth or TSB (BD, Sparks, MD, USA) and allowed to incubate at 37º C for 24 hours. After incubation, a loop of enrichment culture was streaked for isolation on CHROMagar Staph aureus (CHROMagar Microbiology, Paris, France) and allowed to incubate at 37º C for 24 hours. Two to three well-isolated typical (mauve-colored) colonies were picked, using a sterile disposable needle, into test tubes containing ten mL volumes of sterile TSB and incubated for 24 hours at 37º C prior to storage at refrigeration temperatures (2-5º C).

Fecal swabs were collected from chickens, ducks, pigs, cattle, a snake, a llama and a camel. Also, swabs were collected from the intestines of a wild boar. Sterile swabs were used to collect the feces and/or intestinal contents and placed in tubes containing ten milliliters of TSB which were incubated at 37º C for 24

hours. Presumptive *S. aureus* organisms were then isolated from the enrichment and stored as described above.

Nasal swabs were collected from various lab personnel and from people working with or around livestock. A sterile swab was inserted into the nose as far as comfortably possible and rotated to ensure contact with nasal surfaces. The swab was then placed in a test tube containing ten milliliters of TSB and allowed to incubate for 24 hours at 37º C. The enrichment culture was then processed and stored as previously described. All isolates were maintained by weekly subculturing in TSB using a 1% inoculum and incubation at 37º C for 18 to 24 hours. Cultures were subcultured for three consecutive days prior to being used in any experiments and were stored at refrigeration temperatures between uses.

Each isolate underwent a series of identification tests including Gram stain, the coagulase test, the catalase test, the hemolysis test and biochemical testing using the API Staph identification system (bioMérieux, Hazelwood, MO, USA).

Gram stains were performed on all isolates to help confirm purity and to observe the morphology and Gram reaction of the cells. Isolates were then streaked onto Tryptic Soy Agar (TSA), TSB supplemented with 1.5% granulated agar (BD, Sparks, MD, USA), and incubated for 24 hours at 37º C. To test for the presence of coagulase, several colonies from the TSA plates were picked into a tube containing 0.5 milliliters of reconstituted lyophilized rabbit plasma (BD, Sparks, MD, USA) and incubated in a 37º C waterbath for 4 hours. Tubes were examined for the formation of a complete or partial clot which indicated the

presence of coagulase. Following the removal of colonies for the coagulase test, TSA plates were flooded with 3% hydrogen peroxide where bubbling indicated the presence of the enzyme catalase. To test for β-hemolysis, which is typical of *S. aureus*, isolates were streaked for isolation onto Blood Agar Plates containing 5% sheep blood (BD, Sparks, MD, USA) and allowed to incubate for 48 hours at 37º C. Plates were observed for the formation of clear or transparent zones around the streak caused by lysis of the red blood cells in the medium.

Isolates that were found to be Gram positive cocci, tested positive for the production of both coagulase and catalase, and exhibited β-hemolysis were presumed to be *S. aureus* and subjected to identification testing. Isolates were streaked onto Colombia Blood Agar (BD, Sparks, MD, USA) and allowed to incubate for 24 hours at 37º C. Colonies were used to prepare a bacterial suspension in API Staph Medium (bioMérieux, Hazelwood, MO, USA) equal to a 0.5 McFarland Standard. This bacterial suspension was then used to inoculate the API Staph identification strips. In accordance with manufacturer instructions, the ADH and URE cupules were filled with mineral oil to ensure an anaerobic environment; strips were then incubated at 37º C for 18-24 hours. After incubation, results were developed in accordance with manufacturer instructions and recorded on the provided results sheets. Identification of isolates was carried out using Bergey's Manual of Systematic Bacteriology (Sneath and others 1986) and apiweb® identification software (bioMérieux, Hazelwood, MO). Twenty-one isolates were determined to be *S. aureus* and were, along with the commercial strains, subjected to testing for enterotoxin production.

Enterotoxin Production

Production of staphylococcal enterotoxin types A, B, C, D and E by commercial and freshly isolated strains of *S. aureus* was detected using VIDAS® Staph Enterotoxin II test strips with the miniVIDAS system (bioMérieux, Hazelwood, MO, USA). Isolates were subcultured 3 times prior to the experiment in TSB using a 1% inoculum and incubation at 37º C for 18 hours. Calibration of the miniVIDAS was performed using the standard and controls provided with the kit and the assay was carried out in accordance with manufacturer instructions.

The test was carried out automatically by the miniVIDAS, and the results were analyzed mechanically by its computer. The instrument calculated a test value for each isolate where a value equal to or above 0.13 indicated the presence of staphylococcal enterotoxin. Isolates that tested positive for the production of staphylococcal enterotoxin were then subjected to enterotoxin typing.

Enterotoxin Typing

Toxin typing was carried out using the Set-RPLA Toxin Detection Kit (Oxoid, Hampshire, UK) in accordance with manufacturer instructions. The kit is capable of detecting staphylococcal enterotoxin types A, B, C and D. Isolates were subcultured 3 times into TSB, using the previously detailed procedure, prior to use. Cultures of *S. aureus* that produced staphylococcal enterotoxin A were retained and used for subsequent experiments.

DNA Extraction

Isolates were subcultured in 10 milliliters of TSB (1% inoculum incubated at 37º C for 18-24 hours) 3 times prior to use. Each fresh culture was centrifuged at 4000 rpm for 10 minutes; the supernatant was discarded and each cell pellet was resuspended in 500 µl of PBS. The DNA was then extracted from the cells using the High Pure PCR Template Preparation Kit (Roche Applied Science, Indianapolis, IN, USA) in accordance with manufacturer instructions. Extracted DNA was stored at -20º C for future use.

16s rDNA Sequencing

The DNA extracted from each isolate was used to amplify, via PCR, a 1500 base pair 16s rDNA fragment with BacT0008F 5'-AGAGTTTGATCCTGGCTGAG-3' and BacT1510R 5'-GGTTACCTTGTTACGACTT-3' primers using a MJ Dyad thermocycler (MJ Research, Waltham, MA). The PCR reaction contained 25 µl that consisted of 16 µl deionized water, 5 µl 5X buffer (Promega, Madison, WI, USA), 1.5 µl of 25 mM $MgCl_2$ (Promega, Madison, WI, USA), 0.5 µl 10 mM dNTPs (Fisher Scientific, Pittsburgh, PA, USA), 0.5 µl 25 mM primers, 100 ng/µl BSA (Roche Applied Science, Indianapolis, IN, USA), 1.25 U Taq polymerase (Promega, Madison, WI, USA) and 1-2 µl template DNA. The PCR cycling conditions were as follows: 4 minutes at 95º C, followed by 35 cycles of 30 seconds at 95º C, 52º C for 30 seconds and 1 minute and 45 seconds at 72º C; the reaction was then held at 4º C until removed. To ensure the quality of the DNA, the PCR product was evaluated using gel electrophoresis with a 1.2% agarose gel where 10 µl of PCR

product was mixed with 2 µl of 6X loading dye and evaluated against a 1 Kb DNA ladder (Invitrogen, Carlsbad, CA, USA).

After the initial PCR reaction, samples underwent a DNA clean-up step prior to the sequencing reaction. In a new PCR tube, 10 µl of PCR product was mixed with 2 µl of a shrimp alkaline phosphatase and exonuclease (USB Corp, Cleveland, OH, USA) mixture and put through a thermal cycle consisting of 30 minutes at 37º C followed by 15 minutes at 85º C. The PCR product resulting from this DNA clean-up process was then used in the sequencing reaction.

In the 16s rDNA sequencing reaction, 3 primers were used: BacT0008F, BacT1510R and BacT0805R 5'-GGACTACCAGGGTATCTAATCC-3'. A master mix was prepared for each primer consisting of 4 µl deionized water, 1 µl 5X sequencing buffer (Applied Biosystems, Foster City, CA, USA), 1 µl 25 mM individual primer, 2 µl Big Dye (Applied Biosystems, Foster City, CA, USA) and 2 µl PCR product from each sample. The samples underwent thermal cycling conditions as follows: 2 minutes at 96º C, followed by 61 cycles of 30 seconds at 96º C, 30 seconds at 50º C and 2 minutes at 60º C. Prior to sequencing, ethanol precipitation of the DNA was performed. Twelve microliters of deionized water, 5 µl 3M ammonium acetate and 57 µl of 100% ethanol were added to each tube and mixed before centrifugation at 4000 rpm for 30 minutes. After discarding the supernatant, 70 µl of 70% ethanol was added to each tube, and they were once again centrifuged at 4000 rpm for 15 minutes. The supernatant was discarded, and 10 µl of deionized water was added to the sample which was vortexed to bring the DNA into solution. The DNA was sequenced by the Oklahoma State

University Recombinant DNA/Protein Core facility using an ABI Model 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

EntA DNA Sequencing

Using *entA* sequences from the National Center for Biotechnology (NCBI) database and a primer-building program, PrimerQuest (Available from: www.idtdna.com/Scitools/Applications/Primerquest), two sets of primers for the *entA* gene were developed: primer set 1 consised of *S. aureus* Forward 1 5'-CATTGCCCTAACGTGGACAACAAG-3' and *S. aureus* Reverse 1 5'-GCCACCATCTATCCAACTTGCTCA-3', while primer set 2 consisted of *S. aureus* Forward 2 5'-AGGTGTCATTTGCTAATCCAACTG-3' and *S. aureus* Reverse 2 5'-ACCTCATGACCATACTCACCA-3'. The DNA extracted from all samples was PCR amplified separately with each primer set using a reaction that contained 14.75 µl deionized water, 2 µl 10X PCR Buffer (Roche Applied Science, Indianapolis, IN, USA), 1.2 µl 25 mM $MgCl_2$ (Roche Applied Science, Indianapolis, IN, USA), 0.4 µl 25 mM primers, 0.4 µl 10 mM dNTPs, 1.25 U Taq polymerase and 2 µl of template DNA. Cycling conditions consisted of: 2 minutes at 94º C, followed by 34 cycles of 30 seconds at 94º C, 30 seconds at 60º C and 72º C for 1 minute and 30 seconds; the reaction was held at 4º C until it was removed. Quality of the PCR product was assessed using gel electrophoresis as previously described.

