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GENETIC ANALYSIS OF TOXIC SHOCK SYNDROME TOXIN-1 PRODUCTION
BY STAPHYLOCOCCUS AUREUS STRAINS

University of Hawaii

Ph.D. 1985

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GENETIC ANALYSIS OF TOXIC SHOCK SYNDROME TOXIN-1

PRODUCTION BY STAPHYLOCOCCUS AUREUS STRAINS

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE
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By

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ABSTRACT

The relationship between nutritional requirement and toxic shock syndrome toxin-1 (TSST-1) production by Staphylococcus aureus strains associated with toxic shock syndrome (TSS) was investigated. Auxotyping of S. aureus strains obtained from TSS patients, patients with staphylococcal infections other than TSS and from asymptomatic carriers revealed that a requirement for tryptophan was significantly associated with TSST-1 production ($p < 0.001$, chi square). This association appeared not to be confounded by disease status of the patient from whom the isolate was obtained, or by the anatomic site of isolation. Based on this finding it was hypothesized that tryptophan auxotrophs must satisfy their nutritional requirement by forming close relationships with other organisms in vivo to obtain required nutrients to grow. Escherichia coli strains co-isolated with TSST-1-producing S. aureus strains were able to provide the necessary nutrients for tryptophan auxotrophs to grow and produce TSST-1. In addition, the TSST-1-producing/tryptophan auxotrophs were found to have an identical genetic lesion at the tryptophan synthase gene site leading to the hypothesis that the TSST-1 genetic determinant (tst) may be found on a movable element that preferentially inserts into the tryptophan gene cluster.

Chromosomal mapping by protoplast fusion between a TSST-1-producing S. aureus strain and a non-TSST-1 multiple marker deficient marked S. aureus strain located tst in the tryptophan-tyrosine (trp-tyrB) chromosomal region. Using S. aureus strains with multiple deficiencies in the trp-tyrB region to transform TSST-1-producing strains mapped the tst determinant to the trp site for a tryptophan auxotroph, and at another site near the tyrB region for a non-tryptophan requiring but a slow tyrosine responding strain. The results of this investigation provide a genetic explanation of how TSST-1 production by S. aureus strains is controlled by the availability of required nutrients provided by other organisms found to occupy the same ecological niche.

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INTRODUCTION

Epidemiological and laboratory data have implicated Staphylococcus aureus as the cause of toxic shock syndrome (TSS). A marker protein, Toxic Shock Syndrome Toxin-1 (TSST-1) (12) has been identified in more than 90% of S. aureus isolates from menstrual TSS cases (2,11,29,103) and from at least 62% of non-vaginal TSS isolates (40,46). TSST-1 administered to baboons (21) and to rabbits (4,13,30,31,71,106) induces symptoms of clinical TSS which can be prevented by administration of serum with anti-TSST-1 activity (13,71). Human seroprevalence data indicate that anti-TSST-1 antibody protects against TSS: although 88% of adults >20 years old have circulating antibody to TSST-1, less than 15% of TSS patients have detectable antibody at onset. Only 45% of women with menstrual TSS develop an antibody titer greater than 1:100 by one year after an initial episode of illness (21). Recurrent TSS episodes are identified in patients who fail to seroconvert to TSST-1 (11,21). It has been demonstrated that the incidence of TSS in a region is inversely proportional to the seroprevalence of anti-TSST-1 (120).

Studies of S. aureus strains isolated from patients with TSS suggest that these strains are genotypically different from other staphylococci isolated from asymptomatic carriers or from patients with non-TSS staphylococci infections. TSS-associated strains are predominantly lysed by group I phage types 29/52/80 (2,52,117), more likely to be resistant to penicillin, resistant to heavy metals, more proteolytic, more susceptible to bacteriocins, less

hemolytic, and less pigmented (7,57,117). These unique genetic characteristics suggest TSST-1 production by TSS-related S. aureus strains is not plasmid-associated (52,117), but likely to be chromosomally inserted. A genomic segment that directs TSST-1 production has been cloned (31,51), but none of the phenotypic characteristics associated with TSS isolates have been linked directly to TSST-1 production. For other staphylococcal toxins (15,56,62 73,108), however, a relation between nutritional requirement and toxin production is documented. For example, in a chemically defined medium to identify nutritional conditions required for production of staphylococcal beta-hemolysin, Sharma et al (108) noted a specific requirement for certain amino acids in production of the hemolysin. Likewise, formation of staphylococcal alpha toxin is linked to the biosynthesis of phenylalanine (15).

To find out if an analogous nutritional requirement was linked to TSST-1 production, 154 S. aureus isolates which represented a spectrum of disease severity from TSS to severe staphylococcal disease other than TSS and asymptomatic carriage of S. aureus were obtained and studied.

Specific aims

The objective of this study is to evaluate the relationship of S. aureus nutritional requirements to TSST-1 production by:

1. defining the nutritional requirements of TSST-1-producing S. aureus strains with a micromethod auxotyping system.
2. detecting TSST-1 production in a sensitive enzyme-linked immunoassay.

Upon detecting an association between nutritional requirement(s) and TSST-1 production, this association will then be characterized by:

1. defining the relationship of nutritional auxotrophy and TSST-1 production.
2. identifying conditions for TSST-1-producing S. aureus auxotrophic growth and TSST-1 production.
3. defining the genetic biosynthetic block in TSST-1-producing auxotrophs.
4. locating the site of the TSST-1 genetic determinant on the S. aureus chromosome and providing a genetic explanation for the association between toxin-production and auxotrophism.

LITERATURE REVIEW

Toxic shock syndrome history

The term Toxic Shock Syndrome (TSS) was introduced by Todd et al in 1978 to describe newly identified disease associated with the isolation of Staphylococcus aureus (117). Todd proposed that a new staphylococcal toxin was involved since the syndrome was characterized by evidence of toxemia: acute onset of fever, absence of bacteremia, headache, confusion, generalized erythroderma, vomiting, diarrhea, oliguria, shock, and often followed by desquamation of the palms and soles. Uniform criteria for clinical diagnosis of TSS were established after initial observation of 52 cases (107) (Table 1). Within 2 years, over 400 cases had been reported to the Centers for Disease Control (CDC) (21).

TSS-like syndromes have been described in earlier reports, including a pediatric syndrome observed in 8-12 year old children with erythroderma, toxicity and mild desquamation that was associated with local staphylococcal infections (113). Aranow and Wood (3) described a syndrome indistinguishable from scarlet fever but associated with staphylococcal infection. Three women, ages 19-21 years, described by Dunnet and Schallibaum in 1960 (33) had a scarlet fever-like illness with localized staphylococcal infection. The Staphylococcus pyogenes (sic) isolates from these three women produced erythrotoxic factor(s) that were believed to

Table 1

Toxic shock syndrome case definition (21)

-
1. Fever (temperature >38.9 C or 102 F).
 2. Rash (diffuse macular erythroderma).
 3. Desquamation, 1-2 weeks after onset of illness, particularly of palms and soles.
 4. Hypotension (systolic blood pressure <90 mm Hg for adults of <5th percentile by age for children <16 years of age, or orthostatic syncope).
 5. Involvement of three or more of the following organ systems:
 - A. Gastrointestinal (vomiting or diarrhea at onset of illness).
 - B. Muscular (severe myalgia or creatine phosphokinase level >2 x upper limits of normal).
 - C. Mucous membrane (vaginal, oropharyngeal, or conjunctival hyperemia).
 - D. Renal (blood urea nitrogen or creatinine level >2 x upper limits of normal, or >5 white blood cells per high power field-in the absence of a urinary tract infection).
 - E. Hepatic (total bilirubin, SGOT or SGPT >2 x upper limit of normal).
 - F. Hematologic changes (platelets <100,000/mm³).
 - G. Central nervous system (disorientation or alterations in consciousness without focal neurologic signs when fever and hypotension are absent).
 6. Negative results of the following tests, if obtained:
 - A. Blood, throat, or cerebrospinal fluid cultures.
 - B. Serologic tests for Rocky Mountain spotted fever, leptospirosis, or measles.
-

contribute to the collective symptoms of fever, generalized rash and desquamation of the palms and soles. In another case report of a TSS-like illness (68), a 23 year old woman presented with confusion, erythroderma, fever, hypotension and a strawberry tongue. A penicillin-resistant S. aureus was isolated from the patient's abscess culture, and filtered culture broth from the isolate produced fever and scarlatiniform rash in a human volunteer.

TSS epidemiology

Surveillance and incidence. In a co-operative tri-state surveillance (the tri-state study), the Departments of Health of Wisconsin, Iowa, and Minnesota (29,79) identified 80 cases of TSS between June, 1979 and September, 1980. Most of the cases had onset of symptoms in 1979. Seventy-six cases (95%) were associated with menses, and 10 out of 35 of Wisconsin patients (29%) had at least one recurrent episode during subsequent menstrual periods (79). Epidemiologic investigations indicated that most of cases identified since 1979 were in young, healthy women who used tampons and became ill during the first few days of their menstrual periods. There was a high association between tampon usage, particularly continuous usage, and TSS. By June, 1980, case-control studies statistically linked the onset of menstrual TSS with tampons, leading to a manufacturer's recall of Rely brand tampons (Proctor and Gamble Co.) (107). However, continued active surveillance in Minnesota and Wisconsin after the Rely tampon

recall indicated that the incidence of TSS did not drop; in fact, it has remained constant, with new cases divided equally between those associated with menses and those associated with abscess/wounds (80). TSS is not a common syndrome: even at the height of media publicity the incidence rate was considered to be between 6-14 cases per 100,000 menstruating women and considerably less in men and children (21).

Risk factors: menstrual and non-menstrual. The principal risk factor for developing vaginal TSS is the use of highly absorbant tampons (79). Ninety-nine percent of women with menstrual-related TSS are tampon users (50,107). Such women were also more likely to have had a history of vaginitis in the year before TSS onset (74). Non-menstrual TSS is associated with S. aureus cutaneous/subcutaneous infections, post-surgical complications, localized abscesses and postpartum infections (21,40,119). Both menstrual and non-menstrual TSS patients are young, in the range of 19-27 years (21). The age similarity between case groups associated with different risk factors suggests a shared susceptibility to TSS. Hormone-related factors may also be involved since the majority of TSS occurs in women (21).

Toxic Shock Syndrome Toxin-1 (TSST-1) is considered a reliable marker for S. aureus strains isolated from TSS patients (11,26,91,103,117). Antibody level against TSST-1 varies inversely with susceptibility to TSS (11,120). Only 15% of TSS patients have detectable antibody to TSST-1 at onset of symptoms, and only 45% of

menstrual TSS cases develop anti-TSST-1 antibody one year after initial onset (80). However, ninety-seven percent of adults over 40 years old have high levels of antibody, decreasing to 88% for those 20-40 years old, and 30% for children over 2 years of age (21). Men and women have identical prevalence of anti-TSST-1 antibody (5,79,120).

Recurrences. Recurrent TSS is predominantly associated with menstrual cases. In the tri-state study, 80 patients were noted to have had at least one recurrent episode of TSS with 34% having one or more confirmed recurrences (79). When these 80 patients were stratified by treatment received, it was shown that anti-staphylococcal antibiotics and discontinuance of tampon use was associated with significantly fewer recurrences.

Subsequent serologic studies that prospectively followed patients recovering from TSS showed conclusively that failure to treat with antibiotics and failure to develop anti-TSST-1 antibody were both risk factors for development of recurrent episodes (21,28).

Staphylococcus aureus associated with TSS

Isolation frequency and prevalence of colonization. S. aureus is the predominant organism isolated from vaginal or wound sites in patients with TSS. The isolation rate is 62% (91) from non-menstrual cases and up to 97% from menstrual TSS (11,21). Thus both the isolation of S. aureus from a patient with clinical TSS, and the detection of TSST-1 production are of diagnostic

importance. Up to 10% of healthy menstruating women carry S. aureus without signs of TSS (2,22,38). The identification of TSST-1 as unique marker for TSS strains has simplified case detection. Bergdoll's report that 92% of 142 TSS strains produced TSST-1 has been confirmed by other studies (11,21,117). TSST-1 production by non-TSS strains is found less frequently, both in vaginal (4-40%) and non-vaginal (36%) isolates from healthy carriers (2,22,67).

It is difficult to determine whether the absence of S. aureus strain isolation from confirmed TSS cases and the absence of TSST-1 production in isolated strains reflect actual colonization site situations since the methods of culturing and detection methods vary (7,26,121). In most studies of vaginal cultures, unless the suspect organism was especially looked for, S. aureus is often overlooked because the media used were inappropriate for its isolation; furthermore, prior to the knowledge that TSS-associated strains were less hemolytic (7) and less pigmented (57), and prior to having the ability to detect for TSST-1 directly, a TSST-1-producing colony may have been easily cast aside as being "atypical" S. aureus.

Phage types. S. aureus phage typing groups the staphylococci into standard sets useful for classification and epidemiological studies. The association of toxin production with inclusion in a particular phage type has been well documented including the association of group II phage types with the production of

epidermolytic toxin (ET) in Staphylococcal Scalded Skin Syndrome (SSSS) (72). The TSS isolates described by Todd et al (116) were of phage group I (types 29/52/80). Subsequent epidemiologic studies indicate that 50% of TSS-associated isolates are of the same phage types within group I, the rest belonging either to group III, or being non-typable (52,117). Production of TSST-1 has not been associated with any particular phage group or phage group subset.

Phenotypic characteristics. S. aureus associated with TSS are different from other known staphylococcal isolates. A study of 33 vaginal isolates obtained from confirmed TSS cases, from women with vaginitis, and from healthy controls indicated that most TSS-associated strains were arsenate and cadmium resistant, were penicillin-resistant, were less hemolytic on sheep blood agar, and produced an exoprotein with pI of 7.0 (7). These observations were confirmed by a second comparative study of 20 TSS-isolates and 20 strains from patients with staphylococcal infection other than TSS (117). In addition, TSS-associated strains were often devoid of plasmids, less pigmented, capable of producing one or more of the enterotoxins A-E, more proteolytic and susceptible to bacteriocins.

Markers isolated from TSS-associated S. aureus. The initial description of TSS by Todd et al (116) described S. aureus isolates as predominantly of phage group I, and capable of producing a serologically distinct epidermal toxin that induced desquamation

in newborn mice. These observations established TSS as an unique staphylococcal-toxin-related disease. Cohen and Falkow (25) identified two cell-associated antigenic markers unique to TSS S. aureus isolates by reacting TSS convalescent serum with lysed cell proteins obtained from both TSS-associated and control strains. The presence of unique 30,000 and 33,000 dalton proteins was demonstrated in 78% of 32 TSS isolates and in 5 of 20 (25%) control strains. These proteins, which reacted with anti-TSST-1 serum, were suggested to be cellular precursors of TSST-1 (21).

As confirmed independently by different methods in three laboratories, TSS-associated S. aureus isolates were significantly more likely to produce TSST-1 than non-TSS isolates (11,102,103,117). TSST-1 has become the most reliable marker and the most likely candidate responsible for the development of clinical TSS (13,31,71,90). TSST-1, isolated independently by Schlievert et al (103) as staphylococcus pyrogenic toxin-C, and by Bergdoll et al (11) as enterotoxin F, is a protein of 24,000 daltons with an isoelectric point of pH 7.2. Because of varied methods employed for TSST-1 purification, the molecular weight estimates and reported isoelectic points have been slightly different (7,11,26,47,70,76,101); however, different preparations submitted for biochemical characterization contained the same immunologically unique protein (16,26) with similar or identical biological properties (12).

Biological properties of TSST-1. TSST-1 alone is capable of inducing most of the clinical symptoms of TSS in animal models (21,71); it is capable of inducing enhancement of laboratory-controlled endotoxic shock (32,96), alteration of immune functions (97), production of interleukin-1 (48,81), is subject to receptor-mediated endocytosis (54), and is toxic for human epithelial tissue cells (53).

TSST-1 binding to human epithelial cells. Strains of S. aureus isolated from TSS patients are non-invasive (59), and do not adhere to vaginal epithelial cells obtained from TSS patients nor to cells obtained from healthy women (9). The identity of the target cells, the cell receptors, and the mode of cellular action of TSST-1 are unknown. Recent studies by Kushnaryov et al (53,54) using an in vitro tissue culture cell system have shown that TSST-1 binds specifically to Chang conjunctival cells (human epithelial type) and prolongs their generation time in culture. Kinetic studies using radiolabelled TSST-1 demonstrated that Chang cells were saturated with 10-20 ng TSST-1 per 5×10^5 cells, leading to an estimate of 1×10^4 TSST-1 receptor sites per cell, similar to the binding capacity of other ligands (53). Furthermore, ferritin-labelled TSST-1 was located in coated pits by electron microscopy, implying that TSST-1 is internalized by receptor-mediated endocytosis in a manner similar to that of other bacterial toxins, hormones and interferons. These data are consistent with the possibility that the human epithelium transports TSST-1 into the systemic circulation (54).

Animal models for TSS. A primate animal model for TSS was developed in baboons by Qumiby (21) and by Melish (M. Melish, personal communication). Infusion in adult baboons of up to 100 ug TSST-1 per kg produced nearly all the signs and symptoms of human TSS, including marked decrease in blood pressure, orthostatic hypotension, rash, conjunctival injection, thrombocytopenia and evidence of damage to muscle, liver, and kidney.

Rabbits have provided the most useful model of TSS, including the simulated abscess model using implanted polyethylene practice golf balls, "whiffle balls" (4). Several weeks before experimentation, the balls are surgically implanted in the subcutaneous tissue and allowed to develop a capsule of granulation tissue and fill with fluid. The membraneous tissue around the whiffle ball allows for direct inoculation of live organisms into the chamber without bacterial dissemination.

In this rabbit model, TSS-associated isolates can be differentiated from non-TSS strains: log phase cultures of 11 of 16 TSS-associated S. aureus strains were lethal to experimental animals, whereas no deaths occurred in rabbits injected with control strains (4). Genetic transfer of TSST-1 production into a non-TSST-1 producing, non-lethal S. aureus strain led to increased fatality in the abscess model (90). The most prominent TSS-like signs in the rabbit model are fever, diarrhea, labored breathing, increase in BUN, creatinine, and liver enzyme levels, decrease in serum calcium, and conjunctival hyperemia.

A rabbit genital tract-TSS model has been reported (31). Isogenic strains of S. aureus differing only in the presence of a cloned plasmid-borne TSST-1 determinant (tst) were inoculated into diffusion chambers consisting of tubing sealed with 0.22 u filters that had been implanted into rabbit uteri. The tst-bearing strain produced signs identical to those of rabbits injected with purified TSST-1, while both the non-TSST-1 parental strain and the strain carrying an inactivated tst plasmid produced no severe symptoms. In the same study, implantation with six TSST-1-producing and five non-producing strains revealed that only the TSST-1-producing strains produced disease, including lethal TSS-like effects (4/6) and milder toxic disease (2/6).

Twelve to fifteen week old male New Zealand rabbits were found to be more susceptible to lethal infections with TSST-1-producing strains than female and neutered animals (13,106). However, male rabbits treated with synthetic estrogen were less likely to die than controls (13). These data suggest that poorly defined hormonal factors may play a role in the development of TSS. In the same study, if the rabbits were pretreated with anti-TSST-1 serum the lethal effect of TSST-1 was neutralized (13).

In various experiments with the rabbit TSS model, it became apparent that not all TSS strains were lethal and not all were TSST-1 producers. It was suggested, therefore, that another biologically active substance was responsible for TSS (31). However, more recent studies that control for breed of rabbit (4),

age (30), sex (13), and experimental pre-conditioning (13) suggest that only TSST-1 is responsible for all of the TSS-like signs, including rabbit lethality.

Enhancement of host susceptibility to lethal endotoxic shock.

TSST-1 biological activity was initially reported to be similar to the streptococcal pyrogenic toxins (103). Pyrogenic toxins are defined by a capacity to induce high fever, enhance susceptibility to endotoxins and produce a transient suppression of RNA synthesis in liver cells that leads to a block in clearance of endotoxin. It was therefore postulated that the combination of staphylococcal TSST-1 and host-derived endotoxin may induce many of the features of TSS. Healthy rabbits sequentially administered small amounts of TSST-1 developed TSS-like signs and subsequent gram-negative bacteremia (96). These rabbits demonstrated a greater than 50,000-fold increase in susceptibility to endotoxin, in dose-dependent fashion, after TSST-1 pre-treatment. Schlievert et al (96) noted that rabbits not pretreated for gram-negative pulmonary infection were highly susceptible to TSST-1: approximately 50% were killed by a single injection of TSST-1 at either 10 or 30 ug/kg, and 100% of the survivors died upon subsequent challenge with 30 ug/kg TSST-1. In comparison, rabbits pre-treated with tetracycline for pulmonary infection with Pasteurella multocida survived identical TSST-1 doses, suggesting that the TSS-like disease and death in non-treated animals resulted

from enhanced susceptibility to Pasteurella endotoxin as a consequence of TSST-1 immunosuppression and reticulendothelial blockade.

de Azavedo et al (32) have subsequently demonstrated TSST-1 potentiation of endotoxin effects in chicken embryo assays: TSST-1 itself had no effect on chicken embryos, but at low doses acted synergistically with 0.45 ug/ml endotoxin to produce fatal disease.

Four strains of mice differing in sensitivity to endotoxin were examined for hematologic changes following intra-peritoneal injection of 50 ug of TSST-1 (20). Strain A/HeJ, most sensitive to endotoxin, was also most sensitive to TSST-1, showing decreases in hematocrit, depletion of lymphocytes and thrombocytopenia. Mouse strains intermediate in endotoxin sensitivity showed a direct decrease in blood chemistry changes, while an endotoxin resistant strain, C3H/HeJ, had no significant changes after exposure to TSST-1.

In contrast, the lethal effects of TSST-1 in rabbit abscess model were not eliminated by pretreating rabbits with anti-J5 endotoxin anti-serum, a pretreatment directed at inactivating endogenous endotoxin components (71). Pretreatment of rabbits with anti-TSST-1 serum, however, blocked the signs of TSS (13,71). The issue of TSST-1-enhanced lethal endotoxic shock is complex and may not be relevant to human TSS since the effects of enhanced lethal toxicity has only been observed in laboratory models; moreover, the pathologic aspects of TSS differ from those of endotoxic shock (21).

Alteration of immune function. Proposed effects of TSST-1 include toxin-induced activation of lymphocytes, including T-suppressor cells, to depress the humoral immune response to other antigens, such as those of endogenous normal flora or opportunistic organisms (97). In vitro experiments show that TSST-1 caused suppression of synthesis of IgM antibody to sheep erythrocytes in a plaque-forming cell assay consistent with TSST-1-stimulation of a population of regulatory cells to suppress the humoral immune response to second antigens (97). Since many patients with TSS fail to make antibodies to TSST-1 and it has been suggested that the toxin alters T-cell responses based in part upon data from rabbit experiments in which 50% of animals failed to develop an antibody response to TSST-1 but were able to develop antibody to sheep erythrocytes.

The speculative role of TSST-1 as an immunoregulatory agent is also based upon in vitro demonstration of TSST-1-proliferation of lymphocytes (97). TSST-1-induced proliferation of lymphocytes from three animal species (human, rabbit, and mouse) suggested that TSST-1 functions as a nonspecific T-cell mitogen in each of these three systems (87). The experiments demonstrating TSST-1-induced alteration of T-cell immune functions in fact do not correlate with in vivo observation that fatally ill TSS patients exhibit evidence of lymphopenia, and autopsy reveals lymphocyte depletion in the lymph nodes and spleen (21).

Interleukin-1 induction. Interleukin-1 (IL-1) is a single substance, or a closely-related group of substances also known as endogenous pyrogen in leukocytic pyrogen assay (LP) and as lymphocyte activating factor (LAF). IL-1 is synthesized by and released from mononuclear phagocytes following exposure to a variety of microbial products and to various substances that induce inflammation. IL-1 production which continues long after the removal of the inducing agent, causes fever. In rabbits IL-1 production can be detected both by development of fever, by LP, and by its mitogenic activity for thymocytes in LAF.

Culture filtrates of TSS-associated S. aureus strains induce circulating human monocytes to secrete IL-1 (48,81). Ikejima et al (48) clearly demonstrated that 5 TSS-associated S. aureus isolates, and a TSST-1-producing wound isolate were significantly better inducers of IL-1 when compared to 8 control strains of S. aureus isolated from the vaginal flora of healthy women without TSS. Purified TSST-1 incubated with adherent monocytes induced production of LAF that enhanced mitogen-induced proliferation of murine thymocytes (81). The product generated in these assays was shown to be IL-1 by addition of human anti-IL-1 to the culture fluids: both LP and LAF activities were abolished (48). The LAF-induced proliferation was neither due to direct TSST-1 stimulation of thymocytes, nor to contamination with endotoxin since co-incubation of TSST-1 with polymyxin B, a known inactivator of endotoxin, did not attenuate IL-1 induction.

On the basis of these experiments, purified TSST-1 could be the "exogenous pyrogen" required to induce IL-1 production and the consequent febrile response seen in TSS patients without bacteremia or severe local inflammation. Furthermore, it has been suggested that IL-1 activity is mediated by arachidonic acid (48) leading to production of prostaglandins and leukotrienes, which may contribute to the diarrhea and hypotension associated with TSS. However, since IL-1 production may be induced non-specifically, the relationship of TSST-1 to IL-1-induced effects in TSS remains to be clarified, as does the possibility of direct interactions between TSST-1, the toxin-producing S. aureus strain, and the host.

Other TSS-associated bacterial products. Nakashima and Wuepper have isolated a protein similar in molecular weight and pI to TSST-1. Though this protein does not appear to be identical to TSST-1, this protein elicits nonspecific mitogenic activity and is presumed to be an IL-1 inducer as well (21).

Staphylococcal enterotoxins are a group of proteins in the 25,000-30,000 dalton molecular weight range with pI's of 6.8-7.2. TSST-1 was originally named enterotoxin F (11) because of its physical and biological similarities to enterotoxins. Later work showed that earlier preparations of TSST-1 contained components of enterotoxin. When such preparations were purified serological similarities between TSST-1 and the known enterotoxins were not found. However, as noted, TSST-1-producing strains are more likely to elaborate one or more of the enterotoxins (117), especially enterotoxin B.

An encapsulated, spontaneous mucoid variant of a TSS-associated isolate was detected by electron microscopy (58). This strain produced a 40-fold increase in LD₅₀ when injected into mice. Viable organisms persisted longer in mice infected with the mucoid strain, and were shown to induce production of anti-capsular antibodies by ELISA. The role, if any, for these capsular antigens in TSS is uncertain since most TSS isolates are not mucoid at the time of isolation.

Todd et al (117) have hypothesized that TSS strains produce a protease which effects post-translational modification of S. aureus proteins. This theory suggest that, though TSST-1 may serve as the detectable marker associated with TSS, other less stable post-proteolytic products are more important in TSS pathogenesis. In fact, it is further suggested that the effects of TSS may be mediated by the acquisition of a staphylococcal genetic alteration affecting proteolysis. The ensuing proteolysis precipitates the production of markers and at the same time, modifies host response to a common bacteria.

Enviromental and nutritional factors influencing TSST-1 production

Vaginal flora and influences of host physiology on S. aureus colonization and TSST-1 production. The types of microorganisms colonizing a particular site are determined by the nature of that microenviroment. The vagina and its microbial flora represent an ecosystem that is constantly changing due to hormonal influences

and to menstruation. Various constituents of the vaginal microenvironment may also be expected to have a role in controlling the flora. In the normal vaginal-cervical flora, lactobacilli dominate the facultative flora of the genital tract in 96% of females (8). Peptococci, peptostreptococci, and bacteriodes comprise the major anaerobic species in the vaginal flora and outnumber facultative aerobes by a ratio of 10 to 1 (55). In sequential quantitative vaginal cultures of healthy women, facultative gram-positive cocci were represented predominantly by Group B streptococci, by S. epidermidis (41%), and uncommonly by S. aureus (<5%) (8). Gram-negative rods found frequently are Enterobacteriaceae species and the most commonly found is Escherichia coli (55).

