THE FREQUENCY AND INDUCIBILITY OF *STAPHYLOCOCCUS EPIDERMIDIS* BACTERIOPHAGES AND THEIR POTENTIAL FOR USE IN A TYPING SERIES

A Thesis

Presented to

the Faculty of the Department of Biology

East Carolina University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts in Biology

by

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July 1976

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ACKNOWLEDGEMENT

Words cannot express the extent of my sincere gratitude for the professional advice, encouragement, guidance and untiring patience given me by Dr. Wendall E. Allen, my thesis advisor, whose efforts made this thesis possible.

Special thanks are given to Mrs. Wende Allen for reading this thesis during the writing.

I would also like to sincerely thank Dr. W. James Smith, Dr. Donald B. Jeffreys and Dr. Robert S. Fulghum for serving on my reading committee.

My wife, Gail, also deserves special thanks for her tireless and letter-perfect typing of this thesis and for enduring me from its beginnings to its completion.

Dedicated

to

My Wife, Gail

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INTRODUCTION

Until recently *Staphylococcus epidermidis* has been considered by clinical laboratories as nonpathogenic and regarded as a contaminant in bacterial isolates from infections. *S. epidermidis* has been increasingly associated with certain diseases such as endocarditis (Holt, 1969), urinary tract infections (Mabeck, 1969), cardiac surgery complications (Blouse et al., 1975), and osteomyelitis (Overturf and Balfour, 1975). Baird-Parker Biotype 1 cultures of *S. epidermidis* have been most often found by Holt (1969) and other workers implicated in infections.

Due to the rising significance of *S. epidermidis* in disease, a phage-typing system similar to the one already used in epidemiological studies of *Staphylococcus aureus* seems desirable. Lysogeny in *S. epidermidis* has been found to be as frequent as in *S. aureus* (Pulverer et al., 1974). Mitomycin C and UV-irradiation have been the most used methods of induction of staphylococci. Verhoef et al. (1972), Dean et al. (1973) and Pulverer et al. (1975) used these methods to obtain phages useful for typing *S. epidermidis* cultures predominately of European origin.

To facilitate development of a phage-typing system for *S. epidermidis*, we have identified a group of *S. epidermidis* cultures particularly sensitive to phages useful in screening induction products. This paper describes the frequency of lysogeny in 241 strains of *S. epidermidis* from the U. S. A., compares the effectiveness of Mitomycin C and UV-irradiation as inducing agents, and presents a group

of S. epidermidis phages that may be of value in phage typing S. epidermidis cultures.

LITERATURE REVIEW

Classification of the Staphylococci

Separation of Micrococci and Staphylococci

Bergey's Manual of Determinative Bacteriology (8th edition, 1974) recognizes 3 genera, Staphylococcus, Micrococcus, and Planococcus, in the family Micrococcaceae. All are gram-positive and spherical cells 0.5-3.5 μm in diameter. The genera Staphylococcus and Micrococcus have posed problems to bacterial taxonomists because of their very close similarities. In fact, early editions of Bergey's Manual included the genus Staphylococcus from 1923 to 1939; however, from 1939 to 1957 the staphylococci were assigned to the genus Micrococcus. It was proposed by Evans, Bradford, and Niven (1955) to separate micrococci from staphylococci by the latter's ability to ferment glucose anaerobically with acid production. This was accepted in the 7th edition of Bergey's Manual (1957) and continued in the 8th edition (1974). Baird-Parker (1963) studied 1250 isolates and confirmed the proposal of Evans et al. (1955). The ICBS (International Committee on Systematic Bacteriology) Subcommittee on the Taxonomy of Staphylococci and Micrococci recommended a standard method for glucose fermentation determination to distinguish staphylococci from micrococci (Cohen, 1972). Evans and Kloos (1972) proposed an improved method of glucose fermentation using semisolid thioglycollate medium.

Schleifer and Kloos (1975) described a simplified system to separate micrococci from staphylococci by identifying the latter's sensitivity to lysostaphin and ability to aerobically produce acid

from glycerol in the presence of erythromycin. This appears to be the simplest, easiest, and most accurate basis for separation of the two genera.

Species Description of Staphylococcus epidermidis

Bergey's Manual (8th edition, 1974) recognizes three species in the genus Staphylococcus: S. aureus, S. epidermidis, and S. saprophyticus. S. saprophyticus was classified as Micrococcus saprophyticus until recently. However, their very slow anaerobic growth in various media, DNA base composition and cell wall composition relegates them to the staphylococci. The main criterion for separating S. aureus from other species of Staphylococcus is their ability to produce coagulase (Cohen, 1972). The need of S. epidermidis for biotin (Gretler et al., 1955) further distinguishes S. aureus and S. epidermidis.

Smith and Farkas-Himsley (1969) studied 46 characters of 21 coagulase-negative pathogenic strains. They found that these strains formed a heterogenous intermediate group which shared many more characters with S. aureus than with S. epidermidis cultures. Their conclusions were that under the classification system then used, coagulase-positive strains could be called S. aureus but coagulase-negative strains could not accurately be called S. epidermidis. They suggested that strains of Staphylococcus formed a "continuous spectrum" between S. aureus and S. epidermidis.

According to Baird-Parker (1965) coagulase-negative S. epidermidis could be separated into four biotypes primarily on their ability to produce acids from various carbohydrates. Sovadina (1975), in classifying 191 coagulase-negative skin isolates of S. epidermidis according

to Baird-Parker's scheme, found 144 were biotype 1, 1 was biotype 2, 4 were biotype 3, and 42 could not be classified.

According to Kloos and Schleifer (1975), the Baird-Parker scheme of identifying staphylococci used so few characters that it was limited in the number of subspecies or species it could separate. In an extensive study they proposed nine different species of coagulase-negative staphylococci and devised a simplified scheme for identifying these Staphylococcus species in the clinical laboratory. According to Kloos and Schleifer (1975), S. epidermidis did not produce coagulase nor acid aerobically from mannitol, D(+)-xylose, L(+)-arabinose and D(+)-trehalose, but did produce acid aerobically from maltose and sucrose. Some species of coagulase-negative staphylococci proposed by them showed preferences for particular regions of the skin. For example, S. capitis was found mainly on the head whereas S. epidermidis was found on all areas tested. Additionally, they pointed out that species variation differed with certain geographical locations. The S. epidermidis cultures described in their studies corresponded closely to biotype 1 strains described by Baird-Parker (1965).

Pathogenicity of Coagulase-Negative Staphylococci

Until recently clinicians have considered coagulase-positive S. aureus as the pathogenic species of the staphylococci and coagulase-negative staphylococci as nonvirulent (Cohen, 1972). Coagulase-negative staphylococci have been increasingly implicated in certain clinical infections.

Human Infections Caused by Coagulase-Negative Staphylococci

Quinn, Cox, and Drake (1966) definitely implicated coagulase-negative staphylococci in bacterial endocarditis. Cluff et al. (1968) found that S. epidermidis caused 10% of the staphylococcal bacteremia cases studied. Holt (1969) isolated strains from endocarditis cases and infections due to contaminated Spitz-holter valves which had been inserted in hydrocephalic patients to shunt spinal fluid to the right atrium. He reported cultures belonging to biotype 1 (Baird-Parker) were often isolated. At the Children's Hospital Medical Center (Boston), 27% of 289 hydrocephalic patients who had cerebrospinal fluid shunts inserted became infected (Schoenbaum, et al, 1975). S. epidermidis was responsible for half of the infections compared to S. aureus being responsible for one-fourth. Coagulase-negative staphylococci also have been implicated with urinary tract infections (Mabeck, 1969), with food poisoning (Breckenridge and Bergdoll, 1971) and with osteomyelitis in infants (Overturf and Balfour, 1975).

Factors Relating to the Virulence of Coagulase-Negative Staphylococci

Gemmell and Roberts (1974) carried out various biological tests (e.g., DNase, coagulase, lipase, gelatinase, mannitol, and mouse pathogenicity) on 129 coagulase-negative isolates from clinical infections of the urinary tract, heart, wounds, abscesses, and skin. They found that coagulase-negative staphylococci killed mice at higher doses than S. aureus and localized in the liver rather than the renal cortex as did S. aureus. The localization in the liver was attributed to the production of Succinic Oxidase Factor (S.O.F.) which has a strong preference for liver mitochondria. Their results indicated these

pathogenic strains were intermediate between S. epidermidis and S. aureus strains and could not be identified with any of the four biotypes of Baird-Parker. They concluded that S.O.F., phosphatase, hemolysins, and DNase were the best indicators of virulence in coagulase-negative staphylococci.