After the initial PCR, samples underwent the DNA clean-up process previously described followed by a PCR sequencing reaction. The same master mix was used as with the 16s rDNA sequencing reaction. Cycling conditions

consisted of 30 seconds at 95º C, followed by 61 cycles of 10 seconds at 96º C, 10 seconds at 50º C and 1 minute at 60º C. The DNA underwent ethanol precipitation and sequencing as previously described.

Analysis of Results

The DNA sequences were aligned and analyzed using MEGA 4: Molecular Evolutionary Genetics Analysis software (Available at: www.megasoftware.net). Phylogenetic trees were created using the MEGA neighbor-joining method with the p-distance matrix. Bootstrapping was employed to assess the accuracy of the phylogenetic trees where a higher bootstrap value was indicative of a higher degree of accuracy.

The DNA sequences were translated into proteins using the Biology Workbench 6-frame translation function (Available at: workbench.sdsc.edu). The longest open-reading frame was used to construct protein-based phylogenetic trees in MEGA using the parsimony method. Once again, bootstrapping was used to ensure the accuracy of the trees.

RESULTS

Identification of *Staphylococcus aureus*

Isolates of *S. aureus* were identified based on Gram stain reactions, the coagulase test, the catalase test, the hemolysis test and the metabolism of various substrates using the API Staph identification system (Appendix A).

Metabolism patterns were based on the ability of the organism to metabolize the substrate in each cupule during incubation where metabolism of the substrate results in a visual color change. *Staphylococcus aureus* is able to metabolize D-glucose, D-fructose, D-mannose, D-maltose, D-lactose, D-trehalose, D-mannitol, D-saccharose, L-arginine and urea (Sneath and others 1986). Based on metabolism patterns and positive results for all other identification tests, all isolates were identified as *S. aureus*. Isolates that match the metabolism patterns found in Bergey's Manual of Systematic Bacteriology (Sneath and others 1986) are summarized in Table 1.

Enterotoxin Production

Cultures of *S. aureus* were tested for their ability to produce staphylococcal enterotoxins A, B, C, D and E. Using an assay based on the binding of staphylococcal enterotoxin in the culture medium to monoclonal anti-staphylococcal enterotoxin antibodies. The formation of this bond catalyzes the hydrolysis of a substrate resulting in the release of fluorescence that is measured at 450nm. A relative fluorescence value (RFV) of 0.13 or above is indicative of a positive result for enterotoxin production by the isolate.

All commercial strains of *S. aureus* tested positive for the production of enterotoxin; however, only 4 freshly isolated strains were shown to produce enterotoxin (Table 2). The strains of *S. aureus* listed in this table were used for further study.

Enterotoxin Typing

Isolates of *S. aureus* that produced enterotoxin were subjected to toxin typing using the Set-RPLA Toxin Detection Kit. Identification of the enterotoxin subtype is based on the agglutination that occurs when antiserum purified with a staphylococcal enterotoxin A, B, C, or D comes into contact with its matching enterotoxin. When agglutination occurs a lattice-like structure is formed that creates a visible layer that can be observed as a positive reaction.

Many of the isolates produced multiple enterotoxin subtypes (Table3). However, because foodborne staphylococcal intoxication is most commonly associated with enterotoxin A, only those isolates capable of producing SEA were chosen for use in further experiments (Holmburg and Blake 1984). Isolates shown to produce SEA include: *S. aureus* subsp *aureus* strains ATCC 13566, ATCC 13565, ATCC 51651, ATCC 19095, 27664, ATCC 8095, ATCC 14458 and PIG-M1.

16s rDNA Sequencing

To ensure that all isolates were *S. aureus*, a 16s rDNA fragment from each isolate was amplified using PCR. To make certain that the amplified DNA fragment was the correct size, 1500 base pairs, the PCR product was evaluated using gel electrophoresis. The placement of the DNA fragments on the gel after

electrophoresis was then compared to a DNA ladder that allows the size of each DNA fragment to be discerned. This process was repeated for all PCR products obtained throughout the study.

The PCR products from all isolates of *S. aureus* displayed the same DNA fragment size and, when compared to the 100 bp DNA ladder, were roughly 1500 base pairs in size (Figure 1). The PCR products were sequenced and the genetic relationships between the 16s rDNA nucleotide fragments from the isolates of *S. aureus* (Appendix B) were observed in the form of a phylogenetic tree (Figure 2).

The 16s rDNA fragments from the commercial isolates of *S. aureus* were shown to be very genetically similar while the laboratory isolate, *S. aureus* PIG-M1, is slightly divergent. The structure of the phylogenetic tree makes it appear as though *S. aureus* PIG-M1 is very different from the nucleotide sequences of the commercial strains; however, when the nucleotide sequences are aligned, there is only a 1-nucleotide substitution within the conserved region of the sequence.

EntA DNA Sequencing

All isolates of *S. aureus* were subjected to PCR using 2 sets of primers: Primer Set 1 (SA1) and Primer Set 2 (SA2). Amplification with SA1 resulted in a PCR product consisting of approximately 800 nucleotides while that resulting from amplification with SA2 consisted of roughly 500 nucleotides. Primer Set 1 amplified the *entA* gene of strains ATCC 13566, ATCC 13565, ATCC 51651, ATCC 19095, ATCC 27664, ATCC 8095 and PIG-M1. In contrast, SA2 amplified

the *entA* gene of only 4 cultures: strains ATCC 51651, ATCC 27664, ATCC 14458 and PIG-M1.

The PCR products were sequenced as previously described (Appendix C) and a dendogram was constructed from the results in order to observe the genetic relationships among the isolates (Figure 3). The regions of the gene amplified by SA1 and SA2 are separate and do not overlap; thus, comparison of isolates is done separately for each primer set. The nucleotide sequences of the *entA* genes amplified with SA1 from strains ATCC 13566 and ATCC 51651 were exactly the same and these sequences differed from those of strains ATCC 8095 and ATCC 13565 by 9 nucleotides. Similarly, the *entA* nucleotide sequence obtained by PCR amplification with SA1 from *S. aureus* PIG-M1 is identical to portions of both strain ATCC 27664 and strain ATCC 19095 amplified with the same primer set. The nucleotide sequences of strains ATCC 8095 and ATCC 13565 resulting from amplification with SA1 only differ by 1 nucleotide while those of strains ATCC 27664 and ATCC 19095 differ by 2 nucleotides. With these exceptions, sequences obtained by amplification with SA1 differed from all other sequences by 100 nucleotides or more.

The *entA* sequences obtained by amplification with SA2 were more divergent from each other than those obtained by amplification with SA1. The SA2 amplified *entA* genes of strain PIG-M1 and strain ATCC 14458 only differed by 2 nucleotides. When all other SA2 amplified DNA sequences are compared, they differ by at least 14 nucleotides with some differing by as many 200 nucleotides. When the sequences obtained using SA2 are compared to those

obtained using SA1, there is an observable difference of at least 100 nucleotides for each comparison.

In order to assess the similarity of the actual enterotoxin produced by each isolate, the *entA* DNA sequences were translated into proteins using the Biology Workbench 6-frame translation function. The protein translation with the longest open reading frame for each isolate (Appendix D) was chosen to construct a phylogenetic tree using the MEGA parsimony method (Figure 4).

As expected, the PCR products from different isolates of *S. aureus* that showed identical nucleotide sequences also exhibited identical amino acid sequences. This was the case for strains: ATCC 13566 and ATCC 51651 amplified with SA1, ATCC 19095 and PIG-M1 amplified with SA1, and ATCC 27664 and PIG-M1 amplified with SA1. Even though amplification with SA1 showed a 2-nucleotide difference, the amino acid sequences of the PCR products from strains ATCC 27664 and ATCC 19095 were also identical, which can be attributed to the redundant nature of the genetic code.