Cervical colonization by S. aureus increases during normal menstruation (8,95). Larsen and Galask (53) sampled vaginal cultures of 31 women studied prospectively during their menstrual cycles and detected 3 women who were culture positive for S. aureus only during menstruation. All 31 women carried a greater variety of organisms during menstruation than at any other time during their menstrual cycles. In another study, 50 women cultured sequentially during their menstrual cycles showed that moderate to heavy growth of S. aureus was only found during menstruation (110); cervical colonization with E. coli and other Enterobacteriaceae also increased during menstruation in women using tampons and absorbant sea sponges (110).

A prospective study (23) of vaginal cultures for 495 healthy women found that those who carried TSST-1-producing staphylococci (54% of the total) also carried E. coli significantly more often than those who carried either non-TSST-1-producing S. aureus (15%) or no staphylococci at all (11%). The relative frequency of co-isolation of E. coli among women carrying TSST-1-producing S. aureus was 8-11 times that of women not carrying a TSST-1-producing S. aureus. E. coli may be the source of endogenous endotoxin that is enhanced by TSST-1 as has been proposed (96), or the organisms may exist with TSST-1-producing S. aureus in an as yet unexplained synergistic relationship.

Association of nasal carriage of TSST-1 producing strains and vaginal colonization. An association between nasal and vaginal carriage of the same phage-type S. aureus led to the hypothesis that individuals with multi-site colonization are at risk for TSS (67). Serial vaginal and nares cultures from 39 women with the same phage types showed that the genital S. aureus were transient while organisms of the nares persisted. In one case, the vagina was repeatedly recolonized during menses with the same toxin-producing strain (61). Human seroprevalence studies have shown that though TSS patients have undetectable levels of TSST-1 antibody, healthy individuals harboring nasal TSST-1-producing strains have significantly higher levels of serum antibody to TSST-1 than carriers of non-TSST-1 strains or individuals without S. aureus colonization (93). Conceivably, TSST-1-strain carriers

with low or undetectable anti-TSST-1 serum antibody may constitute a high risk group for TSS (67,75).

Implications for vaginal and wound TSS. The insertion of a tampon into the vagina during menstruation or packing and dressing into an wound may constitute a foreign body that disrupts the normal flora. Fifteen surgical patients with post-surgical staphylococcal complications were shown to have been co-infected with their own nasal strain as determined by isolation prior to surgery (75), suggesting that the development of vaginal and non-vaginal TSS may be analogous.

Tampons and TSST-1 production. The relationship between tampon constituents, bacterial growth, and toxin production has been extensively studied, including evaluations of the effect of tampons on growth of S. aureus and TSST-1 production in vitro. The earliest studies showed that tampon components neither supported bacterial growth nor stimulated TSST-1 production, but that tampon insertion into the vagina increased the oxygen content of the internal environment, creating necessary conditions for S. aureus growth (49) and expression of TSST-1 (99,100). Tierno et al (115) suggested earlier that in Rely brand tampons, degradation of the carboxymethylcellulose (CMC) to glucose stimulated growth of TSS-associated S. aureus. However, contradictory data of Schlievert et al (100) indicated that increased glucose levels inhibited the action of microbial cellulase and that TSST-1 production was decreased in cultures with increased levels of glucose.

The in vitro fiber content of tampons directly influences the amount of TSST-1 recoverable from culture fluids (74,98). Cotton, viscose rayon, CMC, either alone or in combination had no effect on bacterial growth or toxin production. Polyester foam with CMC chips (Rely tampons) increased toxin production by ten-fold without significantly increasing bacterial growth. Tampons composed of polyacrylate rayon inhibited both bacterial growth and TSST-1 production. The inhibition of growth and suppression of TSST-1 production was reversible by addition of 0.5 ug Mg⁺⁺/ml to chemically defined medium, and by addition of >5 ug Mg⁺⁺/ml to beef-heart medium pre-absorbed with polyacrylate fibers (98). Preincubation of complete defined medium with tampon components indicated that polyacrylate fibers were capable of removing 97% of total Mg⁺⁺ from the medium (from 12.2 ug/ml to 0.4 ug/ml). In contrast, polyester foam/CMC fibers only reduced Mg⁺⁺ levels by 50% (74). These data suggest that tampon fibers differ in their ability to absorb Mg⁺⁺, and that reduced concentrations of Mg⁺⁺ in controlled environments directly affect S. aureus growth and TSST-1 production.

Relationship of nutritional factors and S. aureus protein production. The availability of nutritional factors have been shown to affect staphylococcal exoprotein production. Arvidson et al (6) observed that S. aureus grown in batch cultures in nutrient excess led to reduced synthesis of phosphatase, protease I and

other enzymes. When the production of these enzymes were studied under nutritional limitation, production was increased by three-fold. Continuous culture studies in a chemostat of staphylocoagulase production showed that its production was influenced by low oxygen levels and magnesium limitation (34). Studying the relationship of nutritional factors and TSST-1 production by S. aureus strains have demonstrated that decreased magnesium (74) and glucose levels (100) led to increased TSST-1 production. These studies did not address how other media components like amino acids may affect TSST-1 production by S. aureus. Therefore if TSST-1 production can be shown to be influenced by the availability of necessary amino acids, this would furnish an important clue in understanding how TSST-1 production could be controlled.

Use of genetic manipulations to study S. aureus genes of interest

Understanding of bacterial genetics has advanced with isolation and characterization of many mutant strains having easily identifiable phenotypic characteristics. The recognition and use of systems of genetic transfer has provided the basis for studying functional genomic organization of bacteria. The isolation of DNA from most bacterial species involves lysis or disruption of the cell wall, removal of cell protein by denaturation, removal of RNA, and selective precipitation of DNA with ethanol. However,

isolation and characterization of the staphylococcal genome has not progressed rapidly because staphylococci are relatively resistant to lytic enzymes and mechanical disruption.

Staphylococcal chromosome. Genetic mapping of the S. aureus chromosome has been accomplished by the induction and isolation of mutants in S. aureus NCTC 8325 and the subsequent manipulation of the mutants by transduction, transformation, and protoplast fusion. Mutants were obtained either by selection of naturally-occurring strains or by induction with UV-irradiation, by chemical treatment, or by insertion of translocatable elements. In 1975, the three major S. aureus linkage groups were defined by Pattee and Neveln (85) using nutritional deficiencies as selective phenotypic markers in transformation studies. The three linkage groups were composed of 10 separate functional markers and were further defined by the localization of antibiotic resistance markers, phage attachment sites, and pigment production. Pattee and Glatz (84) divided the 3 linkage groups into overlapping segments, each of which was bound by a pair of easily defined phenotypic markers. Chromosomal mapping was further expanded by insertional mutagenesis using a translocatable element, Tn551 (63,83). Use of protoplast fusion in conjunction with transformation data arranged the S. aureus linkage groups into one continuous circular bacterial chromosome (111). Subsequent transformation data have confirmed many of the linkages established by protoplast fusion (112), and more detailed analysis

of the genetic loci have been completed by transduction mapping with Tn551 inserted mutants (104).

Protoplast fusion. Fusion analyses in S. aureus have been used to determine the probable location of specific markers. The genetic location of the markers can be confirmed by transformation and transduction with the help of Tn551 insertional mutagenesis (63). Protoplast fusion is unique as a mode of genetic exchange in prokaryotes; it is unlike such established modes of genetic exchange as generalized transduction, transformation, and conjugation which are mediated strictly by an unidirectional transfer of fragments from the donor chromosome. Fusion leads to bi-directional transfer of genetic information so that intact chromosomes from each parent have an equal chance for inheritance. Analysis of genetic fusion results in several bacterial systems showed that during fusion, markers closely linked in the parental strain were transferred to the fusion progeny at high frequencies; while markers that were distantly linked were transferred at much lower frequencies.

Transduction. Transduction can be used to map for closely linked markers, and has been achieved for a wide range of chromosomal and plasmid markers. Most of the phages which transduce belong to either serological group B or less frequently to group F. Transducible strains of S. aureus are typically of phage group I or III. The amount of bacterial DNA which can be transferred is limited by the size of the phage particle into which

it must be incorporated. The size of the phage genome is in the range of 28 megadaltons (transducing phage 11) to about 31 megadaltons (typing phage 80), representing about 1% of the total staphylococcal DNA. Therefore, although the amount of DNA carried by the phage particle limits the extent to which transduction can be used to determine a large scale linkage map, transduction studies are ideally suited to detailed mapping of closely placed markers (104).

Transformation. Transformation provides certain advantages over transduction since it is a higher frequency event, and since the fragments of transferred DNA are generally larger. Markers which show less than 20% linkage are detectable by transformation but may be too far apart to be co-transduced by phage 11 (115).

Transformation, which involves the uptake and expression of DNA from a donor strain by a recipient strain, has been used, like transduction, to study both chromosomal and plasmid markers in S. aureus (86). The ability of a strain to be transformed is loosely referred to as "competence". Competence depends on a number of well defined factors (115). Competence-conferring activity may be associated with phage tail fiber protein and is not dependent on phage gene expression. The competence of a strain is also dependent upon the presence of a high concentration of divalent ions, among which Ca^{++} is the most effective. Unsuccessful transformation may be due to nuclease production by the recipient strain or to the presence of host restriction. Experimentally, the

former may be overcome by the use of mutant nuclease-negative recipients and the latter either by heating the recipient at 56 C for 2 mins to effect temporary inactivation of the restriction enzymes or by selection of restriction-negative mutants.

Transposons. Transposon (Tn) elements are defined as small DNA segments capable of insertion at numerous sites in a bacterial genome in the absence of both chromosomal directed recombinant function and extensive base pair homology. Transposons insert neither entirely at random (like phage Mu), nor as site-specific (as lambda phage) elements; rather, Tn elements exhibit either regional or localized specificity for an insertion area. That is, a Tn may integrate as a discrete unit in many sites within a short DNA segment (83), or integrate preferentially into small target DNA sequence "hot spots", or into a limited number of sites with a similar sequence. Transposons are useful in genetic mapping because they carry genomic information encoding for markers that can be easily identified and selected, such as antibiotic resistance, heavy metal resistance, production of metabolic enzymes, or other unique extracellular antigens.

The insertion of a Tn element into a gene of known function usually eliminates the function of that gene (e.g., insertion into a structural gene for tryptophan biosynthesis would result in a tryptophan auxotroph) (104). The mutant phenotype remains intimately associated with the genetic determinant encoded by the inserted transposon, and allows for the selective transfer of this

mutation into a new genetic background. Transposon mutagenesis is easier to perform experimentally without the need to control for multiple mutational events as is the case with traditional methods of inducing mutations by chemical mutagens or by UV-irradiation.

Currently, there are three Tn elements amenable to use in genetic analysis of S. aureus, all of which encode for resistance to erythromycin. However, the nature of erythromycin resistance and the preferential insertion sites are different. Of the three transposons, Tn551, which carries constitutive erythromycin resistance (ermB) with regional specificity has been the most studied. Tn551 is a 5.2 kilobase transposon that was initially detected on plasmid pI258. Definitive evidence that the ermB determinant was a component of a transposon was obtained by construction of a thermosensitive replication-defective mutant of pI258 (78). A S. aureus strain harboring a thermosensitive derivative of pI258 was cultured in the presence of erythromycin at the non-permissive temperature. Under these conditions, only cells containing chromosomal Tn551 inserts could survive, and they could be easily selected because they displayed an erythromycin resistant phenotype (Em^R). Luchansky and Pattee (63) used this temperature sensitive device to place Tn551 near markers of interest.

Genetics in relation to toxin production in S. aureus.

S. aureus extracellular toxin are of several classes including the cytolytic toxins, leucocidin, epidermolytic toxins, and the

enterotoxins. Considerable interest has been directed toward understanding the genetic control of toxin production. Studies on the genetics of staphylococcal toxins have been hampered by the lack of suitable vectors for cloning experiments and by the number of defective prophages in many strains of S. aureus that restrict the exchange of DNA necessary for mapping biological markers of interest.

Rogolsky comprehensively reviewed the genetic basis for epidermolytic production (ET) (94). Results cumulated from many studies strongly suggested that there are two immunologically distinct ET species both of which induce histologically identical separation of cells in the stratum granulosum. Epidermolytic strains of S. aureus may produce either ETA/ETB or both. ETA-producing strains cured of detectable plasmids retained the ability to elaborate ETA while ETB-producing strains could no longer produce ETB when they were grown under conditions which promoted loss of plasmids. Tryptic peptide maps for ETA and ETB revealed only 4 out of 30 spots to be identical, suggesting differences between the two toxins in amino acid composition. The ETB genetic determinant is associated with a 42 kb plasmid and found in strains of differing phenotypes isolated from different parts of the world. The genetic determinant coding for ETA production has yet to be located.

Alpha hemolysin production was mapped by Brown and Pattee (19) to a site between the purine (pur) and the isoleucine/valine (ilv)

complex. Since the genetic determinant for the production of enterotoxin A (entA) has been located in the same chromosomal area (64), this chromosomal region appears to be important to the production of pathogenic extracellular products.

Betley et al (14) cloned the entA determinant in pBR322 in E. coli and found it to be within a 2.5-kb HindIII fragment. The entA element codes for enterotoxin A (SEA) production. This genetic determinant is a heterologous insertion detectable in at least two chromosomal locations and to be absent in entA-negative strains. The size and behaviour of this element suggest that it is similar to a transposon. The entA element seems to be a hot spot for structural rearrangements since a study of 16 strains, there were 6 different DNA-DNA hybridization patterns with respect to the presence, type, and location of the characteristic 2.5 kb fragment. Twenty-four of 29 SEA-producing strains had an identifiable entA element located in the pur-ilv region while the remaining 5 strains contained either an incomplete element at the same site, or an intact copy at some other location.

More recently the genes encoding for resistance to penicillin, cadmium, mercury, lead, arsenate, and arsenite were located on a 56.2 kb plasmid pZA10 from S. aureus strain 6344, that produced both enterotoxin B (SEB) and enterotoxin C₁ (SEC₁) (1). Elimination of pZA10 resulted in the loss of ability to produce both enterotoxins. Transfer of the plasmid DNA into either a standard non-toxigenic recipient (S. aureus RN450) or into a

plasmid-cured S. aureus 6344, resulted in integration of the plasmid and enterotoxin production into the chromosome of the recipient strains. This suggests that pZA10 carries either structural genes for SEB and SEC₁, or essential regulatory genes which switch on inactivated structural genes in the recipients. The ability to produce SEB was correlated with the presence of an 18.1 kb fragment while the genetic information for SEC₁ production was identified on a pZA10 segment adjacent to BglII fragments of 8.6 kb and 6.0 kb.

The majority of TSS-associated S. aureus strains express a series of common phenotypic traits suggesting that these strains may be associated with prophages with similar plaque characteristics (105). These phages were demonstrated by Kreiswirth et al (51) to lack the genetic determinant for TSST-1. The same group cloned and expressed a 10.6 kb chromosomal fragment that contained the purported structural gene for TSST-1, and upon subcloning, it was found that only a small segment containing 300 base pairs was required for TSST-1 expression. Blot hybridization of chromosomal DNA of several S. aureus strains with the TSST-1 positive clone suggested that the TSST-1 determinant is part of a larger segment of DNA that is absent or rearranged in TSST-1 negative strains. However, the blotting patterns were complex, and 2 TSST-1-positive strains each had two homologous fragments while another TSST-1-positive strain and 2 TSST-1-negative strains had a

third larger fragment, all of which hybridized with the probe. Thus the TSST-1 gene segment is variable and localizable to different sites on the staphylococcal chromosome.

In summary, the genetic determinants encoding for the production of plamid-controlled epidermolytic toxin B, enterotoxins A, B and C₁ and TSST-1 have been identified. The location of the chromosomal determinants for alpha-hemolysin, and enterotoxin A were mapped to proximal sites in the pur-ilv region, while the determinant for SEB and SEC₁ has been determined to be on a plasmid which can translocate to chromosomal DNA. All of the possible sites in which each of these toxin genes reside on the staphylococcal genome have not been conclusively determined, and await further analysis by DNA hybridization and molecular sequencing. The complex data of the cloned genetic determinant experiments suggest that in each case there is either a favored site, or hot spot, where the genetic determinant primarily resides, with incomplete or rearranged genes at the same spot in negative and weakly expressing strains, or have complete but unexpressed genes located elsewhere on the staphylococcal chromosome.

MATERIALS AND METHODS

Bacterial strains

All bacterial cultures were stored at -70 C on glass beads in Todd-Hewitt broth (THB, Difco, Detroit, MI) and 50% glycerol.

TSS-associated and control S. aureus strains. One hundred and fifty-four human isolates were selected for study: 78 vaginal isolates, 54 isolates from wounds and abscess, 10 blood isolates, 9 nasopharyngeal isolates and 3 throat isolates. Phenotypic characteristics of the 154 S. aureus strains are listed in Appendix A.

Table 2
Disease association of S. aureus strains used in this study

	<u>TSS</u>	<u>non-TSS</u>	<u>Received from</u>
	10	18	A. Barbour, N.I.A.I.D.
	15	10	A. Reingold, C.D.C.
	10	10	A. Chow, Univ. of British Columbia
	14	17	J. Todd, Univ. of Colorado
	1		M. Bergdoll, Univ. of Wisconsin
	<u>7</u>	<u>42</u>	Department of Tropical Medicine,
<u>Totals</u>	<u>57</u>	<u>97</u>	<u>Univ. of Hawaii</u>

N.I.A.I.D.=National Institute of Allergy and Infectious Diseases.

C.D.C.=Centers for Disease Control

Genotype of *S. aureus* strains selected for genetic mapping.

Eighteen strains were selected for genetic mapping studies. The Iowa State-Pattee (ISP) strains were obtained from or constructed at the laboratory of Dr. P. Pattee, Iowa State University, Ames, Iowa. Each auxotrophic or resistance character is listed after the ISP number with the allele number when available (Table 3). Numbers preceded by Ω X[Tn551] indicate the insertion of an erythromycin (ErmB) resistant marker.

Table 3
S. aureus strains used for genetic mapping

ISP no.	Genotype
2	8325 nov142 pig131
5	8325 thy101 thrB106 ilv129 pig131
8	8325-4 pig131
391	8325 thrA118 pig131 Ω 8[Tn551]
484	8325 thy101 thrB106 ilv129 tmn3106 purB110 pig131 Ω 40[Tn551]
542	8325-4 purC193[Tn551] pig131
552	8325 thy101 pig131 trp159[Tn551]
630	8325 thy101 thrB106 ilv129 pig131 uraB220[Tn551]
646	8325 thy101 thrB106 ilv129 pig131 trp263[Tn551]
681	8325 thy101 thrB106 ilv129 pig131 tyrB282[Tn551]
830	8325 Ω 11[Tn551]
945	8325 thr106 ilv129 mec4916 nov142 pig131 ala126 tmn3106 trpE85 tyrB282[Tn551 ermB321] Ω 40[Tn551] uraB232[Tn551 ermB327]
1074	8325 nov142 pig131 fus149 tet3490 PurC193[Tn551]
1083	8325 nov142 pig131 fus149 Ω 1035[Tn551] tet3490
1526	FRI-1169 tst
1532	FRI-1169 tst nov142
1637	S411 tst trp
mc8	S411 tst trp nov142
1653	8325 lys115 trp103 thrB106 ala126 tmn3106 ilv129 pig131 uraA141 tyrB282[Tn551 ermB321] Ω 1035[Tn551] fus149

Escherichia coli strains. Twenty-nine human urinary tract and vaginal E. coli strains were kindly supplied by Dr. A. Chow, University of British Columbia, Vancouver, British Columbia. Several of the vaginal E. coli were co-isolated with S. aureus from either healthy persons or from TSS patients. These E. coli strains were used in crossfeeding experiments with the 154 S. aureus strains. Crossfeeding results are given in Appendix B.

Table 4
Escherichia coli isolates used in this study

Strain (UBC#)	Isolation site	Characteristic
EC1 (6094)	urine	non-TSS, no <u>S. aureus</u>
EC2 (6095)	urine	non-TSS, no <u>S. aureus</u>
EC3 (6096)	urine	non-TSS, no <u>S. aureus</u>
EC4 (7004)	urine	non-TSS, no <u>S. aureus</u>
EC5 (7005)	urine	non-TSS, no <u>S. aureus</u>
EC6 (7007)	urine	non-TSS, no <u>S. aureus</u>
EC7 (7008)	urine	non-TSS, no <u>S. aureus</u>
EC8 (7009)	urine	non-TSS, no <u>S. aureus</u>
EC9 (7010)	urine	non-TSS, no <u>S. aureus</u>
EC10 (7020)	urine	non-TSS, no <u>S. aureus</u>
EC11 (7346)	vagina	non-TSS, TSST- <u>S. aureus</u>
EC12 (7373)	vagina	non-TSS, TSST- <u>S. aureus</u>
EC13 (7376)	vagina	non-TSS, TSST+ <u>S. aureus</u>
EC14 (7498)	vagina	non-TSS, TSST- <u>S. aureus</u>
EC16 (7529)	vagina	non-TSS, TSST- <u>S. aureus</u>
EC17 (7543)	vagina	non-TSS, no <u>S. aureus</u>
EC18 (7544)	vagina	non-TSS, no <u>S. aureus</u>
EC19 (7603)	vagina	TSS, TSST+ <u>S. aureus</u>
EC20 (7611)	vagina	non-TSS, no <u>S. aureus</u>
EC21 (7632)	vagina	non-TSS, no <u>S. aureus</u>
EC22 (7635)	vagina	non-TSS, no <u>S. aureus</u>
EC23 (7637)	vagina	non-TSS, TSST+ <u>S. aureus</u>
EC24 (7638)	vagina	non-TSS, no <u>S. aureus</u>
EC25 (7648)	vagina	non-TSS, no <u>S. aureus</u>
EC26 (7649)	vagina	non-TSS, no <u>S. aureus</u>
EC27 (7669)	vagina	non-TSS, no <u>S. aureus</u>
EC28 (7670)	vagina	TSS, TSST+ <u>S. aureus</u>
EC29 (7738)	vagina	TSS, TSST+ <u>S. aureus</u>
EC30 (7877)	vagina	non-TSS, no <u>S. aureus</u>

UBC#—Univeristy of British Columbia identification number.

Phenotypic characterization of 154 S. aureus strains

Coagulase. Strains were tested both by slide and tube coagulase tests (69) using Bacto-Coagulase Plasma (Difco). For the slide test, one drop of staphylococcal suspension is mixed with a drop of citrated rabbit coagulase plasma and observed for marked clumping within 5 to 20 seconds. To confirm the slide test, a heavy loopful of staphylococci was mixed with 1 ml of rabbit coagulase plasma, incubated at 37 C and observed for clot formation between 30 mins to 4 hours.

Hemolysis. Each strain was grown on 5% sheep blood agar at 37 C for 24 hrs, beta-hemolysis was recorded as the degree of red blood cell lysis from no change to complete clearing around the staphylococcal growth.

Protease production. Casein proteolysis was studied by the method of Martley et al (66) on yeast extract-sodium caseinate agar. Each plate was divided into 3 sections and each strain was stabbed once into each area. Plates were incubated for 48 hours at 37 C, after which the average diameters of the three zones of casein digestion for each plate were recorded.

DNase production. Standard DNase agar (Difco) plates were each stabbed with 4 strains of staphylococci, and incubated for 24 hours at 37 C. The plates were then flooded with a 0.01% toluidine blue O (Allied Chemical Corp., New York, N.Y.) in saline at room temperature and reactions observed after 30 minutes (69). A

positive rose-pink zone, indicative of DNase production, was measured and recorded.

Toxin production. Enterotoxin B production was identified by modified immunodiffusion on thin-layer trypticase soy agar (TSA) with specific anti-serum and control antigens, kindly supplied by A. Johnson, U.S. Army Medical Research Institute of Infectious Diseases, and with rabbit anti-enterotoxin B (Sigma Chemical, St. Louis, Missouri). Three ml of TSA was placed in a 50 mm petri dish. After the agar solidified, 5 ul of an overnight test culture grown in Todd Hewitt broth (THB, Difco) was spotted in one of four sectors. Plates were incubated for 24 hrs at 37 C, after which time a central well and two peripheral wells were punched. The central well was filled with 8 ul of undiluted anti-serum and the other wells with 8 ul of 50 ug/ml of control toxin. Plates were re-incubated at 37 C for 24 hrs and toxin-producing strains identified by presence of precipitin lines of identity between control toxin and test culture.

Delta toxin production was detected by enhanced synergistic hemolysis at the juncture of a beta-hemolysin-producing strain and a delta toxin-producing strain (17). A beta-hemolysin-producing S. aureus strain was streaked down the center of the plate and four test strains placed perpendicularly up to but not touching the center streak. The plates were incubated at 37 C and after 24 hrs delta toxin production was detected by enhanced hemolysis between the center streak and the test strain.

TSST-1 production and purification

A TSST-1-producing strain FRI-1169 was grown in Todd-Hewitt Broth in a 37 C shaker incubator for 24 hours. The resulting TSST-1 was purified by cation exchange chromatography (CMC-50 Sephadex, Pharmacia, Inc., Piscataway, New Jersey), followed by size separation on a Sephacryl S-200 column (Pharmacia). Protein fractions were further separated by flat-bed preparative isoelectric focusing (LKB). The reactive band in the pH 6.8-7.2 range was pooled and refocused. The focused protein band at pI 7.2 contained the toxin. Purified TSST-1 reacted with hyperimmune rabbit antiserum, stained as a single band at pI 7.2 on a thin-layer isoelectric focusing gel, was visualized as a single band of 24,000 daltons on a 12.5% SDS-PAGE gel by silver staining (Figure 1), and reacted with both TSST-1 hyperimmune rabbit serum and TSST-1-reactive monoclonal antibody AC3 by Western blot (118) (Figure 1).

Anti-TSST-1 serum production and purification

Rabbit anti-TSST-1 serum. Polyclonal rabbit antiserum was prepared by i.m. inoculation of 10 ug of purified TSST-1 mixed with Freund's complete adjuvant into adult New Zealand white rabbits. Rabbits were boosted with 10 ug TSST-1 at multiple sites s.q. without adjuvant at 2, 3, and 4 weeks after initial inoculation. Rabbit serums that reacted by immunodiffusion to purified TSST-1 were pooled. Specificity of immune rabbit serum was determined by

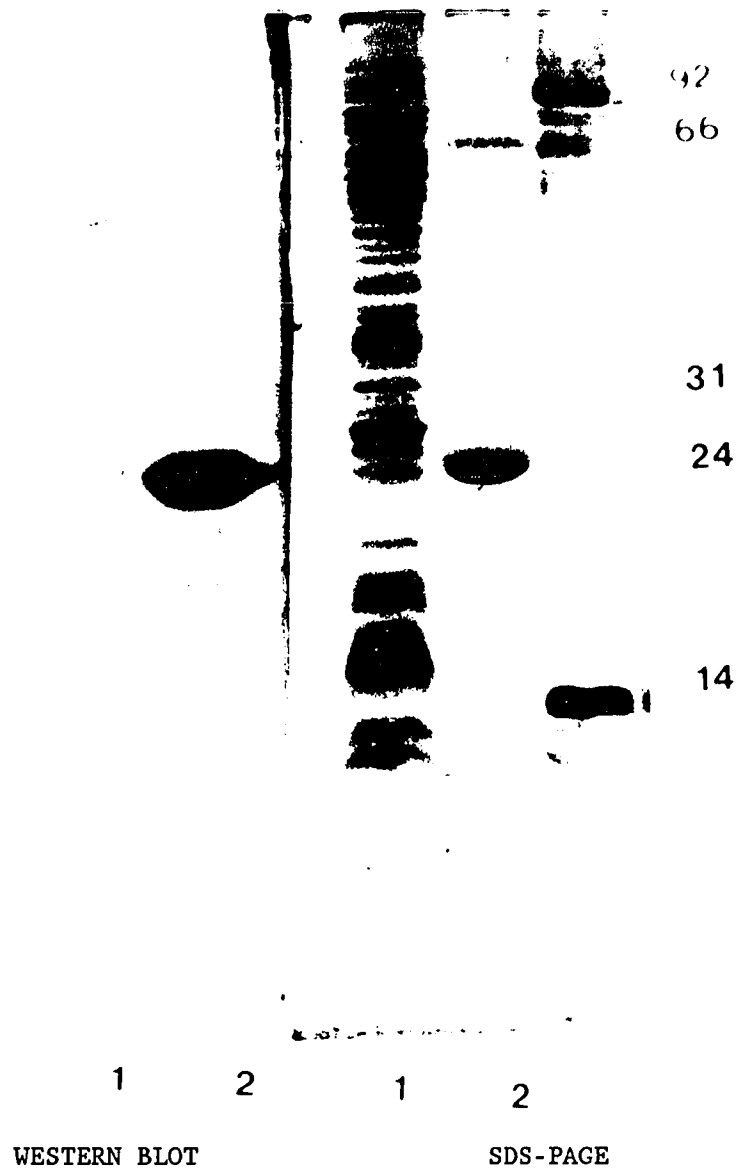


Figure 1
 SDS-PAGE and Western blot results of purified
 TSST-1 reacted with ACS

Lane 1 - crude *S. aureus* FRI 1169 cell lysate.
 Lane 2 - purified TSST-1

the presence of a single identical precipitin line against both crude and purified TSST-1.