Sovadina (1975) tested 191 S. epidermidis strains and observed 81-92% from sources other than pus produced urease. Urease production was significantly higher in strains belonging to biotype 1 (Quoraishi, 1972; Males, et al., 1975). Males et al. (1975) using Baird-Parker's scheme classified 228 S. epidermidis strains from patients. Urine isolates accounted for 39.5% of these strains. Biotype 1 strains represented 63.3% of the 228 strains with biotypes 4, 3, and 2 occurring in decreasing order of incidence. The number of virulence factors based on enzymatic activity (for example, urease, gelatinase, DNase) was greatest in biotype 1 strains followed in decreasing order by biotypes 3, 4, and 2 strains.

Gelosa and Bianchi (1974) tested the sensitivity of 155 pharyngeal mucous isolates of S. aureus, S. epidermidis, and Micrococcus to $HgCl_2$ and desoxycholate. They found nonpathogens were more affected by desoxycholate and virulent strains were more sensitive to mercury.

Kleck and Donahue (1968) confirmed earlier findings that delta hemolysin was the typical hemolysin of cultures of S. epidermidis. They found that more cultures from normal nasal flora produced detectable delta hemolysin than did cultures from clinical sources indicating delta hemolysin may not be the important virulence factor in coagulase-negative staphylococci. Recently Marsik and Parisi (1973)

reported alpha, beta, as well as delta hemolysins produced by S. epidermidis. Since S. aureus was shown to produce any combination of alpha, beta, and delta hemolysins (Kleck and Donahue, 1968), the relationship between hemolysin production and virulence remained unclear.

Antibiotic Resistance in S. epidermidis

Watanakunakorn and Hamburger (1970) found antibiotic therapy to be ineffective for infection following a Starr-Edwards valve implantation. McAllister et al. (1974) reported an infection at the site of insertion of a Starr-Edwards ball valve by S. epidermidis that progressed rapidly in spite of antibiotic therapy.

Marsik and Parisi (1973) showed that antibiotic resistance was more frequent in S. epidermidis cultures than in cultures of S. aureus. Males, et al. (1975) found 54% of the S. epidermidis strains studied were resistant to tetracycline, 81.6% to penicillin and 69.3% to ampicillin. Sovadina (1975) found 64% of 191 coagulase-negative staphylococci studied were resistant to penicillin and 72% to streptomycin. Schlaefer (1972) reported a penicillinase plasmid in S. epidermidis (P-BV strain) in which penicillinase activity was linked with mannitol fermentation.

There have been reports of attempts to transfer genes for antibiotic resistance from S. aureus to S. epidermidis and vice versa. Yu and Baldwin (1971) were successful in transducing novobiocin resistance but not penicillinase production between S. aureus and S. epidermidis. Minshew and Rosenblum (1972) were unable to transduce

tetracycline resistance between S. aureus and S. epidermidis. Dean et al. (1973) were unsuccessful in transducing antibiotic resistance from coagulase-negative staphylococci to S. aureus.

Lysogeny

When a bacterial virus (phage) infects a bacterium, two possible consequences may result: 1) the lytic cycle in which the phage DNA directs the metabolic machinery of the cell to produce progeny viruses with resultant lysis of the bacterium, or 2) lysogeny. Lysogenic bacteria are latently infected with bacteriophages and have two definitive characteristics: 1) they have the potential to produce and release phages carried as a stable heritable trait; 2) they cannot be lysed by the same or closely related phages (Hayes, 1968).

A sensitive indicator strain on which the lysogenizing phage will form plaques is usually necessary for the detection of lysogeny. For example, experimental laboratories had used the K-12 strain of E. coli for many years before the accidental discovery of its lysogenization by phage lambda. This was discovered when plaques were formed when strain K-12 was mixed and plated with a strain of K-12 that had lost the phage (cured).

Lysogeny has been found so widespread among different species of bacteria that it should be considered normal, rather than exceptional (Hayes, 1968). The studies of Rountree (1949), Gorrill and Gray (1956), and Blair and Carr (1961) suggested that temperate bacteriophages are carried by most strains of the coagulase-positive S. aureus. Early studies on lysogeny of coagulase-negative staphylococci showed low frequencies of lysogeny: Karska (1960) detected no phages in 65 strains

tested, Comtois (1962) detected one out of 20 strains examined. Recently, however, Verhoef et al. (1971) using Mitomycin C (Mit C) detected phages released by 45 (26%) of 173 strains. Holmberg (1973) detected 47 (37.3%) of 126 S. epidermidis strains isolated from bovine milk released phages. Pulverer et al. (1974) detected 100% lysogeny using Mitomycin C and/or UV in 127 strains of S. albus (S. epidermidis). The apparent increase in the incidence of lysogeny in S. epidermidis was likely due to better methods of induction and more sensitive indicator strains. It appears that the frequency of lysogeny in coagulase-negative staphylococci is similar to that in S. aureus. In addition, it has been found that many staphylococci are multi-lysogenic (Rountree, 1949, and Pulverer et al., 1974).

Prophage-Bacterium Relationship

Burnet and McKie (1929) were the first to show that lysogenic bacteria did not contain infectious phage particles as no phage were released when these bacteria were artificially disrupted. The hypothesis that lysogeny was a hereditary potentiality of the bacteria to generate phages was confirmed by Lwoff and Gutmann (1950). Using a micromanipulator they separated individual lysogenic cells in micro-drops over many generations. They found: 1) many generations could pass without a single phage particle released; 2) there was a correlation of the appearance of phages in the micro-drop and the rapid disappearance of a bacterium; and 3) in the lysis of each bacterium the mean number of phages released was high.

Since these early studies, the prophage-bacterium relationship has been detailed. Stanier, Adelberg, and Ingraham (1976) summarized

this relationship as follows. The only gene active in lysogenizing phage codes for a repressor protein which prevents expression of the phage genome. Just after infection of a cell by a phage, there is a "race" between viral maturation and repressor production. If repressor action cannot interfere before mature virions and lysozyme are produced the cell is lysed. If the repressor accumulates and shuts off viral replication, the lysogenic state is attained.

The repressor system has been most extensively studied in E. coli K-12 lysogenized with lambda prophage. The C_I gene of a lambda prophage has been shown to produce the repressor protein which binds to the receptor site on the phage genome. When the repressor is bound to the genome vegetative replication and maturation are prevented. It does not, however, interfere with the replication of the prophage in synchrony with cell division. The repressor makes the lysogenic cell immune to temperate or lytic phages sensitive to that repressor. A cell can, however, be lysogenized by several different prophages because of differing specificities of their particular repressors. If an infecting phage is resistant to the repressor of a phage lysogenizing a cell, the cell will be lysed. Two different types of virulent mutants have been isolated: those in which the receptor will no longer bind the repressor and those in which the repressor is no longer produced by the C_I gene.

Two major types of lysogeny have been discovered. The first and most studied is lysogeny of the lambda type. Recombination experiments have shown that lambda prophage occupies a discrete site on the E. coli K-12 chromosome between the gal and bio genes. According to the

"insertion hypothesis," a continuous structure is formed from a single recombination event in which the phage genome (prophage) becomes integrated into the bacterial chromosome (Campbell, 1962). The lambda prophage replicates in synchrony with the bacterial chromosome. Jacob and Wollman (1961) suggested that the prophage should be considered an episome alternating between a state of autonomy and a stable state of attachment to the chromosome. E. coli phage, P_1, is the most studied of the second type. Recombination experiments indicate there is not a discrete chromosomal location for prophages of this type. It is thought that prophages of the P_1 type are attached to cell membrane attachment sites and replicate autonomously as do plasmids (extrachromosomal genetic elements found in bacteria). Plasmids able to integrate with the host chromosome are called episomes.