The amino acid sequences of the proteins translated from the SA1 PCR products of strains ATCC 13566 and ATCC 51651 differed from strains ATCC 13565 and ATCC 8095 by 3 amino acids. Amplification with SA1 resulted in a difference of only 1 amino acid in the sequences of strains ATCC 13565 and ATCC 8095.

None of the proteins translated from the PCR products obtained by amplification with SA2 were identical. Strains ATCC 27664 and ATCC 51651 differed by 3 amino acids. The amino acid sequence from strain ATCC 14458

differed from those of ATCC 27664 and PIG-M1 by 4 amino acid residues. Comparison of all other amino acid sequences resulted in at least 5, and often more than 50, amino acid differences.

DISCUSSION

Foods associated with staphylococcal intoxication are typically those that require hands-on preparation which allows for contamination of the food by preparers carrying the microorganism. These foods include milk and egg products, sandwich fillings, cream-filled pastries, salads, cheese, meats and poultry (Freeman-Cook and Freeman-Cook 2006; Bennet 2001). Incubation of the organism in the food results in the production of enterotoxins that are harmful to the consumer (Holmburg and Blake 1984).

Foods contaminated with *S. aureus* often undergo thermal processes at high enough temperatures to kill any vegetative cells of the organism. The USDA recommends that hot foods be held at 60º C; however, vegetative cells of the bacterium are only able to survive at temperatures up to 50º C (USDA 2008; Baird-Parker 1965). The absence of vegetative cells of *S. aureus* in contaminated foods poses a challenge to food manufacturers and investigators because, at the current time, there are no methods by which a staphylococcal enterotoxin can be traced to its vegetative source at the strain-level. The ability to trace an enterotoxin to the specific strain of *S. aureus* that produced it would be especially important in the event that purified enterotoxin were introduced into the food supply as an act of bioterrorism.

This study has shown that it may be possible to trace a staphylococcal enterotoxin to its vegetative source using only the nucleotide or amino acid sequence of the toxin. There were observable differences among many of the

PCR products used in this study, indicating that the nucleotide and amino acid composition of staphylococcal enterotoxin A is somewhat variable.

The *entA* gene of strains ATCC 51651 and ATCC 13566 amplified with SA1 were shown to have identical nucleotide and amino acid sequences. However, the length of the protein translations from these isolates differed by nearly 80 amino acids indicating that there may have been differences outside of the amplified region of the gene. This is also the case for the PCR product obtained from amplification of *S. aureus* PIG-M1 with SA1. The *S. aureus* PIG-M1 PCR product was shown to be identical to those of strains ATCC 19095 and ATCC 27664; however, the sequence from strain PIG-M1 was more than 200 nucleotides shorter. The variable length of the PCR products could be easily overcome by developing and employing primers that allow the entire gene to be amplified.

In order to build a staphylococcal enterotoxin database, all enterotoxin subtypes from all known strains of *S. aureus* would need to be processed in accordance with the methods described in this study. This would require the development of primers for each subtype of enterotoxin and the PCR amplification of multiple genes from each bacterial strain.

In order to employ a staphylococcal enterotoxin database in determining the vegetative source of an enterotoxin, it would need to be extracted from the contaminated food product, purified and typed. Once purified, the amino acid sequence of the enterotoxin would need to be determined. The amino acid sequence of the enterotoxin in question would then be electronically compared to

the sequences all ready acquired using the methods described in this study. In the event that the enterotoxin were purposely introduced into the food supply and a match were found in the database, investigators could survey culture companies who sell that specific strain of *S. aureus* to see who had recently purchased the culture, thus narrowing the field of suspects.

Further research must be conducted to develop primers that allow for the amplification of the entire staphylococcal enterotoxin A gene, to develop similar primers for all enterotoxin subtypes and to ensure that observable variation does exist when the nucleotide and amino acid sequences of other enterotoxin subtypes are compared.

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TABLE 1

ISOLATES DETERMINED TO BE *STAPHYLOCOCCUS AUREUS* BASED ON METABOLISM PATTERNS[1,2]

S. aureus CHK-B3	*S. aureus* MLK-1046
S. aureus CHK-S2	*S. aureus* MLK-1021
S. aureus PIG-M1	*S. aureus* MLK-1125
S. aureus PIG-M2	*S. aureus* MLK-1034
S. aureus PIG-M3	*S. aureus* MLK-159
S. aureus PIG-M4	*S. aureus* JC
S. aureus Camel	*S. aureus* SM
S. aureus WB-2	*S. aureus* JG
S. aureus MLK-78	*S. aureus* IT
S. aureus MLK-182	*S. aureus* JB
S. aureus MLK-1041	

[1]All isolates were Gram (+), Coagulase (+), Catalase (+), β-hemolysis (+) coccus-shaped bacteria

[2]Metabolism patterns compared to *S. aureus* as listed in Bergey's Manual of Systematic Bacteriology (Sneath and others 1986)

TABLE 2

ENTEROTOXIN TYPES PRODUCED BY CULTURES OF *STAPHYLOCOCCUS AUREUS* [1,2]

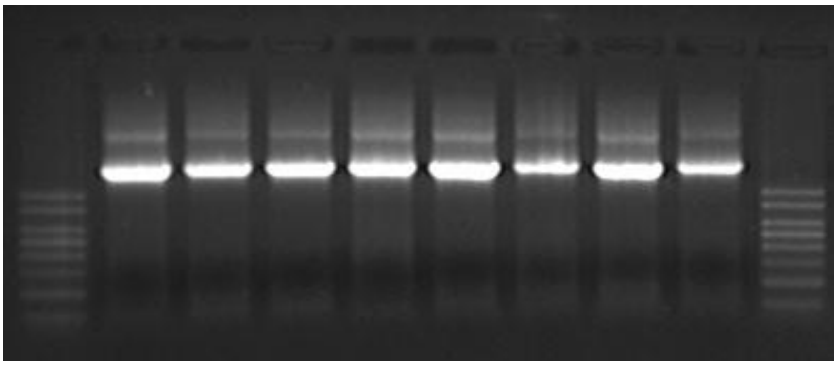
	Enterotoxin Subtype			
Strain	A	B	C	D
ATCC 14458	+	+	-	-
ATCC 8095	+	-	-	-
ATCC 51650	-	-	+	-
ATCC 51651	+	-	-	-
ATCC 13565	+	-	-	+
ATCC 13566	+	+	-	-
ATCC 27664	+	-	-	-
ATCC 19095	+	-	+	-
ATCC 51740	-	-	+	+
PIG-M1	+	-	-	-
MLK-159	-	-	-	-
JG	-	-	+	-
JB	-	-	-	-

[1]Enterotoxin production determined using the miniVIDAS and Staph Enterotoxin II test strips

[2]Enterotoxin types determined using the SET-RPLA Staphylococcal Enterotxoin Test Kit

FIGURE 1

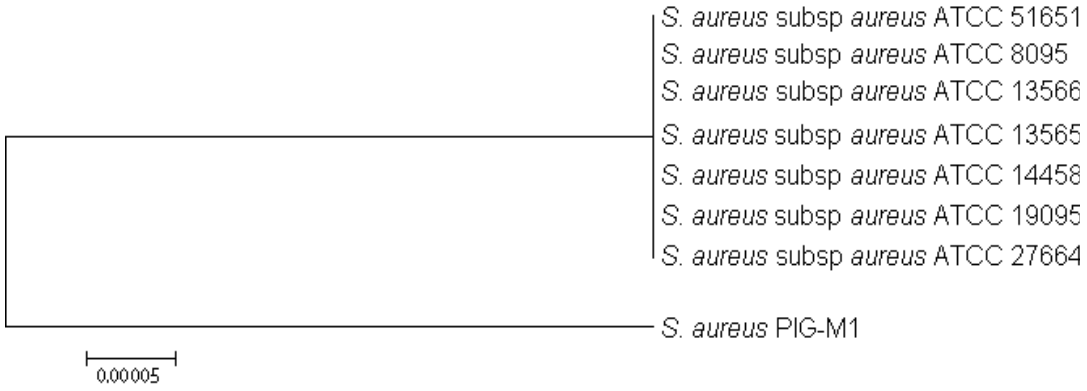
GEL ELECTROPHORESIS OF 16s rDNA FRAGMENTS FROM STAPHYLOCOCCAL ENTEROTOXIN A PRODUCING ISOLATES OF *STAPHYLOCOCCUS AUREUS* [1]



[1]Lane Descriptions: 1) 100 bp DNA ladder, 2) *S. aureus* subsp *aureus* ATCC 13566, 3) *S. aureus* subsp *aureus* ATCC 13565, 4) *S. aureus* subsp *aureus* ATCC 51651, 5) *S. aureus* subsp *aureus* ATCC 19095, 6) *S. aureus* sbusp *aureus* ATCC 27664, 7) *S. aureus* subsp *aureus* ATCC 8095, 8) *S. aureus* subsp *aureus* ATCC 14458, 9) *S. aureus* PIG-M1, 10) 100 bp DNA ladder