Rabbit anti-TSST-1 total immunoglobulin was purified (41) by precipitation with 50% saturated ammonium sulfate, followed by dissolving the precipitate in 0.1 M phosphate buffer (pH 8.0), desalting and concentrating to 1/10 starting serum volume in a nitrogen-pressure chamber at 4 C (Amicon, Lexington, Mass.) with an XM-50 filter (Amicon). The globulin fraction was clarified by centrifugation at 10,000 x g (8,000 rpm) for 20 mins at 4 C, and the supernatant adsorbed to Protein A-Sepharose CL-4B (Pharmacia) in 0.1 M phosphate buffer (NaH_2PO_4 - Na_2HPO_4 , pH 8). Total immunoglobulins were eluted by desorption from Protein A in 0.1 M citrate buffer (Sodium citrate-citric acid, pH 3). The pH 3 fractions were pooled and returned to neutral pH with 1 M Na_2CO_3 . The fractions were then concentrated 10-fold in a nitrogen-pressure chamber. Purified immunoglobulin was adjusted to 2 mg/ml protein by Bradford assay (18) in 0.01 M phosphate buffered saline (PBS) (0.01 M Na_2HPO_4 - NaH_2PO_4 , 0.15 M NaCl, 0.01% thimersol and 0.05% Tween-20, pH 7.5) and stored at 4 C.

Hybridoma cell culture. Anti-TSST-1 monoclonal cells were prepared by the University of Hawaii Monoclonal Service. Hybridoma cells were kept at -90 C in liquid nitrogen storage at a concentration of 3×10^6 viable cells with 10% DMSO in 1 ml of Dulbecco's minimal essential glucose media (DMEM-glucose, Gibco, Los Angeles, CA) supplemented with 20 mM glutamine, 200 units of

penicillin G, 200 ug of streptomycin, and 10% heat-inactivated fetal calf serum (Gibco). Frozen cells recovered from nitrogen were washed twice with supplemented DMEM-glucose by centrifuging at 600 x g (1200 rpm) at 4 C. Cells were resuspended in 1 ml DMEM-glucose and 100 ul aliquots dropped into wells of a 96-well polystyrene microtiter plate each containing 1×10^4 washed peritoneal mononuclear cells obtained from Balb/C mice.

Hybridoma cultures were routinely grown in 75 cm² tissue polystyrene culture flasks (Costar, Cambridge, MA). To harvest culture supernatant, the contents of each tissue culture flask were transferred to 50 ml sterile conical polypropylene centrifuge tubes (Costar) and spun at 600 x g (1200 rpm) for 20 mins. Supernatant fluids were pooled and kept at 4 C prior to concentration. New flasks were seeded with 1×10^7 cells and incubated at 37 C with 5% CO₂ until the population of cells reached $3-5 \times 10^8$.

Monoclonal culture fluid preparation. The monoclonal antibody raised by the University of Hawaii Monoclonal Service was of isotype IgG_{2a} when assayed by solid phase enzyme-linked immunosorbent assay (ELISA) using rabbit anti-mouse Ig subclass sera (Cappel, Cooper Biomedical, Malvern, PA.). Because anti-TSST-1 monoclonal AC3 frequently induced solid tumors in mice and because ascites fluids contained interfering anti-staphylococcal antibodies as well as anti-TSST-1, AC3 monoclonal antibody was collected and concentrated from 85 liters of hybridoma culture supernatant fluids. Hybridoma culture supernatant fluids

were concentrated in 8 liter batches to 0.4 L in a hollow-fiber concentrator (Amicon, model CH4) with a 10,000 m.w. cut-off filter core, and the globulin fraction collected by 50% ammonium sulfate precipitation. The IgG was purified as described for rabbit anti-TSST-1 serum. However, since AC3 is of IgG_{2a} isotype, desorption from Protein A-Sepharose (Pharmacia) was with citrate buffer at pH 4.5 (41). Specificity of the monoclonal antibody AC3 was determined by Western blot (118) (Figure 1), which detected a band at 22 kd with trace bands located at 60 kd and near 20 kd. Blot results with crude TSST-1 preparations also picked up lines in the 30 and 60 kd range. Irrelevant mouse IgG_{2a} monoclonals (PC5, a mineral oil plasmacytoma line, and 4G2, a dengue 2 virus monoclonal) did not react with TSST-1 preparations, and did not block AC3 activity by liquid-phase ELISA. Protein concentration of the purified Ig peak was determined with the Bradford assay (18), and was adjusted to 2 mg/ml in 0.01 M PBS saline pH 7.5. Two milligram aliquots of AC3 was lyophilized for storage.

TSST-1 detection

Immunodiffusion. TSST-1 was detected by immunodiffusion assay (93). Briefly, a 1 ul drop of test strain culture diluted in TSB was placed on a thin layer of THB containing 1.5% agar in a 50 mm petri dish. Plates were incubated for 24 h at 37 C, after which a central well was punched and filled with 20 ul of hyperimmune rabbit serum diluted 1:4 in saline. Two peripheral wells were also punched and filled with 8 ul of preparation containing 0.8 ug of

TSST-1. The plates were reincubated for 24 h at 25 C and examined for identical precipitin lines between the test culture and purified TSST-1.

Radioimmunoassay. Radioimmunoassay (RIA) for quantitation of TSST-1 was done by the laboratory of M. Melish at Department of Pediatrics, University of Hawaii. The competitive binding assay consisted of reacting test samples of culture supernatants with rabbit anti-TSST-1 serum, followed by addition of iodinated TSST-1 (42). The immune-complex was precipitated with goat anti-rabbit serum (Cappel) and the amount of radioactivity in the precipitate, as recorded by gamma counter, was inversely related to the amount of TSST-1 in the test sample.

Solid-phase ELISA screen. To screen multiple *S. aureus* strains for TSST-1 production, solid phase enzyme-linked immunoassay on nitrocellulose filters (0.45 u, Millipore Corp., Bedford, MA) (121) was performed in one of two ways. The first method used double nitrocellulose filters (0.45u, Millipore) placed over trypticase agar, with 1 ul of culture spotted onto the top filter. After incubation for 24 h at 37 C, the bottom filter was reacted with rabbit anti-TSST-1 at 2 ug/ml in 0.1 M PBS pH 7.5, followed by Protein A-peroxidase complex (Sigma) at 1:1000, and reactive spots identified by dark purple color with the addition of 600 ug/ml 4-chloro-1-naphthol substrate and 0.004% hydrogen peroxide.

The second method employed was that of individual colony blot detection of TSST-1 production by reaction with AC3 and avidin-biotin-peroxidase (Figure 3). Immunoglobulin reagents used

in the test were pre-incubated to eliminate false-positive reactions due to endogenous staphylococcal protein A. Prevention of protein A reaction was accomplished in two ways. First, 50 ug each of AC3 and biotinylated horse anti-mouse Ig (Vecstain ABC kit, Vector, Burlingame, CA) were pre-incubated with 200 ug of unlabelled staphylococcal protein A (Sigma) to block available Fc sites; secondly, the BSA-blocked nitrocellulose blot was incubated with a solution containing unlabelled avidin (Vector, diluted 1:20 in 3% bovine serum albumin-PBS) with rabbit anti-Protein A (Sigma) diluted 1:100. Endogenous biotin was blocked by the addition of unlabelled avidin during blocking with anti-Protein A followed by incubation of unlabelled biotin (Vector) diluted 1:20 in 1.5% BSA-5% skim milk/PBS added to the solution of AC3.

Colony blot for TSST-1 production was performed at 25 C as follows: nitrocellulose paper (0.45 u Millipore) was placed over the growth area and proteins adsorbed for 15 mins. Bacterial growth was washed off the filters with deionized water and blocked with 3% bovine serum albumin (BSA, Sigma) in 0.01 M PBS, pH 7.5. After BSA blocking, the filter was incubated for 30 mins with a blocking solution of rabbit anti-Protein A (Sigma, 1:100) and unlabelled avidin (Vector, 1:20) in 3% BSA-PBS with gentle agitation. The filter was washed with deionized water and placed in 1:1000 diluted AC3 plus 1:20 dilution of unlabelled biotin (Vector) for 2 hrs. Again the filter was washed with deionized

COLONY BLOT ELISA FOR TSST-1 DETECTION

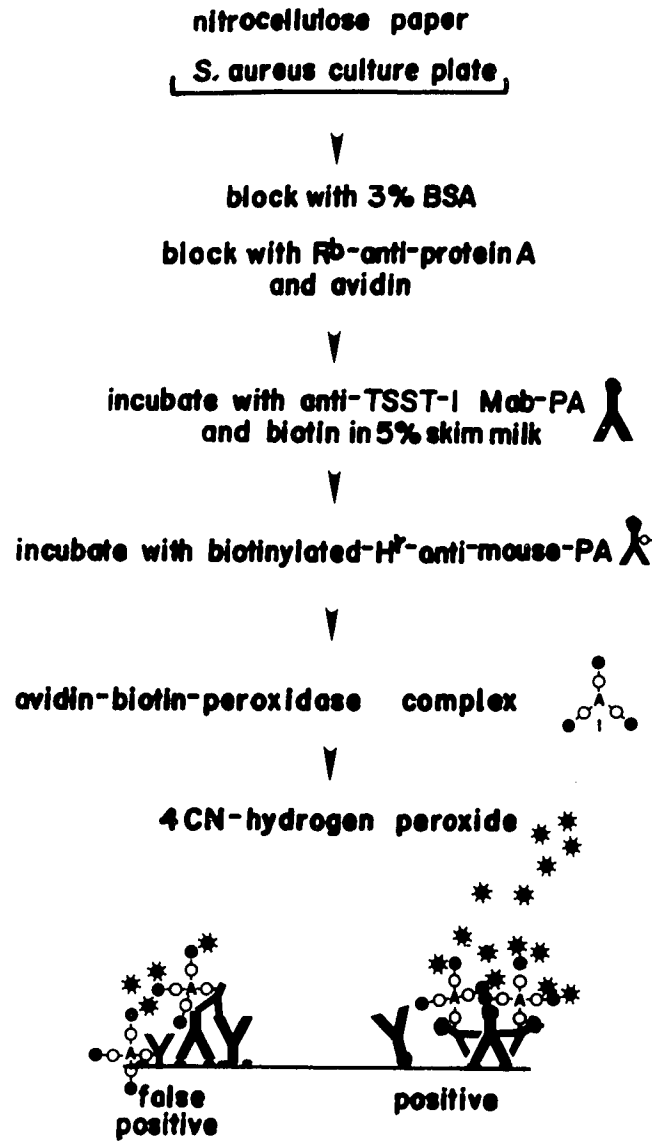


Figure 2
Colony blot ELISA for TSST-1 detection

1 2 3
4 5 6 7 8
 9 10 11 12 13 14 15
 16 17 18 19 20 21 22 23
 24 25 26 27 28 29 30 31
32 33 34 35 36 37 38 39
 40 41 42 43 44 45 46
 47 48 49 50 51 52
 53 54 55 56

Figure 3A
 Colony blot template for 56 colonies
 ___-positive for TSST-1 (see blot result in Figure 3B).



Figure 3B
 Result of colony blot ELISA detecting for TSST-1
 on nitrocellulose filter
 dark spot - TSST-1-producing colony.

water and incubated for 1 hr with a 1:200 dilution of biotinylated horse anti-mouse Ig in 5% skim milk-PBS, followed by incubation with 1:100 diluted peroxidase-labelled ABC complex in PBS. Visualization of peroxidase-bound TSST-1-producing colonies was recorded after addition of a substrate solution consisting of 600 ug/ml 4-cholor-1-naphthol (Sigma) with 0.004% hydrogen peroxide in PBS (Figures 3A and 3B).

Liquid-phase ELISA. Liquid phase ELISA (81,93) was carried out in a 96-well polyvinyl microtiter plate (MicroELISA, Dynatech, Arlington, Virginia). One microgram of TSST-1 in 50 ul of PBS was adsorbed to each well for 1 h at 37 C. Plates were then blocked for 15 mins with 1% BSA-PBS. Two microgram per ml of AC3 diluted in 1% BSA-PBS was added to each well, incubated at 25 C for 1 h and, following PBS wash, test wells reacted with 50 ul of secondary biotinylated antibody for 30 mins and incubated 30 mins with avidin-peroxidase complex. Reaction was visulaized by the addition of 20mM ABTS (Sigma) and 0.004% hydrogen peroxide in PBS and results recorded by ELISA microtiter plate-reading spectrophotometer at 490 nm (Dynatech).

S. aureus auxotyping

Chemically defined auxotyping medium (Table 5) was adapted to liquid micromethods from the medium used by Emmett and Kloos (35). For auxotyping, a suspension of 10^8 cfu/ml of staphylococci was made in buffered salts, washed twice by centrifugation at 600 x g (1200 rpm) for 10 mins, and resuspended to 1 ml in buffered salts.

Five microliters of the washed cells were added to 200 ul of medium lacking one of the 18 amino acids in a 96-well sterile flat-bottom polystyrene plate (Costar) (Figure 4). Control wells included those without niacin, without thiamine, without B-glycerophosphate, without glucose, with complete medium, and with only buffered salts and phenol red. After 24 h incubation at 37 C, the wells were examined for growth by visualized turbidity, and for acid production by color change of phenol red indicator from red to yellow.

Nutritional-limiting cultures in the chemostat. Chemically defined medium were made with varying concentrations of tryptophan or valine to study the effects of the limiting of available nutrients on TSST-1 production. Chemostat reservoirs were filled with 14 L of chemically defined medium with 5 ug/ml of tryptophan or valine. The amount of nutrient limitation was controlled by the rate of delivery of fresh medium from the reservoir into the culture flask. Washed 10^8 cells of a TSST-1-producing/tryptophan auxotroph (Harrisburg strain) was inoculated into the chemostat culture flask (Bio-Flo model C30, New Brunswick Scientific Co., Inc., New Brunswick, NJ) at 35 C with aeration at 240 cycles/min. Cultures were grown to steady-state as determined by the dilution rate for each generation time. When steady-state was reached, 20 ml of culture supernatant was harvested and cell-free proteins precipitated with 80% ethanol for TSST-1 quantitation by RIA.

Table 5

Chemically defined medium used for auxotyping

	mg/liter*
Buffered salts:	
Na ₂ HPO ₄	2,480
KH ₂ PO ₄	410
salts solution.....	5 ml
1% MgSO ₄	
1% MnCl ₂	
0.32% Na citrate	
0.32% FeSO ₄	
Amino acids: @	
L-alanine.....	140
L-arginine.....	120
L-aspartic acid.....	120
L-cysteine.....	60
L-glutamine.....	180
L-glycine.....	120
L-histidine.....	80
L-isoleucine.....	80
L-leucine.....	80
L-lysine.....	120
L-methionine.....	40
L-phenylalanine.....	100
L-proline.....	120
L-serine.....	80
L-threonine.....	80
L-tryptophan.....	80
L-tyrosine.....	80
L-valine.....	120
Carbohydrates: @	
glucose.....	20,000
Beta-glycerophosphate.....	20,000
Vitamins: @	
niacin.....	0.10
thiamine.....	0.10
Indicator:	
phenol red.....	10
pH	7.0

*=fresh glass-distilled water.

@=10 ml aliquots of each component was sterilized by filtration and stored separately at -70 C.

All components were 0.45 u filter-sterilized.

Auxotype profiles were repeated blindly and independently on 3-5 different occasions for each strain.

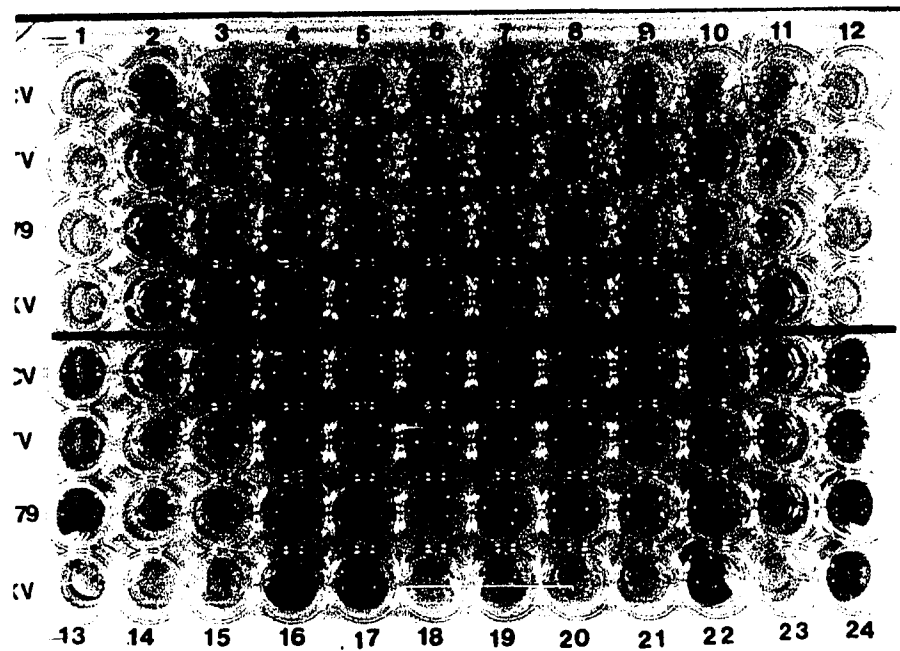


Figure 4
 Micromethod for auxotyping *S. aureus* strains

1=no alanine, 2=no arginine, 3=no aspartic acid, 4=no cysteine, 5=no glutamine, 6=no glycine, 7=no histidine, 8=no isoleucine, 9=no leucine, 10=no lysine, 11=no methionine, 12=no phenylalanine, 13=no proline, 14=no serine, 15=no threonine, 16=no tryptophan, 17=no tyrosine, 18=no valine, 19=no niacin, 20=no thiamine, 21=no B-glycerophosphate, 22=no glucose, 23=complete medium, and 24=buffered salt solution and phenol red indicator.

Tryptophan genetic block characterization

Tryptophan-requiring strains were characterized for response to tryptophan synthesis pathway intermediates, L-tryptophan, and tryptophan analogs (65,88,109). Semi-defined medium consisted of 20 g of tryptophan-free casamino acids (Difco), 20 g of B-glycerophosphate, 2 g of D-glucose, and 15 g of Bacto-agar (Difco) dissolved in 1 liter of buffered salts solution and autoclaved. Ten micrograms each of niacin and thiamine were added to the autoclaved medium. Washed staphylococci suspended to 1×10^8 cfu/ml in buffered salts solution were streaked onto the semi-defined medium plate using a sterile cotton swab. Stock solutions at concentration of 200 ug/ml for each intermediary substrate and tryptophan analog were prepared in buffered salts and filter-sterilized. Five mm filter Whatman paper discs soaked with 10 ul of one of the prepared solutions was placed on top of the lawn of bacteria. Plates were incubated overnight at 37 C. Response to the exogenous substrate was noted as growth around the filter disc (Figure 5).

Tryptophan pathway intermediates tested included shikimic acid, chorismic acid, anthranilic acid, and indole. Tryptophan analogs included 7-azotryptophan, 4-methyltryptophan, 5-methyltryptophan, 6-methyltryptophan, and 5-fluorotryptophan. All the above reagents were obtained from Sigma.

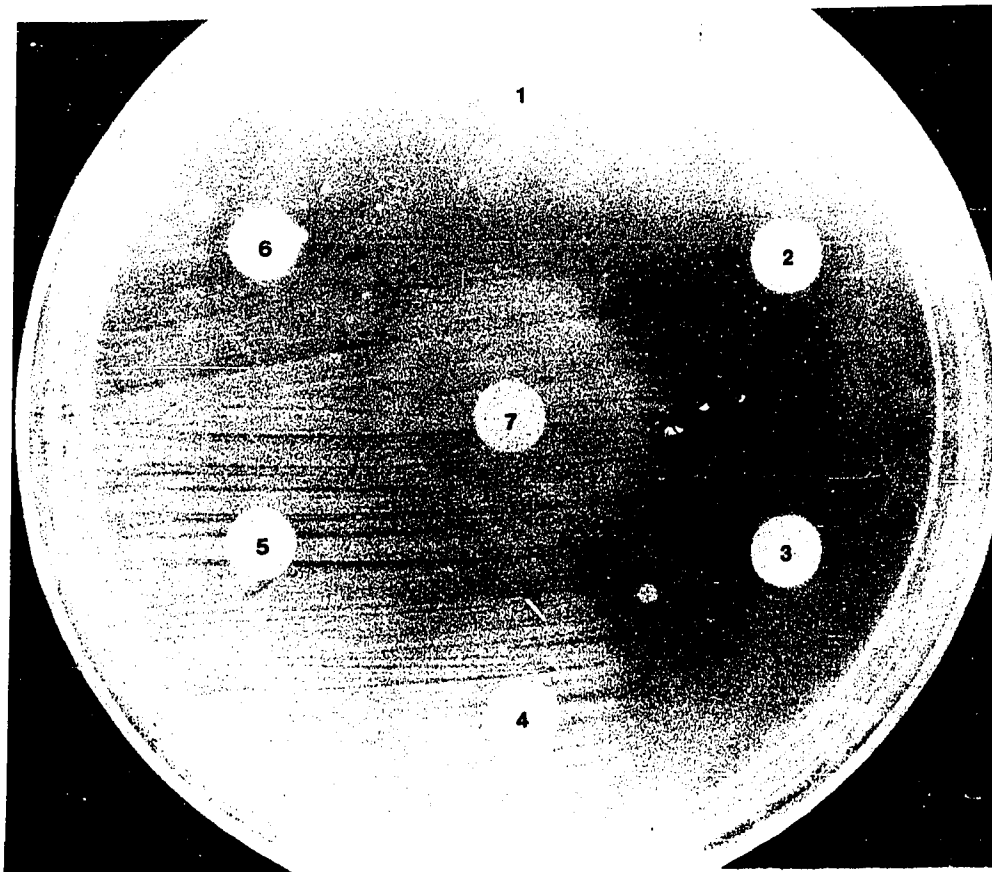


Figure 5
Auxonographic plate for testing the response of a
S. aureus strain to exogenous tryptophan biosynthesis
pathway substrates and analogs

Clockwise from top: 1-shikimic acid, 2=chorismic acid,
3-anthranilic acid, 4=indole, 5=5-fluorotryptophan,
6=6-methyltryptophan, and 7=tryptophan. Growth is evident
around indole, 5FT, and tryptophan discs.

Crossfeeding of *S. aureus* with *E. coli*

S. aureus strains were tested in auxonographic fashion (described above) but with 1 ul of washed *E. coli* spotted over the staphylococcal lawn instead of defined chemical substrates. Reaction of *S. aureus* strains to *E. coli* were considered to be inhibited if there was a zone of no growth, as stimulated if there was increased growth on *S. aureus* strains not requiring tryptophan, and if there was satellite growth around the *E. coli* spot on *S. aureus* tryptophan auxotrophs.

Genetic mapping for TSST-1 gene

Transductions. Phage 55 (a helper phage used to increase competence of cells) and phage 80 (a phage used to transfer desired genotypic characteristics) were propagated and maintained on ISP 8. ISP 2 was the donor strain for transduction of novobiocin resistance to TSST-1+ strains. ISP 2 was grown overnight on brain heart infusion agar (BHIA) slant at 35 C. ISP 2 cells were suspended in 1 ml of trypticase soy broth, 0.1 ml of which was added to a tube of melted soft TSA (TSB + 0.5% agar, 4 ml/tube). Each tube of soft agar received 10^{10} - 10^{11} pfu/ml of phage 80, and each agar tube was poured onto a fresh trypticase soy agar plate (TSA) with 5 mM CaCl_2 . The solidified plate was incubated for 6 h at 35 C. Propagated phage were harvested by removing the soft agar layer from the TSA plate, and suspending it in 10 ml of TSB with vigorous mixing. The phage-containing material was centrifuged and the supernatant filter-sterilized (0.22u,

Zeta-probe, Millipore) and stored at 4 C. Harvested phage was titrated by making serial ten-fold dilutions of the material in TSB, mixed with a tube of soft agar containing 10^{10} cfu/ml of ISP 8 and poured onto a TSA plate. Titration plates were incubated at 35 C for 18 hrs and plaque forming units (pfu) were determined by counting the number of plaques of a dilution plate and that number multiplied by the fold-dilution.

Recipient cells (TSST-1-producing strains) were harvested from an overnight BHIA slant (about 10^{10} cfu/ml) with 1 ml of TSB, the prepared phage-containing material was added to the recipient cells at a multiplicity of infection ratio of 1 pfu to 1 cfu, and the mixture incubated for 20 mins at 35 C. Infection with the phage preparation was stopped by adding 1 ml of 0.02 M sodium citrate at 4 C. Transduced cells (recipient cells) were plated on complete synthetic medium (CDS) (98) supplemented with 10 ug/ml novobiocin. Novobiocin-CDS plates were incubated until colonies could be easily picked and these plates were then replicated by use of sterile velveteen cloth squares onto other selective media to score for additional markers associated with both donor and recipient strains. All selective plates were incubated at 35 C.

