Grouping of Staphylococci on the Basis of Their Bacteriolytic-Activity Patterns: a New Approach to the Taxonomy of the *Micrococcaceae*

II. Main Characters of 1,054 Strains Subdivided into "Lyogroups"

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A number of characters (colony features, biochemical and metabolic properties, susceptibility to antimicrobial agents) were determined on 1,054 staphylococci previously placed in six "lyogroups" on the basis of bacteriolytic activity. A great deal of uniformity was found among the characteristics of strains of the same lyogroup. Virtually 100% agreement was found between four of the six lyogroups and four nomen species or biotypes: i.e., lyogroup I was found to equate with *Staphylococcus aureus*; lyogroups II and III were found to equate with the recently described species *S. simulans* and *S. capitis*, respectively; and lyogroup V was found to equate with *S. epidermidis* sensu stricto. The occurrence of a relationship between the bacteriolytic activities of staphylococci and their cell wall compositions is shown. Such a correlation may be an expression of a specific mechanism of cell wall growth. The taxonomic significance of bacteriolytic activity and the relationship of this criterion and the resultant classificatory scheme to other criteria and schemes are discussed.

It was shown in a companion paper (26) that staphylococci can be subdivided into six different groups (lyogroups) on the basis of their bacteriolytic activities, each group showing a distinctive bacteriolytic-activity pattern. Since the bacteriolytic-activity pattern was the only property taken into consideration for grouping the staphylococci, we determined additional characteristics of the same 1,054 isolates to ascertain whether any other properties, in particular biochemical and physiological, are shared by the members of the various lyogroups. The isolates were identified at the specific level by the classificatory schemes of Baird-Parker (2) and Schleifer and Kloos (10, 19).

The results obtained indicate that the members of one lyogroup share important biochemical and physiological properties that are different from those shared by the members of other lyogroups. A precise correlation was found between four of the six lyogroups and four nomen species and biotypes of the genus *Staphylococcus*. The remaining two lyogroups each contained a cluster of closely related nomen species.

MATERIALS AND METHODS

Bacterial strains. The same 1,054 staphylococcal strains that were grouped by their bacteriolytic-activity patterns in a previously reported study (26) were also used in this study.

Colonial characters. Organisms were streaked on plates of M9 medium of the following composition: 3%

Peptonum Siccum (A. Costantino & C., Favria, Torino, Italy), 0.2% glucose, 0.5% NaCl, 1.5% agar (pH 6.9). Plates were incubated for 4 days at 37°C, after which pigment production, size, and other colonial characteristics were recorded; each characteristic was determined on observations made on three, four, or five isolated colonies.

Coagulase production. The production of free coagulase was tested on rabbit plasma. A 0.2-ml sample of an overnight culture (brain heart infusion broth [Difco]) of each strain to be examined was added to 0.3 ml of plasma. Tubes were incubated at 37°C and examined for clot formation after 1, 4, and 24 h.

Phosphatase production. Phosphatase production was determined by a modification of the procedure of Barber and Kuper (3). Phenolphthalein diphosphate sodium salt (Serva) was added from a Seitz-filtered solution to sterile M9 medium, melted and cooled to 45°C. The final concentration of phenolphthalein diphosphate was 200 µg/ml. Plates of this medium were streaked with the organisms to be examined and were incubated for 24 h at 37°C. Cultures that turned deep pink after exposure to ammonia vapors were recorded as positive.

Acetoin production. The Voges-Proskauer test for acetoin production was carried out by the procedure of Barritt (4). One milliliter of a 48-h-old culture in MR-VP broth (Difco) was added to 0.6 ml of a 5% α-naphthol solution in ethanol and 1 ml of 16% potassium hydroxide. Tubes were shaken, allowed to stand for 30 to 45 min, and then examined. The appearance of an orange-red color in the upper portion of the mixture was regarded as a positive reaction.

Urease production. Urease production was determined on urea agar base medium (Difco) by the

method of Christensen (6). Inoculated tubes were incubated at 37°C and examined after 24 and 48 h. Cultures that turned pink were recorded as positive.

DNase production. The production of deoxyribonuclease (DNase) was tested using the toluidine blue-deoxyribonucleic acid agar mixture described by Lachica et al. (12). Paper disks (7 mm in diameter) were dipped in 24-h-old broth cultures of the strains to be tested and were then placed on a toluidine blue-deoxyribonucleic acid-agar overlay (6 to 9 disks per plate). After incubation for 5 h at 37°C, the appearance of a zone of decolorization around the disk was regarded as a positive reaction.