Induction

Lwoff and Gutmann (1950) suggested that external factors may induce phage production with subsequent release. Using lysogenic Bacillus megaterium, Lwoff, Siminovitch, and Kjelgaard (1950) showed that UV-irradiation exposure could result in induction of lysis of an entire culture. They found induction of lysis occurred maximally in rich growth medium and no induction occurred in lysogenic cells grown in a minimal medium. Gorrill and Gray (1956) suggested inducibility was mainly a property of the prophage; whereas Adams (1959) suggested that the physiological condition of the host affected the response to inducing agents. Many effective inducing agents and methods have been found: X-Rays, gamma rays, nitrogen mustards, hydrogen peroxides,

organic peroxides, 6-azauracil, fluorodeoxyuridine, Mitomycin C and thymine deprivation (Hayes, 1968). UV-irradiation was used by Gorrill and Gray (1956) for induction of lysogenic S. aureus cultures. UV and Mitomycin C have been the two most common methods of induction of coagulase-negative staphylococci (Verhoef et al., 1971; Pulverer et al., 1974). Many prophages have been induced by UV-irradiation. Others were poorly induced by UV exposure but were inducible by other methods as exposure to alkylating agents such as Mitomycin C (Davis et al., 1973).

There was no general agreement in the literature as to the action of Mitomycin C and UV-irradiation as inducing agents. Hong et al. (1971) reported DNA synthesis was temporarily inhibited followed by the accumulation of an "active inducer" that prevented binding of repressor to the phage operons in cells exposed to sub-lethal doses of UV-irradiation. The inducer was an adenine derivative made in normal metabolism. The inducer inactivated the repressor; the phage genome became functional and the lytic cycle occurred. Mitomycin C was metabolically converted in cells into an agent producing cross-linkage of complementary DNA strands causing cessation of DNA synthesis (Davis et al., 1973). That portion of the DNA containing cross-links was subsequently deleted from the DNA. The relation of the effect of Mitomycin C to phage induction was unclear.

Stanier et al. (1976) described an alternate hypothesis that accounted for Mitomycin C and UV-irradiation activity as agents producing phage induction. Treatments with these agents proposedly produced lesions in DNA that directly bound the repressor. This reduced the

repressor concentration to the point of ineffectivity and resulted in the viral lytic cycle.

Phage Typing of the Staphylococci

Phage Typing of S. aureus

Blair and Carr (1952) in the United States, and Williams and Rippon (1952) in England practically simultaneously proposed phage typing systems that established the value of typing in clinical laboratories. The basic techniques for phage typing coagulase-positive staphylococci of Blair and Williams (1961) involved testing the susceptibility of a S. aureus culture to a group of phages with narrow host ranges. The typing phages lysed only coagulase-positive staphylococci. Wentworth (1963) stated that almost all pathogenic strains produced coagulase, but not all strains producing coagulase were pathogenic. According to Wentworth (1963), the epidemiology of infections caused by staphylococci has been aided by phage typing. Certain phage types called "epidemic types," the most well known example being the 80/81 phage types, have been identified with outbreaks of staphylococcal disease.

The Subcommittee on Phage Typing of Staphylococci of the International Committee on Nomenclature of Bacteria was established in 1954 to standardize phage-typing techniques, to establish the basic set of typing phages, and to keep standardized stocks of typing phages and their propagating strains. The recommended typing set for S. aureus contained 22 phages divided into four lytic groups. In addition, some laboratories used other phages in phage typing.

Rountree and Rheuben (1956) found correlations between strains lysed by particular lytic groups and antibiotic resistance (e.g., Lytic Group III and penicillin-resistance) as well as certain infections.

Rountree (1949) and Rippon (1956) serologically grouped staphylococcal phages. Serological groups A, B, F, and L were contained in the recommended typing set (Blair and Williams, 1961). According to Pariza and Iandolo (1974), lytic groupings were an arbitrary means of separating, whereas serological groupings referred more to genome similarities. Cohen (1972) stated that serotyping of staphylococcal strains along with phage-typing studies could more completely determine epidemics and food-poisoning outbreaks.

Phage Typing of Coagulase-Negative Staphylococci

Until recently there had been little research of phage typing of coagulase-negative staphylococci. However, with its role as an emerging pathogen, attempts have been made by a few workers to investigate the feasibility of developing a typing system similar to that of S. aureus.

Verhoef et al. (1972) in the Netherlands tested 18 S. epidermidis phages against 240 coagulase-negative strains from patients and the normal population using the standardized techniques of Blair and Williams (1961) developed for S. aureus. They found 75.5% of the strains were lysed by one or more of the phages. Twenty-four lytic patterns were observed; however, the majority were of seven main patterns. Their findings suggested that strains from patients possibly belonged to different "types" than strains from the normal population. They stated that epidemiological studies would be fairly difficult

due to the variability of the S. epidermidis flora of the nose. They reported some strains were carried transitorily and others were carried for several months in the same person.

Dean et al. (1973) typed 1517 strains of coagulase-negative staphylococci and micrococci from staff and patients from London with 19 phages (8 obtained from Verhoef et al, 10 isolated in their lab, and 1 from Colindale culture collection). They reported 56% (855) of the total were typable and that 72% of biotype 1 strains were typable. They also reported 36% of the strains were lysed by 6 or more phages and that 17% were lysed by 10 or more phages. They stated this sensitivity would be a serious drawback in the development of a universal set of typing phages.

Pulverer et al. (1975) used a set of 16 S. epidermidis phages for typing 183 S. epidermidis strains from clinical sources from Canada, Denmark, United States, the Netherlands, Germany and Czechoslovakia. They found that 71.6% (131) of the strains were lysed by at least one phage. These strains were of 42 phage patterns; 92 of the strains belonged to seven main patterns. There was some correlation between origin of a strain and lysis of the strain by at least one phage. They were unable to confirm the findings of Verhoef et al. (1971) that S. epidermidis phages could be grouped into those that lysed only S. epidermidis strains and those that initially lysed only S. aureus strains but acquired lytic activity for S. epidermidis strains after adaptation to growth on S. epidermidis.

Blouse et al. (1975) used the 18 provisional typing phages of Verhoef et al. (1972) in "typing" 118 epidemiologically related strains

from hospital staff, patients, and fomites from the U. S. A. Seventy or 59.3% of these strains were typable by the phages. They found a correlation of "phage types" and antimicrobial resistance. Strains of four particular phage types were found resistant to penicillin and ampicillin and frequently to methicillin and erythromycin. Certain phage types were frequently associated with endocarditis complicating prosthetic valve replacements. Similar strains were found in the same category of patients by Dean and her co-workers (1973).

Verhoef et al. (1971) confirmed the findings of Rountree (1949) and Rippon (1956) that serological differences existed between phages lysing coagulase-negative staphylococci and those lysing S. aureus. The 18 phages of Verhoef et al. (1972) all belonged to a serological group distinct from the A, B, F, and L serological groups of S. aureus phages.

The findings of Rose and McDonald (1973) suggested the feasibility of a phage-typing system for S. epidermidis of bovine origin. Holmberg (1975) used the 18 phages of Verhoef et al. (1972) to type 218 strains of bovine origin. Only 3% could be typed suggesting human and bovine S. epidermidis strains were essentially different. Alternately, approximately 23% were typable by a set of 11 phages isolated from bovine strains of S. epidermidis.

These results indicated that a phage-typing system for S. epidermidis might be feasible and could be of value in identifying cultures from clinical sources. Pulverer et al. (1975) suggested that phage-typing results on various S. epidermidis cultures with certain phages in different laboratories was needed. They also stated that

phages lytic on heretofore phage-resistant staphylococci needed to be sought.

MATERIALS AND METHODS

Cultures

One hundred and seventy-seven cultures of Staphylococcus epidermidis were obtained from Dr. Wesley Kloos (N. C. State University, Raleigh, North Carolina). These cultures were isolated from 20 individuals sampled monthly for 6 to 13 months in Raleigh, North Carolina, and from a group sampled once living in New Jersey. The samples were from 11 different sites on the human body. Twenty-five cultures were obtained from the nostrils of faculty and staff at East Carolina University. Additionally cultures were isolated from the skins of student nurses in Kinston, North Carolina. All of the above 241 cultures were numerically coded to mask their source during the study. Seven S. epidermidis propagating strains and their 7 S. epidermidis phages were obtained from Dr. Eugene Rosenblum (Southwestern Medical School, Dallas, Texas). These phages and their propagating strains had been obtained from Dr. J. Verhoef (The University of Utrecht, the Netherlands). Two other S. epidermidis phages used had been previously isolated in our lab from the lysogenic strains EC 10 and EC 16.