Protoplast fusion. Growth rates of the two fusion strains were pre-calibrated so that each would reach an optical density (O.D.) at 0.65 (540 nm) at the same time to start protoplast preparation and fusion (111) (Figure 6). Each parental strain was harvested from an overnight BHIA slant with saline and suspended in 100 ml

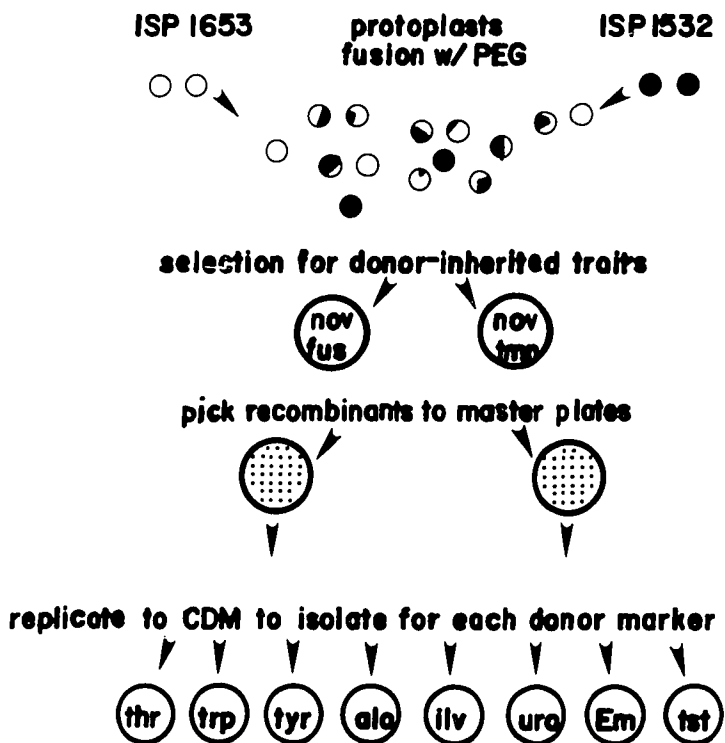
TSB/flask to O.D. of 0.1 at the start of the fusion procedure. The flasks were incubated at 35 C in a shaker water bath, harvested by centrifugation (10,000 x g, 30 mins @ 4 C), and washed once with saline. Pooled cells from each strain were resuspended separately in 10 ml of sucrose-magnesium-tris buffer (100 mM Tris, 40 mM MgSO₄, 0.8 M sucrose, pH 7.6) (SMTB). DNase (15 ug/ml, DNase I, Sigma) and lysostaphin (30 ug/ml, Sigma) were added, mixed, and transferred to 50-ml screw-capped erlenmeyer flasks. The flasks were incubated at 35 C on a rotary mixer for 45 mins and protoplasts harvested by centrifugation (3,400 x g, 10 mins at room temperature). Each pellet was resuspended in 1 ml of SMTB containing DNase (15 ug/ml) and divided into appropriate 0.1 ml aliquots. Experimental and cell controls were carried out as follows: one aliquot of each parental protoplasts was added together (fusion tube), one tube each containing only one parental protoplast (reversion tube), one tube containing both parental non-protoplasted cells (cell tube), and to these 4 tubes 1.8 ml of polyethylene glycol (PEG 400, 60% in SMTB, Sigma) was added for 1 min at 20 C; in addition, a fifth tube containing both parental protoplasts without PEG served as the fusion control. Fused cells and controls were then spread onto regeneration (R) agar plates (30 g TSB, 273 g sucrose, 25 g agar, 0.5 g sodium citrate, 3.0 g starch per liter of deionized water), plates were held at 35 C under 60% humidity for 7 days.

Cells were harvested by washing R plates with saline and pooled. The regenerated cells were evenly dispersed by sonication for 1 min at 20 Kcps (Biosonik II). Cells were titrated on selective media (CDS containing 10 ug/ml each of fusidic acid/novobiocin and tetracycline/ novobiocin) and incubated at 35 C. Based on the titration that yielded 100 cfu/plate, fresh selective plates were seeded and incubated at 35 C. After incubation for 48 hrs, 672 colonies (12 plates each with 56 colonies) were picked for each set of selected markers, and these master plates were incubated for 24 hrs at 35 C. The master plates were replicated by velveteen imprint transfer to fresh selective plates (sub-masters). When the sub-masters have grown, they were replicated to appropriate media to score for unselected markers among the recombinants that represented inheritance of known traits of the parental donors. For ISP 1532, selection was for TSST-1 production, threonine, thymine, tryptophan, tyrosine, alanine, isoleucine/valine, and uracil prototrophy. For ISP 1635, selection was for erythromycin resistance and auxotrophism for all markers listed for ISP 1532 and for TSST-1 production (Figure 6). Each set of unselected markers was scored together either at 24 or 48 hrs, at which time growth responses were recorded with all like-selective plates stacked in sequential order (#1-12) identical to the master plates and results entered into the fusion analysis program (Commodore model 8032, program courtesy of P. Pattee, Iowa State University). The closer a pair of markers are located on the

GENOTYPE OF FUSION STRAINS

	thrB	trp	tyrB	ala	ilv	ura	pig	Em	tmn	fus	nov	tst
ISP1653	-	-	-	-	-	-	-	R	R	R	S	-
ISP1532	+	+	+	+	+	+	+	S	S	S	R	+

PROTOPLAST FUSION



**Computer sort the number of recombinants
to calculate the co-inheritance frequency
of each marker**

Figure 6
Protoplast fusion procedure

chromosome, the more often they would be inherited together. The data was sorted into a frequency distribution for each type of recombinant observed. By arbitrarily selecting a marker as the starting point, in this fusion it was threonine (thr), inheritance of this marker will be set at 100%. The computer program will then rank the rest of the markers relative to this starting point so that the closest marker to thr will be highest in percent and the farthest being the lowest percent inherited.

Transformation. Standard transforming DNA (114) from donor strains were prepared by growing the cells overnight in 150 ml of TSB in a shaker water bath at 35 C. Cells were harvested by centrifugation at 10,000 x g (8,000 rpm) for 10 min and washed in 0.1 M Tris-EDTA-saline, pH 7.5 and resuspended in 5 ml of the same buffer. The cells were placed in a 50-ml screw cap erlenmeyer flask, and 0.3 ml of lysostaphin (1 mg/ml) (Sigma) was added and gently mixed for 30 m at 35 C in a shaker water bath. One ml of preincubated DNase-free proteolytic enzyme (Protease type XIV, Sigma) was added for 5 min with shaking, followed by a 55 min incubation without shaking in the 35 C water bath. SDS-ethanol was added to each flask and shaken vigorously on a wrist-action shaker (Burrell) at 25 C for 30 min. The foamed mixture was then extracted with equal volumes of re-distilled phenol (saturated with 0.01 M Tris buffer, pH 8.1) and roller-extracted for 60 min at 35 C. The extract was transferred to a glass centrifuge tube and spun at 10,000 x g (8,000 rpm) for 60 min at 4 C. The upper aqueous

layer was removed and the phenol-roller extraction repeated. Residual phenol was removed by adding equal volumes of ethyl ether (U.S.P., Mallinckrodt, Los Angeles, CA.) to the extract and shaking vigorously. The upper ether layer was then removed and the leftover ether vaporized with nitrogen gas. DNA was precipitated with 2 volumes of cold 95% ethanol, and the precipitate was spooled up on a glass rod. DNA was further rinsed in cold ethanol and then stored in 5 ml of saline-citrate (SSC, 6.1 g NaCl, 4.44 g trisodium citrate per liter, pH 7.2) at 4 C.

Transformation with prepared DNA was carried out by harvesting recipient cells from an overnight BHIA slant with 5 ml of normal saline. Harvested cells were used to seed a 100 ml volume of trypticase soy broth (BBL) to achieve an O.D. of 0.2 at 540 nm. Ten ml of the seeded broth was used to inoculate each 100 ml of fresh trypticase soy broth and these flasks were placed in a 35 C water bath shaker until an O.D. of 0.2 is reached (late log phase growth), at which time the cells were harvested by centrifugation at 10,000 x g (8,000 rpm), for 30 min at 4C. Harvested cells were artificially made competent by the addition of normal rabbit serum (Pel-Freez Biologicals, Rogers, Arizona) (114) and by addition of helper phage 55 lysates if the recipient cells were not derived from S. aureus strain NCTC 8325. After the phage-cell mixture was allowed to adsorb at 35 C for 5 mins with gentle shaking, the cells were washed with cold Tris-maleate (TM) buffer, resuspended in cold TM buffer with 0.1 M CaCl₂ and 1 ml aliquots immediately

transferred to tubes containing either 0.1 ml of prepared DNA or TM-CaCl₂. The tubes were then heat-shocked, first by incubating at 20 C for 2.5 mins, and then by incubating for 2.5 min at 35 C. Cells were re-equilibrated by incubation in 1 ml of BHI with gentle shaking at 35 C for 1 hr, and centrifuged and resuspended in saline. Transformants were then assayed by plating 0.1 ml of the cells on selective media prepared according to the desired donor and recipient characteristics. The transformants were characterized for unselected markers by velvetreen imprint transfer onto appropriate selective media. For example, DNA from ISP mc8, a TSST-1-producing, novobiocin-resistant, tryptophan-requiring strain was used to transform ISP 646, a strain with Tn551 erythromycin-resistance marker inserted in the tryptophan gene, and a thymine, threonine, isoleucine/valine, and tryptophan auxotroph. To identify transformation by ISP mc8, lack of growth on erythromycin medium is first established, then the transformant is evaluated for repair of auxotrophic markers by observing growth on media without thymine, without threonine, and without isoleucine/valine, and without tryptophan.

RESULTS

Phenotypic profile of 154 *S. aureus* strains

All strains were gram-positive cocci, were coagulase positive, fermented glucose, produced acid on mannitol salt agar, and produced DNase. There were no apparent differences among the TSS and non-TSS strains in their ability to produce delta toxin: 17 of 49 of TSS strains (35%) v. 21 of 78 of non-TSS strains (27%) produced delta toxin. Nor were there differences in enterotoxin B production: 5 of 53 of TSS strains (9%) v. 6 of 79 of non-TSS strains (8%) produced enterotoxin B.

Characteristics of TSS isolates. TSS-associated strains were less hemolytic, and less pigmented than non-TSS associated *S. aureus* strains. Immunodiffusion assay with polyclonal rabbit serum identified 62 of 154 strains (40%) as TSST-1 producers. TSST-1 monoclonal antibody AC3 confirmed all 62 strains as TSST-1 positive, and detected an additional 7 TSST-1 producing strains not identified by immunodiffusion. Forty-five of 62 TSST-1-producing strains (73%) were associated with TSS disease, a figure consistent with TSST-1 production by TSS-associated *S. aureus* as reported by other studies (21).

Proteolysis was significantly less in TSST-1-producing strains than controls: 95% of the 62 TSST-1-producing strains v. 53% of 92 TSST-1 non-producing strains produced casein digestion zones <21 mm ($p < 0.001$, F.E.T.).

Nutritional requirements

Composite amino acid requirements of the 154 S. aureus strains (Figure 7) were typical of those reported by other investigators (35), including normal requirements for arginine (83%), valine (80%), and proline (69%). Cysteine and tyrosine requirement was variable among the respective auxotrophic strains, and such strains exhibited partial requirement for the amino acids by growing weakly in the absence of cysteine or tyrosine after 48-72 hours of incubation. Comparison of single amino acid requirement of each strain with TSST-1 production identified four amino acids whose relative frequency of requirement differed with respect to TSST-1 production: histidine ($p < 0.1$), threonine ($p < 0.1$), tryptophan ($p < 0.001$), and tyrosine ($p < 0.001$) (Table 6). However, the proportion of strains requiring histidine (18 of 154 = 11%), threonine (6 of 154 = 9.6%), and tyrosine (17 of 154 = 11%) were within the expected range for naturally occurring auxotrophs in S. aureus as reported by other investigators (35,73).

The detection of tryptophan requirement by 49 of 154 (32%) strains was unexpected. Other investigators had reported that less than 12% of S. aureus isolated from human skin require tryptophan (35). Forty-one of these 49 tryptophan auxotrophs (82%) had been isolated from persons with TSS. This suggested that tryptophan auxotrophism may be a phenotypic trait that differentiates TSS-associated strains. Moreover, the data further suggested that tryptophan requirement was related to TSST-1 production since 44 of

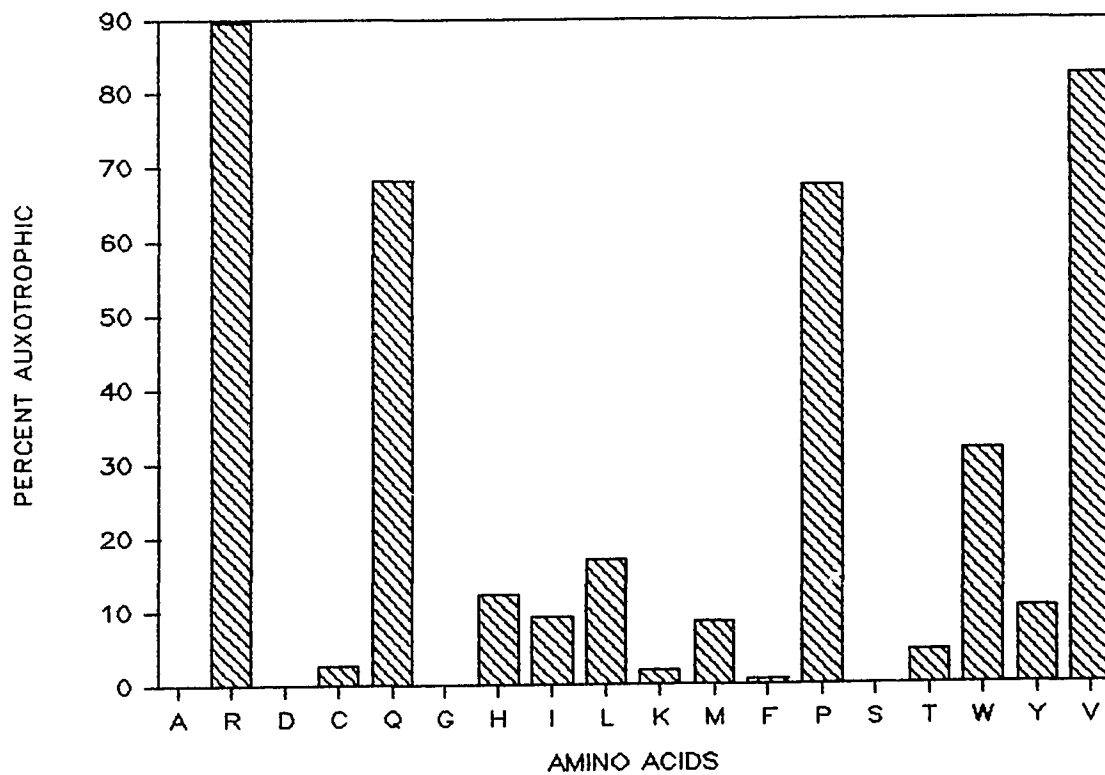


Figure 7
 Nutritional requirement of 154 *S. aureus* strains

A=alanine, R=arginine, D=aspartic acid, C=cysteine,
 Q=glutamine, G=glycine, H=histidine, I=isoleucine, L=leucine,
 K=lysine, M=methionine, F=phenylalanine, P=proline, S=serine,
 T=threonine, W=tryptophan, Y=tyrosine, V=valine.

62 TSST-1 producers (71%) required tryptophan for growth while only 5 of 92 non-TSST-1 producers (5%) were tryptophan auxotrophs, $p < 0.001$, chi square (Figure 8).

Table 6
Association of amino acid requirement and TSST-1 production

	TSST-1+ N = 62(%)	TSST-1- N = 92(%)	p [@]
Alanine	0	0	n.d.
Arginine	54 (87)	74 (80)	n.s.
Aspartic acid	0	0	n.d.
Cysteine	1 (2)	3 (3)	n.s.
Glutamine	0	4 (4)	n.s.
Histidine	10 (16)	8 (9)	<0.1
Isoleucine	4 (6)	7 (8)	n.s.
Leucine	5 (8)	22 (23)	n.s.*
Lysine	1 (2)	2 (2)	n.s.
Methionine	0	1 (1)	n.s.
Phenylalanine	4 (6)	9 (10)	n.s.
Proline	49 (79)	58 (63)	n.s.
Serine	0	0	n.d.
Threonine	4 (6)	2 (2)	<0.1
Tryptophan	44 (71)	5 (5)	<0.001
Tyrosine	13 (21)	4 (4)	<0.001
Valine	53 (85)	70 (76)	<0.25

[@] = by corrected chi square or by Fisher's exact test.

* = significant association of leucine requirement with no TSST-1 production.

n.s. = no significant association of amino requirement with TSST-1 production.

n.d. = not done, inadequate sample numbers.

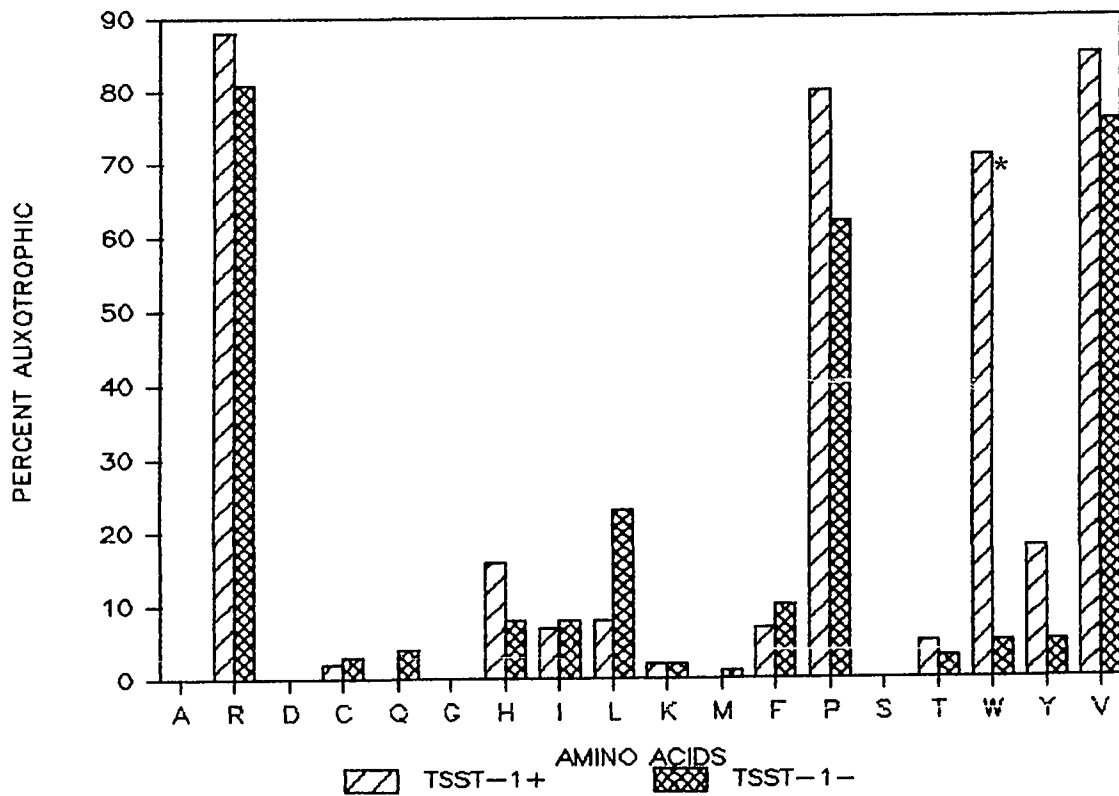


Figure 8
 Relationship of amino acid requirement to TSST-1
 production by 154 *S. aureus* strains.

Amino acid abbreviations same as Figure 4.
 *=p<0.001 by chi square.

Influence of limiting nutrients on TSST-1 production. Thus the data suggested that the levels of available tryptophan may control production of TSST-1. A TSST-1-producing tryptophan auxotroph, Harrisburg strain, was grown in chemically defined medium with varying levels of tryptophan. A medium limiting valine but not tryptophan was used as control. When either tryptophan or valine (at 5 ug/ml) was the limiting nutrient, growth was reduced to 50% of that obtained with complete medium as recorded by spectrophotometric measurement of O.D. When tryptophan was the limiting nutrient, TSST-1 production by immunodiffusion using rabbit anti-TSST-1 serum produced a precipitin line of identity with purified TSST-1. Under the same concentration of valine, TSST-1 could not be detected. The limiting-chemically defined medium was placed under continuous culture in a chemostat which controlled the generation time of the Harrisburg strain. Culture supernatants harvested at generation times of 2.48, 3.85, and 5.33 hours demonstrated that, under tryptophan-limiting conditions and prolonging in vitro generation time, significantly more TSST-1 was produced (Table 7).

Table 7
Chemostat controlled bacterial generation-times and
TSST-1 production in a chemically-limiting defined medium

Generation Time	Tryptophan 5 ug/ml		Valine 5 ug/ml	
	I.D.	RIA(ng)	I.D.	RIA(ng)
2.33 hours	+	204.3	-	58.3
3.85 hours	+	219.0	+	87.0
5.33 hours	+	72.4	-	not done

I.D.-immunodiffusion with rabbit anti-TSST-1 serum,
RIA-radioimmunoassay quantitation with rabbit anti-TSST-1
serum.

Epidemiological analysis of tryptophan requirement in TSS

Relationship of TSS disease to TSST-1 production by, and tryptophan requirement of, disease-associated strain isolates. The 154 S. aureus strains represented a spectrum of disease severity, including TSS, non-TSS staphylococcal infections, and asymptomatic healthy carriers. S. aureus tryptophan requirement was unrelated to the disease status of the patient from whom the isolate came when TSST-1 production was controlled ($p < 0.001$, chi square), as was TSST-1 production when tryptophan requirement was controlled ($p < 0.001$) (Table 8). Thus tryptophan requirement and TSST-1 production covaried regardless of the disease status of the patient from whom the isolate came.

Table 8
Relationship of TSS and non-TSS isolates to
TSST-1 production and tryptophan auxotrophy

	TSS		NON-TSS	
	TSST-1+	TSST-1-	TSST-1+	TSST-1-
TRP-	32 (71)*	0	12 (71)*	5 (6)
TRP+	13 (29)	8 (100)	5 (29)	79 (94)
Total	45	8	17	84

()=%; TRP-, tryptophan requiring; TRP+, non-tryptophan requiring.

*= $p < 0.001$, chi square.

Relationship of tryptophan auxotrophy to colonization site.

Both TSST-1-producing and non-producing strains were represented by isolates from various anatomic sites. Regardless of the site of isolation, TSST-1 production was associated with tryptophan requirement, whereas most toxin-negative strains were associated with tryptophan prototrophy ($p < 0.001$, 1 df for isolates obtained from vagina, wound/abscess, and blood) (Table 9). Tryptophan requirement and TSST-1 production thus covaried irrespective of isolation site.

Table 9
Relationship of tryptophan auxotrophy
to colonization site

SOURCE	N	TSST-1+		TSST-1-		P*
		TRP-	TRP+	TRP-	TRP+	
Vaginal	78	36	8	2	32	<0.001
Wound	54	5	7	2	40	<0.001
Blood	10	2	2	1	5	<0.001
Nares	9	1	0	0	8	n.s.
Throat	<u>3</u>	<u>0</u>	<u>0</u>	<u>0</u>	<u>3</u>	n.s.
Totals	154	44	17	5	88	

*=p value by chi square and Fisher's exact test.

Tryptophan genetic block characterization

Response to exogenous substrates. None of the 49 tryptophan auxotrophs responded to the exogenous tryptophan analogs 4-, 5-, and 6-methyltryptophan or to 7 azotryptophan, nor to the pathway intermediates shikimic acid or chorismic acid (87,88,98) (Figure 9). However, all 44 TSST-1-producing tryptophan auxotrophs grew in response to exogenous indole, tryptophan, and 5-fluorotryptophan (5 FT). Only 2 of 5 non-TSST-1 producing tryptophan auxotrophs responded to these 3 compounds; 2 responded only to indole, and the other to anthranilic acid (Table 10). Thus all of the TSST-1-producing strains had a similar or identical metabolic lesion at the tryptophan synthase gene site, while the non-TSST-1 producing tryptophan auxotrophs appeared to have defects at different sites along the tryptophan synthesis pathway.

Table 10
Growth response of 49 tryptophan requiring *S. aureus* strains
to tryptophan biosynthesis intermediary substrates
and to tryptophan analogs

Substrate	TSST-1+	TSST-1-
Shikimic acid	0	0
Anthranilic acid	0	1
Indole only [^]	0	0
Ind/Trp/5FT	44*	2
Tryptophan only	<u>0</u>	<u>2</u>
Totals	44	5

[^]=indole, tryptophan and 5-fluorotryptophan.

*=p<0.001 by Fisher's exact test.

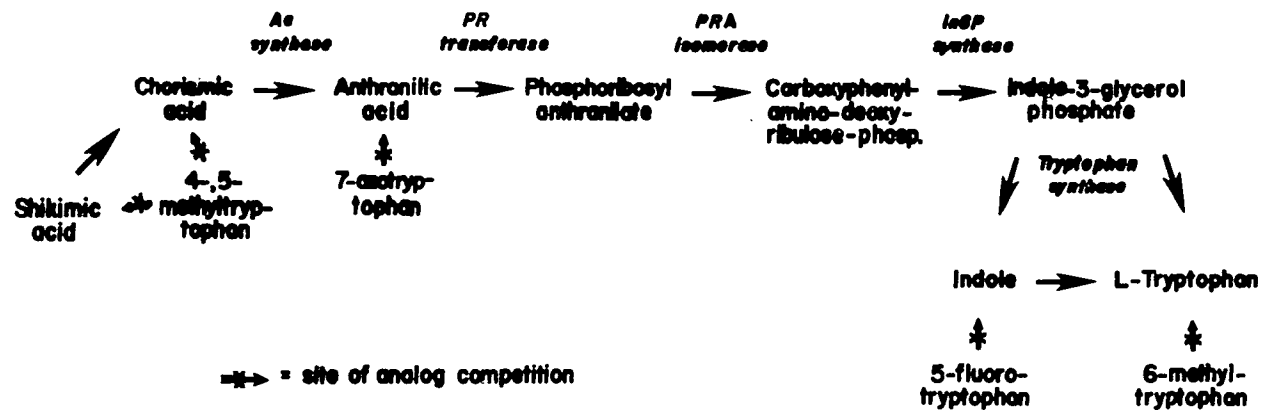


Figure 9

S. aureus tryptophan biosynthesis pathway
(56,65,88,92,109)

Since the tryptophan analog 5FT competes with indole in the tryptophan synthesis pathway (92) (Figure 9). Growth of strains augmented by 5FT is presumably associated with a by-passing of the tryptophan synthesis pathway at the indole site. TSST-1-producing and non-producing strains were plated on semi-defined medium upon which were placed paper discs saturated with either tryptophan, indole, or 5FT. Tryptophan auxotrophs demonstrated satellite growth around all 3 of the substrate discs, but TSST-1 was only detected by colony blot ELISA around the tryptophan and indole discs, and not around the 5 FT (Figure 10). The non-tryptophan requiring/TSST-1-producing strains grew to a confluent lawn, without inhibition of TSST-1 production by 5FT.

Growth augmentation of tryptophan auxotrophs by E. coli

As noted, TSST-1-producing S. aureus strains were often co-isolated with E. coli from vaginal washings of TSS patients and healthy controls (23). Twenty-nine indole-producing E. coli isolates obtained from vaginal co-isolation with TSST-1-producing and non-producing S. aureus strains, from vaginal washings that did not contain S. aureus, and from urinary tract specimens that did not contain S. aureus were used as exogenous nutrient sources in crossfeeding auxonographic experiments with the 154 S. aureus strains. All of the indole- and tryptophan-requiring TSST-1 strains not only grew as satellite colonies around the E. coli growth but produced TSST-1 as well. E. coli strains reacted with non-tryptophan requiring and TSST-1 negative strains by inhibiting or stimulating growth (Table 11).