FIGURE 2

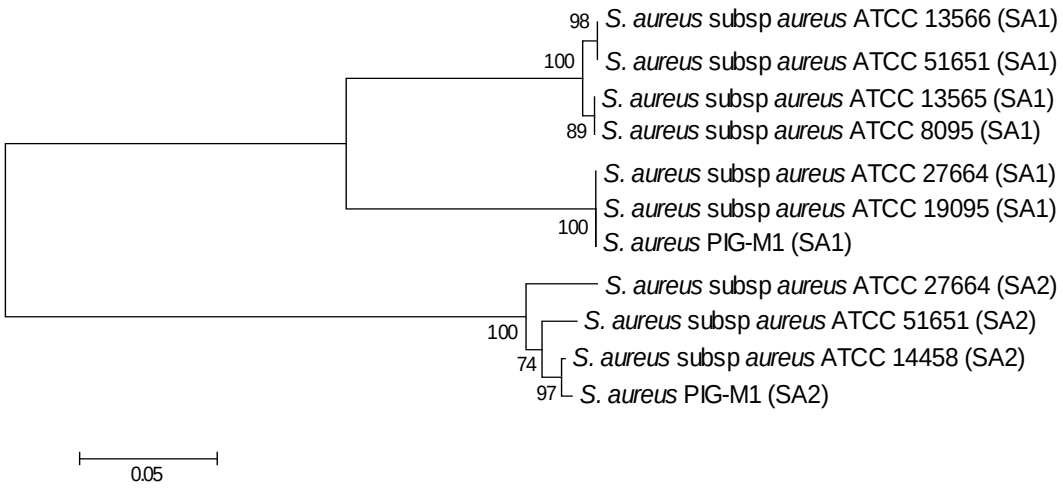
PHYLOGENETIC ANALYSIS OF 16s rDNA NUCLEOTIDE SEQUENCES FROM ISOLATES OF *STAPHYLOCOCCUS AUREUS* [1]



[1]Tree constructed using the MEGA neighbor-joining method with the p-distance matrix.

FIGURE 3

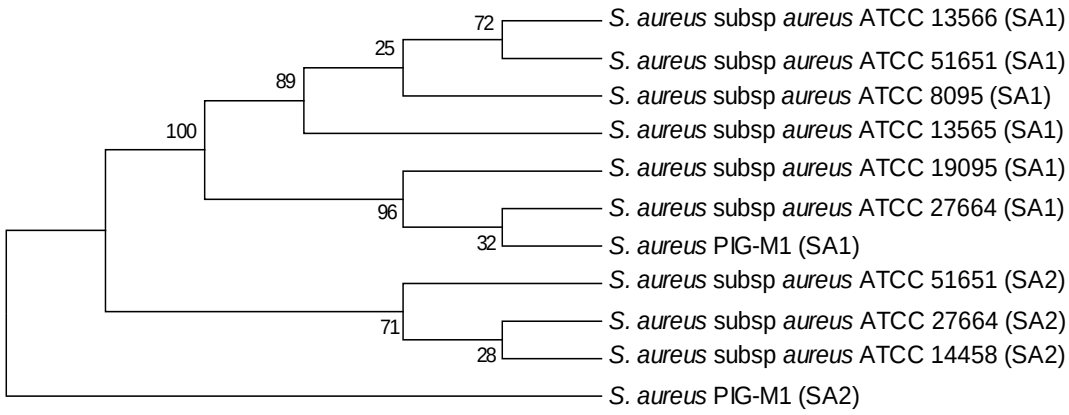
PHYLOGENETIC ANALYSIS OF *EntA* NUCLEOTIDE SEQUENCES FROM ISOLATES OF *STAPHYLOCOCCUS AUREUS* [1]



[1]Tree constructed using the MEGA neighbor-joining method with the p-distance matrix.

FIGURE 4

PHYLOGENETIC ANALYSIS OF ENTEROTOXIN A AMINO ACID SEQUENCES FROM ISOLATES OF STAPHYLOCOCCUS AUREUS[1]



[1]Tree constructed using the MEGA parsimony method.

APPENDICES

APPENDIX A

TABLE 1

IDENTITY CHARACTERISTICS OF ISOLATES OF *STAPHYLOCOCCUS AUREUS*

IDENTITY CONFIRMATION OF *STAPHYLOCOCCUS AUREUS*

	Staphylococcus aureus							
Test	Bergey[1]	CHK-B3	CHK-S2	PIG-M1	PIG-M2	PIG-M3	PIG-M4	Camel
D-Glucose	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+
D-Maltose	+	+	+	+	+	+	+	+
D-Lactose	+	+	+	+	+	+	+	+
D-Trehalose	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+
Xylitol	-	-	-	-	-	-	-	-
D-Melibiose	-	-	-	-	-	-	-	-
Potassium Nitrate	?	+	+	+	+	+	+	+
β-Naphthyl Phosphate	?	+	+	+	+	+	+	+
Sodium Pyruvate	?	+	+	+	+	+	+	+
D-Raffinose	-	-	-	-	-	-	-	-
D-Xylose	-	-	-	-	-	-	-	-
D-Saccharose	+	+	+	+	+	+	+	+
Methyl-αD-Glucopyranoside	?	-	-	-	-	-	-	-
N-Acetyl-Glucosamine	?	+/-	+/-	-	+/-	+/-	+/-	-
L-Arginine	+	+	+	+	+	+	+	+
Urea	+	+	+	+	+	+	+	+
Lysostaphin Resistance	?	+	+	+	+	+	+	+

[1]Reaction for *S. aureus* as listed in Bergey's Manual of Systematic Bacteriology

Test	*Staphylococcus aureus*							
	Bergey[1]	WB-2	MLK-78	MLK-182	MLK-1041	MLK-1046	MLK-1021	MLK-1125
D-Glucose	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+
D-Maltose	+	+	+	+	+	+	+	+
D-Lactose	+	-	+	+	+	+	+	+
D-Trehalose	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+
Xylitol	-	-	-	-	-	-	-	-
D-Melibiose	-	-	-	-	-	-	-	-
Potassium Nitrate	?	+	+	+	+	+	+	+
β-Naphthyl Phosphate	?	+	+	+	+	+	+	+
Sodium Pyruvate	?	+	+	+	+	+	+	+
D-Raffinose	-	-	-	-	-	-	-	-
D-Xylose	-	-	-	-	-	-	-	-
D-Saccharose	+	+	+	+	+	+	+	+
Methyl-αD-Glucopyranoside	?	-	-	-	-	-	-	-
N-Acetyl-Glucosamine	?	+/-	+/-	+/-	+/-	+	+	+/-
L-Arginine	+	+	+	+	+	+	+	+
Urea	+	+	+	+	+	+	+	+
Lysostaphin Resistance	?	+	+	+	+	+	+	+

[1]Reaction for *S. aureus* as listed in Bergey's Manual of Systematic Bacteriology

	Staphylococcus aureus							
Test	Bergey[1]	MLK-1034	MLK-159	JC	SM	JG	IT	JB
D-Glucose	+	+	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+	+	+
D-Maltose	+	+	+	+	+	+	+	+
D-Lactose	+	+	-	+	-	+	+	+
D-Trehalose	+	+	+	+	+	+	+	+
D-Mannitol	+	+	+	+	+	+	+	+
Xylitol	-	-	-	-	-	-	-	-
D-Melibiose	-	-	-	-	-	-	-	-
Potassium Nitrate	?	+	+	+	+	+	+	+
β-Naphthyl Phosphate	?	+	+	+	+	+	+	+
Sodium Pyruvate	?	-	-	+	+	+	+	+
D-Raffinose	-	-	-	-	-	-	-	-
D-Xylose	-	-	-	-	-	-	-	-
D-Saccharose	+	+	+	+	+	+	+	+
Methyl-αD-Glucopyranoside	?	-	-	-	-	-	-	-
N-Acetyl-Glucosamine	?	+	+/-	+/-	+	+	+	+
L-Arginine	+	+	+	+	+	+	+	+
Urea	+	+	+	+	+	-	+	+
Lysostaphin Resistance	?	+	+	+	+	+	+	+

1Reaction for *S. aureus* as listed in Bergey's Manual of Systematic Bacteriology