The same procedure was used to test for the production of thermostable DNase, but the broth cultures were heated for 15 min at 100°C before testing.

Hemolytic activity. The hemolytic abilities of the strains were determined on bovine blood agar plates. The strains to be tested were inoculated on the plates as spots, up to 12 per plate. The plates were incubated at 37°C and examined after 24 and 48 h. The appearance of a zone of hemolysis around the spot of growth was regarded as a positive reaction.

Lysostaphin susceptibility. Groups of 100 sterile paper disks (6 mm in diameter) were imbued with 1 ml of a sterile 40-μg/ml solution of lysostaphin (Schwarz/Mann, Orangeburg, N.Y.). A small drop of an overnight broth culture of each strain was distributed on half or a third of the surface of a plate of DST agar (Oxoid), so that two or three strains were tested on one plate. One disk was placed in the middle of each zone inoculated in this way, and plates were then incubated for 24 h at 37°C. A zone of growth inhibition around the disk denoted susceptibility to lysostaphin.

Novobiocin susceptibility. Oxoid sensitivity disks containing 5 μg of novobiocin were employed. The test procedure was the same as that described above for determining lysostaphin susceptibility.

Carbohydrate reactions. Acid production from carbohydrates was determined by an agar tube method in which the medium of Mossel and Martin (13) was employed. Tubes containing 7 ml of the melted medium were inoculated with 0.5 ml of an overnight broth culture. The following carbohydrates were tested under aerobic conditions: D-(+)-glucose, D-(+)-xylose, maltose, lactose, sucrose, trehalose, D-(-)-mannitol, and xylitol. D-(+)-Glucose and D-(-)-mannitol were also tested under anaerobic conditions by adding 7 ml of sterile, melted paraffin onto the inoculated medium in the tubes. Tubes were incubated at 37°C and examined for acid production after 1, 2, and 4 days.

Strain identification. Based on the tests performed, the identification of the strains was attempted by the schemes of Baird-Parker (2, 5) and Schleifer and Kloos (10, 11, 19). Some strains could not be satisfactorily identified by either scheme.

RESULTS

Table 1 summarizes the characteristics of the strains studied, arranged according to lyogroup. The strain identification and other findings that cannot be deduced from the table are reported below.

Lyogroup I. All strains of lyogroup I could clearly be identified as *Staphylococcus aureus*. Seven isolates that did not produce coagulase were also identifiable as coagulase-negative *S. aureus* strains on the basis of the other tests.

Lyogroup II. Due to carbohydrate reactions and nonproduction of both phosphatase and acetoin, the strains of lyogroup II could not be satisfactorily included in any of the Baird-Parker biotypes (2). On the contrary, they could be recognized as virtually identical to the strains described by Kloos and Schleifer as forming the new species *S. simulans* (10), on the basis of both carbohydrate reactions (mainly no acid production from maltose) and biochemical characters (weak nuclease activity, nonproduction of acetoin). This finding confirms our original identification and definition of such a new staphylococcal group (25). Most members of the species *S. simulans* have been reported as weak phosphatase producers (10, 11), whereas no strain of lyogroup II was found to produce phosphatase. The technique we used to test phosphatase activity, however, was intentionally not as sensitive as that of Pennock and Huddy (15) used by Kloos and Schleifer; on the other hand, Oeding and Digraanes (14) have recently reported that the technique of Pennock and Huddy produce a considerable number of false-positive weak reactions.

The new species *S. simulans* was so named for having similarities to coagulase-positive staphylococci (10). Further similarities of the strains of lyogroup II to *S. aureus* are shown in this study: acid production from mannitol also under anerobic conditions; production of a thermostable DNase, although weak and nonconstant. The bacteriolytic activity type-pattern of lyogroup II, moreover, does not differ considerably from that of lyogroup I, particularly with respect to the effect of ionic strength (26).

Lyogroup III. Most strains of this group could be identified as belonging to the Baird-Parker *S. epidermidis* biotype 4; only three strains failing to produce acid aerobically from mannitol had to be included in biotype 3 (2). According to Kloos and Schleifer (10), most or probably all strains could be identified as *S. capitis*, on the basis of colonial characteristics (in particular a usually chalk-white colony color), moderate DNase production, and the carbohydrate reaction pattern (in particular a null or weak acid production from trehalose, maltose, and lactose).