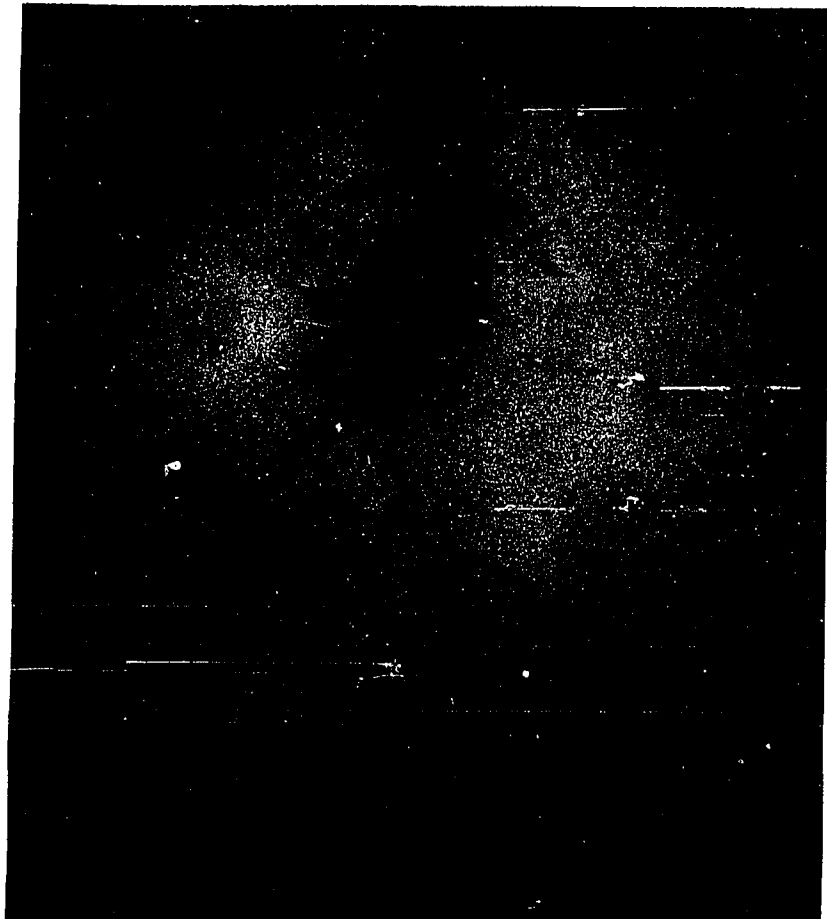


Figure 10
Detection of TSST-1 production by solid-phase ELISA
of a TSST-1-producing/tryptophan auxotroph in response to
exogenous addition of tryptophan, indole, and 5FT.
1-tryptophan, 2-5FT, and 3-indole

Table 11
Effect of E. coli strains crossfeeding S. aureus

Strain #	TSST+/TRP- N = 44	TSST+/TRP+ N = 18	TSST-/TRP- N = 5	TSST-/TRP+ N = 87
EC 1	2.20 ±1.06 [^]	0.72 ±1.04	2.00 ±1.06	0.70 ±1.24
EC 2	2.39 ±0.96	0.78 ±1.03	1.80 ±1.17	0.60 ±1.12
EC 3	2.20 ±1.12	0.67 ±1.00	1.60 ±1.36	0.53 ±1.07
EC 4	1.91 ±1.00	0.78 ±1.08	1.60 ±1.36	0.53 ±1.09
EC 5	2.02 ±0.84	0.72 ±1.04	1.40 ±1.02	0.45 ±0.99
EC 6	1.80 ±1.22	0.50 ±0.96	1.80 ±1.17	0.26 ±0.67
EC 7	2.02 ±1.36	0.56 ±0.96	1.60 ±1.50	0.40 ±1.00
EC 8	2.09 ±0.79	0.61 ±0.95	1.00 ±1.10	0.53 ±1.13
EC 9	1.86 ±0.97	0.78 ±1.03	1.40 ±1.02	0.77 ±1.21
EC 10	2.09 ±0.90	0.50 ±0.83	1.20 ±1.17	0.57 ±1.03
EC 11	1.73 ±0.99	0.50 ±0.90	1.20 ±1.17	0.51 ±1.03
EC 12	2.18 ±1.01	0.67 ±0.94	1.40 ±1.02	0.64 ±1.12
EC 13	2.00 ±0.85	0.67 ±1.05	1.40 ±1.02	0.55 ±1.07
EC 14	1.64 ±1.13	0.56 ±0.90	1.00 ±1.10	0.43 ±0.87
EC 16	1.70 ±0.97	0.56 ±0.83	1.40 ±0.80	0.81 ±1.23
EC 17	2.39 ±1.11	0.72 ±1.04	1.60 ±0.80	0.68 ±1.17
EC 18	2.27 ±1.05	0.72 ±1.10	1.60 ±0.80	0.49 ±1.05
EC 19	1.64 ±1.21	0.44 ±0.68	1.40 ±1.50	0.40 ±0.91
EC 20	2.34 ±1.36	0.56 ±0.83	2.20 ±0.98	0.49 ±0.99
EC 21	2.23 ±0.85	0.61 ±0.89	1.40 ±0.49	0.60 ±1.10
EC 22	2.27 ±1.07	0.78 ±1.08	1.80 ±0.75	0.60 ±1.07
EC 23	2.25 ±0.98	0.67 ±1.15	1.60 ±0.80	0.40 ±0.89
EC 24	2.11 ±1.03	0.72 ±1.04	1.80 ±0.75	0.51 ±1.01
EC 25	2.18 ±0.98	0.89 ±1.15	1.60 ±1.02	0.74 ±1.18
EC 26	2.16 ±1.11	0.72 ±1.15	1.20 ±1.17	0.66 ±1.06
EC 27	1.68 ±1.10	0.78 ±1.13	0.80 ±0.75	0.53 ±1.01
EC 28	2.02 ±1.08	0.67 ±1.05	1.20 ±1.17	0.49 ±0.96
EC 29	1.84 ±1.17	0.50 ±0.90	2.00 ±1.10	0.53 ±1.05
EC 30	2.30 ±1.08	0.67 ±1.05	1.40 ±1.02	0.36 ±0.84

[^]=average of strains tested ± standard deviation; <1, inhibitory; 1-4 slightly stimulatory to highly stimulatory.

Results of the E. coli crossfeeding are recorded with as inhibited (<1), and stimulated to a degree graded from 1 to 4. Indole non-producing Enterobacteriaceae strains commonly isolated from vaginal washings were also tested. Enterobacter aerogenes, Klebsiella pneumoniae, and Serratia marcescens did not stimulate or inhibit the growth of S. aureus strains when compared to the E. coli test strains and to stimulation with tryptophan alone (Table 12). In addition, stimulation of S. aureus growth was the highest among vaginal isolates irrespective of toxin-production, whereas non-vaginal isolates were all inhibited by the E. coli strains (Table 13).

Table 12
Effect of crossfeeding of S. aureus strains
with Enterobacteriaceae strains

	TSST+/TRP- N=5	TSST+/TRP+ N=7	TSST-/TRP- N=1	TSST-/TRP+ N=12
<u>Ent. aerogenes</u>	<1.0 *	NR	NR	NR
<u>K. pneumoniae</u>	NR	NR	NR	<1.0
<u>S. marcescens</u>	NR	NR	NR	<1.0
<u>E. coli</u> (EC 1)	2.20	0.72	2.00	0.72
<u>E. coli</u> (EC 2)	2.39	0.78	1.80	0.60
Tryptophan 1 ug	3.00	0.00	2.00	<1.0

NR=no reaction; neither stimulatory nor inhibitory.
*=average of stimulation or inhibitory level.

Table 13
 Effect of E. coli crossfeeding S. aureus strains
 obtained from vaginal and non-vaginal sources

E. coli strain #	<u>S. aureus</u> strains*	
	Vaginal N=29	Non-vaginal N=47
EC 1	1.14 ±1.17 [^]	0.71 ±1.14
EC 2	1.14 ±1.17	0.69 ±1.08
EC 3	0.79 ±1.00	0.58 ±1.03
EC 4	0.93 ±1.08	0.61 ±1.08
EC 5	0.72 ±1.05	0.55 ±1.01
EC 6	0.66 ±0.99	0.26 ±0.74
EC 7	0.48 ±0.97	0.48 ±1.00
EC 8	0.98 ±1.00	0.58 ±1.05
EC 9	1.24 ±1.13	0.74 ±1.03
EC 10	1.21 ±1.16	0.53 ±1.01
EC 11	0.93 ±1.01	0.58 ±1.02
EC 12	0.97 ±1.07	0.65 ±1.09
EC 13	0.86 ±0.97	0.60 ±1.06
EC 14	0.86 ±0.97	0.46 ±0.90
EC 16	1.14 ±1.11	0.75 ±1.11
EC 17	1.10 ±1.27	0.70 ±1.01
EC 18	1.17 ±1.29	0.50 ±1.27
EC 19	0.96 ±0.34	0.42 ±0.75
EC 20	1.10 ±1.21	0.53 ±0.95
EC 21	1.00 ±1.14	0.60 ±1.10
EC 22	0.62 ±1.00	0.68 ±1.07
EC 23	1.00 ±1.20	0.51 ±0.90
EC 24	0.79 ±0.96	0.62 ±1.01
EC 25	0.69 ±0.95	0.79 ±1.16
EC 26	1.30 ±1.03	0.69 ±1.07
EC 27	0.93 ±1.20	0.65 ±1.01
EC 28	0.69 ±0.95	0.50 ±0.96
EC 29	0.79 ±1.09	0.51 ±1.00
EC 30	0.79 ±1.00	0.36 ±0.90

*-S. aureus strains are TSST-1-negative and tryptophan non-requiring.

[^]-average of strains tested ± standard deviation; <1, inhibitory; 1-4 slightly stimulatory to highly stimulatory.

Genetic mapping for TSST-1 determinant

Selection of suitable strains for fusion and insertion of selective marker. S. aureus chromosomal mapping studies have been accomplished by using multiply-marked derivatives of strain NCTC 8325. Of the 62 TSST-1-producing strains, phage typing indicated that 14 strains were likely to be phage compatible with NCTC 8325. Only 4 of these strains (FRI 1169, 82026, S396 and S411) could be efficiently transduced and thus useful for chromosomal mapping. Naturally occurring TSST-1-producing strains, with the exception of tryptophan and TSST-1 production, were prototrophic for most nutritional and antibiotic markers. Therefore, the 4 TSST-1-producing strains were transduced with phage 80a (which contained the novobiocin-resistance genomic determinant acquired from ISP 2), to confer novobiocin resistance to them. The introduction of novobiocin-resistance marker was then used as phenotypic evidence of TSST-1 strain genetic transfer in protoplast fusion.

Protoplast fusion. TSST-1-producing strain ISP 1532 (novobiocin resistant) was fused with a multiple-deficient marked S. aureus NCTC 8325 strain, ISP 1653 (fusidic acid- and tetracycline-resistant). Fusion recombinants were selected twice: once for novobiocin and tetracycline resistance (Table 14), and once for novobiocin and fusidic acid resistance (Table 15). Fusion recombinant-phenotypes were analyzed by a computer fusion program which predicted the site of TSST-1 marker (tst) relative to co-inherited linkages between known markers.

Table 14
Co-inheritance frequency* of markers#
Novobiocin and tetracycline selection

	<u>thr</u>	<u>trp</u>	<u>tst</u>	<u>tyr</u>	<u>ala</u>	<u>tmn</u>	<u>ilv</u>	<u>ura</u>	<u>nov</u>	<u>fus</u>
<u>thr</u>	100	93.5	93.1	82.1	41.4	84.8	18.0	63.8	15.2	75.8
<u>trp</u>	93.5	100	88.7	78.7	40.0	81.1	20.3	67.0	18.9	73.6
<u>tst</u>	93.1	88.7	100	81.5	46.0	82.4	20.4	66.5	17.6	78.5
<u>tyr</u>	82.1	78.7	81.5	100	47.0	87.1	15.8	62.8	12.9	70.3
<u>ala</u>	41.4	40.0	46.0	47.0	100	49.1	53.2	48.0	51.0	38.0
<u>tmn</u>	84.8	81.1	82.4	87.1	49.1	100	2.85	55.3	0.00	67.9
<u>ilv</u>	18.0	20.3	20.4	15.8	53.2	2.85	100	47.3	97.2	33.2
<u>ura</u>	63.8	67.0	66.5	62.8	47.9	55.3	47.3	100	44.7	65.8
<u>nov</u>	15.2	18.9	17.6	12.9	50.9	0.00	97.2	44.7	100	32.1
<u>fus</u>	75.8	73.6	78.5	70.3	38.0	67.9	33.2	65.8	32.1	100

*co-inheritance frequency=closely linked markers are transferred at high frequencies.

#markers: thr=threonine, trp=tryptophan, tst=TSST-1, tyr=tyrosine, ala=alanine, tmn=tetracycline, ilv=isoleucine/valine, ura=uracil, nov=novobiocin, and fus=fusidic acid.

Table 15
Co-inheritance frequency* of markers#
Novobiocin and fusidic acid selection

	<u>thr</u>	<u>trp</u>	<u>tst</u>	<u>tyr</u>	<u>ala</u>	<u>tmn</u>	<u>ilv</u>	<u>ura</u>	<u>nov</u>	<u>fus</u>
<u>thr</u>	100	95.5	79.4	73.7	74.7	63.1	39.0	7.17	6.43	93.6
<u>trp</u>	95.5	100	81.2	74.6	75.6	61.3	39.3	7.47	6.73	93.3
<u>tst</u>	79.4	81.2	100	67.4	72.1	58.0	41.1	17.6	16.0	84.0
<u>tyr</u>	73.7	74.6	67.4	100	87.0	80.4	60.8	28.7	25.9	74.1
<u>ala</u>	74.7	75.6	72.1	87.0	100	75.8	55.3	27.7	24.8	75.2
<u>tmn</u>	63.1	61.3	58.0	80.4	75.8	100	72.4	39.3	40.1	59.9
<u>ilv</u>	39.0	39.3	41.1	60.8	55.3	72.4	100	66.7	63.5	36.5
<u>ura</u>	7.17	7.47	17.6	28.7	27.7	39.3	66.7	100	96.9	3.14
<u>nov</u>	6.42	6.73	16.0	25.9	24.8	40.1	63.5	96.9	100	0.00
<u>fus</u>	93.6	83.3	84.0	74.1	75.2	59.9	36.5	3.14	0.00	100

*, #: see legend on Table 14.

The percentage of linkage between recombinants obtained by fusion showed that tst was linked between tryptophan (trp) and tyrosine (tyrB) markers on the S. aureus chromosome (Tables 14,15). Because tetracycline (tmm) resistance and fusidic acid (fus) resistance were the selective factors for strain ISP 1653, these linkage numbers were artificially higher than expected; however, these resistance markers did not interfere with the analysis because they are unlinked and each marker resides in separate areas far from trp-tyrB region. Internal fusion frequency controls showed that the frequency between known pairs of markers were within acceptable limits: threonine and tryptophan genes are known to be closely linked with a co-inheritance frequency difference of 4-6% (98) while the frequency difference for tryptophan and tyrosine is 12% (83).

Transformation with Tn551 inserted strains. The fusion results suggest that the tst determinant could be finely mapped by transformation with strains multiply-marked in the trp-tyrB region. The strains selected for mapping consisted of ISP S. aureus strains with Tn551 inserts: 1) in tryptophan locus (ISP 646 and 552), 2) in tyrB locus (ISP 681), 3) in flanking areas on the outside ends of trp-tyrB markers (ISP 484, 1083, and 542), 4) within the trp-tyrB region (ISP 391 and 830), 5) and one flanking each side of trp locus (ISP 5, not a Tn551-containing strain) (Figure 11).

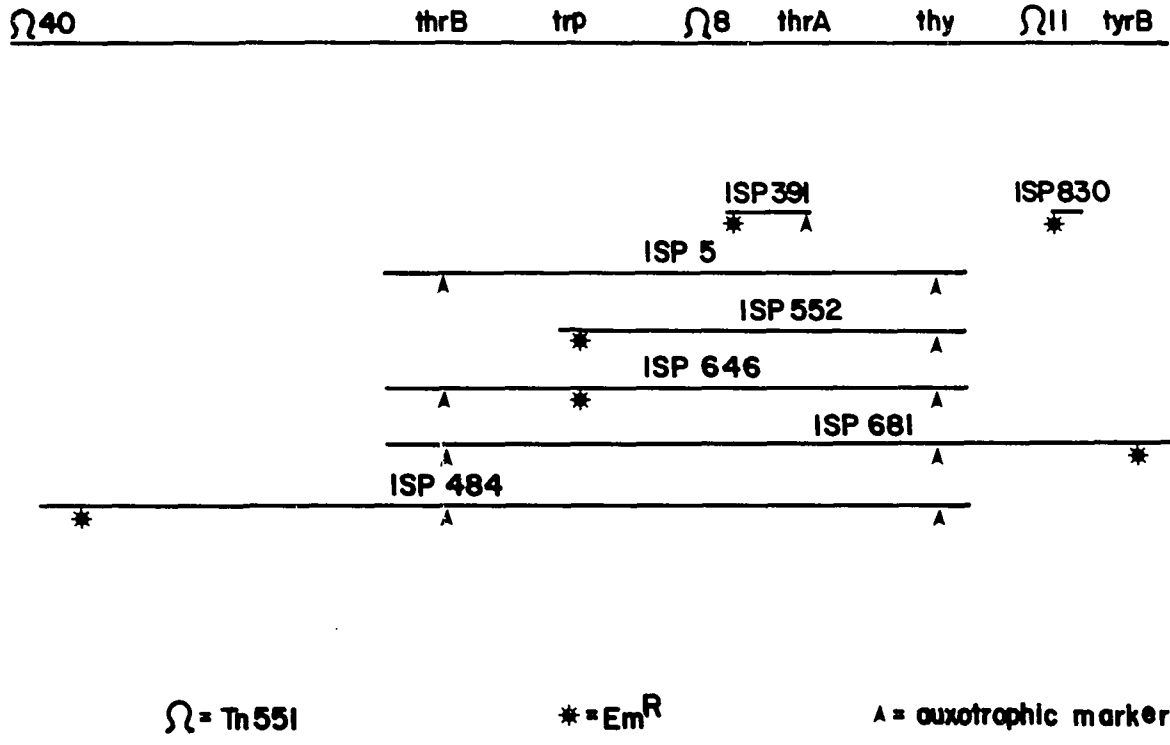


Figure 11
 Marker loci of ISP *S. aureus* strains selected
 for transformation mapping of the *tst* determinant

DNA extracted from each of the Tn551 strains and ISP 5 was used to transform 2 TSST-1-producing strains, ISP 1532 and 1637, a non-tryptophan/partial tyrosine-requiring strain and a tryptophan auxotroph, respectively. Transformation results for both strains located tst to two different sites. Reciprocal experiments using DNA extracted from the 2 TSST-1-producing strains confirmed these observations.

The TSST-1 determinant in ISP 1532, a slow tyrosine responder, was mapped to the left flank but close to the tyrosine marker. ISP 1532 DNA transformed ISP 5 (Table 16) and showed a 0.5% repair with thymine (thy), but not with threonine (thrA), which suggested that the TSST-1 determinant resided somewhere to the right of thy and to the left of tyrB.

Table 16
Transformation results obtained by using
DNA from ISP 1532 into ISP 5

	thy	thr	tst
Donor 1532	+	+	+
Recip 5	-	-	-
<u># of phenotype (%)</u>			
4 (0.5)	+	-	+
11 (1.5)	+	+	-
713 (98.0)	+	-	-

Transformation with ISP 681, a strain that has Tn551 inserted in the tyrosine gene, showed that a total of 52% of transformants express TSST-1, with 50% showing the repair of tyrosine with TSST-1 production, suggestive of a strong linkage between ISP 1532 tst and tyrB (Table 17).

Table 17
Transformation results obtained by using
DNA from ISP 1532 into ISP 681

	tyr	thy	thr	Em	tst
Donor 1532	+	+	+	s	+
Recip 681	-	-	-	r	-
<u># of phenotype (%)</u>					
14 (2)	+	+	-	s	+
34 (4)	+	+	-	s	-
367 (44)	+	-	-	s	-
417 (50)	+	-	-	s	+

Transformation with DNA from ISP 646 (TSST-1 non-producing, with Tn551 inserted into tryptophan gene) into ISP 1637 recipients showed that 22 transformants (100%) became TSST-1 negative (Table 18), resistant to erythromycin, and tryptophan non-requiring; suggesting that the TSST-1 gene was exchanged with the Tn551 portion of the donor and that the two strains had complementary tryptophan defects that could be repaired. Reciprocal experiments using DNA from ISP 1637 (Table 19) confirmed the exchange of the TSST-1 gene segment with erythromycin resistance, but did not show a tryptophan gene repair, indicating that the tryptophan defect between the two strains may be dependent on the sequential order of genetic material transferred.

Table 18
Transformation results obtained by using
DNA from ISP 646 into ISP 1637

	thr	trp	thy	Em	tst
Donor 646	-	-	-	r	-
Recip 1637	+	-	+	s	+
<u># of phenotype (%)</u>					
22 (100)	-	+	-	r	-

Table 19
Reciprocal transformation results obtained by
using DNA from ISP 1637 into ISP 646

	thr	trp	thy	Em	tst
Donor 1637	+	-	+	s	-
Recip 646	-	-	-	r	-
<u># of phenotype (%)</u>					
1 (2)	+	-	-	s	+
58 (98)	+	-	-	r	-

Therefore, protoplast fusion and transformation studies have located the tst determinant at two separate sites in the trp-tyrB chromosomal region of S. aureus (Figure 12). The tst for a tryptophan auxotroph was located in the trp site, and for a slow tyrosine responder, the tst was located very near to the tyrB site.

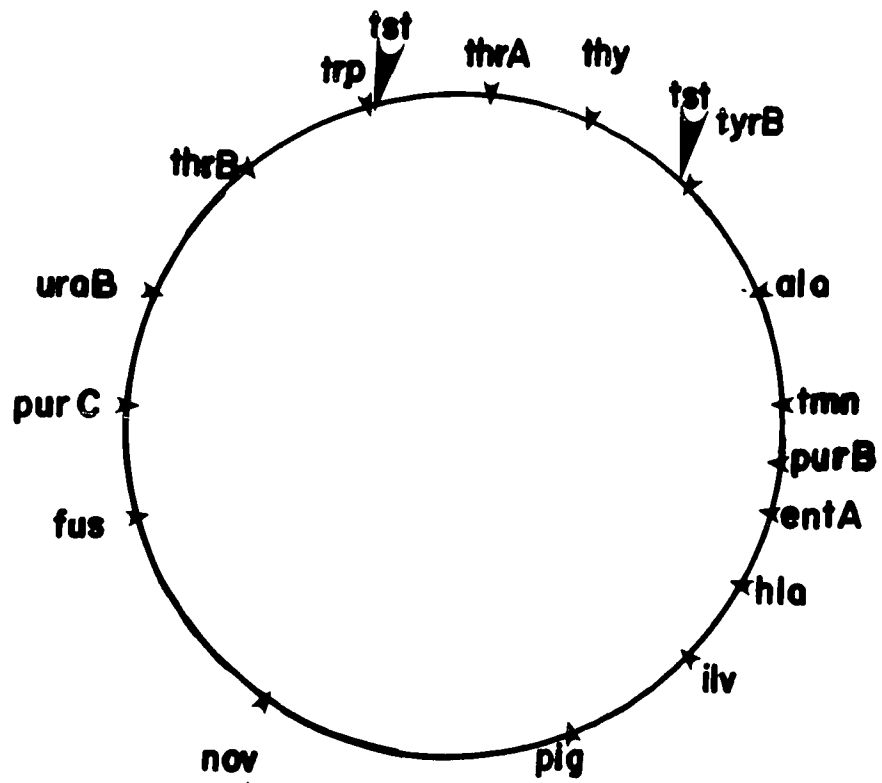


Figure 12

Mapped locations of *tst* on the *S. aureus* chromosome

DISCUSSION

Summary of findings

Documenting an association between S. aureus nutritional requirement and TSST-1 production fulfills the basic goal of this study. Two of four TSS-associated isolates first selected for auxotyping were found to require tryptophan for growth. Since such a requirement was uncommon for human S. aureus isolates (35) investigation of the association of tryptophan auxotrophy to TSST-1 production was undertaken. The association appeared not to be confounded by the disease status of the patient from whom the isolate was obtained, or by the site of isolation. Furthermore, the tryptophan-requiring/TSST-1-producing strains had an identical lesion at the tryptophan synthase gene site. Tryptophan auxotrophs were able to use exogenous indole or tryptophan to circumvent the genetic lesion at the indole site, stimulating growth and toxin production. In contrast 5FT, an indole competitor, stimulated growth but not toxin production.

In the absence of other plausible explanations for the colonization by nutritionally restricted TSST-1-producing/tryptophan auxotrophs, an hypothesis was proposed: that tryptophan auxotrophs must satisfy their tryptophan or indole requirement by forming close relationship with other organisms in the same ecologic niche that produce the required nutrients. TSST-1-producing S. aureus from vaginal washings is most frequently coisolated with E. coli. Twenty-nine indole-producing E. coli

strains obtained from urine or vaginal cultures co-cultured with tryptophan auxotrophs suggest that E. coli strains were capable of furnishing growth requirements for nutritionally deficient staphylococci. The E. coli strains enhanced growth of TSST-1-producing strains regardless of their auxotrophism, enhanced significantly more vaginal S. aureus strains, and overwhelmingly suppressed growth of non-TSST-1/non-vaginal S. aureus control strains.

Since most of the TSST-1-producing strains were tryptophan auxotrophs, and since the evidence cited above suggested that the association was genetically determined, another hypothesis was formulated: that the TSST-1 genetic determinant is found on a movable element that preferentially inserts into the tryptophan synthase gene site and thereby disrupts S. aureus tryptophan biosynthesis. Chromosomal mapping by protoplast fusion between a TSST-1-producing S. aureus, and a non-TSST-1 multiply-deficient marked S. aureus strain located the TSST-1 determinant between the trp-tyrB region of the staphylococcal chromosome. Further mapping by transformation suggest that the TSST-1 genetic determinant is located at the tryptophan gene site in a tryptophan requiring/TSST-1 strain, while in a non-tryptophan/partial-tyrosine requiring strain, the TSST-1 determinant is located in the neighboring tyrosine gene area. Because only 71% of TSST-1-producing strains are tryptophan auxotrophs, the finding that the TSST-1 determinant is located at more than one site is not surprising. Genetic

mapping for other staphylococcal toxin determinants have also shown similar multiple-site results (14,19).

Auxotyping of *S. aureus* strains

The design of a micromethod for auxotyping *S. aureus* strains was crucial to this study. Auxotyping *S. aureus* strains is impeded by technical difficulties in maintaining multiple chemically-defined media agar plates. Emmett and Kloos (35) had successfully used auxotyping to differentiate staphylococcal species based on their auxotrophic profiles; however, this auxotyping system could not be efficiently adapted to screen multiple strains.

Adapting auxotyping medium to liquid phase in micro-volumes enabled the simultaneous testing of multiple strains. To make this micro-method auxotyping a reliable assay: 1) only fresh glass-distilled water was used for making the defined media, since the normal laboratory deionized water was found to support viable staphylococci; 2) all of the amino acid stocks were made at 100-fold concentration, aliquoted, and kept at -70 C so that each set of tests were made from an identical stock; 3) each *S. aureus* strain to be tested was washed thoroughly with buffered salts so that there would be no residual nutrients carried over from the inoculating culture; and 4) the addition of phenol red as an acid indicator to the media enabled immediate interpretation of both growth and acid production.

Detection of TSST-1

The method of preparing anti-TSST-1 mouse monoclonal antibody was by concentrating hybridoma culture supernatant fluids. This method was adopted because AC3 monoclonal often elicited solid tumor formation instead of mouse ascites fluids, and because mouse ascites fluids harvested contained anti-staphylococcal antibodies as well as immunoglobulin directed against TSST-1.

Detecting TSST-1 production by colonies of S. aureus strains was complicated by the endogenous production of staphylococcus biotin and protein A. Biotin-avidin amplification was used as the secondary system to detect the binding of anti-TSST-1 serums since it proved to be the most sensitive detection method for individual colony blots on nitrocellulose paper; neither peroxidase, nor alkaline phosphatase-labelled secondary antibody were as sensitive in detecting TSST-1. Non-specific staining was prevented by a blocking procedure of incubation with unlabelled avidin followed by excess biotin prior to incubation of biotinylated-antibody.