APPENDIX B

STAPHYLOCOCCUS AUREUS 16s rDNA NUCLEOTIDE SEQUENCES OBTAINED VIA PCR

Staphylococcus aureus subsp *aureus* ATCC 13566

5′- CTTAGACAGTTTAGCACTTAAGATTGTTATCTGATTACACGATTCTGCTTTCAGG
CGCAATTGAATTTCACTAGGTTAGACTTCAGTAAGGTCGCTCGGTTGTGGATTTCCATC
TTACATACTTAGTTATGAGGAGCATGCTTAGCGTCCTTTGTTCTTCTCACGGATGTACA
TGATCACAGTGTTATAGACGTCAGGATCGGTTGAATATTTTGGGTGATCGTTATATACA
AAAAATTTTCGCGCAGAGCTGCGTGGGCGCGTTTTTTACTTTTTATTTTAAACCCCACG
GGTTTATCTCGCAGGTGGATTGGTTTATTGCTTATTTGCTCATAAGAGGAAGTTGGACT
CTTATCGTTGCGTTCATTGTGCAAGCATTAGAGTTTTGATCCTGGCTCAGGATGAACGC
TGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGAT
GTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTT
CGGGAAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGA
CGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACG
GCTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTG
AGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAA
GCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTAT
TAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGC
CACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAA
TTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCT
CAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATT
CCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTT
TCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACC
CTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGT
GCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCA
AAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGC
GAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCCTTCCCCTT
CGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACT
CTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGC
CCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACCG
CGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGAC
TACATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAATACGTTCCC
GGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAAGCCGGTGGA
GTAACCTTTTAGGAGC –3′

Staphylococcus aureus subsp *aureus* ATCC 13565

5′– TAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAG
TCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACA
CGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGAT
AATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGG
ATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGC
CGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGA
GGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAG
TGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAA
CTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGG
CGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACT
GGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAG
AGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGT
GCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG
AGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTC
CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACAT
CCTTTGACAACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGC
ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC
CTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACC
GGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGT
GCTACAATGGACAATACAAAGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGT
TGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAA
TCGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCAC
ACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCTTTTAGGAGC -3′

Staphylococcus aureus subsp *aureus* ATCC 51651

5′- GATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGA
ACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATA
ACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTT
GAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGC
TGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGA
GAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG
TAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAG
GTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCAC
ATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTT
TAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACT
TGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGG
AGGAACACCAGTGGCGAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCG
TGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAG
TGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGG
AGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAG
CATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACA
ACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTTGTC
GTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTT
AGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGG
TGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTGCTACAATG
GACAATACAAAGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAG
TTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGTAGATC
AGCATGCTACGGTGAATACGTTCCCGGGTC -3′

Staphylococcus aureus subsp *aureus* ATCC 19095

5′- CAATCAGATAGACGTGCGCCTCGTTGTGAGCGTTTTCTACCTCCAACCTCTCTGT
TGCGAGAAACATTCTTCGCCTGCTCTTTTTTTCTCCCGAGGAGAGGTATATATACACAG
GTGTTAAAAGATCCAGAATTGGTGTAATATTTGTGCTGCTGTTATTTCAAACACATTGT
GCCCACCTCTGGGGGGGCCTTTTTTTTATTTTGTGTTTAAACCCCCGGCTGCACCCCCC
CCGGGGCTTTGATATATGTAATACTGTGCCATATAGAGGGAGGTAAACTTCCTATAGTT
ATGCGCGTAAATTTTGTCAAGCTTATAGAGTTTGATCCTGGCTCAGGATGAACGCTGGC
GGCGTGCCTAATACATGCAAGTCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTA
GCGGCGGACGGGTGAGTAACACGTGGATAACCTACCTATAAGACTGGGATAACTTCGGG
AAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGT
CTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTT
ACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGAC
ACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCT
GACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGG
GAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACG
GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTAT
TGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAAC
CGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCAT
GTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTG
GTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGG
TAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTG
CAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGG
AATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAG
AACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGAGCCTTCCCCTTCGGG
GGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAA
GTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCT
TATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGGCAGCGAAACCGCGAG
GTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACA
TGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGT
CTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACACCCGAA -3′

Staphylococcus aureus subsp *aureus* ATCC 27664

5′- TCCACTGACATGTAGCACTAGATTGACTCAGGTAGACACGTCCCGTTCCGAGCAC
GAATTGAAGTATCCCGTAGGTAGAACTAGTCAGGATCGCCTGCGTTGGAGTTACGACGT
CTACTACGCTGATGAGAGAAAGCATCATCACTGTCACGTTGATCCTCGCGAGGGAATGT
ACTATACCCAAGCGTCTACAGAGCCGCAGACTCGGTATAGTGTGTGTTGTTCCTCGTGA
TTTAAAAAACTTTGTGCCCCACGTCGGGGGTGGGGCGTTTTATATTTTTTGTTTAAACC
CCCCCGGTCTTACCCTCCCAGGGGATTGATAATGTGCAAACTTGGGTCCGTCAGAGGGA
GGTGGCCTTCCTACTGTTTGGCCTAATTTTTTCTACATTGTAGAGTTTGATCCTGGCTC
AGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAACGGACGAGAAGCTT
GCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGATAACCTACCTATAAGAC
TGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGGTTCA
AAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGGATCCGCGCTGCATTAGCTAGTTG
GTAAGGTAACGGCTTACCAAGGCAACGATGCATAGCCGACCTGAGAGGGTGATCGGCCA
CACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGC
AATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTCTTCGGATCGTAA
AACTCTGTTATTAGGGAAGAACATATGTGTAAGTAACTGTGCACATCTTGACGGTACCT
AATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGC
GTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGGCGGTTTTTTAAGTCTGATGTGAA
AGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGAAAACTTGAGTGCAGAAGAGG
AAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACCAGTGGC
GAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGTGCGAAAGCGTGGGGATCAAACAG
GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGGGGGTTTC
CGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAG
GTTGAAACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT
CGAAGCAACGCGAAGAACCTTACCAAATCTTGACATCCTTTGACAACTCTAGAGATAGA
GCCTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCG
TGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAAGCTTAGTTGCCATCATTA
AGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCA
AATCATCATGCCCCTTATGATTTGGGCTACACACGTGCTACAATGGACAATACAAAGGG
CAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGTTGTTCTCAGTTCGGATTGTAGTC
TGCAACTCGACTACATGAAGCTGGAATCGCTAGTAATCGTAGATCAGCATGCTACGGTG
AATACGTTCCCGGGTCTTGTACACACCGCCCGTCACACCACGAGAGTTTGTAACA -3′

Staphylococcus aureus subsp *aureus* ATCC 8095

5′- TAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAG
TCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACA
CGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGAT
AATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGG
ATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGC
CGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGA
GGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAG
TGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAA
CTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGG
CGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACT
GGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAG
AGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGT
GCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG
AGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTC
CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAGGAATTGACGGGGACCCGCACAAG
CGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACATC
CTTTGACAACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCA
TGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCC
TTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACCG
GAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGTG
CTACAATGGACAATACAAAGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGTT
GTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAAT
CGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCACA
CCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCTTTTAGGAGC -3′

Staphylococcus aureus subsp *aureus* 14458

5′- TAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAG
TCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACA
CGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGAT
AATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGG
ATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGC
CGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGA
GGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAG
TGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAA
CTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGG
CGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACT
GGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAG
AGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGT
GCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG
AGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTC
CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACAT
CCTTTGACAACTCTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGC
ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC
CTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACC
GGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGT
GCTACAATGGACAATACAAAGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGT
TGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAA
TCGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCAC
ACCACGAGAGTTTGTAACACCCGAAGCCGGTGGAGTAACCTTTTAGGAGCTAGCCGTTG
AATGTTGGCCGAACGCTTTGGTTC -3′

Staphylococcus aureus PIG-M1

5′- TAGAGTTTGATCCTGGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAG
TCGAGCGAACGGACGAGAAGCTTGCTTCTCTGATGTTAGCGGCGGACGGGTGAGTAACA
CGTGGATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGAT
AATATTTTGAACCGCATGGTTCAAAAGTGAAAGACGGTCTTGCTGTCACTTATAGATGG
ATCCGCGCTGCATTAGCTAGTTGGTAAGGTAACGGCTTACCAAGGCAACGATGCATAGC
CGACCTGAGAGGGTGATCGGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGA
GGCAGCAGTAGGGAATCTTCCGCAATGGGCGAAAGCCTGACGGAGCAACGCCGCGTGAG
TGATGAAGGTCTTCGGATCGTAAAACTCTGTTATTAGGGAAGAACATATGTGTAAGTAA
CTGTGCACATCTTGACGGTACCTAATCAGAAAGCCACGGCTAACTACGTGCCAGCAGCC
GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGTAGG
CGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACT
GGAAAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGCAG
AGATATGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGATGT
GCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG
AGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTC
CGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGGGACCCGCACAA
GCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAAATCTTGACAT
CCTTTGACAACTCTAGAGATAGAGCTTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGC
ATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACC
CTTAAGCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGTTGACTGCCGGTGACAAACC
GGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCTTATGATTTGGGCTACACACGT
GCTACAATGGACAATACAAAGGGCAGCGAAACCGCGAGGTCAAGCAAATCCCATAAAGT
TGTTCTCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGCTGGAATCGCTAGTAA
TCGTAGATCAGCATGCTACGGTGAATACGTTCCCGGGTCTTGTACACACCGCCCGTCAC
ACCACGAGAGTTTGTAACACCCGAA -3′