All cultures were maintained at 4 C on Trypticase Soy Agar, (TSA; Baltimore Biological Laboratories, BBL; Baltimore, Maryland) containing 0.5% (w/v) Yeast Extract (BBL) and 400 ug/ml $CaCl_2$. In this study, all media containing $CaCl_2$(CA) or Yeast Extract (YE) in the concentrations specified above will be indicated by the abbreviation of the added ingredient following the appropriate abbreviation for the medium, e.g.,

the medium just described will be called TSA-YE-CA. Incubation temperature was 37 C unless otherwise specified.

Identification of Cultures

Separation of Staphylococci from Micrococci

Using the methods of Schleifer and Kloos (1975), the gram-positive cocci isolated were identified as staphylococci by their ability to produce acid aerobically from glycerol in the presence of erythromycin (Sigma, St. Louis, Missouri) (0.4 µg/ml), their resistance to lysozyme (Sigma, St. Louis, Missouri) (400 µg/ml) and their sensitivity to lysostaphin (Schwarz/Mann, Division of Becton, Dickinson, and Company, Orangeburg, New York) (50 ug/ml and 200 µg/ml). As controls, cultures known to be members of the genus Micrococcus and Staphylococcus were tested. Micrococci were resistant to lysostaphin, resistant to lysozyme, did not produce acid from glycerol, and were inhibited by erythromycin. Staphylococci were sensitive to lysostaphin, resistant to lysozyme, produced acid from glycerol, and were resistant to erythromycin.

Identification of S. epidermidis

Cultures previously found to be staphylococci were determined to be S. epidermidis using the methods of Kloos and Schleifer (1975). Cultures of S. epidermidis were not able to produce coagulase nor acid aerobically from mannitol, D(+) xylose, L(+) arabinose and D(+) trehalose. They were able to produce acid aerobically from maltose and sucrose.

To determine coagulase production and acid from mannitol, Coagulase Mannitol Broth (BBL) was prepared with the addition of 10% (v/v) sterile rabbit Bacto-Coagulase Plasma (Difco, Detroit, Michigan). Two-ml amounts of the above complete medium were dispensed aseptically into 13x100mm tubes. Tubes were inoculated with several loopfuls of each culture grown overnight on Trypticase Soy Broth (TSB, BBL)-YE-CA, incubated and examined after two and four hours for coagulase activity and after overnight growth for acid production from mannitol. The remaining carbohydrate tests were performed using the methods of Kloos and Schleifer (1975).

Selection of Indicator Strains

Phages were propagated by the methods of Blair and Williams (1961). Phage concentrations used in sensitivity testing were sufficient to produce evidence of plaque formation. Phage preparations were sterilized by filtration (0.45 μ pore size; Nalge, Sybron Corporation; Rochester, New York).

All 248 strains were tested for sensitivity to the 9 S. epidermidis phages. A 0.5-ml volume of a 24-hour TSB culture of the strain to be tested was evenly spread over the surface of a petri plate containing TSA-YE-CA. One one-hundredth-milliliter amounts of each phage suspension were then dropped onto labeled positions on the surface of the plates. After the virus suspensions had been absorbed into the surface of the medium, the plates were inverted, incubated for twenty-four hours, and examined for evidence of plaque formation within the areas where the drops containing phages were placed.

Twenty-one strains were chosen from the strains sensitive to lysis by all nine phages. These particularly sensitive strains were used as indicator strains to detect phages released following induction of lysogenic strains.

Induction of Bacteriophages

Two hundred and forty-one cultures previously described were exposed to Mitomycin C and/or UV-irradiation by a modification of the methods of Pulverer et al.(1974) to determine the effectiveness of these inducing agents separately and in combination, in inducing lysogenic strains to release phages. Cultures to be subjected to induction procedures were incubated for twenty-four hours in 4.5 ml of TSB-YE-CA in 16x150mm test tubes. The induction of known lysogenic S. epidermidis strain EC 16 was used as a control in all induction attempts.

Mitomycin C Induction

A 0.5-ml volume of a 5 µg/ml Mitomycin C (Sigma, St. Louis, Missouri) solution was added to each 24-hour broth culture which was then incubated four hours at 37 C in a shaking water bath (Precision Scientific, Model 50) at 80 cycles/minute. The contents of each tube were transferred into a sterile, 40-ml, plastic centrifuge tube and centrifuged at 1100 x g for 20 minutes (Sorvall, Model SS-3; Newtown, Connecticut). The supernatant from each was poured into a sterile 16x100mm test tube and stored at 4 C until tested for presence of phages. The sediment was discarded.

UV Induction

Ten milliliters of fresh TSB-YE-CA was added to each 24-hour culture grown in 4.5 ml TSB-YE-CA. The contents were incubated in a water bath four hours with shaking as above. After incubation, each culture was transferred into a sterile, 40-ml, plastic centrifuge tube and centrifuged at 3000 x g for 15 minutes. The supernatant was discarded and the sediment resuspended in 2.5 ml of 0.066 M sterile phosphate buffer (Sorensen) at pH 7.5. The cell suspension was then poured into a glass petri dish and shaken gently during irradiation. UV-irradiation exposure was for 45 seconds to an 8-watt germicidal lamp (Sylvania G815) at 254 nm that delivered 0.66×10^4 ergs/cm^2-sec as measured with a radiometer (Model 65, Yellow Springs Instrument Company, Yellow Springs, Ohio). The contents of each were transferred into a sterile 16x100mm test tube and 2.5 ml double strength TSB-YE-CA was added. The irradiated culture suspension was incubated with agitation for two hours and sedimented in a clinical centrifuge (Model CL, International Equipment Company). The supernatant was treated as in the Mitomycin C induction procedure. The sediment was discarded.

UV Plus Mitomycin C Induction

This procedure was the same as the UV induction except 0.5 ml Mitomycin C solution (5 µg/ml) was added after the addition of 2.5 ml TSB-YE-CA to the irradiated culture. After a two-hour incubation with agitation in a water bath, the cultures induced with both UV + Mitomycin C were sedimented in a clinical centrifuge. The supernatant

was poured into a sterile 16x100mm test tube and stored at 4 C until tested for the presence of phages. The sediment was discarded.

Detection of Phages

Each petri plate of TSA-YE-CA medium was inoculated by spreading 0.1 ml of an 18-hour TSB-YE-CA culture of one of the 21 indicator strains over the entire surface. Plates were prepared for all 21 indicator strains. The plates were placed over a pattern containing 33 numbered squares. A volume of 0.01 ml of the supernatant of each induction product was placed on a designated square as follows: supernatants of 10 different cultures subjected to Mitomycin C induction were placed on squares 1-10; supernatants from the same 10 cultures subjected to UV induction were placed on squares 11-20; and supernatants from these cultures subjected to UV and Mitomycin C inductions were placed on squares 21-30. Induction products from the known lysogenic culture, treated as above, were placed on squares 31-33. After the induction products had been absorbed into the surface, the plates were incubated in an inverted position for 24 hours and inspected for plaque formation. Any samples producing confluent lysis were diluted and retested. Formation of plaques on any of the indicator strains was considered evidence of phage present in that induction product.

RESULTS

Selection of Indicator Strains

Table 1 shows results of exposure of 248 S. epidermidis strains to lysis by the 9 S. epidermidis phages. Two hundred twenty-two strains or 89.5% typed into 44 lytic patterns. Twenty-six strains or 10.5% were not lysed by any of the 9 phages. The numbers of the 248 strains lysed by a particular phage ranged from 87.1% by phage 130A to 23.4% by phage 16. Twenty-one indicator strains were chosen from the strains lysed by all 9 phages. Table 2 is a listing of the indicator strains and their source.