Protein A produced by S. aureus strains can be found in both excreted and cellular forms. For example, S. aureus Cowan I strain is a prolific cellular protein A producer, Wood 46 strain does not produce cell-associated protein A but does excrete protein A (37). Nearly all S. aureus strains produce one or both forms of protein A (77), therefore when adsorbing staphylococcal proteins to nitrocellulose filters, endogenous protein A must be blocked. Since protein A specifically binds to Fc sites on immunoglobulins,

enzymatic cleavage of Fc sites from the detecting antibodies would be expected to prevent binding. In this study, Fab' fragments were made by papain digestion from the monoclonal antibody AC3, but antibody activity was reduced. To resolve this problem, all detecting antibodies (primary and secondary) were incubated with excess unlabelled protein A prior to testing, and endogenous staphylococcal protein A was blocked by using rabbit anti-protein A serum (Sigma). The combination of blocking endogenous protein A that attached to nitrocellulose filter and of blocking Fc receptor sites on the immunoglobulins prevented non-specific attachment of immunoglobulins.

Phenotypic characteristics of TSST-1-producing strains

Since thirty-four of the 57 TSS-associated S. aureus were the same strains used for phenotypic characterization by Todd (117) and by Barbour (7), it is not surprising that most of the phenotypic traits identified in this study were comparable to those previously reported. The other 23 strains not included in the previous phenotype studies were also similar to the 34 tested strains in phage susceptibility, antibiotic resistance, hemolysis, and pigmentation.

The only phenotypic characteristic found to be different in one of these studies (117) was the decreased proteolytic ability of TSS-associated, TSST-1-producing strains. It had been suggested by Todd (117) that TSS S. aureus strains were more proteolytic than other strains ($p < 0.03$), and that proteolytic activity was the

result of a primary genetic event affecting post-translational modification of normal staphylococcal proteins to create unique new markers associated with pathogenesis of TSS. However, in this study increased proteolysis could not be confirmed under in vitro conditions of aeration that optimize TSST-1 production.

Proteolysis was, in fact, slightly less in TSST-1-producing strains than controls. This difference in proteolysis could be attributed to the different conditions under which the casein plates were incubated: under 10% CO₂ in Todd's studies, and under the conditions optimal for in vitro TSST-1 production in this investigation.

Genetic implications of nutritional requirement in TSST-1-producing *S. aureus*

Before this study was undertaken, the genetic implications of nutritional requirements associated with TSST-1-producing *S. aureus* strains were not known. Previous studies on nutritional requirements of TSS-associated *S. aureus* isolates were designed only to control for growth and TSST-1 production.

S. aureus isolated from human skin were found to be predominantly auxotrophic for arginine, proline, and valine; very few strains required other amino acids for growth, with some strains having partial requirements for histidine, leucine, tryptophan and valine (35). Of the 154 strains studied, the nutritional requirement profile was similar with the notable exception of tryptophan. In analyzing the association of amino

acid requirement with TSST-1 production, only tryptophan was directly related to toxin production and an association of partial tyrosine requirement to TSST-1 production was noted.

The issue of the stability of tryptophan auxotrophism was examined. Generally, in nutritional studies, auxotrophic strains may exhibit reduced background growth after prolonged incubation, indicating "partial" requirement for the nutritional element in question. In other cases, strains spontaneously revert to prototrophic growth upon serial propagation (35). The tryptophan auxotrophs associated with TSST-1 production exhibited neither reversion nor partial requirement for tryptophan after multiple laboratory passages, indicating stability of the site mutation and suggesting that there is a deletion or insertion of a genomic segment or rearrangement at the site.

Characterization of the tryptophan gene defect

The site of a genetic biosynthetic lesion may be detected by auxonographic tests in which biosynthesis pathway intermediate substrates are added exogenously to repair the requirement (56,88). The 49 tryptophan auxotrophs were tested to characterize their defect; 44 TSST-1-producing strains responded to the same exogenous pathway intermediates, and to tryptophan analog 5FT, indicating an identical lesion located at the tryptophan synthase gene site. Of the 5 non-TSST-1 producing tryptophan auxotrophs, 3 apparent separate lesions were detected suggesting that they were different from the TSST-1/tryptophan auxotrophs.

Amino acid analogs can become incorporated into proteins in place of the natural amino acids, and may either inhibit growth, or successfully compete with its natural counterpart in restoring growth of microorganisms (92,109). Restoration of growth by an amino acid analog effectively by-passes the lesion site without activation of the gene at the lesion site. 5FT is a tryptophan analog that becomes incorporated during the conversion of anthranilic acid to indole; when substituted for indole it can restore growth at the tryptophan synthase gene site (109). Tryptophan auxotroph growth was restored in tryptophan-deficient medium by indole, tryptophan, and 5FT; however, TSST-1 production was detected with addition of indole or tryptophan but not with 5FT, suggesting that 5FT successfully restores auxotrophic growth without de-repressing the tryptophan synthase gene site. In addition, TSST-1-producing/non-tryptophan requiring strains continue to produce detectable TSST-1 in the presence of 5FT indicating that 5FT itself does not alter the toxin protein antigenically.

Environmental association of tryptophan requirement, TSST-1 production, to E. coli co-isolation

It is important to consider what impact the in vivo culture environment have on auxotrophic/toxin-producing S. aureus strains, and what environmental factors may contribute to TSST-1 production. Exogenous nutrients must be available to tryptophan requiring/TSST-1-producing strains for its growth and for toxin production to

occur. In the vaginal environment, S. aureus colonies increase at the time of menses, indicating that nutritional factors may be abundant then. It is also during menses that TSS onset occurs in women colonized with toxin-producing strains. Co-isolation of E. coli with TSST-1-producing S. aureus strains suggests the possibility that E. coli strains supply necessary nutrients to S. aureus. Prototrophic E. coli and other enterobacteriaceae are prolific tryptophan- and indole-excreting bacteria (60). The crossfeeding experiments in this study showed that the E. coli strains were indole producers and could stimulate growth of both S. aureus tryptophan auxotrophs and other vaginal non-tryptophan requiring S. aureus strains. This suggests that not only are the E. coli able to support indole/tryptophan auxotrophs, they are in a commensal, supportive relationship with other vaginal-derived staphylococci as well.

Mixed bacterial studies provide a more realistic basis for analyzing the metabolic and ecologic aspects in which selection, antagonism, synergism and competition may all play a role in initiating and promoting disease in the host. Experiments on the interaction of Pseudomonas aeruginosa and S. aureus thymine and tryptophan auxotrophs demonstrated that either mixed cultures or culture filtrates of P. aeruginosa, were able to supply the essential metabolites required to ensure growth of the auxotrophic S. aureus strains (39). Mice, infected with a mixed culture of P. aeruginosa and nutritionally deficient S. aureus, exhibited reduced host phagocytosis of staphylococci because of Pseudomonas endotoxin

damage to leucocytes (27,39). Thus, possibilities for survival and growth of nutritionally dependent strains of a bacterial species may be improved by the metabolic activities of another species in mixed cultures and infections. Associated organisms may therefore exert control over pathogenicity of other auxotrophic bacteria.

Genetic basis for TSST-1 determinant

Stable integration of a transposable element carrying a TSST-1 determinant into the S. aureus chromosome is the most likely explanation for TSST-1 production in S. aureus. A derivative of RP1 plasmid carrying Tn1 (carbenicillin resistance) was selectively integrated at high frequency ($>10^{-3}$) into the trpA, trpB gene cluster of Pseudomonas aeruginosa chromosome, resulting in high numbers of tryptophan auxotrophs (44). The same phenomenon has been observed in E. coli systems: integration of Tn1 also produced 3% auxotrophic clones inserting in non-random sites producing stable non-reverting auxotrophs (reversion frequency at $<10^{-10}$) (45).

The tryptophan-tyrosine area where the TSST-1 genetic determinant inserts is considered a "hot spot" for insertion of Tn551, and may also be important for insertion of a TSST-1 containing movable element with regional specificity. Because TSS strains lack detectable plasmids yet carry penicillin and heavy metal resistance markers that are associated with extrachromosomal plasmids, TSS-associated isolates may already carry such an integrated genetic element. Southern blot hybridization with the

cloned TSST-1 segment suggest that TSST-1 determinant can be present in more than one copy, partial copies, or present but unexpressed (51). The phenotypic distinctiveness of TSS-associated strains, the lack of extrachromosomal elements, and multiple blot patterns all suggest that the TSST-1 genetic determinant is carried on an integrated segment capable of insertion in several sites.

As yet, there is no physical evidence for the presence of an integrated segment that carries a TSST-1 determinant. However, such a segment is plausible: 1) the tryptophan to tyrosine region on the staphylococcal chromosome occupies no more than 15% of the total genome (personal communication, P.A.Pattee), 2) the distance between mapped sites of TSST-1 determinant is not more than 300 kilo base pairs apart, 3) successful transduction of a TSST-1 determinant by phage 80a (90), defined a TSST-1 copy to be <1% of the total staphylococcal chromosome, and this size would be small enough to be carried by a transposon (78).

Concluding statement

TSST-1 genetic determinant has been mapped to the tryptophan-tyrosine region of the S. aureus genome. This finding was based on the discovery of a genetic association between tryptophan requirement and TSST-1 production. Because not all TSST-1-producing S. aureus strains were tryptophan auxotrophs, it was expected that the tst determinant could be located at more than one site. The presence of more than one genetic loci for tst is

analogous to the 6 genomic mapped sites for entA which preferentially resides in the purB-ilv region of the staphylococcal chromosome (14).

Why some TSST-1-producing strains are more lethal than others in the animal model system may be explained by the fact that when all possible loci for tst determinants are found, the preferential sites would be those found downstream from a strong gene promoter sequence. The tryptophan biosynthesis gene cluster is known to have a strong promoter gene (60), therefore the insertion of a TSST-1 determinant downstream of the tryptophan promoter would turn the S. aureus strain into a prolific TSST-1 producer. If the tst determinant is inserted at a site away from the influence of a strong promoter, the result would be a weak or cryptic TSST-1-producing strain. This then may explain why there are multiple Southern blot patterns when TSST-1-producing strains were hybridized with the cloned tst determinant and why the amount of detectable TSST-1 varies in culture..

Future perspectives of TSST-1 genetic research

Since tst is located in the trp-tyrB region there is a need to isolate and sequence this segment of the S. aureus chromosome. The segment would need to be analyzed by nucleotide sequence and by restriction endonuclease fragments so that: 1) a comparison can be made with the cloned tst determinant isolated by Kreiswirth et al (51), and 2) a comparison be made with isolated trp-tyrB sequence from a non-TSST-1 producing strain to identify an insertional element that is related to TSST-1 production.

LEGEND FOR S. AUREUS STRAINS PROFILES

Nutrient studies: + = growth, not auxotrophic; - = no growth, auxotrophic; +/- = variable growth, partial requirement

TSST: I.D. = immunodiffusion using rabbit anti-TSST-1 serum
N.C. = nitrocellulose colony ELISA with mouse monoclonal anti-TSST-1

Source: ATTC = American type culture
Blood = blood culture isolate
Nasal/NP = nasopharyngeal site
Throt = throat culture isolate
Vag = vaginal culture isolate
W/AB = wound and abscess sites
Cellu = cellulitis
Ear = ear drainage fluid
Em fl = chest drainage fluid
Furnc = furuncle
Joint = joint fluid
Lesn = lesion
Osteo = osteomyelitis
Pleur = pleural fluid
Surg = surgical wound

Disease: Disease state of patient from whom the isolate came
ASYMP = asymptomatic
ILL = ill, disease not defined
SSSS = staphylococcal scalded skin syndrome
SYMP = symptomatic for TSS but not diagnosed
TSS = toxic shock patient
VGNTS = vaginitis, not defined as TSS

Tryptophan substrates and analogs:

Shikimic = shikimic acid	G = growth, no requiring
Anthran = anthranilic acid	E = enhanced growth
Indole = indole	N = no growth, no reaction
Tryp = tryptophan	R = satellite growth, auxo-
5 F.-tryp = 5 fluorotryptophan	trophic
6 M.-tryp = 6 methyltryptophan	
7 AZ.tryp = 7 azotryptophan	

Dnase: + = Dnase production

Ent A: + = enterotoxin A producer; NR = no reaction

Delta Tox: - = no enhanced hemolysis; 1-3 = enhanced hemolysis

Hemolysis: +1 = low hemolysis; +2-+4 = increased hemolysis

Protease: proteolysis diameter zone (mm)

Coagulase: + = coagulase-positive

STAPHYLOCOCCUS AUREUS STRAINS PROFILES

	D1209	D1233	D1470	D1513	D1515	D4159	D4160	D4161	D4212	D4214	D4215	D4217	D4218	D4219	D4220	S444
NUTRIENT																
no ala.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no arg.	-	-	-	+/-	+/-	-	-	-	-	-	-	-	-	-	-	-
no asp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no cys.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no glu.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no gly.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no his.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+/-
no isl.	+	+	+/-	+	+	+	-	-	+	+	+	+	+	+	+	+
no leu.	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-
no lys.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no met.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no phe.	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+
no pro.	-	-	-	+	-	+	+	-	+	-	-	+	-	+	-	-
no ser.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no thr.	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
no trp.	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-
no tyr.	+	+	+	+	+/-	+	+	+	+	+	+	+	+	+	+	-
no val.	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
no vit.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
no glc.	-	-	-	-	-	-	-	+	-	-	+/-	-	-	-	-	-
all	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
buffer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSST(ID)	+	+	+	+	+	-	-	-	-	-	+	-	-	-	+	+
TSST(NC)	+	+	+	+	+	-	-	-	-	+	+	-	-	-	+	+
SOURCE	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	FURNC
DISEASE	TSS	TSS	TSS	TSS	TSS	ASYMP	ASYMP	ASYMP	ASYMP	ASYMP	ASYMP	ASYMP	ASYMP	ASYMP	ASYMP	TSS
SHIKIMIC	N	N	N	N	N	G	N	G	G	G	N	G	G	G	N	N
ANTHRAN.	N	N	N	N	N	G	N	G	G	G	N	G	G	G	N	N
INDOLE	R	R	R	R	R	G	R	G	G	G	R	G	G	G	N	R
TRYP.	R	R	R	R	R	G	R	G	G	G	R	G	G	G	R	R
5 F.-TRYP	R	R	R	R	R	G	N	G	G	G	R	G	G	G	N	R
6 M.-TRYP	N	N	N	N	N	G	N	G	G	G	N	G	G	G	N	N
7 AZ. TRYP	N	N	N	N	N	G	N	G	G	G	N	G	G	G	N	N
DNASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENT A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
DELTA TOX	+1	-	-	-	-	+2	+2	-	+2	-	-	-	+1	+2	-	-
HEMOLYSIS	+1	+2	+1	-	+2	+1	+3	+3	+1	+1	+1	+3	+1	+4	+1	+1
PROTEASE	16.0	22.1	18.0	13.8	19.6	20.3	21.1	21.0	15.2	12.75	14.0	20.0	20.0	19.3	10.0	19.0
COAGULASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Set I.D. C.D.C SET																

66

STAPHYLOCOCCUS AUREUS STRAINS PROFILES

	S445	S446	S447	S448	S449	S450	S451	S452	S453	R179	3092	TV-1	NJ-2	HV-1	BP-1	HRSBG		
NUTRIENT																		
no ala.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
no arg.	-	-	-	-	-	+/-	+	-	-	-	-	+/-	+	-	-	-		
no asp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
no cys.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
no glu.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
no gly.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
no his.	+/-	+	+	+	+	-	+	+/-	+	-	+	-	+	+	+	+		
no isl.	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+		
no leu.	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+		
no lys.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
no met.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
no phe.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
no pro.	-	-	-	+/-	-	-	+/-	-	-	-	-	-	+/-	-	-	-		
no ser.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
no thr.	+/-	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+		
no trp.	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-		
no tyr.	+	+	+	+	+	+	+	+	+	-	+	-	+	+/-	+	-		
no val.	-	-	-	+/-	-	-	+	+/-	+/-	-	+	-	+	-	-	-		
no vit.	-	-	-	-	-	+	+	-	-	-	+	-	-	-	-	+/-		
no glc.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
all	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
buffer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
TSST(ID)	S445	S446	S447	S448	S449	S450	S451	S452	S453	R179	3092	TV-1	NJ-2	HV-1	BP-1	HRSBG		
TSST(NC)	+	+	+	+	-	-	-	-	-	+	-	+	+	+	+	+		
SOURCE	W/AB	W/AB	W/AB	LESN	EM.FL.	SURG	SURG	LESN	W/AB	VAG	VAG	VAG	VAG	VAG	VAG	VAG		
DISEASE	TSS	TSS	TSS	TSS	TSS	TSS	TSS	TSS	TSS	TSS	TSS	TSS	TSS	TSS	TSS	TSS		
SHIKIMIC	G	G	G	G	G	G	G	G	G	N	G	N	G	N	N	N		
ANTHRAN.	G	G	G	G	G	G	G	G	G	N	G	N	G	N	N	N		
INDOLE	G	E	E	G	G	G	G	G	G	R	G	R	G	R	R	R		
TRYP.	G	E	E	E	G	G	G	G	G	R	G	R	G	R	R	R		
5 F.-TRYP	G	G	G	G	G	G	G	G	G	N	G	R	G	R	R	R		
6 M.-TRYP	G	G	G	G	G	G	G	G	G	N	G	N	G	N	N	N		
7 A2-TRYP	G	G	G	G	G	G	G	G	G	N	G	N	G	N	N	N		
DNASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
ENT A	NR	NR	NR	NR	+	+	NR	NR	NR	NR	NR	NR	NR	+	NR	NR		
DELTA TOX	-	-	-	-	-	-	-	-	-	+2	+2	-	-	-	-	+1		
HEMOLYSIS	+1	+1	+1	+1	+1	+1	+3	+1	+1	+1	+1	+1	+1	+1	+1	+1		
PROTEASE	17.0	14.25	18.5	17.2	22.7	22.8	22.4	20.3	14.2	14.75	15.6	19.2	16.0	19.0	16.3	17.4		
COAGULASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Set I.D. C.D.C. SET										N.I.A. I.D. SET								

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STAPHYLOCOCCUS AUREUS STRAINS PROFILES

	82035	82028	123V	82008	82024	A7029	A7315	A7317	A7383	A7470	A7493	A7494	A7516	A7528	A7533	A7537
NUTRIENT																
no ala.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no arg.	-	+	+	-	+	-	-	-	-	-	+/-	+	+	+	+	+
no asp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no cys.	-	+	+	+	+	+	+	+	+/-	+	+	+	+	+	+	+
no glu.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no gly.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no his.	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
no isl.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no leu.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no lys.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no met.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no phe.	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
no pro.	+	-	-	+	+/-	-	-	-	-	+	+	+/-	-	-	+	+
no ser.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no thr.	+	+	+	+	+	+	+	+/-	+	+	+	+	-	+	+	+
no trp.	-	+	-	-	+	-	+	-	-	+	+	+	-	+	+	+
no tyr.	+/-	+	+	+	+	+	+	+	+	+	+	+	+/-	+	+	+
no val.	-	-	-	-	-	-	+	-	-	+/-	-	+	+	-	+/-	-
no vit.	-	+/-	+	-	+/-	-	-	-	-	-	-	-	-	-	-	-
no glc.	-	+	+	+	+	-	-	-	+	-	-	-	-	-	-	-
all	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
buffer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSST(ID)	-	-	+	+	-	+	+	+	+	-	-	-	+	-	-	-
TSST(NC)	+	+	+	+	+	+	+	+	+	-	-	-	+	-	-	-
SOURCE	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG	VAG
DISEASE	ASYMP	ASYMP	ASYM	ASYMP	ASYMP	TSS	TSS	TSS	TSS	ASYMP	ASYMP	ASYMP	TSS	ASYMP	ASYMP	ASYMP
SHIKIMIC	N	G	N	N	G	N	G	N	N	G	G	G	N	G	G	G
ANTHRAN.	N	E	N	N	G	N	G	N	N	G	E	G	N	G	G	G
INDOLE	R	E	R	R	G	R	G	R	R	G	E	G	R	G	G	G
TRYP.	R	E	R	R	G	R	G	R	R	G	E	G	R	G	G	E
5 F.-TRYP	R	E	R	R	G	R	G	R	R	G	G	G	R	G	G	G
6 M.-TRYP	N	G	N	N	G	N	G	N	N	G	G	G	N	G	G	G
7 AZ. TRYP	N	G	N	N	G	N	G	N	N	G	G	G	N	G	G	G
DNASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENT A	NR	NR	+	NR	+	NR	NR	NR	NR	NR	NR	NR	NR	NR	+	NR
DELTA TOX	-	+2	-	-	-	+1	-	-	+1	-	-	-	-	+1	-	-
HEMOLYSIS	+3	+1	+1	+1	+1	+1	+1	+1	+1	+1	+2	+1	+1	+2	+3	+1
PROTEASE	15.4	18.3	18.1	15.5	23.5	17.0	15.0	17.3	15.85	21.5	21.4	20.7	17.0	17.25	20.5	18.8
COAGULASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Set I.D. N.I.R.I.D. SET																

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UNIV. OF BRITISH COLUMBIA SET

STAPHYLOCOCCUS AUREUS STRAINS PROFILES

	A7545	A7592	A7601	A7602	A7614	A7618	A7660	A7662	A7671	S374	S375	S378	S379	S380	S381	S382
NUTRIENT																
no ala.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no arg.	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	-
no asp.	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
no cys.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no glu.	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
no gly.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no his.	+	+	+	+/-	+	+	+	+	-	+	+	+	+	+	+	-
no isl.	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
no leu.	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	-
no lys.	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
no met.	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
no pha.	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
no pro.	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-
no ser.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+/-
no thr.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no trp.	+	-	-	-	+	-	+	-	-	-	-	+	+	-	-	+
no tyr.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no val.	+	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-
no vit.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
no glc.	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
all	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
buffer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	A7545	A7592	A7601	A7602	A7614	A7618	A7660	A7662	A7671	S374	S375	S378	S379	S380	S381	S382
TSST(ID)	-	+	+	+	-	+	-	-	+	-	+	-	+	+	+	-
TSST(NC)	-	+	+	+	+	+	-	-	+	-	+	-	+	+	+	-
SOURCE DISEASE	VAG ASYMP	VAG TSS	VAG TSS	VAG TSS	VAG ASYMP	VAG TSS	VAG ASYMP	VAG ASYMP	VAG TSS	BLOOD ASYMP	BLOOD ASYMP	BLOOD TSS	VAG TSS	VAG TSS	BLOOD TSS	W/AB ASYMP
SHIKIMIC	G	G	N	N	G	G	G	N	N	N	N	G	G	N	N	G
ANTHRAN.	G	G	N	N	G	G	G	R	N	N	N	G	G	N	N	G
INDOLE	G	G	R	R	G	G	G	R	R	N	R	G	G	R	R	G
TRYP.	G	G	R	R	G	G	G	R	R	R	R	G	G	R	R	G
5 F.-TRYP	G	G	R	R	G	G	G	R	R	R	R	G	G	R	R	G
6 M.-TRYP	G	I	N	N	G	G	G	N	N	N	N	G	G	N	N	G
7 AZ. TRYP	G	I	N	N	G	G	G	N	N	N	N	G	G	N	N	G
DNASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENT A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	+	NR	NR	NR
DELTA TOX	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HEMOLYSIS	+3	+1	+2	+1	+3	+1	+1	+1	+1	+3	+1	+1	+1	+1	+1	+3
PROTEASE	20.57	16.25	17.3	17.5	19.6	14.6	21.0	21.0	16.8	15.0	11.6	11.0	13.4	21.6	17.3	9.5
COAGULASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Set I.D. UNIV. OF BRITISH COLUMBIA SET										UNIV. OF COLORADO SET						

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STAPHYLOCOCCUS AUREUS STRAINS PROFILES

	S439	S440	S443	5916	SA93	SA94	SA103	SA104	4022	4023	4041	4043	4063	4100	4106	4132
NUTRIENT																
no ala.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no arg.	-	-	-	-	-	-	-	-	-	+/-	-	-	-	-	-	-
no asp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no cys.	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
no glu.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no gly.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no his.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no isl.	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
no leu.	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
no lys.	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+
no met.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no phe.	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
no pro.	-	-	-	-	+	+	+	+	-	+/-	-	-	-	-	-	-
no ser.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no thr.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no trp.	+	+	+	-	+	+	+	-	+	+	-	+	+	+	-	+
no tyr.	+	+	+	+/-	+	+	+	+	+	+	+	+	+	+	+/-	+
no val.	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
no vit.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
no glc.	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+/-
all	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
buffer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSST(ID)	S439	S440	S443	5916	SA93	SA94	SA103	SA104	4022	4023	4041	4043	4063	4100	4106	4132
TSST(NC)	-	-	-	+	-	-	+	+	-	-	-	-	-	-	+	-
SOURCE	W/AB	W/AB	W/AB	VAG	VAG	VAG	VAG	VAG	W/AB	W/AB	W/AB	W/AB	W/AB	THROT	W/AB	THROT
DISEASE	SSSS	SSSS	SSSS	TSS	TSS	TSS	TSS	TSS	ILL	ILL	ILL	ILL	ILL	ILL	ILL	ILL
SHIKIMIC	G	G	G	N	G	G	G	N	G	G	N	G	N	G	N	G
ANTHRAN.	G	G	G	N	G	G	G	N	G	G	N	G	N	G	N	G
INDOLE	G	G	G	R	G	G	G	R	G	G	N	G	N	G	R	G
TRYP.	G	G	G	R	G	G	G	R	G	G	R	G	R	G	R	G
5 F.-TRYP	G	G	G	R	G	G	G	R	G	G	N	G	N	G	R	G
6 M.-TRYP	G	G	G	N	G	G	G	N	G	G	N	G	N	G	N	G
7 A2. TRYP	G	G	G	N	G	G	G	N	G	G	N	G	N	G	N	G
DNASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENT A	NR	NR	NR	NR	+	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
DELTA TOX	-	+1	-	-	-	+1	+1	-	-	-	-	-	-	-	-	-
HEMOLYSIS	+1	+1	+3	+1	+3	+1	+1	+1	+1	+1	+1	+3	+1	+3	+1	+1
PROTEASE	12.0	22.0	20.0	20.8	16.6	22.0	13.5	15.6	21.0	25.0	26.0	25.0	21.0	12.0	20.0	22.0
COAGULASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Set I.D. UNIV. OF HAWAII SET

STAPHYLOCOCCUS AUREUS STRAINS PROFILES

	4135	4155	4157	4158	4508	4509	4512	4513	4543	4558	4578	4580	4635	4660	4662	4684
NUTRIENT																
no ala.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no arg.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
no asp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no cys.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no glu.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no gly.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no his.	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+
no isl.	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
no leu.	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	-
no lys.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no met.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no pha.	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
no pro.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
no ser.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no thr.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no trp.	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
no tyr.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
no val.	-	+/-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
no vit.	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-
no glc.	+	-	-	-	-	+/-	-	-	-	-	-	-	-	-	-	-
all	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
buffer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TSST(ID)	4135	4155	4157	4158	4508	4509	4512	4513	4543	4558	4578	4580	4635	4660	4662	4684
TSST(NC)	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
SOURCE	W/AB	W/AB	W/AB	W/AB	W/AB	W/AB	NP	W/AB	W/AB	W/AB	THROT	W/AB	W/AB	W/AB	W/AB	W/AB
DISEASE	ILL	ILL	ILL	ILL	ILL	ILL	ILL	ILL	ILL	ILL	ILL	ILL	ILL	ILL	ILL	ILL
SHIKIMIC	G	G	G	G	G	G	G	G	G	G	G	G	G	N	G	G
ANTHRAN.	G	G	G	G	G	G	G	G	G	G	E	G	E	N	G	G
INDOLE	G	G	G	G	G	G	G	G	G	G	E	G	E	R	G	G
TRYP.	G	G	G	G	G	G	G	G	G	G	E	G	E	R	G	G
5 F.-TRYP	G	G	G	G	G	G	G	G	G	G	E	G	E	R	G	G
6 M.-TRYP	G	G	G	G	G	G	I	G	G	G	G	G	G	N	G	G
7 A2. TRYP	G	G	G	G	G	G	I	G	G	G	G	G	G	N	G	G
DNASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENT A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	+	+	NR	NR
DELTA TOX	-	-	-	-	-	-	-	+1	-	-	-	-	-	-	-	-
HEMOLYSIS	+2	+3	+3	+2	+2	+3	+2	+2	+1	+1	+1	+	+1	+1	+1	+2
PROTEASE	25.0	25.0	25.0	25.0	20.0	24.0	25.0	15.0	25.0	25.0	24.0	24.0	13.0	18.0	23.0	21.0
COAGULASE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Set I.D. UNIV. OF HAWAII SET																