APPENDIX C

STAPHYLOCOCCUS AUREUS EntA NUCLEOTIDE SEQUENCES OBTAINED VIA PCR

Staphylococcus aureus subsp *aureus* ATCC 13566 with Primer Set 1

5′- ACGTGGACAACAAGTCCACTTGTAAATGGTAGCGAGAAAAGCGAAGAAATAAA
TGAAAAAGATTTGCGAAAAAAGTCTGAATTGCAGGGAGCAGCTTTAGGCAATCTTAAAC
AAATCTATTATTACAATGAAAAAGCTAAAACTGAAAATAAAGAGAGTCACGATCAATTT
TTACAGCATACTATATTGTTTAAAGGCTTTTTTACAAATCATTCATGGTATAACGATTT
ATTAGTAGATTTTGATTCAAAGGATATTGTTGATAAATATAAAGGGAAAAAAGTAGACT
TATATGGTGCTTATTATGGTTATCAATGTGCGGGTGGTACACCAAACAAAACAGCTTGC
ATGTATGGTGGTGTAACGTTACATGATAATAATCGATTGACCGAAGAGAAAAAAGTGCC
AATCAATTTATGGCTAGACGGTAAACAAAATACAGTACCTTTGGAAACGGTTAAAACGA
ATAAGAAAAATGTAACTGTTCAGGAGTTGGATCTTCAAGCAAGACGTTATTTACAGGAA
AAATATAATTTATATAACTCTGATGTTTTTGATGGGAAGGTTCAGAGGGGATTAATCGT
GTTTCATACTTCTACAGAACCTTCGGTTAATTACGATTTATTTGGTGCTCAAGGACAGA
ATTCAAATACACTATTAAGAATATATAGAGATAATAAAACGATTAACTCTGAAAACATG
CATATTGATATATATTTATATACAAGTTAAACATGGTAGTTTTGAACACGTAATGTTCA
GATTATTATGAACCGAGAATAATCTGAAAGTTTACAAGCAGTAAAAAAAGTATATGTGC
TATAATATGCTTTGAGCAAGTTGGATA -3′

Staphylococcus aureus subsp *aureus* ATCC 13565 with Primer Set 1

5′- CCTAACGTGGACAACAAGTCCACTTGTAAATGGTAGCGAGAAAAGCGAAGAAA
TAAATGAAAAAGATTTGCGAAAAAAGTCTGAATTGCAGGGAACAGCTTTAGGCAATCTT
AAACAAATCTATTATTACAATGAAAAAGCTAAAACTGAAAATAAAGAGAGTCACGATCA
ATTTTTACAGCATACTATATTGTTTAAAGGCTTTTTTACAGATCATTCGTGGTATAACG
ATTTATTAGTAGATTTTGATTCAAAGGATATTGTTGATAAATATAAAGGGAAAAAAGTA
GACTTGTATGGTGCTTATTATGGTTATCAATGTGCGGGTGGTACACCAAACAAAACAGC
TTGTATGTATGGTGGTGTAACGTTACATGATAATAATCGATTGACCGAAGAGAAAAAAG
TGCCGATCAATTTATGGCTAGACGGTAAACAAAATACAGTACCTTTGGAAACGGTTAAA
ACGAATAAGAAAAATGTAACTGTTCAGGAGTTGGATCTTCAAGCAAGACGTTATTTACA
GGAAAAATATAATTTATATAACTCTGATGTTTTTGATGGGAAGGTTCAGAGGGGATTAA
TCGTGTTTCATACTTCTACAGAACCTTCGGTTAATTACGATTTATTTGGTGCTCAAGGA
CAGTATTCAAATACACTATTAAGAATATATAGAGATAATAAAACGATTAACTCTGAAAA
CATGCATATTGATATATATTTATATACAAGTTAAACATGGTAGTTTTGACCACGTAATG
TTCAGATTATTATGAACCGAGAATAATCTAAAAGTTTACAAGCAGTAAAAAAAGTATAT
GTGCTATAATATGCTTTGAGCAAGTTGGATAGAGGGTGGC -3′

Staphylococcus aureus subsp aureus ATCC 51651 with Primer Set 1

5′- CCTAACGTGGACAACAAGTCCACTTGTAAATGGTAGCGAGAAAAGCGAAGAAA
TAAATGAAAAAGATTTGCGAAAAAAGTCTGAATTGCAGGGAGCAGCTTTAGGCAATCTT
AAACAAATCTATTATTACAATGAAAAAGCTAAAACTGAAAATAAAGAGAGTCACGATCA
ATTTTTACAGCATACTATATTGTTTAAAGGCTTTTTTACAAATCATTCATGGTATAACG
ATTTATTAGTAGATTTTGATTCAAAGGATATTGTTGATAAATATAAAGGGAAAAAAGTA
GACTTATATGGTGCTTATTATGGTTATCAATGTGCGGGTGGTACACCAAACAAAACAGC
TTGCATGTATGGTGGTGTAACGTTACATGATAATAATCGATTGACCGAAGAGAAAAAAG
TGCCAATCAATTTATGGCTAGACGGTAAACAAAATACAGTACCTTTGGAAACGGTTAAA
ACGAATAAGAAAAATGTAACTGTTCAGGAGTTGGATCTTCAAGCAAGACGTTATTTACA
GGAAAAATATAATTTATATAACTCTGATGTTTTTGATGGGAAGGTTCAGAGGGGATTAA
TCGTGTTTCATACTTCTACAGAACCTTCGGTTAATTACGATTTATTTGGTGCTCAAGGA
CAGAATTCAAATACACTATTAAGAATATATAGAGATAATAAAACGATTAACTCTGAAAA
CATGCATATTGATATATATTTATATACAAGTTAAACATGGTAGTTTTGAACACGTAATG
TTCAGATTATTATGAACCGAGAATAATCTGAAAGTTTACAAGCAGTAAAAAAAGTATAT
GTGCTATAATATGCTTTGAGCAAGTTGGATAGA -3′

Staphylococcus aureus subsp aureus ATCC 51651 with Primer Set 2

5′- GCTAATCCAACTGATCTTGAAAATAAAAAAACAAATGATAGACTGTTGAAGCA
TGATTTGTTATTTCATGACATGTTCTTAAATGATGCTTGGAAAAAAGATTTAAAAGTTG
AATTCGAAAATGAGGCACTTTCAAAGAAATTTATAAATAAGAATATTGATGTATATGCT
GGAAGCTATAGCTATGAATGTCATGGGGGAGAAACCAATAAAACGCAATGTAGTTATGG
TGGTGTTACTTTAAGTGACAATAATAAATACGATGATTATAAGAATATACCTTGTAATT
TATGGATTGACGGACATCAAACAGAAATAGAACTAACTGCAGTGAAAACGAAAAAGTAA
ATTGTTACCATTCAAGAATTAGATGTTCAATTAAGAAATTATTTGAATGAGAAGTATAA
GTTGTACGAACAAGGTGGCGACATTGTTAAAGGGTATGTTAAATATCATAATGATGATG
AAAAAAATGTAGAATATGATTTTTATAATTTAAATGGTGAGTATGGT -3′

Staphylococcus aureus subsp *aureus* ATCC 19095 with Primer Set 1

5′- CATTGCCCTAACGTGGACAACAAGTCCACTTGTAAATGGTAGCGAGAAAAGCG
AAGAAATAAATGAAAAAGATTTGCGAAAAAAGTCTGAATTACAAAGAAATGCTTTAAGC
AATCTTAGGCAAATTTATTATTATAATGAAAAAGCTATAACTGAAAACAAAGAGAGTGA
TGATCAGTTTTTAGAGAATACTTTGTTATTTAAAGGTTTTTTCACAGGTCATCCATGGT
ATAACGATTTATTAGTAGATCTTGGTTCAAAAGATGCTACTAATAAATATAAAGGGAAA
AAAGTAGACTTATATGGTGCTTATTATGGATATCAATGTGCTGGAGGCACACCAAATAA
AACAGCATGTATGTACGGGGGTGTAACATTACATGATAATAACCGATTGACCGAAGAAA
AAAAAGTACCAATTAACTTGTGGATAGACGGAAAACAAACTACAGTACCTATAGATAAA
GTTAAAACAAGCAAAAAAGAAGTAACTGTTCAAGAGCTAGATCTTCAGGCAAGGCATTA
TTTACACGGAAAATTTGGTTTATATAACTCAGACAGCTTTGGCGGTAAGGTGCAAAGAG
GCTTGATTGTGTTTCATTCTTCTGAAGGGTCCACGGTAAGTTATGATTTGTTTGATGCT
CAAGGGCAATATCCAGATACATTATTAAGAATTTACAGAGATAATAAAACTATTAATTC
AGAGAACCTCCATATTGATTTGTATTTATACACAACTTGAGCATTATTTTG -3′