Induction Results

Thirty-nine or 16.2% of the 241 strains released phages detectable by the indicator strains. Indicator strains I-18, I-19, I-20, and I-21 obtained from J. Verhoef were not tested for lysogeny. Indicator strains I-1 through I-17 were tested for lysogeny by the three methods and no evidence of phage was detectable on any indicator strain. Table 3 shows the abilities of the supernatants from the three methods of induction of each of the 39 lysogenic strains to lyse the 21 indicator strains. All 39 of the cultures yielding phages were induced by Mitomycin C, 33 by UV-irradiation (UV), and 30 by a combination of the two methods. Five cultures (87, 116, 120, 164, and 185) were exclusively induced by Mitomycin C. Separate plaques were noted in 98 (18.5%) of 527 lytic activities observed when Mitomycin C-induction lysates were tested on the indicator strains.

Similarly, separate plaques were observed in 142 (65.7%) of the 216 lytic activities observed with UV-induction lysates and 135 (63.4%) of 213 lytic activities observed with UV plus Mitomycin C lysates (Table 3).

Table 4 shows the comparative effectiveness of the three methods to induce phages and of the capacity of the indicator strains to detect phages released. For example, I-1 detected 36 of the 39 phages induced by Mitomycin C, 28 of 33 induced by UV, and 27 of 30 induced by UV plus Mitomycin C while I-8 detected 16 of the 39 phages induced by Mitomycin C, 2 of 33 induced by UV, and 1 of 30 induced by UV plus Mitomycin C. The capacity of the 21 indicator strains to detect viruses released by the induction methods is given at the bottom of Table 4. This value was determined by totaling the numbers of phages induced by each induction method detectable by the indicator strains (i.e., 527 induced by Mitomycin C, 216 induced by UV and 213 induced by UV plus Mitomycin C).

The total lytic activity detectable by the indicator strains of UV and UV plus Mitomycin C inductions was almost identical; however, differences were noted in the abilities of the phages induced by these two methods to lyse the indicator strains (Table 3). Thirty-nine of the 216 lytic activities produced by UV inductions detected on the indicator strains were not detected by the indicator strains after UV plus Mitomycin C induction. Thirty-five of the 213 lytic activities produced by UV plus Mitomycin C inductions detected on the indicator strains were not detected after UV induction. For example, lysogenic strain 81 was not induced using UV and UV plus Mitomycin C (Table 3). Indicator strain 20 detected lysogeny with UV plus Mitomycin C

induction and not with UV induction and I-14 detected UV induction but not UV plus Mitomycin C induction of phage strain 81. Twenty-nine phage strains were induced with both UV and UV plus Mitomycin C (e.g., phage strains 29, 31, 62, etc.). Phage strains 117, 145, 184, and 186 were induced by UV and not by UV plus Mitomycin C. However, phage strain 137 was induced using UV plus Mitomycin C and not UV.

Differences were also noted in comparing the phages induced by Mitomycin C and UV and phages induced by Mitomycin C and UV plus Mitomycin C. Thirty-three phages were induced with both Mitomycin C and UV. Phages 87, 116, 120, 137, 164, and 185 were induced by Mitomycin C and not by UV. Thirty phages were induced with both Mitomycin C and UV plus Mitomycin C (Table 3). Phages 87, 116, 117, 120, 145, 164, 184, 185, and 186 were induced by Mitomycin C and not UV plus Mitomycin C. Induction products from UV and UV plus Mitomycin C showed a loss or reduction of lysis for the indicator strains compared to Mitomycin C induction products. For example, the Mitomycin C induction product from S. epidermidis strain 117 lysed 16 of the indicator strains whereas the UV induction product lysed only 7 of these 16 and the UV plus Mitomycin C induction produce lysed none.

In table 5 the indicator strains were arranged according to increasing sensitivity to lysis by each phage (down) and the viruses arranged according to decreasing lytic activity for the indicator strains (across). The lytic activity of the bacteriophages for the indicator strains ranged from phage strains 10, 16, and 186 lytic for all 21 indicator strains, to phage strain 87 lytic for two of the indicator strains. The indicator strains varied in their susceptibility to lysis by the 39 phages from I-8, lysed by 16 phages, to I-3, lysed by 38 of 39 viruses.

Table 1. Lytic Patterns for 248 Strains of *Staphylococcus epidermidis* with 9 *S. epidermidis* Phages.

Number of Strains Per Lytic Pattern	Bacteriophage									Number of Phage(s) Lysing in Pattern
	130A	71	471	1548	456	82A	10	48	16	
37	+	+								2
28	+	+	+	+	+	+	+	+	+	9
29	+									1
24	+	+	+	+	+	+	+		+	8
12	+	+	+	+	+	+	+	+		8
8	+	+	+	+	+	+				6
8	+	+	+							3
7	+	+	+	+	+	+		+		7
5	+	+	+	+			+			5
5	+	+	+	+						4
4	+	+	+	+	+	+	+			7
4	+	+	+	+	+					5
4	+	+						+		3
3	+	+	+	+	+		+	+		7
3	+	+	+	+	+		+			6
3	+	+	+				+			4
3	+	+	+					+		4
3		+								1
2	+	+	+	+	+	+			+	7
2	+	+	+	+	+		+		+	7
2	+	+	+	+	+			+		6
2	+			+	+		+			4
2	+			+	+	+				4
2	+	+		+						3
1	+	+	+		+	+	+	+		7
1	+	+	+	+			+	+		6
1	+	+	+	+		+	+			6
1	+		+	+	+	+	+			6
1	+		+		+	+	+		+	6
1	+	+	+		+		+	+		6
1	+	+		+	+	+				5
1	+	+	+		+	+				5
1	+	+	+		+		+			5
1	+	+				+		+	+	5
1		+	+	+	+	+				5
1	+		+	+	+		+			5
1	+	+			+	+				4
1	+	+		+	+					4
1		+	+				+			3
1	+	+				+				3
1	+	+			+					3
1	+				+	+				3
1	+	+								2
1			+	+						2
26	0	0	0	0	0	0	0	0	0	0
Total Number of Strains Tested	216	184	135	123	116	98	95	63	58	
	(87.1)	(74.2)	(54.4)	(49.6)	(46.8)	(39.5)	(38.3)	(25.4)	(23.4)	
	Number (& %) of Strains Lysed by Each Phage									

Table 2. Identification and Source of S. epidermidis Phage-Sensitive Cultures Used as Indicator Strains in Induction Experiments

Indicator Strain	S. epidermidis Culture
I-1	WK 211[a]
I-2	WK 12[a]
I-3	PM 67[a]
I-4	KH 170[a]
I-5	LK 282[a]
I-6	LK 74[a]
I-7	RM 234[a]
I-8	RM 233[a]
I-9	RM 172[a]
I-10	TM 17[a]
I-11	CM 22[a]
I-12	B-7[b]
I-13	JL 245[a]
I-14	C 10[b]
I-15	C 3[b]
I-16	B 3[b]
I-17	EC 9[c]
I-18	41[d]
I-19	98[d]
I-20	240[d]
I-21	368[d]

Source: [a]Dr. W. E. Kloos, North Carolina State University, Raleigh, North Carolina.

[b]Nursing students, Lenoir Community College, Kinston, North Carolina.

[c]East Carolina University faculty, Greenville, North Carolina.

[d]Dr. J. Verhoef, The University of Utrecht, The Netherlands. Kindly supplied by Dr. E. D. Rosenblum, Southwestern Medical School, Dallas, Texas.