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STAPHYLOCOCCUS AUREUS STRAINS PROFILES

	4711	4712	SAC	KP	MCT	DMM	KL	JK	CO	12598	12228
NUTRIENT											
no ala.	+	+	+	+	+	+	+	+	+	+	+
no arg.	-	-	+	-	-	-	-	-	-	-	-
no asp.	+	+	+	+	+	+	+	+	+	+	+
no cys.	+	+	+	+	+	+	+	+	+	+	+
no glu.	+	+	+	+	+	+	+	+	+	+	+
no gly.	+	+	+	+	+	+	+	+	+	+	-
no his.	+	+	+	+	+	+	+	-	-	+	+
no isl.	+	+	+	+	+	+	+	+	+	+	+
no leu.	+	+	+	+	+	+	+	-	-	+	-
no lys.	+	+	+	+	+	+	+	+	+	+	+
no met.	+	+	+	+	+	+	+	+	+	+	+
no phe.	+	+	+	+	+	+	+	-	+	+	+
no pro.	-	-	+/-	-	+	-	-	-	-	+	-
no ser.	+	+	+	+	+	+	+	+	+	+	+
no thr.	+	+	+	+	+	+	+	+	+	-	+
no trp.	+	+	+	+	+	+	-	+	+	+	-
no tyr.	+	+	+	+	+	+	+	+	+	+	+
no val.	-	-	+	-	-	-	-	-	-	+	-
no vit.	-	-	-	-	-	-	-	-	-	+	+
no glc.	-	-	-	-	-	-	-	-	-	-	-
all	+	+	+	+	+	+	+	+	+	+	+
buffer	-	-	-	-	-	-	-	-	-	-	-
TSST(ID)	4711	4712	SAC	KP	MCT	DMM	KL	JK	CO	12598	12228
TSST(NC)	-	-	-	-	-	-	+	-	-	-	+
SOURCE	W/AB	W/AB	ATTC	NASAL	NASAL	NASAL	NASAL	NASAL	NASAL	ATTC	ATTC
DISEASE	ILL	ILL	ILL	ASYMP	ASYMP	ASYMP	ASYMP	ASYMP	ASYMP	ILL	ILL
SHIKIMIC	G	G	G	G	G	G	N	G	G	G	N
ANTHRAN.	E	G	G	G	G	G	N	G	G	G	N
INDOLE	E	G	G	G	G	G	R	G	G	G	N
TRYP.	E	G	G	G	G	G	R	G	G	G	R
5 F.-TRYP	E	G	G	G	I	G	R	G	G	G	N
6 M.-TRYP	G	G	G	G	G	G	N	G	G	G	N
7 AZ. TRYP	G	G	G	G	G	G	N	G	G	G	N
DNASE	+	+	+	+	+	+	+	+	+	+	+
ENT A	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
DELTA TOX	-	-	-	-	-	+2	-	-	-	-	-
HEMOLYSIS	+3	+1	+2	+2	+1	+1	+1	+1	+1	+1	+1
PROTEASE	24.0	24.0	4.37	15.0	10.3	15.0	13.0	25.0	25.0	22.3	16.0
COAGULASE	+	+	+	+	+	+	+	+	+	+	+
Set I.D. UNIV. OF HAWAII SET											

801

E. COLI CROSSFEEDING S. AUREUS STRAINS

	D1208	D1233	D1470	D1513	D1515	D4159	D4160	D4161	D4212	D4214	D4215	D4217	D4218	D4219	D4220	S444
EC1	12.00	10.00	12.00	12.00	11.00	2.00	10.00	5.00	5.00	3.00	4.00	6.00	4.00	4.00	5.00	12.00
EC2	12.00	11.00	12.00	12.00	10.00	2.00	5.00	5.00	5.00	3.00	4.00	3.00	4.00	4.00	5.00	12.00
EC3	12.00	11.00	12.00	12.00	5.00	2.00	5.00	5.00	5.00	4.00	11.00	3.00	4.00	4.00	5.00	12.00
EC4	12.00	10.00	12.00	12.00	10.00	4.00	5.00	5.00	5.00	5.00	11.00	4.00	5.00	4.00	5.00	10.00
EC5	12.00	10.00	12.00	11.00	10.00	4.00	5.00	5.00	5.00	5.00	12.00	4.00	4.00	4.00	5.00	11.00
EC6	12.00	12.00	11.00	5.00	5.00	3.00	5.00	5.00	5.00	4.00	10.00	4.00	4.00	4.00	5.00	5.00
EC7	13.00	10.00	11.00	10.00	5.00	4.00	5.00	5.00	5.00	4.00	5.00	4.00	4.00	4.00	5.00	10.00
EC8	12.00	12.00	12.00	12.00	11.00	1.00	6.00	5.00	5.00	2.00	11.00	3.00	1.00	2.00	5.00	12.00
EC9	12.00	12.00	12.00	11.00	10.00	2.00	5.00	5.00	5.00	3.00	12.00	4.00	2.00	2.00	5.00	11.00
EC10	12.00	12.00	12.00	11.00	10.00	4.00	5.00	5.00	5.00	4.00	12.00	4.00	4.00	4.00	5.00	11.00
EC11	12.00	11.00	11.00	4.00	5.00	2.00	5.00	5.00	5.00	4.00	12.00	3.00	3.00	2.00	5.00	10.00
EC12	13.00	12.00	12.00	11.00	10.00	4.00	5.00	5.00	5.00	4.00	12.00	4.00	5.00	4.00	5.00	10.00
EC13	12.00	11.00	12.00	11.00	11.00	4.00	5.00	5.00	5.00	4.00	12.00	4.00	2.00	4.00	5.00	11.00
EC14	12.00	11.00	12.00	5.00	5.00	4.00	5.00	5.00	5.00	4.00	12.00	4.00	4.00	3.00	5.00	10.00
EC15																
EC16	12.00	11.00	12.00	11.00	5.00	4.00	5.00	5.00	5.00	4.00	12.00	4.00	4.00	3.00	5.00	10.00
EC17	13.00	11.00	12.00	12.00	10.00	5.00	5.00	5.00	5.00	5.00	11.00	5.00	5.00	4.00	5.00	12.00
EC18	13.00	12.00	12.00	12.00	10.00	4.00	5.00	5.00	5.00	5.00	12.00	5.00	4.00	4.00	6.00	12.00
EC19	12.00	11.00	10.00	4.00	4.00	4.00	5.00	5.00	5.00	4.00	5.00	5.00	4.00	4.00	5.00	5.00
EC20	13.00	12.00	12.00	11.00	5.00	4.00	5.00	5.00	5.00	4.00	4.00	4.00	4.00	4.00	5.00	12.00
EC21	12.00	11.00	12.00	11.00	10.00	4.00	5.00	5.00	5.00	4.00	12.00	4.00	4.00	4.00	5.00	12.00
EC22	13.00	12.00	12.00	12.00	10.00	4.00	5.00	5.00	5.00	4.00	12.00	4.00	4.00	3.00	5.00	12.00
EC23	12.00	11.00	12.00	12.00	11.00	2.00	6.00	3.00	5.00	2.00	12.00	3.00	2.00	2.00	5.00	11.00
EC24	12.00	10.00	12.00	11.00	5.00	4.00	5.00	5.00	5.00	4.00	11.00	6.00	4.00	4.00	5.00	12.00
EC25	12.00	11.00	12.00	12.00	5.00	4.00	5.00	5.00	5.00	4.00	10.00	4.00	4.00	4.00	5.00	12.00
EC26	13.00	11.00	12.00	4.00	5.00	2.00	5.00	5.00	5.00	4.00	12.00	4.00	4.00	4.00	5.00	12.00
EC27	12.00	10.00	11.00	11.00	5.00	4.00	5.00	5.00	5.00	5.00	4.00	6.00	5.00	4.00	5.00	4.00
EC28	12.00	10.00	11.00	11.00	5.00	4.00	5.00	5.00	5.00	5.00	12.00	4.00	5.00	4.00	5.00	11.00
EC29	12.00	10.00	11.00	5.00	5.00	4.00	5.00	5.00	5.00	4.00	5.00	4.00	4.00	4.00	5.00	10.00
EC30	12.00	10.00	12.00	11.00	5.00	4.00	5.00	5.00	5.00	4.00	10.00	4.00	4.00	4.00	5.00	12.00
	D1208	D1233	D1470	D1513	D1515	D4159	D4160	D4161	D4212	D4214	D4215	D4217	D4218	D4219	D4220	S444

Sat I.D. C.D.C. SET

LEGEND 1-4=INHIBITED GROWTH, 5=NO REACTION, 6-9=STIMULATED GROWTH/NON-TRP REQUIRING, 10-13=SATELLITE GROWTH

E. COLI CROSSFEEDING S. AUREUS STRAINS

	S445	S446	S447	S448	S449	S450	S451	S452	S453	R179	3092	TV-1	NJ-2	HV-1	BP-1	HRSBG
EC1	2.00	3.00	11.00	12.00	3.00	4.00	2.00	4.00	5.00	11.00	7.00	12.00	7.00	12.00	12.00	12.00
EC2	3.00	3.00	11.00	12.00	3.00	4.00	2.00	4.00	5.00	11.00	7.00	12.00	7.00	12.00	13.00	12.00
EC3	4.00	4.00	11.00	12.00	4.00	4.00	3.00	4.00	3.00	12.00	7.00	12.00	6.00	12.00	12.00	12.00
EC4	4.00	4.00	11.00	12.00	4.00	4.00	4.00	4.00	4.00	11.00	7.00	12.00	7.00	12.00	12.00	11.00
EC5	4.00	4.00	11.00	12.00	3.00	4.00	4.00	4.00	5.00	11.00	7.00	12.00	7.00	11.00	12.00	10.00
EC6	3.00	3.00	5.00	12.00	3.00	4.00	3.00	3.00	4.00	12.00	7.00	12.00	6.00	12.00	12.00	12.00
EC7	4.00	3.00	5.00	12.00	4.00	4.00	4.00	4.00	4.00	12.00	7.00	12.00	6.00	13.00	13.00	11.00
EC8	1.00	2.00	11.00	12.00	1.00	1.00	1.00	2.00	4.00	11.00	7.00	11.00	7.00	11.00	11.00	11.00
EC9	2.00	2.00	10.00	10.00	2.00	4.00	2.00	4.00	5.00	10.00	7.00	12.00	8.00	11.00	12.00	10.00
EC10	4.00	4.00	10.00	11.00	4.00	5.00	4.00	4.00	5.00	11.00	7.00	12.00	8.00	12.00	12.00	11.00
EC11	1.00	2.00	10.00	12.00	2.00	4.00	2.00	2.00	5.00	11.00	7.00	11.00	7.00	11.00	11.00	11.00
EC12	4.00	5.00	10.00	12.00	5.00	6.00	6.00	6.00	5.00	11.00	7.00	12.00	7.00	12.00	12.00	11.00
EC13	3.00	4.00	10.00	12.00	2.00	4.00	6.00	4.00	5.00	11.00	6.00	12.00	7.00	12.00	12.00	11.00
EC14	4.00	4.00	5.00	11.00	4.00	4.00	6.00	4.00	4.00	11.00	7.00	12.00	7.00	12.00	11.00	11.00
EC15																
EC16	4.00	4.00	5.00	10.00	4.00	4.00	4.00	4.00	5.00	10.00	7.00	12.00	8.00	11.00	11.00	10.00
EC17	4.00	5.00	11.00	12.00	4.00	5.00	4.00	4.00	5.00	10.00	7.00	12.00	8.00	13.00	13.00	11.00
EC18	5.00	6.00	11.00	12.00	4.00	4.00	4.00	4.00	5.00	11.00	7.00	12.00	8.00	12.00	12.00	11.00
EC19	5.00	4.00	4.00	11.00	3.00	4.00	2.00	4.00	4.00	10.00	5.00	12.00	6.00	12.00	12.00	10.00
EC20	4.00	4.00	11.00	11.00	4.00	4.00	4.00	4.00	4.00	11.00	7.00	13.00	7.00	13.00	12.00	11.00
EC21	4.00	4.00	11.00	10.00	2.00	4.00	2.00	4.00	5.00	11.00	7.00	12.00	8.00	11.00	12.00	10.00
EC22	4.00	4.00	11.00	12.00	2.00	4.00	2.00	4.00	4.00	11.00	8.00	13.00	7.00	12.00	12.00	11.00
EC23	2.00	2.00	11.00	11.00	1.00	2.00	1.00	2.00	5.00	11.00	8.00	12.00	8.00	11.00	12.00	10.00
EC24	4.00	4.00	12.00	12.00	4.00	4.00	4.00	4.00	5.00	10.00	7.00	12.00	7.00	12.00	12.00	11.00
EC25	4.00	4.00	12.00	12.00	4.00	4.00	3.00	4.00	4.00	11.00	8.00	12.00	7.00	12.00	12.00	11.00
EC26	2.00	4.00	12.00	12.00	4.00	4.00	2.00	4.00	3.00	11.00	8.00	13.00	8.00	12.00	12.00	11.00
EC27	4.00	4.00	11.00	12.00	4.00	4.00	4.00	5.00	4.00	10.00	8.00	11.00	8.00	11.00	11.00	10.00
EC28	2.00	4.00	11.00	12.00	4.00	4.00	4.00	4.00	4.00	10.00	7.00	12.00	7.00	12.00	12.00	11.00
EC29	2.00	4.00	4.00	12.00	3.00	4.00	2.00	4.00	4.00	12.00	7.00	12.00	7.00	12.00	12.00	10.00
EC30	1.00	2.00	11.00	12.00	2.00	3.00	3.00	3.00	4.00	12.00	7.00	12.00	8.00	12.00	12.00	10.00
	S445	S446	S447	S448	S449	S450	S451	S452	S453	R179	3092	TV-1	NJ-2	HV-1	BP-1	HRSBG

Sat I.D. C.D.C SET

N.I.A.I.D. SET

LEGEND 1-4=INHIBITED GROWTH, 5=NO REACTION, 6-9=STIMULATED GROWTH/NON-TRP REQUIRING, 10-13=SATELLITE GROWTH/

E. COLI CROSSFEEDING S. AUREUS STRAINS

	MV-1	KV-1	CV-1	30-5	26-2	27-13	92143	30-2	23-5	831	3916	82017	82026	82010	112V	82038
EC1	12.00	12.00	12.00	3.00	7.00	12.00	5.00	7.00	7.00	12.00	8.00	7.00	8.00	6.00	6.00	7.00
EC2	12.00	12.00	12.00	3.00	6.00	12.00	5.00	7.00	7.00	12.00	8.00	7.00	8.00	6.00	6.00	7.00
EC3	11.00	12.00	12.00	3.00	5.00	12.00	5.00	5.00	7.00	12.00	7.00	6.00	8.00	6.00	6.00	6.00
EC4	11.00	11.00	12.00	4.00	6.00	12.00	5.00	7.00	7.00	12.00	8.00	6.00	6.00	6.00	6.00	7.00
EC5	12.00	11.00	11.00	4.00	6.00	11.00	5.00	6.00	6.00	12.00	8.00	6.00	6.00	5.00	5.00	7.00
EC6	12.00	11.00	12.00	4.00	5.00	12.00	5.00	6.00	5.00	12.00	7.00	6.00	7.00	5.00	5.00	5.00
EC7	12.00	12.00	12.00	4.00	5.00	13.00	5.00	5.00	6.00	11.00	6.00	5.00	5.00	5.00	5.00	6.00
EC8	11.00	12.00	11.00	3.00	5.00	12.00	5.00	6.00	7.00	11.00	7.00	7.00	7.00	6.00	6.00	7.00
EC9	12.00	11.00	11.00	6.00	6.00	12.00	5.00	6.00	7.00	12.00	8.00	8.00	8.00	7.00	7.00	8.00
EC10	11.00	11.00	12.00	6.00	6.00	12.00	5.00	7.00	7.00	11.00	7.00	8.00	8.00	6.00	6.00	6.00
EC11	11.00	11.00	10.00	2.00	5.00	11.00	5.00	7.00	7.00	12.00	7.00	7.00	8.00	6.00	6.00	7.00
EC12	12.00	11.00	12.00	6.00	6.00	12.00	5.00	6.00	6.00	12.00	7.00	7.00	9.00	5.00	5.00	8.00
EC13	11.00	11.00	11.00	6.00	5.00	12.00	5.00	6.00	6.00	11.00	6.00	7.00	9.00	5.00	5.00	7.00
EC14	12.00	11.00	10.00	6.00	5.00	12.00	5.00	6.00	5.00	11.00	6.00	7.00	9.00	5.00	5.00	9.00
EC15																
EC16	10.00	10.00	12.00	6.00	6.00	11.00	5.00	8.00	7.00	12.00	8.00	7.00	6.00	6.00	6.00	7.00
EC17	12.00	10.00	12.00	4.00	6.00	12.00	5.00	8.00	7.00	13.00	8.00	7.00	7.00	5.00	5.00	7.00
EC18	12.00	10.00	12.00	4.00	6.00	12.00	5.00	8.00	7.00	13.00	8.00	7.00	7.00	6.00	6.00	7.00
EC19	12.00	10.00	11.00	4.00	5.00	12.00	5.00	5.00	5.00	12.00	5.00	5.00	7.00	5.00	5.00	5.00
EC20	13.00	12.00	13.00	4.00	6.00	13.00	5.00	8.00	6.00	13.00	7.00	7.00	8.00	6.00	6.00	6.00
EC21	12.00	12.00	11.00	4.00	6.00	11.00	5.00	8.00	6.00	11.00	8.00	7.00	9.00	6.00	6.00	7.00
EC22	12.00	12.00	12.00	4.00	5.00	12.00	5.00	8.00	5.00	12.00	6.00	6.00	8.00	5.00	5.00	5.00
EC23	11.00	12.00	12.00	1.00	6.00	11.00	5.00	9.00	5.00	12.00	8.00	6.00	9.00	5.00	5.00	6.00
EC24	11.00	12.00	12.00	4.00	5.00	12.00	5.00	7.00	6.00	12.00	6.00	6.00	9.00	5.00	5.00	6.00
EC25	11.00	12.00	12.00	4.00	5.00	12.00	5.00	7.00	6.00	12.00	7.00	6.00	9.00	5.00	5.00	5.00
EC26	11.00	12.00	12.00	4.00	7.00	12.00	5.00	8.00	6.00	12.00	6.00	7.00	9.00	6.00	6.00	6.00
EC27	10.00	12.00	11.00	4.00	5.00	11.00	5.00	9.00	5.00	11.00	6.00	6.00	9.00	5.00	5.00	5.00
EC28	13.00	12.00	12.00	4.00	5.00	12.00	5.00	5.00	6.00	12.00	6.00	5.00	7.00	5.00	5.00	5.00
EC29	11.00	12.00	12.00	3.00	5.00	11.00	5.00	6.00	6.00	12.00	6.00	6.00	9.00	5.00	5.00	5.00
EC30	12.00	12.00	12.00	2.00	5.00	12.00	5.00	5.00	6.00	12.00	6.00	7.00	7.00	5.00	5.00	6.00
	MV-1	KV-1	CV-1	30-5	26-2	27-13	92143	30-2	23-5	831	3916	82017	82026	82010	112V	82038

Set I.D. N.I.A.I.D. SET

LEGEND 1-4=INHIBITED GROWTH, 5=NO REACTION, 6-9=STIMULATED GROWTH/NON-TRP REQUIRING, 10-13=SATELLITE GROWTH/

E. COLI CROSSFEEDING S. AUREUS STRAINS

	82035	82028	123V	82008	82024	A7029	A7315	A7317	A7383	A7470	A7493	A7494	A7516	A7528	A7533	A7537
EC1	12.00	6.00	12.00	13.00	6.00	2.00	6.00	12.00	12.00	5.00	6.00	5.00	11.00	7.00	6.00	9.00
EC2	12.00	7.00	12.00	12.00	8.00	12.00	6.00	11.00	12.00	5.00	6.00	6.00	12.00	7.00	7.00	9.00
EC3	12.00	6.00	12.00	11.00	7.00	12.00	6.00	10.00	12.00	5.00	5.00	6.00	12.00	7.00	6.00	9.00
EC4	12.00	6.00	12.00	12.00	6.00	11.00	6.00	10.00	11.00	5.00	5.00	6.00	11.00	7.00	6.00	9.00
EC5	12.00	6.00	11.00	12.00	6.00	11.00	6.00	10.00	11.00	5.00	5.00	5.00	11.00	7.00	6.00	9.00
EC6	12.00	5.00	11.00	11.00	5.00	12.00	6.00	10.00	12.00	5.00	6.00	6.00	11.00	7.00	6.00	9.00
EC7	12.00	6.00	13.00	12.00	6.00	12.00	6.00	11.00	12.00	5.00	5.00	5.00	12.00	6.00	5.00	9.00
EC8	11.00	5.00	11.00	12.00	7.00	10.00	6.00	10.00	11.00	5.00	6.00	5.00	11.00	7.00	7.00	8.00
EC9	12.00	5.00	11.00	12.00	8.00	10.00	6.00	10.00	10.00	5.00	7.00	6.00	11.00	7.00	7.00	8.00
EC10	12.00	5.00	12.00	12.00	8.00	12.00	6.00	10.00	12.00	5.00	7.00	6.00	11.00	7.00	7.00	9.00
EC11	12.00	5.00	11.00	12.00	7.00	11.00	6.00	10.00	11.00	5.00	7.00	5.00	11.00	6.00	7.00	8.00
EC12	12.00	5.00	12.00	12.00	7.00	11.00	6.00	11.00	11.00	5.00	6.00	6.00	11.00	7.00	7.00	9.00
EC13	12.00	5.00	12.00	12.00	7.00	11.00	6.00	10.00	11.00	5.00	6.00	6.00	11.00	7.00	7.00	8.00
EC14	12.00	5.00	12.00	12.00	7.00	10.00	6.00	5.00	11.00	5.00	6.00	6.00	10.00	7.00	7.00	9.00
EC15																
EC16	12.00	6.00	11.00	12.00	8.00	10.00	6.00	5.00	10.00	5.00	6.00	6.00	10.00	7.00	7.00	7.00
EC17	12.00	7.00	13.00	13.00	7.00	12.00	6.00	10.00	12.00	5.00	6.00	6.00	12.00	7.00	6.00	9.00
EC18	12.00	5.00	13.00	13.00	8.00	12.00	6.00	10.00	12.00	5.00	6.00	6.00	12.00	7.00	7.00	9.00
EC19	13.00	5.00	12.00	12.00	5.00	10.00	6.00	5.00	11.00	5.00	5.00	5.00	11.00	5.00	5.00	9.00
EC20	12.00	5.00	13.00	13.00	7.00	12.00	6.00	11.00	12.00	5.00	6.00	6.00	13.00	7.00	7.00	9.00
EC21	11.00	5.00	11.00	11.00	7.00	12.00	6.00	10.00	11.00	5.00	5.00	5.00	11.00	7.00	6.00	9.00
EC22	12.00	5.00	12.00	12.00	6.00	12.00	6.00	10.00	12.00	5.00	5.00	5.00	12.00	7.00	6.00	8.00
EC23	12.00	5.00	12.00	11.00	6.00	12.00	6.00	10.00	11.00	5.00	6.00	6.00	12.00	7.00	7.00	9.00
EC24	12.00	5.00	12.00	12.00	6.00	12.00	6.00	10.00	11.00	5.00	6.00	6.00	12.00	7.00	7.00	7.00
EC25	12.00	5.00	12.00	12.00	6.00	12.00	6.00	10.00	11.00	5.00	5.00	5.00	12.00	7.00	6.00	8.00
EC26	11.00	5.00	12.00	12.00	7.00	12.00	6.00	10.00	11.00	5.00	7.00	6.00	12.00	7.00	7.00	8.00
EC27	11.00	5.00	11.00	11.00	7.00	11.00	6.00	10.00	11.00	5.00	5.00	6.00	11.00	8.00	7.00	8.00
EC28	12.00	5.00	12.00	12.00	6.00	12.00	6.00	10.00	11.00	5.00	6.00	6.00	12.00	7.00	7.00	8.00
EC29	11.00	6.00	12.00	12.00	7.00	11.00	6.00	10.00	12.00	5.00	5.00	5.00	11.00	7.00	6.00	9.00
EC30	12.00	5.00	12.00	12.00	6.00	12.00	6.00	11.00	12.00	5.00	7.00	6.00	12.00	7.00	7.00	8.00
	82035	82028	123V	82008	82024	A7029	A7315	A7317	A7383	A7470	A7493	A7494	A7516	A7528	A7533	A7537

Set I.D. N.I.A.I.D. SET UNIV. OF BRITISH COLUMBIA SET
 LEGEND 1-4=INHIBITED GROWTH, 5=NO REACTION, 6-9=STIMULATED GROWTH/NON-TRP REQUIRING, 10-13=SATELLITE GROWTH/