Lyogroup IV. Mainly on the basis of the carbohydrate reaction pattern, colonial characteristics, and phosphatase reaction, 17 strains were identifiable as *S. saprophyticus*, 16 (which included all strains of this lyogroup producing

TABLE 1. *Characterization of the staphylococci studied arranged according to lyogroup*

Lyo-group	Total no. of strains studied	Colony characteristics								Biochemical characters													
		Diam (mm)	Pigment ^a		Edge		Profile			Coagulase		Phosphatase			DNase			Thermostable DNase			Acetoin		
			Yes	No	Entire	Cre-nate	Flat	Low con- vex	Con- vex	+ ^b	-	+	±	-	+	±	-	+	±	-	+	±	
I	413	5-8	84 ^c	16	100		99	1		98	2	100			100			100			97	3	
II	39	5-7		100	100		100				100			100		77	23		77		23	8	92
III	24	2-4	4	96	100			8	92		100			100		88	12			100	79	21	
IV	42	5-8	55	45	74	26	48	40	12		100	24	5	71			100			100	48	26	26
V	267	2-4		100	100		97	3			100	89		11		2	98			100	99	1	
VI	255	3-6	6	94	100		8	74	18		100			100		4	96			100	53	27	20

Lyo-group	Biochemical characters						Susceptibility to:						Aerobic acid from:							
	Urease			Hemolysis			Lysostaphin			Novobiocin			Glucose		Xylose			Maltose		
	+	±	-	+	±	-	+	±	-	+	±	-	+	±	+	±	-	+	±	+
I	12	69	19	82	8	10	100			99	<1		100				100	100		
II	97		3		44	56	100			100			100				100			100
III	29	25	46		17	83			100	100			100				100		21	79
IV	91	7	2	2	5	93		7	93		5	95	67	33	28	5	67	36	43	21
V	82	17	1		24	76			100	99	<1		100				100	100		
VI	1	12	87	60	26	14			100	98	2		100				100	97	3	

Lyo-group	Aerobic acid from:												Anaerobic acid from:							
	Lactose			Sucrose			Trehalose			Mannitol			Xylitol			Glucose			Mannitol	
	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-	+	±	-	+	-
I	90	7	3	100			100			96		4			100	100			96	4
II	100			100			79	8	13	97		3			100	100			97	3
III		29	71	96		4		8	92	71	17	12			100	100				100
IV	36	28	36	69	12	19	60	31	9	50	31	19	7	36	57	17	40	43		100
V	83	1	16	100				1	99			100			100	100				100
VI	29	32	39	90	10		95	2	3	68	1	31			100	100				100

^a Colony pigment: Yes, pigmented colonies (orange, yellow-orange, yellow-cream, or cream); no, unpigmented colonies (gray-white or white).^b Reactions: +, positive; ±, weak; -, negative.^c Data are given as percentages of strains.

phosphatase and/or showing irregularly edged colonies) as *S. xylosus*, and 7 as *S. cohnii* according to Schleifer and Kloos (19). The remaining two strains, for which identification was rather uncertain, should probably be also identified as *S. saprophyticus*. According to Baird-Parker (2), most strains had to be identified as *S. saprophyticus* (mainly biotype 3) or as micrococci.

S. saprophyticus strains have been reported as being consistently devoid of lytic activity (19), whereas the strains belonging to lyogroup IV are among the strongest producers of such activity. In a study on staphylococci and micrococci, however, Holt (9), by utilizing assay conditions that were not very sensitive, reported strains of the Baird-Parker *Micrococcus* subgroup 3 as the only other producers of strong lytic activity besides *S. aureus* strains. These strains have been subsequently reclassified as typical *S. saprophyticus* strains (2, 7). These different findings may depend on the different conditions used for the testing of lytic activity. In fact, it has been shown previously that conditions for detecting the bacteriolytic activity of several coagulase-negative strains are rather critical (17).

Lyogroup V. The strains of lyogroup V have been subdivided into two subgroups according to their bacteriolytic-activity patterns (26). As regards the conventional characters tested in the present study, however, significant differences between the two subgroups were not found, aside from a different incidence of phosphatase production (92% in subgroup A and 57% in subgroup B). The phosphatase-negative strains were the same, showing no bacteriolytic-activity decrease on TP2P medium in comparison with TP2 (26).