Bacteriophage[a]

Indicator Strain	29 MAW 278			31 MAW 19			62 PM 336			81 LK 118			87 BK A12			91 KES F12			94 GS F11			96 DGS F22			103 RM 351			113 GH 171		
	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um
I-1	+	+/−	+	+	+	+	+	+	+	+	+	+				+	+	+	+	+	+	+	+	+/−	+	+	+/−	+	+	+
I-2	+			+		+/−				+	+	+	+			+	+	+				+/−	+	+/−	+	+	+/−	+		
I-3	+		+	+	+/−	+/−	+			+	+/−	+/−				+	+/−	+/−	+	+/−	+/−	+/−	+/−	+/−	+	+/−	+/−	+		
I-4	+/−			+/−						+	+/−	+/−	+				+/−	+/−				+/−	+/−	+/−	+	+/−	+/−	+		+/−
I-5	+/−			+/−			+	+/−	+/−		+/−								+	+/−	+/−	+/−	+/−		+	+/−	+/−	+		
I-6	+/−									+						+/−	+/−	+				+/−	+/−	+/−	+	+/−	+/−	+/−		
I-7							+			+						+						+/−			+			+		
I-8							+			+						+						+/−			+					
I-9										+						+												+		
I-10	+	+/−	+	+	+/−	+	+												+		+/−	+			+	+/−	+/−	+		
I-11	+	+/−	+/−	+	+/−	+/−	+			+	+/−	+/−				+	+	+	+	+/−	+/−	+/−	+/−	+/−	+	+/−	+/−	+	+	+
I-12																+						+			+			+		
I-13	+	+/−	+	+	+/−	+	+	+/−	+	+	+	+/−				+	+/−	+	+	+/−	+/−	+/−	+/−	+/−	+	+/−	+/−	+		
I-14	+/−			+/−						+	+/−														+					
I-15	+/−			+/−						+	+/−	+/−				+	+/−	+/−	+/−						+					
I-16	+/−			+/−						+						+	+/−	+/−	+/−			+			+					
I-17	+/−		+	+/−	+	+				+						+	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+/−	+	+/−	+/−			
I-18	+/−			+/−												+						+/−	+/−	+/−	+					
I-19	+/−			+/−		+/−				+	+/−	+/−				+	+	+				+/−	+/−	+/−	+	+/−	+/−	+		
I-20										+		+/−				+	+/−	+/−				+/−	+/−	+/−	+	+/−	+/−	+		
I-21										+						+	+/−	+/−							+					

Table 3 (Cont'd.)

Bacteriophage[a]

Indicator Strain	114 GH 37			115 GH 56			116 GH 237			117 GH 111			120 GH 250			128 AW 259			137 MK 54			144 MK 197			145 MK 214			152 HK 217		
	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um
I-1	+	+	+	+	+	+	±			+	+		±			+	±	±	+			+		±	+			+	±	+
I-2	+			+			+			+			±			±		±				+	±	±				±		±
I-3	+		+	+	+	+	±			+	+		±			±	±	+	+		±	+	±		+			±		±
I-4	+	±	±	+	±	+	±			+	+		±			+						+	±	±				±		±
I-5	±			±												+	+	+					±	±				±	±	±
I-6	±			±						+	±		±			+						+		+				+		±
I-7	+			+						+												+	+	+						
I-8										+																				
I-9	+			+						+						±						+	±	±						
I-10	+			+						+						±	+	+	±		±		±		+	±				
I-11	+	+	+	+	+	+	±			+	+		±			+	±					+	±	±				+	±	±
I-12	+			+						+						+	±													
I-13	+		+	+			±			+			±			+						+		±						
I-14										±						+						+		±						
I-15																±						+								
I-16																±														
I-17																±	±					+								
I-18										+						+														
I-19	+			+						+	+		±			±			±			+						+		
I-20	+			+	+	+	±			+	±		±			±						+	±	±						
I-21																±														

+ = lysis, ± = separate plaques, m = Mitomycin C induction, u = UV induction, um = UV + Mitomycin C induction

[a]Phage obtained by induction are identified by a phage strain number over the S. epidermidis culture from which the phages were obtained.

Bacteriophage[a]

Indicator Strain	158 TM 195			162 CM 196			164 DW 145			165 DW 76			166 DW 104			167 DBM 376			156 TM 52			179 SL 160			181 TW 147			182 TW 256		
	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um	m	u	um
I-1	+	+	±	+	+	+				+	+	+	+	+	±	+	±	±	+	+	+	+	±	±	+			+		
I-2				+	+	+				+	±	±	+			+	+		+			+			+			+		
I-3	+	±	±	+	+	+	+			+			+	+	±	+			+	±		+	±	±	+			+		
I-4	±			+	±	±				+			±			+			+			+						+		
I-5	±	±	±							+	+	+	±	+					+			+	+	±				+	+	
I-6	±			+	±	±				+			+						+			+		±	+			+		
I-7				+						+			+			+			+			+		±	+					
I-8																			+			+		±	+					
I-9																			+			+		±	+					
I-10	+	+	±	+	+	+				+	+	+	+	+	±	+			+									+	±	±
I-11	+	±	±	+	+	+				+	±	+	+	±	±	+	±		+	±	±	+	±	±	+			+		
I-12	+			+										±					+	±		+		±	+					
I-13	±			+			+			+			+						+			+	±	±	+	±	±	+	±	
I-14				+			+			+			+						+						+			+		
I-15	±									+			+	±		+			+			+						±		
I-16	±						+			+			+									+			+			±		
I-17	±	±					+			+			+	±					+			+	±		+			±		
I-18				+	+	±	+						+						+			+			+			+		
I-19				+	±	±										+			+	±	±	+	±	±				+		
I-20				+	±	±				+			+			+			+			+		±	+			+		
I-21										+			+									+						±		

Table 3 (Cont'd.)

Indicator Strain	Bacteriophage[a] 183 TW 145 m	u	um	186 TW 257 m	u	um	184 TW 153 m	u	um	185 TW 118 m	u	um	198 ASF 2 m	u	um	200 LMS F22 m	u	um	213 B 8 m	u	um	10 EC 10 m	u	um	16 EC 16 m	u	um
I-1	+	+	+	+			+	+					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-2	+	+	+	+			+						+	+	+	+		+	+	+	+	+	+	+	+	+	+
I-3	+	+	+	+			+	+		+			+	+	+	+	+	+	+	+	+	+	+	+	+		
I-4	+	+		+			+						+	+	+	+		+	+	+	+	+	+	+	+		
I-5	+			+	+		+						+			+		+				+			+	+	+
I-6	+		+	+			+	+		+			+	+	+	+		+	+	+	+	+			+		
I-7	+			+			+									+			+	+	+	+			+		
I-8	+			+			+	+								+			+	+	+	+			+		
I-9	+	+		+			+												+	+	+						
I-10	+		+	+			+			+			+	+	+	+		+	+	+		+	+	+	+	+	+
I-11	+	+	+	+	+		+	+					+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
I-12	+			+			+									+			+	+		+			+		
I-13	+		+	+						+			+	+	+	+	+	+	+	+	+	+	+	+	+		+
I-14	+			+										+	+	+						+			+		
I-15				+												+			+			+			+		
I-16	+			+						+			+	+	+	+			+	+	+	+			+		
I-17	+	+	+	+	+					+			+	+	+	+	+	+	+	+	+	+	+		+		
I-18	+			+			+									+			+	+	+						
I-19	+	+	+	+	+		+	+					+	+	+	+	+	+	+	+	+	+	+	+	+		+
I-20	+	+	+	+			+						+	+	+	+	+	+	+	+	+	+		+	+		
I-21	+	+		+									+	+	+	+			+	+	+	+			+		

+ = lysis, ± = separate plaques, m = Mitomycin C induction, u = UV induction, um = UV + Mitomycin C induction

[a]Phage obtained by induction are identified by a phage strain number over the S. epidermidis culture from which the phages were obtained.

Table 4. Sensitivity of the Indicator Strains to the Products of Induction of the Lysogenic S. epidermidis Cultures

Indicator Strain	Method of Induction			
	Mitomycin C	UV	UV + Mitomycin C	
I-1	36	28	27	
I-2	31	13	15	
I-3	38	21	21	
I-4	31	13	14	
I-5	25	12	10	
I-6	30	8	11	
I-7	24	2	3	
I-8	16	2	1	
I-9	15	3	3	
I-10	29	14	16	
I-11	34	29	24	
I-12	20	2	1	
I-13	32	15	18	
I-14	19	2	2	
I-15	20	3	2	
I-16	23	3	3	
I-17	24	14	10	
I-18	19	2	2	
I-19	28	15	14	
I-20	28	11	13	
I-21	15	4	3	
	527	216	213	Total Number of Lytic Activities

Table 5. Selection of S. epidermidis Indicator Strains and Possible Typing Phages