E. COLI CROSSFEEDING S. AUREUS STRAINS

	A7545	A7592	A7601	A7602	A7614	A7618	A7660	A7662	A7671	S374	S375	S378	S379	S380	S381	S382
EC1	6.00	12.00	12.00	12.00	7.00	7.00	7.00	11.00	11.00	12.00	11.00	3.00	4.00	4.00	12.00	5.00
EC2	6.00	12.00	12.00	12.00	7.00	7.00	7.00	11.00	11.00	12.00	12.00	4.00	4.00	4.00	12.00	5.00
EC3	6.00	12.00	12.00	12.00	7.00	6.00	6.00	12.00	12.00	11.00	10.00	3.00	3.00	12.00	12.00	4.00
EC4	6.00	12.00	11.00	12.00	7.00	7.00	6.00	11.00	11.00	12.00	10.00	4.00	4.00	12.00	12.00	5.00
EC5	6.00	12.00	12.00	11.00	6.00	7.00	6.00	11.00	11.00	5.00	11.00	5.00	4.00	12.00	12.00	5.00
EC6	7.00	12.00	12.00	12.00	7.00	6.00	5.00	11.00	11.00	12.00	12.00	5.00	4.00	12.00	10.00	2.00
EC7	5.00	12.00	12.00	12.00	7.00	6.00	6.00	11.00	12.00	13.00	10.00	3.00	4.00	12.00	11.00	5.00
EC8	7.00	12.00	11.00	11.00	7.00	7.00	7.00	5.00	11.00	10.00	10.00	4.00	1.00	12.00	12.00	5.00
EC9	7.00	12.00	12.00	10.00	7.00	7.00	7.00	10.00	10.00	11.00	10.00	4.00	4.00	11.00	11.00	5.00
EC10	7.00	12.00	12.00	10.00	7.00	7.00	7.00	5.00	10.00	11.00	10.00	4.00	4.00	11.00	11.00	5.00
EC11	6.00	12.00	12.00	11.00	7.00	7.00	6.00	11.00	11.00	10.00	10.00	5.00	2.00	12.00	12.00	5.00
EC12	6.00	12.00	12.00	11.00	6.00	7.00	7.00	10.00	11.00	11.00	11.00	5.00	4.00	12.00	12.00	5.00
EC13	7.00	12.00	12.00	10.00	7.00	7.00	7.00	10.00	11.00	11.00	11.00	5.00	4.00	11.00	12.00	5.00
EC14	7.00	12.00	12.00	10.00	5.00	7.00	7.00	10.00	11.00	10.00	11.00	5.00	4.00	10.00	5.00	4.00
EC15																
EC16	7.00	10.00	11.00	10.00	7.00	7.00	7.00	10.00	10.00	10.00	10.00	6.00	4.00	12.00	10.00	4.00
EC17	7.00	13.00	11.00	11.00	7.00	7.00	7.00	10.00	10.00	11.00	11.00	3.00	4.00	12.00	11.00	5.00
EC18	7.00	13.00	11.00	11.00	5.00	7.00	7.00	10.00	11.00	11.00	11.00	3.00	4.00	12.00	10.00	5.00
EC19	5.00	13.00	10.00	10.00	7.00	6.00	5.00	5.00	11.00	11.00	11.00	5.00	3.00	11.00	11.00	4.00
EC20	7.00	13.00	12.00	12.00	7.00	7.00	7.00	12.00	11.00	12.00	11.00	5.00	4.00	12.00	4.00	4.00
EC21	7.00	10.00	11.00	11.00	7.00	7.00	7.00	11.00	11.00	10.00	10.00	5.00	4.00	12.00	11.00	5.00
EC22	5.00	12.00	12.00	11.00	7.00	7.00	6.00	11.00	11.00	11.00	11.00	3.00	3.00	12.00	5.00	5.00
EC23	7.00	12.00	12.00	11.00	7.00	7.00	7.00	10.00	10.00	11.00	10.00	5.00	2.00	12.00	11.00	5.00
EC24	6.00	12.00	12.00	11.00	7.00	7.00	6.00	11.00	10.00	11.00	11.00	4.00	4.00	12.00	11.00	5.00
EC25	6.00	12.00	12.00	11.00	7.00	7.00	6.00	11.00	11.00	11.00	11.00	4.00	4.00	11.00	10.00	5.00
EC26	7.00	12.00	12.00	11.00	7.00	8.00	6.00	10.00	11.00	11.00	11.00	2.00	4.00	11.00	11.00	4.00
EC27	6.00	11.00	12.00	11.00	7.00	8.00	6.00	10.00	10.00	10.00	10.00	2.00	5.00	11.00	4.00	4.00
EC28	7.00	12.00	12.00	11.00	7.00	7.00	6.00	10.00	10.00	12.00	10.00	2.00	4.00	11.00	11.00	4.00
EC29	6.00	12.00	12.00	12.00	7.00	7.00	7.00	11.00	11.00	11.00	11.00	2.00	3.00	4.00	5.00	3.00
EC30	7.00	13.00	12.00	11.00	7.00	7.00	7.00	10.00	12.00	11.00	12.00	2.00	2.00	12.00	11.00	4.00
	A7545	A7592	A7601	A7602	A7614	A7618	A7660	A7662	A7671	S374	S375	S378	S379	S380	S381	S382

Sat I.D. UNIV. OF BRITISH COLUMBIA SET
 LEGEND 1-4=INHIBITED GROWTH, 5=NO REACTION, 6-9=STIMULATED GROWTH/NON-TRP REQUIRING, 10-13=SATELLITE GROWTH/

UNIV. OF COLORADO SET

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E. COLI CROSSFEEDING S. AUREUS STRAINS

	S383	S384	S385	S386	S387	S388	S389	S390	S393	S394	S395	S396	S397	S400	S401	S402
EC1	5.00	5.00	10.00	5.00	5.00	13.00	5.00	5.00	11.00	5.00	7.00	5.00	5.00	3.00	5.00	5.00
EC2	5.00	5.00	10.00	6.00	5.00	12.00	6.00	5.00	10.00	5.00	6.00	5.00	5.00	4.00	5.00	5.00
EC3	5.00	5.00	5.00	1.00	5.00	10.00	4.00	5.00	5.00	5.00	8.00	5.00	5.00	3.00	5.00	5.00
EC4	5.00	5.00	5.00	5.00	5.00	11.00	4.00	5.00	5.00	5.00	8.00	5.00	5.00	2.00	5.00	5.00
EC5	4.00	5.00	10.00	5.00	5.00	12.00	5.00	5.00	10.00	5.00	6.00	5.00	5.00	5.00	5.00	3.00
EC6	5.00	5.00	5.00	1.00	5.00	10.00	4.00	5.00	5.00	4.00	4.00	5.00	5.00	2.00	4.00	5.00
EC7	5.00	5.00	5.00	5.00	5.00	10.00	5.00	5.00	5.00	5.00	4.00	5.00	5.00	2.00	5.00	5.00
EC8	5.00	5.00	10.00	5.00	5.00	12.00	5.00	5.00	10.00	5.00	7.00	5.00	5.00	2.00	5.00	5.00
EC9	5.00	5.00	10.00	5.00	5.00	13.00	5.00	5.00	10.00	5.00	9.00	4.00	5.00	6.00	5.00	5.00
EC10	4.00	5.00	10.00	5.00	5.00	12.00	5.00	5.00	10.00	5.00	6.00	5.00	5.00	4.00	5.00	5.00
EC11	5.00	5.00	5.00	1.00	5.00	5.00	5.00	5.00	5.00	4.00	6.00	4.00	5.00	4.00	5.00	5.00
EC12	5.00	5.00	10.00	5.00	5.00	11.00	5.00	5.00	10.00	5.00	9.00	5.00	5.00	4.00	5.00	5.00
EC13	4.00	5.00	10.00	5.00	5.00	13.00	5.00	5.00	10.00	5.00	6.00	5.00	5.00	3.00	5.00	5.00
EC14	5.00	5.00	5.00	1.00	5.00	5.00	4.00	5.00	5.00	4.00	7.00	4.00	5.00	3.00	4.00	5.00
EC15																
EC16	5.00	5.00	10.00	1.00	5.00	11.00	4.00	5.00	11.00	4.00	6.00	4.00	5.00	2.00	5.00	5.00
EC17	5.00	5.00	10.00	5.00	5.00	13.00	5.00	5.00	11.00	5.00	8.00	5.00	5.00	2.00	5.00	5.00
EC18	5.00	5.00	10.00	5.00	6.00	13.00	5.00	5.00	11.00	5.00	4.00	5.00	5.00	2.00	5.00	5.00
EC19	5.00	5.00	5.00	1.00	5.00	10.00	4.00	5.00	5.00	5.00	4.00	4.00	5.00	6.00	5.00	5.00
EC20	5.00	5.00	5.00	2.00	5.00	10.00	5.00	5.00	5.00	5.00	6.00	4.00	5.00	3.00	5.00	5.00
EC21	5.00	5.00	10.00	5.00	6.00	13.00	5.00	5.00	11.00	5.00	4.00	5.00	5.00	4.00	5.00	5.00
EC22	5.00	5.00	5.00	4.00	5.00	11.00	4.00	5.00	10.00	5.00	4.00	5.00	5.00	4.00	5.00	5.00
EC23	5.00	5.00	10.00	5.00	6.00	13.00	5.00	5.00	11.00	5.00	5.00	4.00	5.00	5.00	5.00	5.00
EC24	5.00	5.00	10.00	5.00	5.00	12.00	5.00	5.00	5.00	5.00	7.00	4.00	5.00	3.00	5.00	5.00
EC25	4.00	5.00	10.00	4.00	5.00	12.00	5.00	5.00	5.00	5.00	9.00	4.00	5.00	8.00	5.00	5.00
EC26	4.00	5.00	5.00	1.00	5.00	11.00	4.00	5.00	5.00	4.00	8.00	4.00	5.00	6.00	5.00	5.00
EC27	5.00	5.00	5.00	1.00	5.00	11.00	4.00	5.00	5.00	5.00	9.00	4.00	5.00	4.00	5.00	5.00
EC28	4.00	4.00	5.00	1.00	5.00	10.00	4.00	5.00	5.00	5.00	8.00	5.00	5.00	4.00	5.00	5.00
EC29	4.00	4.00	5.00	1.00	4.00	11.00	4.00	5.00	5.00	5.00	8.00	4.00	5.00	4.00	5.00	5.00
EC30	5.00	5.00	5.00	4.00	5.00	12.00	5.00	5.00	5.00	5.00	4.00	5.00	5.00	3.00	5.00	5.00
	S383	S384	S385	S386	S387	S388	S389	S390	S393	S394	S395	S396	S397	S400	S401	S402

Set I.D. UNIV. OF COLORADO SET

LEGEND 1-4=INHIBITED GROWTH, 5=NO REACTION, 6-9=STIMULATED GROWTH/NON-TRP REQUIRING, 10-13=SATELLITE GROWTH/

E. COLI CROSSFEEDING S. AUREUS STRAINS

	S403	S404	S408	S409	S410	S411	S412	S414	S108	S210	S244	S256	1169	S373	S431	S436
EC1	5.00	12.00	5.00	2.00	10.00	11.00	10.00	11.00	5.00	5.00	12.00	3.00	6.00	6.00	5.00	2.00
EC2	5.00	12.00	5.00	4.00	10.00	12.00	10.00	10.00	5.00	5.00	11.00	5.00	6.00	7.00	5.00	4.00
EC3	2.00	12.00	5.00	3.00	5.00	11.00	5.00	10.00	5.00	5.00	12.00	4.00	6.00	5.00	4.00	5.00
EC4	5.00	12.00	5.00	3.00	5.00	11.00	5.00	10.00	5.00	5.00	11.00	4.00	6.00	5.00	5.00	4.00
EC5	5.00	12.00	5.00	4.00	10.00	11.00	10.00	10.00	5.00	5.00	5.00	5.00	6.00	6.00	5.00	4.00
EC6	2.00	12.00	5.00	4.00	5.00	10.00	5.00	4.00	5.00	5.00	11.00	4.00	6.00	5.00	6.00	5.00
EC7	5.00	12.00	5.00	3.00	5.00	10.00	5.00	4.00	5.00	5.00	12.00	4.00	6.00	5.00	5.00	5.00
EC8	5.00	11.00	5.00	3.00	10.00	10.00	10.00	5.00	5.00	5.00	12.00	4.00	6.00	6.00	5.00	5.00
EC9	1.00	11.00	5.00	7.00	10.00	10.00	10.00	10.00	5.00	5.00	10.00	6.00	6.00	6.00	5.00	5.00
EC10	2.00	4.00	5.00	4.00	5.00	11.00	10.00	5.00	5.00	5.00	13.00	4.00	6.00	6.00	5.00	5.00
EC11	4.00	5.00	5.00	5.00	5.00	12.00	5.00	4.00	5.00	5.00	5.00	5.00	4.00	5.00	5.00	5.00
EC12	5.00	11.00	5.00	4.00	10.00	10.00	5.00	10.00	5.00	5.00	11.00	4.00	6.00	5.00	5.00	6.00
EC13	4.00	12.00	5.00	5.00	10.00	10.00	10.00	10.00	5.00	5.00	10.00	3.00	6.00	6.00	5.00	5.00
EC14	3.00	12.00	5.00	5.00	5.00	11.00	5.00	10.00	5.00	5.00	12.00	3.00	6.00	5.00	6.00	5.00
EC15																
EC16	4.00	10.00	5.00	4.00	10.00	11.00	10.00	4.00	5.00	5.00	10.00	3.00	6.00	5.00	5.00	6.00
EC17	5.00	11.00	5.00	3.00	10.00	11.00	10.00	10.00	5.00	5.00	12.00	3.00	6.00	6.00	5.00	5.00
EC18	5.00	12.00	5.00	3.00	10.00	12.00	10.00	10.00	5.00	5.00	10.00	5.00	5.00	6.00	5.00	5.00
EC19	5.00	5.00	5.00	3.00	5.00	12.00	5.00	10.00	5.00	5.00	12.00	4.00	6.00	5.00	6.00	5.00
EC20	5.00	11.00	5.00	4.00	5.00	11.00	10.00	4.00	5.00	5.00	13.00	4.00	6.00	5.00	5.00	5.00
EC21	5.00	11.00	5.00	4.00	10.00	13.00	10.00	10.00	5.00	5.00	13.00	5.00	6.00	7.00	5.00	6.00
EC22	5.00	12.00	5.00	3.00	10.00	12.00	10.00	10.00	5.00	5.00	10.00	5.00	6.00	7.00	5.00	6.00
EC23	4.00	12.00	5.00	4.00	10.00	11.00	10.00	10.00	5.00	5.00	13.00	5.00	5.00	6.00	5.00	6.00
EC24	5.00	11.00	5.00	3.00	5.00	11.00	10.00	4.00	5.00	5.00	12.00	3.00	6.00	7.00	5.00	6.00
EC25	5.00	12.00	5.00	7.00	10.00	10.00	10.00	10.00	5.00	5.00	13.00	6.00	6.00	6.00	5.00	5.00
EC26	5.00	12.00	5.00	5.00	10.00	12.00	5.00	4.00	4.00	5.00	5.00	5.00	6.00	7.00	5.00	5.00
EC27	5.00	12.00	5.00	4.00	5.00	13.00	5.00	10.00	4.00	5.00	10.00	4.00	6.00	5.00	5.00	5.00
EC28	5.00	12.00	5.00	4.00	5.00	12.00	5.00	10.00	4.00	5.00	10.00	6.00	6.00	6.00	5.00	6.00
EC29	4.00	11.00	5.00	3.00	5.00	10.00	5.00	10.00	4.00	5.00	12.00	5.00	6.00	6.00	4.00	5.00
EC30	5.00	11.00	5.00	4.00	5.00	12.00	5.00	10.00	4.00	5.00	12.00	5.00	6.00	7.00	3.00	6.00
	S403	S404	S408	S409	S410	S411	S412	S414	S108	S210	S244	S256	1169	S373	S431	S436

Sat I.D. UNIV. OF COLORADO SET UNIV. OF HAWAII SET
 LEGEND 1-4=INHIBITED GROWTH, 5=NO REACTION, 6-9=STIMULATED GROWTH/NON-TRP REQUIRING, 10-13=SATELLITE GROWTH/

E. COLI CROSSFEEDING S. AUREUS STRAINS

	S439	S440	S443	5916	SA93	SA94	SA103	SA104	4022	4023	4041	4043	4063	4100	4106	4132
EC1	3.00	5.00	3.00	11.00	5.00	6.00	3.00	5.00	2.00	2.00	6.00	8.00	7.00	5.00	10.00	2.00
EC2	4.00	5.00	5.00	12.00	5.00	6.00	4.00	5.00	5.00	3.00	5.00	8.00	8.00	5.00	11.00	3.00
EC3	3.00	5.00	4.00	13.00	2.00	5.00	5.00	11.00	3.00	2.00	5.00	8.00	7.00	5.00	11.00	2.00
EC4	5.00	5.00	5.00	12.00	5.00	5.00	3.00	10.00	3.00	2.00	5.00	5.00	7.00	5.00	11.00	7.00
EC5	5.00	5.00	5.00	11.00	5.00	5.00	4.00	5.00	2.00	2.00	6.00	5.00	7.00	5.00	11.00	7.00
EC6	5.00	5.00	2.00	10.00	1.00	5.00	4.00	4.00	1.00	1.00	6.00	5.00	6.00	5.00	10.00	5.00
EC7	5.00	5.00	3.00	12.00	4.00	5.00	4.00	1.00	2.00	3.00	5.00	5.00	7.00	5.00	12.00	3.00
EC8	3.00	5.00	5.00	12.00	4.00	6.00	5.00	4.00	2.00	3.00	5.00	5.00	7.00	5.00	10.00	3.00
EC9	2.00	5.00	7.00	10.00	5.00	6.00	4.00	5.00	7.00	7.00	5.00	7.00	7.00	5.00	10.00	8.00
EC10	4.00	5.00	4.00	12.00	5.00	6.00	4.00	5.00	3.00	4.00	5.00	7.00	7.00	5.00	10.00	5.00
EC11	5.00	5.00	5.00	10.00	2.00	5.00	4.00	10.00	6.00	4.00	5.00	7.00	6.00	5.00	10.00	6.00
EC12	5.00	5.00	4.00	12.00	5.00	5.00	5.00	10.00	4.00	3.00	6.00	7.00	6.00	7.00	10.00	6.00
EC13	5.00	5.00	3.00	11.00	2.00	5.00	4.00	10.00	3.00	2.00	5.00	5.00	8.00	6.00	10.00	4.00
EC14	6.00	5.00	5.00	11.00	2.00	5.00	4.00	5.00	5.00	4.00	5.00	5.00	8.00	5.00	11.00	6.00
EC15																
EC16	6.00	5.00	5.00	11.00	5.00	5.00	5.00	11.00	2.00	2.00	6.00	5.00	7.00	5.00	10.00	6.00
EC17	3.00	6.00	7.00	12.00	5.00	6.00	4.00	5.00	6.00	3.00	6.00	6.00	8.00	6.00	11.00	8.00
EC18	6.00	5.00	7.00	10.00	5.00	5.00	4.00	5.00	5.00	3.00	6.00	5.00	8.00	6.00	10.00	6.00
EC19	3.00	6.00	4.00	13.00	4.00	6.00	2.00	10.00	5.00	6.00	6.00	5.00	9.00	5.00	11.00	8.00
EC20	5.00	5.00	2.00	12.00	5.00	6.00	4.00	10.00	4.00	3.00	6.00	6.00	8.00	5.00	11.00	8.00
EC21	3.00	4.00	2.00	12.00	5.00	5.00	4.00	5.00	4.00	3.00	6.00	5.00	8.00	5.00	10.00	8.00
EC22	4.00	6.00	3.00	12.00	6.00	5.00	4.00	5.00	4.00	3.00	6.00	6.00	8.00	5.00	12.00	9.00
EC23	4.00	5.00	2.00	13.00	4.00	3.00	4.00	5.00	5.00	4.00	6.00	5.00	7.00	5.00	10.00	8.00
EC24	5.00	6.00	5.00	11.00	4.00	3.00	4.00	11.00	4.00	2.00	6.00	5.00	7.00	5.00	11.00	6.00
EC25	5.00	5.00	8.00	12.00	5.00	5.00	2.00	11.00	8.00	7.00	5.00	5.00	7.00	5.00	10.00	7.00
EC26	5.00	5.00	7.00	10.00	4.00	5.00	2.00	11.00	6.00	5.00	5.00	6.00	7.00	5.00	12.00	5.00
EC27	5.00	5.00	6.00	11.00	4.00	5.00	4.00	11.00	5.00	3.00	5.00	5.00	7.00	5.00	12.00	7.00
EC28	5.00	5.00	4.00	11.00	5.00	5.00	4.00	11.00	5.00	3.00	5.00	5.00	7.00	5.00	12.00	6.00
EC29	6.00	5.00	6.00	12.00	8.00	5.00	5.00	12.00	4.00	3.00	8.00	5.00	7.00	5.00	12.00	6.00
EC30	2.00	5.00	3.00	13.00	6.00	5.00	5.00	12.00	5.00	3.00	6.00	5.00	7.00	5.00	12.00	5.00
	S439	S440	S443	5916	SA93	SA94	SA103	SA104	4022	4023	4041	4043	4063	4100	4106	4132

Sat I.D. UNIV. OF HAWAII SET

LEGEND 1-4=INHIBITED GROWTH, 5=NO REACTION, 6-9=STIMULATED GROWTH/NON-TRP REQUIRING, 10-13=SATELLITE GROWTH/

E. COLI CROSSFEEDING S. AUREUS STRAINS

	4135	4155	4157	4158	4508	4509	4512	4513	4513	4558	4578	4580	4635	4660	4662	4684
EC1	9.00	6.00	7.00	1.00	8.00	8.00	5.00	6.00	5.00	3.00	5.00	5.00	5.00	11.00	8.00	8.00
EC2	6.00	6.00	6.00	3.00	8.00	8.00	3.00	4.00	5.00	3.00	5.00	5.00	4.00	11.00	9.00	8.00
EC3	8.00	5.00	5.00	1.00	8.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	4.00	12.00	8.00	9.00
EC4	7.00	5.00	8.00	2.00	7.00	7.00	5.00	5.00	5.00	4.00	5.00	5.00	5.00	10.00	7.00	8.00
EC5	5.00	6.00	8.00	5.00	7.00	5.00	5.00	3.00	5.00	3.00	5.00	5.00	5.00	10.00	6.00	9.00
EC6	5.00	5.00	8.00	1.00	7.00	5.00	5.00	5.00	5.00	3.00	5.00	5.00	5.00	10.00	3.00	7.00
EC7	5.00	5.00	8.00	1.00	7.00	5.00	7.00	5.00	5.00	2.00	5.00	5.00	5.00	12.00	6.00	9.00
EC8	7.00	5.00	8.00	1.00	8.00	5.00	5.00	5.00	5.00	1.00	4.00	5.00	5.00	12.00	7.00	9.00
EC9	8.00	6.00	8.00	6.00	8.00	7.00	5.00	5.00	5.00	6.00	5.00	5.00	5.00	10.00	9.00	8.00
EC10	6.00	7.00	8.00	4.00	5.00	7.00	5.00	5.00	4.00	7.00	6.00	5.00	5.00	11.00	9.00	8.00
EC11	8.00	5.00	7.00	3.00	8.00	7.00	6.00	4.00	5.00	5.00	6.00	5.00	5.00	10.00	9.00	8.00
EC12	6.00	7.00	5.00	4.00	8.00	7.00	5.00	7.00	6.00	5.00	5.00	5.00	5.00	11.00	9.00	8.00
EC13	9.00	6.00	7.00	2.00	5.00	6.00	6.00	5.00	6.00	5.00	5.00	5.00	5.00	10.00	9.00	6.00
EC14	5.00	5.00	7.00	2.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	11.00	9.00	7.00
EC15																
EC16	9.00	7.00	3.00	2.00	6.00	7.00	5.00	7.00	5.00	6.00	6.00	6.00	6.00	11.00	9.00	8.00
EC17	9.00	5.00	7.00	2.00	6.00	6.00	6.00	5.00	5.00	5.00	5.00	5.00	5.00	12.00	5.00	8.00
EC18	9.00	6.00	7.00	2.00	5.00	6.00	6.00	5.00	5.00	5.00	5.00	6.00	5.00	10.00	9.00	8.00
EC19	5.00	5.00	7.00	1.00	5.00	4.00	3.00	5.00	5.00	3.00	5.00	5.00	5.00	12.00	9.00	9.00
EC20	9.00	6.00	7.00	1.00	6.00	7.00	2.00	5.00	5.00	5.00	5.00	5.00	5.00	11.00	7.00	9.00
EC21	9.00	6.00	7.00	1.00	5.00	7.00	7.00	6.00	4.00	5.00	5.00	5.00	5.00	11.00	9.00	6.00
EC22	9.00	5.00	7.00	1.00	6.00	7.00	6.00	4.00	5.00	4.00	5.00	5.00	5.00	11.00	9.00	9.00
EC23	5.00	5.00	7.00	5.00	6.00	7.00	5.00	6.00	5.00	4.00	5.00	5.00	5.00	12.00	8.00	8.00
EC24	7.00	5.00	7.00	2.00	6.00	7.00	6.00	5.00	6.00	4.00	7.00	5.00	5.00	12.00	9.00	8.00
EC25	6.00	5.00	5.00	7.00	7.00	7.00	5.00	5.00	6.00	5.00	6.00	5.00	5.00	12.00	9.00	9.00
EC26	7.00	6.00	7.00	5.00	6.00	7.00	5.00	5.00	5.00	5.00	6.00	3.00	5.00	10.00	9.00	8.00
EC27	5.00	6.00	7.00	4.00	7.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	11.00	9.00	9.00
EC28	5.00	6.00	7.00	3.00	7.00	7.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	11.00	8.00	9.00
EC29	9.00	6.00	5.00	2.00	5.00	7.00	3.00	5.00	5.00	5.00	5.00	5.00	5.00	12.00	9.00	9.00
EC30	8.00	5.00	2.00	2.00	5.00	7.00	3.00	5.00	4.00	5.00	4.00	5.00	5.00	12.00	6.00	9.00
	4135	4155	4157	4158	4508	4509	4512	4513	4513	4558	4578	4580	4635	4660	4662	4684

Set I.D. LEGEND

UNIV. OF HAWAII SET
 1-4=INHIBITED GROWTH, 5=NO REACTION, 6-9=STIMULATED GROWTH/NON-TRP REQUIRING, 10-13=SATELLITE G

E. COLI CROSSFEEDING S. AUREUS

	4711	4712	SAC	KP	MCT	DMM	KL	JK	CO	12598	12228
EC1	2.00	1.00	5.00	5.00	8.00	5.00	6.00	5.00	5.00	6.00	10.00
EC2	4.00	3.00	5.00	5.00	8.00	5.00	6.00	5.00	5.00	6.00	11.00
EC3	3.00	2.00	5.00	5.00	8.00	5.00	6.00	5.00	5.00	5.00	5.00
EC4	3.00	3.00	5.00	5.00	5.00	7.00	6.00	6.00	5.00	4.00	5.00
EC5	3.00	2.00	5.00	5.00	6.00	7.00	6.00	5.00	5.00	4.00	12.00
EC6	1.00	5.00	5.00	6.00	7.00	5.00	6.00	6.00	5.00	5.00	5.00
EC7	4.00	3.00	5.00	5.00	7.00	5.00	6.00	6.00	5.00	3.00	5.00
EC8	2.00	3.00	8.00	5.00	7.00	5.00	7.00	6.00	5.00	4.00	12.00
EC9	6.00	7.00	5.00	5.00	9.00	5.00	7.00	7.00	5.00	5.00	5.00
EC10	4.00	4.00	5.00	5.00	6.00	5.00	7.00	7.00	5.00	5.00	10.00
EC11	4.00	6.00	5.00	5.00	5.00	5.00	7.00	5.00	5.00	5.00	5.00
EC12	4.00	4.00	5.00	5.00	5.00	5.00	7.00	5.00	6.00	3.00	5.00
EC13	5.00	3.00	7.00	5.00	7.00	5.00	6.00	5.00	5.00	5.00	10.00
EC14	3.00	3.00	7.00	5.00	7.00	6.00	6.00	5.00	5.00	3.00	5.00
EC15											
EC16	3.00	3.00	8.00	6.00	6.00	8.00	6.00	6.00	6.00	5.00	5.00
EC17	3.00	7.00	5.00	5.00	5.00	8.00	6.00	6.00	5.00	2.00	12.00
EC18	3.00	4.00	5.00	5.00	5.00	5.00	6.00	6.00	5.00	3.00	11.00
EC19	3.00	6.00	5.00	5.00	7.00	5.00	7.00	6.00	5.00	5.00	4.00
EC20	2.00	6.00	5.00	5.00	6.00	5.00	7.00	6.00	5.00	5.00	4.00
EC21	3.00	4.00	5.00	5.00	5.00	7.00	6.00	6.00	5.00	5.00	12.00
EC22	3.00	6.00	5.00	8.00	5.00	6.00	6.00	7.00	5.00	5.00	3.00
EC23	5.00	5.00	5.00	7.00	5.00	5.00	6.00	6.00	5.00	4.00	10.00
EC24	4.00	4.00	8.00	7.00	5.00	5.00	6.00	6.00	5.00	4.00	5.00
EC25	5.00	7.00	8.00	7.00	5.00	5.00	6.00	6.00	5.00	4.00	5.00
EC26	5.00	7.00	5.00	7.00	5.00	5.00	6.00	6.00	5.00	4.00	5.00
EC27	5.00	4.00	7.00	5.00	5.00	5.00	6.00	6.00	5.00	4.00	5.00
EC28	4.00	3.00	5.00	7.00	6.00	5.00	6.00	6.00	5.00	3.00	5.00
EC29	3.00	3.00	6.00	6.00	5.00	5.00	6.00	6.00	5.00	5.00	5.00
EC30	3.00	5.00	6.00	6.00	5.00	5.00	6.00	5.00	5.00	3.00	5.00
EC31											
	4711	4712	SAC	KP	MCT	DMM	KL	JK	CO	12598	12228

Set I.D. UNIV. OF HAWAII SET

LEGEND 1-4=INHIBITED GROWTH, 5=NO REACTION, 6-9=STIMULATED GROWTH/NON-TRP REQUIRING, 10-13=SATELLITE GROWTH/

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