On the basis of the carbohydrate reaction pattern and phosphatase production, all strains of this group could be identified as *S. epidermidis* biotype 1 according to Baird-Parker (2), and *S. epidermidis* sensu stricto according to the amended description of Schleifer and Kloos (19).

Lyogroup VI. Considering failure to produce phosphatase, most strains of this group could be identified as Baird-Parker *S. epidermidis* biotype 4 and a minority as biotype 3, depending on their ability to produce acid aerobically from mannitol (2). Mainly on the basis of biochemical characters and the carbohydrate reaction pattern, these strains could mostly be classified, according to Schleifer and Kloos (10, 19), as *S. haemolyticus* or *S. hominis*; the identification of a few strains as *S. warneri*, although likely, was not confirmed.

Ungrouped strains. Fourteen strains of the 1,054 examined for their bacteriolytic-activity patterns had not been included in any of the

previously described six lyogroups (26). Their identification according to both Baird-Parker (2) and Schleifer and Kloos (10, 19) was uncertain in most cases. However, it is worth noting that one of these strains, having bacteriolytic pattern no. 23 (26), could clearly be identified as *S. epidermidis* biotype 2 according to Baird-Parker. Strains of such biotype are known to be of swine origin (1, 5, 22). This suggests that some of the ungrouped strains, although isolated from human specimens, might exhibit unusual patterns resulting from their actual animal origin.

DISCUSSION

The characterization of 1,054 staphylococci previously placed in lyogroups on the basis of their bacteriolytic-activity patterns has indicated that a substantial character homogeneity exists within the strains of a lyogroup whereas major differences in characters are encountered when group-to-group relationships are considered.

Furthermore, it has been shown that a strong correlation exists between the lyogroups and a number of the currently recognized nomen species and biotypes established on completely different criteria. Lyogroups I and V agree perfectly with *S. aureus* and *S. epidermidis*, respectively, which are the only entities which coincide in the two most widely known taxonomic schemes of the staphylococci, namely, the Baird-Parker (2, 5) and the Schleifer and Kloos (10, 19) schemes, and which are thus the most widely recognized and accepted staphylococcal species. Lyogroup II coincides perfectly with *S. simulans*, a well-defined species (10). Our studies have also revealed that lyogroup II strains are quite homogeneous and are unique with regard to their bacteriolytic activity and their salient characters. Lyogroup III virtually coincides with *S. capitis* (10). The strains of this group share many characters, among which are an optimal bacteriolytic activity in media without NaCl and a moderate production of a thermolabile nuclease. Lyogroup IV includes strains each of which was placed by Schleifer and Kloos into one of three closely related species: *S. saprophyticus*, *S. xylosus*, or *S. cohnii* (19). On the other hand, minor differences in bacteriolytic activity were found among lyogroup IV strains (e.g., the sharpness of the outlines of the zones of transparency). Thus, by improving the resolving power of our assay system, further differences within this lyogroup may be found. Lyogroup VI includes strains of *S. haemolyticus* and *S. hominis*, which species are considered closely related to each other by Schleifer and Kloos (10, 19). The identification of strains of *S. warneri*, which also is considered closely related to the two

above-mentioned species (10), proved for us to be rather difficult, as did a reliable separation of the three species.

One interesting finding of this study, and an important theoretical basis for giving the type of bacteriolytic activity a high taxonomic value, is the occurrence of a close relationship between the lytic activities and the cell wall compositions of staphylococci. It should be noted that peptidoglycan types of bacterial cell walls have been recognized to have important taxonomic implications, particularly in gram-positive bacteria (18). Schleifer and Kocur (20) have also shown that cell wall composition, with respect to both peptidoglycan and teichoic acid, is very useful in classifying staphylococci. The above-mentioned relationship between bacteriolytic activity and chemical cell wall composition can be inferred from the identification of staphylococci of known lyogroup according to identification schemes that also take into account cell wall composition. Some data on the cell wall compositions of the species that are recognized by Schleifer and Kloos and that correspond to our lyogroups are summarized in Table 2. A relationship can be noted between the ionic strength requirement for bacteriolytic activity and the type of cell wall peptidoglycan. In particular, strains with a low content of serine in the cell wall exhibit a better bacteriolytic activity in media with low NaCl concentrations (lyogroups I and II), whereas strains with a relatively high content of serine exhibit the most bacteriolytic activity in media