Indicator Strain	Bacteriophage																																						Number of Phages Lysing Each Indicator Strain
	10	16	186	103	183	213	156	128	179	200	91	81	166	182	117	165	198	184	29	96	115	181	31	113	114	144	162	158	167	94	120	62	152	116	164	185	137	145	87	
I-8	+	+	+	+	+	+	+		+		+	+			+			+		+	+	+										+							16
I-21	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+																							16
I-9	+	+	+		+	+	+	+	+		+	+			+			+			+	+		+	+	+													17
I-12	+	+	+	+	+	+	+	+	+	+	+				+			+		+	+	+		+	+		+	+											20
I-14	+	+	+	+	+		+	+		+		+	+	+	+	+	+		+			+	+			+	+							+					20
I-15	+	+	+	+		+	+	+	+	+	+	+	+	+		+			+				+			+		+	+	+									20
I-18	+	+	+	+	+	+	+	+	+	+	+		+	+	+		+		+	+		+	+				+												21
I-16	+	+	+	+	+			+	+	+	+	+	+	+		+	+		+	+		+	+					+		+				+	+				23
I-7	+	+	+	+	+	+	+		+	+	+	+	+		+	+		+		+	+	+		+	+	+	+			+		+							24
I-17	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+	+		+	+			+		+						+	+				25
I-5	+	+	+	+	+	+	+	+	+	+	+		+	+		+	+	+	+	+	+		+	+	+					+			+						26
I-19	+	+	+	+	+	+	+	+	+	+	+	+		+	+		+	+	+	+	+		+	+	+	+	+		+		+		+						28
I-20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+		+	+	+	+												28
I-10	+	+	+	+	+	+	+	+		+			+	+	+	+	+	+	+	+	+		+	+	+		+		+		+			+					29
I-6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		+						+	+		30
I-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+		+						31
I-4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+		+		+					+	32
I-13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+		+	+	+		+	+				33
I-11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+		+						34
I-1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						36
I-3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		38
	21	21	21	20	20	20	20	20	19	19	19	17	17	17	16	16	16	15	15	15	15	15	14	14	14	14	13	12	10	9	9	8	8	6	6	6	4	3	2	

No. Indicator Strains Lysed by Each Bacteriophage

+ = lysis of strain by phage

DISCUSSION

There has been some confusion in the literature as to the identification of staphylococci used by various workers in studying lysogeny in Staphylococcus epidermidis. Verhoef et al. (1972), Dean et al. and Rose and McDonald (1973), Pulverer et al. and Blouse et al. (1975) identified the staphylococci they used as being coagulase-negative or according to biotype. It was not clear if the staphylococci identified by some of these workers based upon their lack of production of coagulase were S. epidermidis as defined by Kloos and Schleifer (1975). Kloos and Schleifer (1975) described 9 different species of coagulase-negative staphylococci, one being S. epidermidis. In the Baird-Parker classification scheme biotype 1 strains produced acid aerobically from maltose, showed phosphatase activity, and did not produce acid aerobically from D-mannitol. In the Kloos and Schleifer (1975) classification scheme only S. epidermidis showed these characteristics and therefore was probably identical to the biotype 1 strains described by Baird-Parker (1965). All of the cultures we used were identified as S. epidermidis by the methods of Kloos and Schleifer (1975).

The results of determining the sensitivity of the 248 S. epidermidis strains to the 9 phages used for initial screening shown in Table 1 approximated those of Verhoef et al. (1972), Dean et al. (1973), and Pulverer et al. (1975) using greater number of phages. Over 64% (160) of the 248 S. epidermidis strains screened in our study belonged to 5 main phage-typing patterns. This was comparable to the

results of Verhoef et al. (1972) and Pulverer et al. (1975) who reported the majority of the phage types observed belonged to a few main patterns. Forty-six percent of the strains in our study were lysed by 6 or more phages. Dean et al. (1973) reported 39% of the strains tested were lysed by 6 or more phages. They stated the sensitivity of many strains to a considerable proportion of the phage series was the main practical difficulty in phage typing with their set of 19 phages. Our results support this.

Workers reporting phage-typing results of S. epidermidis identified from 14 to 41% of their strains as insensitive to lysis by any of their typing phage. Approximately 10% of the strains we tested were not lysable by the nine phages compared to 14% found by Pulverer et al (1975) using 16 phages. This was considerably less than the approximately 25% found unlysable by Verhoef et al. (1972) and Dean et al. (1975) or the 41% of Blouse et al. (1975) using 18, 19, and 18 phages respectively.

The above differences may reflect variations in the method of screening used. High titer preparations of the 9 phages were tested on the 248 cultures in our initial screening experiments. Although the use of concentrated phage stocks increased the possibility of false positive reactions, this concentration also permitted detection of greater numbers of phage-sensitive S. epidermidis cultures than did the use of routine test dilutions (RTD) of phages. Cultures showing confluent lytic effect upon exposure to undiluted phage preparations were retested with a phage dilution sufficient to produce evidence of plaque formation before being considered sensitive to a particular

phage strain. The use of high titer phages to increase the sensitivity of phage screening was consistent with reports of other workers. Verhoef et al. (1972) tested their 240 strains of S. epidermidis at RTD and RTD X 1000; Dean et al. (1973) tested their 972 strains to RTD X 100 and RTD X 1000; Pulverer et al. (1975) tested the sensitivity of their 183 strains to RTD and to RTD X 1000; however, Blouse et al. (1975) tested their 118 strains only to RTD X 100.

The variation among phage-typing studies in the percentage of insensitive strains observed may also be explained on an epidemiological basis. The significantly higher percentage of nontypable cultures from the United States found by Blouse et al. (1975) could have resulted from their use of 18 provisional typing phages Verhoef et al. (1972) isolated primarily from European strains.

None of our resistant strains were lysogenic suggesting a high resistance to phages. The untypable S. epidermidis strains may have produced capsules that prevented infection by phages. Witte (1975) reported capsule formation in S. aureus as a reason for insensitivity to phage infection. Quite possibly the untypable strains represented virus receptor (cell wall) mutants. Schleifer and Steben (1974) specifically showed that S. epidermidis phages isolated by Verhoef et al. (1971) were only adsorbed to cell walls containing glucosylated glycerol teichoic acid characteristic of S. epidermidis. Finally, they may have been physiological variants incapable of supporting growth of or lysis by infecting phages.

Cultures of S. epidermidis from different countries and sources (i.e., normal populations or patients) have also showed different

frequencies of lysis by the same set of phages. Four of the 9 phages (130A, 71, 82A and 48) we used were the same used by Verhoef et al. (1972) in their typing studies. Differences were noted in the frequencies of lysis by the 4 phages of our strains and the strains tested in their laboratory. The main differences were found with phage 71, which lysed the majority of our strains and relatively few of Verhoef et al.'s isolates and phage 82A which lysed a relatively high number of theirs and fewer of ours. This could have been due to a combination of effects of countries of origin and source of the S. epidermidis cultures. The strains we used were skin isolates from healthy persons living in New Jersey and North Carolina. Conversely, their strains were from Europe and approximately 50% included cultures from hospital staff and patients. This was in accord with the findings of Pulverer et al. (1975) who reported some correlation of country of origin of strain and sensitivity to phages. Interestingly, phage strains 10 and 16 isolated from our lysogenic cultures lysed fewer S. epidermidis strains than 6 of the 7 phages obtained from Verhoef et al. Further work will be required on the epidemiological significance of the phage-typing patterns of S. epidermidis.

Indicator strains sensitive to lysis by the lysogenizing phages were required to detect lysogeny. Twenty-one indicator strains were chosen from the 28 strains lysed by all nine phages (Table 1 and 2) for their high sensitivity to phages. These indicator strains were used to screen the Mitomycin C-, UV-irradiation- and UV-irradiation plus Mitomycin C-induced induction products from 241 S. epidermidis strains.

The 16.2% (39 of 241) frequency of lysogeny we found in S. epidermidis compared favorably, though somewhat lower, with the 26% frequency of lysogeny in coagulase-negative staphylococci (using Mitomycin C to induce phages) found by Verhoef et al. (1971). Although we followed basically the same procedures, our study could not confirm the findings of Pulverer et al. (1974) of 100% lysogeny in S. albus (S. epidermidis) using Mitomycin C and UV separately. These authors attributed their findings to induction techniques and to high sensitivity of their indicator strains to phages. A universal indicator strain, Q239, detected phages released by all 127 strains they tested. There was the possibility that this strain was itself unstably lysogenic and the lytic effect observed was due to the spontaneous induction by a contained prophage.