Staphylococcus aureus subsp *aureus* ATCC 27664 with Primer Set 1

5′- CCTACGTGACAACAAGTCCACTTGTAAATGGTAGCGAGAAAAGCGAAGAAATA
AATGAAAAAGATTTGCGAAAAAAGTCTGAATTACAAAGAAATGCTTTAAGCAATCTTAG
GCAAATTTATTATTATAATGAAAAAGCTATAACTGAAAACAAAGAGAGTGATGATCAGT
TTTTAGAGAATACTTTGTTATTTAAAGGTTTTTTCACAGGTCATCCATGGTATAACGAT
TTATTAGTAGATCTTGGTTCAAAAGATGCTACTAATAAATATAAAGGGAAAAAAGTAGA
CTTATATGGTGCTTATTATGGATATCAATGTGCTGGAGGCACACCAAATAAAACAGCAT
GTATGTACGGGGGTGTAACATTACATGATAATAACCGATTGACCGAAGAAAAAAAAGTA
CCAATTAACTTGTGGATAGACGGAAAACAAACTACAGTACCTATAGATAAAGTTAAAAC
AAGCAAAAAAGAAGTAACTGTTCAAGAGCTAGATCTTCAGGCAAGGCATTATTTACACG
GAAAATTTGGTTTATATAACTCAGACAGCTTTGGCGGTAAGGTGCAAAGAGGCTTGATT
GTGTTTCATTCTTCTGAAGGGTCCACGGTAAGTTATGATTTGTTTGATGCTCAAGGGCA
ATATCCAGATACATTATTAAGAATTTACAGAGATAATAAAACTATTAATTCAGAGAACC
TCCATATTGATTTGTATTTATACACAACTTGAGCATTATTTTGAAATGAGAAACTTATT
AAATTGATAAATAAAATGCCATCAGTCATTTATTACTATACTTAAAATATTAACTTTTT
ATTTTCATTTGTAAATTTCACAAAATATTGCTGCGGATATTTTGAA -3′

Staphylococcus aureus subsp *aureus* ATCC 27664 with Primer Set 2

5′- TAGGTGTCATTTGCTAATCCAACTGATCTTGAAAATAAAAAAACAAACGATAG
ACTGTTGAAGCATGATTTGTTATTTCATGACATGTTCGTAAATGATGATTGGAAAAAAG
ATTTTAAAGTTGAATTCGAAAATGAGGCACTTTCAAAGAAATTTATAAATAAGGATATC
GATATATTTGCTGGAAATTATGGATACGGATGTCATGGGGGAGAAACCAATAAAACGCA
ATGTAGTTATGGAGGGGTTACTTTAAGTGACAATAATAAGTACGATAATGATAAGAATA
TACCTTGTAATTTATGGATTGACGGACATCAAACAGAAATAGAACTAACTGCAGTAAAA
ACGAAAAAGAAAATTGTTACCATTCAAGAATTAGATGTTCAATTAAGAAATTATTTGAA
TGAGAAGTATAAGTTGTACGAACAAGGTGGCGACATTGTTAAAGGGTATGTTAAATATC
ATAATGATGATGAAAAAAATGTAGAATATGATTTTTATAATTTAAATGGTGAGTATGGT
CATGAGGT -3′

Staphylococcus aureus subsp *aureus* ATCC 8095 with Primer Set 1

5′- CCTAACGTGGACAACAAGTCCACTTGTAAATGGTAGCGAGAAAAGCGAAGAAA
TAAATGAAAAAGATTTGCGAAAAAAGTCTGAATTGCAGGGAACAGCTTTAGGCAATCTT
AAACAAATCTATTATTACAATGAAAAAGCTAAAACTGAAAATAAAGAGAGTCACGATCA
ATTTTTACAGCATACTATATTGTTTAAAGGCTTTTTTACAGATCATTCGTGGTATAACG
ATTTATTAGTAGATTTTGATTCAAAGGATATTGTTGATAAATATAAAGGGAAAAAAGTA
GACTTGTATGGTGCTTATTATGGTTATCAATGTGCGGGTGGTACACCAAACAAAACAGC
TTGTATGTATGGTGGTGTAACGTTACATGATAATAATCGATTGACCGAAGAGAAAAAAG
TGCCGATCAATTTATGGCTAGACGGTAAACAAAATACAGTACCTTTGGAAACGGTTAAA
ACGAATAAGAAAAATGTAACTGTTCAGGAGTTGGATCTTCAAGCAAGACGTTATTTACA
GGAAAAATATAATTTATATAACTCTGATGTTTTTGATGGGAAGGTTCAGAGGGGATTAA
TCGTGTTTCATACTTCTACAGAACCTTCGGTTAATTACGATTTATTTGGTGCTCAAGGA
CAGTATTCAAATACACTATTAAGAATATATAGAGATAATAAAACGATTAACTCTGAAAA
CATGCATATTGATATATATTTATATACAAGTTAAACATGGTAGTTTTGACCACGTAATG
TTCAGATTATTATGAACCGAGAATAATCTAAAAGTTTACAAGCAGTAAAAAAAGTATAT
GTGCTATAATATGCTTTGAGCAAGTTGGATAGATG -3′

Staphylococcus aureus subsp *aureus* ATCC 14458 with Primer Set 2

5′- ATTTGCTAATCCAACTGATCTTGAAAATAAAAACACAAACGATAGACTGTTGA
AGCATGATTTGTTATTTCATGACATGTTCGTAAATGTTGCTTCGAAAAAGGATTTTAAA
GTTGAATTCGAAAATGAGGCACTTTCAAAGAAATTTATAAATAAGAATATCGATATATA
TGCTGGAAGCTATAGCTATGAATGTCATGGGGGAGCAACCAATAAAACGCAATGTAGTT
ATGGTGGTGTTACTTTAAGTGACAATAATAAATAAGATGATTATAAGAATATACCTTGT
AATTTATGGATTGACGGACATCAAACAGAAATAGAACTAACTGCAGTAAAAACGAAAAA
GAAAATTGTTACCATTCAAGAATTAGATGTTCAATTAAGAAATTATTTGAATGAGAAGT
ATAAGTTGTACGAACAAGGTGGCGACATTGTTAAAGGGTATGTTAAATATCATAATGAT
GATGAACAAAATGTAGAATATGATTTTTATAATTTAAATGGTGAGTATGGTCATGAGGT
-3′

Staphylococcus aureus PIG-M1 with Primer Set 1

5′- TTTATTAGTAGATCTTGGTTCAAAAGATGCTACTAATAAATATAAAGGGAAAA
AAGTAGACTTATATGGTGCTTATTATGGATATCAATGTGCTGGAGGCACACCAAATAAA
ACAGCATGTATGTACGGGGGTGTAACATTACATGATAATAACCGATTGACCGAAGAAAA
AAAAGTACCAATTAACTTGTGGATAGACGGAAAACAAACTACAGTACCTATAGATAAAG
TTAAAACAAGCAAAAAAGAAGTAACTGTTCAAGAGCTAGATCTTCAGGCAAGGCATTAT
TTACACGGAAAATTTGGTTTATATAACTCAGACAGCTTTGGCGGTAAGGTGCAAAGAGG
CTTGATTGTGTTTCATTCTTCTGAAGGGTCCACGGTAAGTTATGATTTGTTTGATGCTC
AAGGGCAATATCCAGATACATTATTAAGAATTTACAGAGATAATAAAACTATTAATTCA
GAGAACCTCCATATTGATTTGTATTTATACACAACTTGAGCATTATTTTGAAATGAGAA
ACTTATTAAATTGATAAATAAAA -3′

Staphylococcus aureus PIG-M1 with Primer Set 2

5′- TAGGTGTCATTTGCTAATCCAACTGATCTTGAAAATAAAAACACAAACGATAG
ACTGTTGAAGCATGATTTGTTATTTCATGACATGTTCGTAAATGTTGCTTCGAAAAAGG
ATTTTAAAGTTGAATTCGAAAATGAGGCACTTTCAAAGAAATTTATAAATAAGAATATC
GATATATATGCTGGAAGCTATAGCTATGAATGTCATGGGGGAGCAACCAATAAAACGCA
ATGTAGTTATGGTGGTGTTACTTTAAGTGACAATAATAAATAAGATGATTATAAGAATA
TACCTTGTAATTTATGGATTGACGGACATCAAACAGAAATAGAACTAACTGCAGTAAAA
ACGAAAAAGAAAATTGTTACCATTCAAGAATTAGATGTTCAATTAAGAAATTATTTGAA
TGAGAAGTATAAGTTGTACGAACAAGGTGGCGACATTGTTAAAGGGTATGTTAAATATC
ATAATGAGAGAACAAAAGTAGAATATGATTTTTATAATTTAAATGGTGAGTATGGTCAT
GAGGTA -3′