with higher NaCl concentrations (lyogroups V and VI). Aside from the apparent exceptions of lyogroups III and IV, it should be pointed out that the serine/glutamic acid ratio is most likely not the only cell wall characteristic exhibiting a relationship to the properties of specific lytic enzymes. For example, the strains of lyogroup III (i.e., *S. capitis* of Kloos and Schleifer), which are the only staphylococci exhibiting optimal lytic activity in the absence of NaCl, although presenting a peptidoglycan composition similar to that of other groups, are the only strains found to bear a glutamic acid polymer in the cell wall. Another component of the cell wall of gram-positive bacteria which should be considered is teichoic acid, which in some species has been found to influence the activity of autolysins and has been suggested as playing a significant role in physiological functions related to the cell wall, such as susceptibility to penicillins, cell shape, and cell division (8, 24). Staphylococci with different bacteriolytic activities but with the same peptidoglycan composition may have teichoic acids of different compositions; this is the case, for example, with lyogroups I and II.

It is now becoming more and more evident that bacteriolytic enzymes must play a key role in cell wall growth, cell septation, and separation of daughter cells (16, 21, 23) of bacteria. The above-mentioned relationship between a particular lytic activity and a special cell wall composition might be the expression of a somewhat specific mechanism of cell wall growth, cell sep-

TABLE 2. Relationship of bacteriolytic activity to the cell wall composition of staphylococci

Lyogroup	Optimal NaCl concn (%) for bacteriolytic activity ^a	Species according to the classification scheme of Schleifer and Kloos	Cell wall composition ^b				
			Serine content in peptidoglycan	Glycine content in peptidoglycan	Glutamic acid polymer	Ribitol teichoic acid	Glycerol teichoic acid
I	0.5-1.0	<i>Staphylococcus aureus</i>	0-0.3	5.0-6.0	-	+	-
II	0-1.0	<i>S. simulans</i>	0-0.2	4.7-6.0	-	-	+
III	0	<i>S. capitis</i>	0.8-1.2	3.5-4.3	+	-	+
IV	0.5-3.0	<i>S. saprophyticus</i>	0.6-0.8	4.1-5.1	-	+	+
		<i>S. xylosus</i>	0-0.1	4.9-5.7	-	+	+
		<i>S. cohnii</i>	0-0.2	4.9-6.0	-	-	+
V	2.0-3.0	<i>S. epidermidis</i>	0.7-1.5	4.0-5.0	-	-	+
VI	2.0-3.0	<i>S. haemolyticus</i>	0.9-1.4	3.3-4.0	-	-	+
		<i>S. hominis</i>	0.6-1.3	3.3-4.3	-	-	+
		<i>S. warneri</i> ^c	0.6-1.4	3.3-4.4	-	-	+

^a From investigations only partially reported in this paper.

^b From reports of Schleifer and Kloos (10, 19). The serine and glycine contents in the peptidoglycan are given as moles per mole of glutamic acid.

^c The identification of some lyogroup VI strains as strains of *S. warneri*, although likely, was not confirmed.

tation, or cell division in each lyogroup, and may thus be of particular value in species resolution and definition.

In another study (P. E. Varaldo and G. Satta, manuscript in preparation), we analyzed the main properties of purified bacteriolytic enzymes from representative strains of each lyogroup and concluded that such lytic enzymes differ from one another with respect to some main properties. This fact, in addition to the above-mentioned relationship between lytic activity and cell wall composition, suggests that the sharing of the same bacteriolytic activity is more significant in staphylococcal taxonomy than the sharing of any other single character.