Some isolates from the same person were lysogenized with the same phage, as evidenced by identical lytic activity of induction products for the indicator cultures, whereas others from that person harbored different phages. This was shown by the lytic activity of induction products from the lysogenic group of strains from "GH" source (Table 3). Strains 113, 114, and 115 contained the same phages wereas strains 116, 117, and 120 contained different phages. This was also shown by the "DW" and "TW" isolates (Table 3).

In comparing the source of the indicator strain with the pattern of lysis of the phages isolated, it was noted in some instances that strains from the same person were sensitive to different phages (Table 3). Indicator strain numbers 7, 8, and 9 from source "RM" were shown to have different virus-sensitivity patterns indicating an

individual may carry different S. epidermidis cultures. The results of Verhoef et al. (1972) indicated that some people temporarily carry one or more strains of S. epidermidis in the nose, others harbor the same strain for months.

Strains 368 (I-21) and 240 (I-20) used as indicator strains in our studies were not as effective as had been reported by Pulverer et al. (1974). In their studies indicator strain 368 detected 124 of 127 cultures tested (97.6%) to be carrying phages and strain 249 was able to detect 119 of 127 (93.7%) carrying phages. As shown in Table 5 strain 368 (I-21) detected only 16 of our 39 (41%) lysogenic cultures and strain 240 (I-20) detected 28 of 39 (71.8%). This discrepancy was possibly due to differences in the origins of the isolates. All cultures used by Pulverer et al. (1974) were isolated from various types of clinical infections (e.g., endocarditis, urinary tract infections) and most were obtained from Europe and Canada, while our cultures were obtained from healthy persons living in the United States.

The indicator strains were found to have different sensitivities to the phages induced. Some indicator strains (e.g., I-11, I-1, and I-3, Tables 3 and 4) were highly sensitive to lysis by phages comparable to the universal indicator strain reported by Pulverer et al. (1974) in contrast to two indicator strains (I-8 and I-21) lysed by by only 16 phages. Verhoef et al. (1972) and Pulverer et al. (1975) noted variations in duplicate phage-typing experiments of S. epidermidis on the same day. To reduce the possibility of a change in phage sensitivity by the indicator strains, all of our indicator strains were

tested at the same time with the products of the cultures exposed to the 3 induction procedures.

The greatly reduced numbers of indicator strains sensitive to induction products from UV and UV plus Mitomycin C treatment as compared to those sensitive to lysis by induction products from Mitomycin C induction (Tables 3 and 4) could not have been due only to variation normally occurring in sensitivity testing. Mitomycin C and UV-irradiation have different modes of action in induction. The differences in lytic activity on the indicator strains of induction products from these two methods could have been due to their selective induction of prophages in multiply lysogenic cultures. Pulverer et al. (1974) concluded that approximately 30% of their lysogenic strains were multi-lysogenic. Exposure to small doses of UV light was reported by Davis et al. (1973) to induce many prophages; however, UV-irradiation was a poor inducer of prophages that were inducible by agents inhibiting DNA synthesis such as Mitomycin C while some prophages were shown to be noninducible. This was supported by the results of Pulverer et al. (1975) who found some phages obtained from the same culture by different induction methods lysed different indicator strains and that some indicator strains detected some phages after induction only by Mitomycin C, or only by UV-irradiation or by both methods. Mitomycin C could therefore have been a less specific induction method than UV-irradiation and induced two or more viruses in a multiply-lysogenic culture while UV-irradiation induced only one. Mitomycin C may also have been able to induce prophages without affecting the virulence of the phages produced. Ultraviolet-irradiation induction and UV plus

Mitomycin C induction may have been able to induce the same prophage as Mitomycin C but the phage lysate had reduced virulence resulting in lysis of fewer indicator strains as well as production of individual plaques.

Mitomycin C induction of a strain following UV induction had no greater effect than use of UV induction alone. The differences between UV and UV plus Mitomycin C inductions may have been due to differences in normal sensitivity testing since the variations observed usually involved lysates containing low titers of phages. Our results (Tables 3 and 4, phage strains 117, 145, 184, and 186) indicated UV-irradiation interfered with induction of some prophages or rendered some prophages resistant to induction by Mitomycin C subsequently added. It would otherwise have been expected that Mitomycin C added after UV-irradiation would induce as many as would Mitomycin C alone.

We are interested in determining whether or not our phages will show a change in lytic pattern in response to propagation. The 39 virus strains tested against the 21 indicator strains (Table 5) were present in induction lysates and had not been propagated. Propagation necessary to get RTD X 100 or 1000 titers could have resulted in host modification of the lytic patterns of the phages. Verhoef et al (1971) observed that modification induced by the propagating strains profoundly affected the lytic pattern of many of their phages.

The results in Table 5 have been arranged to show the value of the 39 phages isolated as possible typing phages and of the 21 indicator strains to detect phages in lysogeny studies. Due to their high

sensitivity to phages the group of 10 indicator strains including strain I-19 lysed by 28 phages through strain I-3, lysed by 38 phages (Table 5) would be most useful to detect lysogeny in S. epidermidis. The finding that none of the indicator strains were detected to be lysogenic may have accounted for their high sensitivity to phages obtained in this study. These non-lysogenic strains would be best for use as propagating strains for phages used in a typing series. Phage recombinants could be produced if a lysogenic propagating strain was used to propagate a phage strain used in a typing series. The recombinants may have different lytic activity from the parent phages. The 31 viruses listed including phage strain 10 through phage strain 120 are proposed as being of possible value in phage-typing experiments with S. epidermidis due to their lytic activity for the indicator strains. The 8 phages with low lytic activity for the indicator strains would probably have a host range too restricted for typing strains from general populations of S. epidermidis. Phage-typing coagulase-positive staphylococci involved the use of a group of phages with narrow host ranges. The 31 phages infecting the greatest number of indicator strains were chosen in our study as possible typing phages for S. epidermidis. The cultures tested for susceptibility were highly sensitive indicator strains and would not likely represent the usual viral sensitivity of a clinical isolate. To determine their possible value in phage typing further studies need to be done with the 39 phages. The effect of propagation and routine test dilutions on their value as typing phages needs to be determined.

A large number of S. epidermidis strains from different sources (different countries, normal populations, infections, etc.) need to be tested.

SUMMARY

Of 248 *S. epidermidis* cultures tested for sensitivity to 9 *S. epidermidis* phages, 89.5% typed into 44 patterns, and 68.9% of the strains belong to 8 of the 44 patterns observed. Some of these phages had been previously used by other workers in typing *S. epidermidis* cultures predominately from countries other than the United States. Twenty-one strains lysed by all 9 phages were chosen to be used as indicator strains from the 248 cultures tested.

Using Mitomycin C and/or UV-irradiation for induction, 16.2% of 241 *S. epidermidis* strains were found to release phages detectable by the indicator strains. Induction with Mitomycin C resulted in the highest frequency of phage recovery compared to UV or a combination of UV plus Mitomycin C. Various explanations were proposed that could have accounted for the differences observed. Further work will be required to explain the difference in induction frequency.

Ten *S. epidermidis* strains were identified as potentially useful as indicator strains (i.e., highly susceptible to lytic infection by *S. epidermidis* phages) in characterizing lysogeny in *S. epidermidis*. Furthermore, 31 virus preparations were obtained from the induction of 241 strains of *S. epidermidis* that may be useful in phage typing *S. epidermidis* cultures. Studies are underway testing the 31 phages on the 248 strains. We hope to identify phage strains useful in phage typing *S. epidermidis*, including the approximately 10% not lysed by any of the 9 phages used in screening.

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James Edward Bostian. THE FREQUENCY AND INDUCIBILITY OF STAPHYLOCOCCUS EPIDERMIDIS BACTERIOPHAGES AND THEIR POTENTIAL FOR USE IN A TYPING SERIES. (Under the direction of Wendall E. Allen) Department of Biology, East Carolina University, July 1976.

The purpose of this investigation was to: 1) identify a group of Staphylococcus epidermidis cultures for use as "indicator strains", 2) to determine the frequency of lysogeny in 241 strains of S. epidermidis from this country, 3) to compare the effectiveness of various inducing agents and 4) to obtain S. epidermidis bacteriophages useful in phage typing S. epidermidis cultures.

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