APPENDIX D

STAPHYLOCOCCUS AUREUS ENTEROTOXIN A AMINO ACID SEQUENCES

Staphylococcus aureus subsp *aureus* ATCC 13566 with Primer Set 1

LLVDFDSKDIVDKYKGKKVDLYGAYYGYQCAGGTPNKTACMYGGVTLHDNNRLTE
EKKVPINLWLDGKQNTVPLETVKTNKKNVTVQELDLQARRYLQEKYNLYNSDVFDGKVQ
RGLIVFHTSTEPSVNYDLFGAQGQNSNTLLRIYRDNKTINSENMHIDIYLYTS*TW*F*
TRNVQIIMNRE*SESLQAVKKVYVL*YALSKLD

* - Designates stop codon

Staphylococcus aureus subsp *aureus* ATCC 13565 with Primer Set 1

LTWTTSPLVNGSEKSEEINEKDLRKKSELQGTALGNLKQIYYYNEKAKTENKESHDQFL
QHTILFKGFFTDHSWYNDLLVDFDSKDIVDKYKGKKVDLYGAYYGYQCAGGTPNKTACM
YGGVTLHDNNRLTEEKKVPINLWLDGKQNTVPLETVKTNKKNVTVQELDLQARRYLQEK
YNLYNSDVFDGKVQRGLIVFHTSTEPSVNYDLFGAQGQYSNTLLRIYRDNKTINSENMH
IDIYLYTS*TW*F*PRNVQIIMNRE*SKSLQAVKKVYVL*YALSLDRGW

* - Designates stop codon

Staphylococcus aureus subsp *aureus* ATCC 51651 with Primer Set 1

LTWTTSPLVNGSEKSEEINEKDLRKKSELQGAALGNLKQIYYYNEKAKTENKESHDQFL
QHTILFKGFFTNHSWYNDLLVDFDSKDIVDKYKGKKVDLYGAYYGYQCAGGTPNKTACM
YGGVTLHDNNRLTEEKKVPINLWLDGKQNTVPLETVKTNKKNVTVQELDLQARRYLQEK
YNLYNSDVFDGKVQRGLIVFHTSTEPSVNYDLFGAQGQNSNTLLRIYRDNKTINSENMH
IDIYLYTS*TW*F*TRNVQIIMNRE*SESLQAVKKVYVL*YALSKLDR

* - Designates stop codon

Staphylococcus aureus subsp *aureus* ATCC 51651 with Primer Set 2

ANPTDLENKKTNDRLLKHDLLFHDMFLNDAWKKDLKVEFENEALSKKFINKNIDVYAGS
YSYECHGGETNKTQCSYGGVTLSDNNKYDDYKNIPCNLWIDGHQTEIELTAVKTKK*IV
TIQELDVQLRNYLNEKYKLYEQGGDIVKGYVKYHNDDEKNVEYDFYNLNGEYG

* - Designates stop codon

Staphylococcus aureus subsp *aureus* ATCC 19095 with Primer Set 1

IALTWTTSPLVNGSEKSEEINEKDLRKKSELQRNALSNLRQIYYYNEKAITENKESDDQ
FLENTLLFKGFFTGHPWYNDLLVDLGSKDATNKYKGKKVDLYGAYYGYQCAGGTPNKTA
CMYGGVTLHDNNRLTEEKKVPINLWIDGKQTTVPIDKVKTSKKEVTVQELDLQARHYLH
GKFGLYNSDSFGGKVQRGLIVFHSSEGSTVSYDLFDAQGQYPDTLLRIYRDNKTINSEN
LHIDLYLYTT*ALF

* - Designates stop codon

Staphylococcus aureus subsp *aureus* ATCC 27664 with Primer Set 1

DLLVDLGSKDATNKYKGKKVDLYGAYYGYQCAGGTPNKTACMYGGVTLHDNNRLTEEKK
VPINLWIDGKQTTVPIDKVKTSKKEVTVQELDLQARHYLHGKFGLYNSDSFGGKVQRGL
IVFHSSEGSTVSYDLFDAQGQYPDTLLRIYRDNKTINSENLHIDLYLYTT*ALF*NEKL
IKLINKMPSVIYYYT*NINFLFSFVNFTKYCCGYFE

* - Designates stop codon

Staphylococcus aureus subsp *aureus* ATCC 27664 with Primer Set 2

QCSYGGVTLSDNNKYDNDKNIPCNLWIDGHQTEIELTAVKTKKKIVTIQELDVQLRNYL
NEKYKLYEQGGDIVKGYVKYHNDDEKNVEYDFYNLNGEYGHE

* - Designates stop codon

Staphylococcus aureus subsp *aureus* ATCC 8095 with Primer Set 1

LTWTTSPLVNGSEKSEEINEKDLRKKSELQGTALGNLKQIYYYNEKAKTENKESHDQFL
QHTILFKGFFTDHSWYNDLLVDFDSKDIVDKYKGKKVDLYGAYYGYQCAGGTPNKTACM
YGGVTLHDNNRLTEEKKVPINLWLDGKQNTVPLETVKTNKKNVTVQELDLQARRYLQEK
YNLYNSDVFDGKVQRGLIVFHTSTEPSVNYDLFGAQGQYSNTLLRIYRDNKTINSENMH
IDIYLYTS*TW*F*PRNVQIIMNRE*SKSLQAVKKVYVL*YALSKLDR

* - Designates stop codon

Staphylococcus aureus subsp *aureus* ATCC 14458 with Primer Set 2

FANPTDLENKNTNDRLLKHDLLFHDMFVNVASKKDFKVEFENEALSKKFINKNIDIYAG
SYSYECHGGATNKTQCSYGGVTLSDNNK*DDYKNIPCNLWIDGHQTEIELTAVKTKKKI
VTIQELDVQLRNYLNEKYKLYEQGGDIVKGYVKYHNDDEQNVEYDFYNLNGEYGHE

* - Designates stop codon

Staphylococcus aureus PIG-M1 with Primer Set 1

KTSKKEVTVQELDLQARHYLHGKFGLYNSDSFGGKVQRGLIVFHSSEGSTVSYDLFDAQ
GQYPDTLLRIYRDNKTINSENLHIDLYLYTT*ALF*NEKLIKLINK

* - Designates stop codon

Staphylococcus aureus PIG-M1 with Primer Set 2

*VSFANPTDLENKNTNDRLLKHDLLFHDMFVNVASKKDFKVEFENEALSKKFINKNIDI
YAGSYSYECHGGATNKTQCSYGGVTLSDNNK*DDYKNIPCNLWIDGHQTEIELTAVKTK
KKIVTIQELDVQLRNYLNEKYKLYEQGGDIVKGYVKYHNERTKVEYDFYNLNGEYGHEV

* - Designates stop codon

VITA

Mindy M. James

Candidate for the Degree of

Master of Science

Thesis: DEVELOPMENT OF A DIAGNOSTIC METHOD TO ALLOW STRAIN-LEVEL IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS* BASED ON THE NUCLEOTIDE SEQUENCE OF THE ENTEROTOXIN GENE AND THE AMINO ACID SEQUENCE OF ITS ENTEROTOXIN

Major Field: Food Science

Biographical:

Personal Data: Born in Liberal, KS, January 27, 1982, the daughter of Joe and Johnna Caldwell

Education: Graduated from Turpin High School, Turpin, OK, in May, 2000; Received a Bachelor of Science degree in Biology from Oklahoma State University in December, 2004; Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, OK, in December, 2008.

Experience: Student lab assistant at Oklahoma State University, 2005; Research Assistant/Teaching Assistant at Oklahoma State University, 2005-present.

Professional Memberships: Institute of Food Technologists, Sigma Xi: The Scientific Research Society

Name: Mindy M. James Date of Degree: December, 2008

Institution: Oklahoma State University Location: Stillwater, OK

Title of Study: DEVELOPMENT OF A DIAGNOSTIC METHOD TO ALLOW STRAIN-LEVEL IDENTIFICATION OF *STAPHYLOCOCCUS AUREUS* BASED ON THE NUCLEOTIDE SEQUENCE OF THE ENTEROTOXIN GENE AND THE AMINO ACID SEQUENCE OF ITS ENTEROTOXIN

Pages in Study: 101 Candidate for the Degree of Master of Science

Major Field: Food Science

Scope and Method of Study: The purpose of this study was to determine the nucleotide and amino acid sequences of the enterotoxins produced by various strains of *S. aureus* for variations using enterotoxin A as a model. This research was carried out in the hopes that any observed variations would allow the strain of *S. aureus* that produced the enterotoxin to be identified.

Findings and Conclusions: The results of the study indicate that it may be possible to identify a strain of *S. aureus* based on the nucleotide or amino acid sequence of its enterotoxin. Comparison of the nucleotide sequences of the enterotoxin A genes and the amino acid sequences of the translated proteins showed variation between many of the strains of *S. aureus*. The sequences of some strains were identical; however, in these cases the length of 1 sequence was much longer than the other. Because of the discrepancy in length, it is impossible to say that no variations would have been observed had the sequences consisted of the same number of nucleotides.

ADVISER'S APPROVAL: Dr. Stanley E. Gilliland