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REPRINT REQUESTS

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LITERATURE CITED

1. Baird-Parker, A. C. 1965. Staphylococci and their classification. *Ann. N.Y. Acad. Sci.* **128**:4-25.
2. Baird-Parker, A. C. 1974. The basis for the recent classification of staphylococci and micrococci. *Ann. N.Y. Acad. Sci.* **236**:7-14.
3. Barber, M., and S. W. A. Kuper. 1951. Identification of *Staphylococcus pyogenes* by the phosphatase reaction. *J. Pathol. Bacteriol.* **63**:65-68.
4. Barritt, M. M. 1936. The intensification of the Voges-Proskauer reaction by the addition of α -naphthol. *J. Pathol. Bacteriol.* **42**:441-454.
5. Buchanan, R. E., and N. E. Gibbons (ed.). 1974. Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Co., Baltimore.
6. Christensen, W. B. 1946. Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J. Bacteriol.* **52**:461-466.
7. Digranes, A., and P. Oeding. 1975. Characterization of *Micrococcaceae* from the urinary tract. *Acta Pathol. Microbiol. Scand. Sect. B.* **83**:373-381.
8. Herbold, D. R., and L. Glaser. 1975. Interaction of *N*-acetylmuramic acid *L*-alanine amidase with cell wall polymers. *J. Biol. Chem.* **250**:7231-7238.
9. Holt, R. J. 1971. Lysozyme production by staphylococci and micrococci. *J. Med. Microbiol.* **4**:375-379.
10. Kloos, W. E., and K. H. Schleifer. 1975. Isolation and characterization of staphylococci from human skin. II. Descriptions of four new species: *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Staphylococcus simulans*. *Int. J. Syst. Bacteriol.* **25**:62-79.
11. Kloos, W. E., and K. H. Schleifer. 1975. Simplified scheme for routine identification of human *Staphylococcus* species. *J. Clin. Microbiol.* **1**:82-88.
12. Lachica, R. V. F., C. Genigeorgis, and P. D. Hoepflich. 1971. Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. *Appl. Microbiol.* **21**:585-587.
13. Mossel, D. A. A., and G. Martin. 1961. Milieu simplifié permettant l'étude des divers modes d'action des bactéries sur les hydrates de carbone. *Ann. Inst. Pasteur (Lille)* **12**:225-226.
14. Oeding, P., and A. Digranes. 1977. Classification of coagulase-negative staphylococci in the diagnostic laboratory. *Acta Pathol. Microbiol. Scand. Sect. B* **85**:136-142.
15. Pennock, C. A., and R. B. Huddy. 1967. Phosphatase reaction of coagulase-negative staphylococci and micrococci. *J. Pathol. Bacteriol.* **93**:685-688.
16. Rogers, H. J. 1970. Bacterial growth and the cell envelope. *Bacteriol. Rev.* **34**:194-214.
17. Satta, G., P. E. Varaldo, G. Grazi, and R. Fontana. 1977. Bacteriolytic activity in staphylococci. *Infect. Immun.* **16**:37-42.
18. Schleifer, K. H., and O. Kandler. 1972. The peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* **36**:407-477.
19. Schleifer, K. H., and W. E. Kloos. 1975. Isolation and characterization of staphylococci from human skin. I. Amended descriptions of *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*, and descriptions of three new species: *Staphylococcus cohnii*, *Staphylococcus haemolyticus*, and *Staphylococcus xylosus*. *Int. J. Syst. Bacteriol.* **25**:50-61.
20. Schleifer, K. H., and M. Kocur. 1973. Classification of staphylococci based on chemical and biochemical properties. *Arch. Mikrobiol.* **93**:65-85.
21. Shockman, G. D., L. Daneo-Moore, and M. L. Higgins. 1974. Problems of cell wall and membrane growth, enlargement and division. *Ann. N.Y. Acad. Sci.* **235**:161-197.
22. Smith, R. F., and C. L. Bettge. 1972. Comparative characteristics of human and porcine staphylococci and their differentiation in burn xenografting procedures. *Appl. Microbiol.* **24**:929-932.
23. Strominger, J. L., and J. M. Ghuyssen. 1967. Mechanisms of enzymatic bacteriolysis. *Science* **156**:213-221.
24. Tomasz, A., and M. Westphal. 1971. Abnormal autolytic enzyme in a pneumococcus with altered teichoic acid composition. *Proc. Natl. Acad. Sci. U.S.A.* **68**:2627-2630.
25. Varaldo, P. E., G. Grazi, G. Satta, and L. Melica. 1974. *Staphylococcus aureus* toxins. I. Peculiarities of "lysozymes" produced by staphylococci, p. 282-286. In T. Hasegawa (ed.), *Proceedings of the First Intersectorial Congress of International Association of Microbiological Societies*, vol. 4. Science Council of Japan, Tokyo.
26. Varaldo, P. E., G. Satta, G. Grazi, and C. A. Romanzi. 1978. Grouping of staphylococci on the basis of their bacteriolytic-activity patterns: a new approach to the taxonomy of the *Micrococcaceae*. I. Identification of six different "lyogroups." *Int. J. Syst. Bacteriol.* **28**:141-147.