Taxonomic Study of Coagulase-Positive Staphylococci: Bacteriolytic Activity Pattern Analysis

PIETRO E. VARALDO,¹ GIUSEPPE SATTA,¹ AND V. HÁJEK²

Instituto di Microbiologia dell'Università di Genova, School of Medicine, Genova, Italy,¹ and Department of Microbiology, School of Medicine, Palacky University, Olomouc, Czechoslovakia²

The taxonomic problem of coagulase-positive staphylococci of various origins is confronted in the present investigation by means of the bacteriolytic activity pattern analysis. A total of 103 strains isolated from various animal sources have been examined using the same assay system which in previous studies had been employed with staphylococci of human origin and had permitted their subdivision into six lyogroups. Two groups (*a* and *b*) of coagulase-positive staphylococci have been recognized by this method. Group *a* is shown to overlap exactly with the species *Staphylococcus aureus*, whereas group *b* overlaps with the recently described species *Staphylococcus intermedius*. The results obtained are discussed, as related both to most recent data dealing with the taxonomy of coagulase-positive staphylococci and to previous findings on bacteriolytic activity patterns of staphylococci of human origin.

It has been shown recently that coagulase-negative as well as coagulase-positive staphylococci excrete an easily detectable bacteriolytic activity (7). Properties of such activities have been shown to differ in various strains. Based on the analysis of these differences by a particular assay system, staphylococci of human origin have been subdivided into six different groups (lyogroups) (11). The strains included in each lyogroup were found to share the major biochemical and metabolic properties. In particular, a significant overlapping of the staphylococcal lyogroups with the staphylococcal species identified according to Schleifer and Kloos (5, 8) has been demonstrated (10). This analysis also brought to light the important finding that strains producing different lytic enzymes generally belong to species with a different chemical cell wall composition. Based on this fact, it has been suggested that association between a specific cell wall chemical composition and a specific lytic enzyme can be the biochemical basis for events of primary importance in cell physiology, such as cell wall growth, cell septation, or cell division. Therefore, the analysis of the bacteriolytic activity pattern has been proposed as a new and valuable taxonomic method, suggesting extensive applications.

In the above-mentioned studies, coagulase-positive strains were all found to fall into one lyogroup, i.e., lyogroup 1 (10). This finding was consistent with the known physiological and taxonomic homogeneity of coagulase-positive staphylococci of human origin (1). Significant differences, on the contrary, have been reported to occur among coagulase-positive staphylococci

of animal origin, depending on the animal source of the strains (1, 3, 4). Therefore the problem is to establish whether such differences are either expression of host-dependent minor variations or are major differential characteristics among different species of coagulase-positive staphylococci. Coagulase-positive staphylococci have already been subdivided into six biotypes (A to F) (3). Recently, the traditional species *Staphylococcus aureus*, previously thought to coincide with coagulase-positive staphylococci, has been limited to biotypes A to D, whereas biotypes E and F have been included into a new species named *Staphylococcus intermedius* (2).

In the present study, the bacteriolytic activity pattern analysis is used as a new way of approaching the taxonomic problem of coagulase-positive staphylococci of various origins. Two groups of coagulase-positive staphylococci have been recognized by this method; they are shown to overlap with *S. aureus* and *S. intermedius*, respectively.

MATERIALS AND METHODS

Bacterial strains. A total of 103 coagulase-positive staphylococci have been investigated. These strains have been obtained from various animal sources, using sheep or horse blood agar (blood agar base no. 2, Oxoid) and staphylococcus medium no. 110 (Oxoid) as isolating media. All strains had been analyzed previously for their physiological characteristics. The strains, their sources, classification, and the relative references are listed in Table 1.

Determination of the bacteriolytic activity pattern. The bacteriolytic activity pattern has been determined using the same assay system, composed of eight testing media, which in a previous study had

TABLE 1. *List of strains used*

Strain(s)	Source	Taxonomic position	Reference
Cowan I	Human	<i>Staphylococcus aureus</i> (biotype A)	
Wood 46	Human	<i>S. aureus</i> (biotype A)	
AT12, BD16, BE8, BZ20, O10	Human	<i>S. aureus</i> (biotype A)	10
B30, B61, B84	Human	<i>S. aureus</i> (biotype A)	4
S18, S24, S26, S31, S36 S42, S45, S50, S70, S83	Poultry	<i>S. aureus</i> (biotype B)	4
V1, V12, V18, V22, V26, V29, V32, V36, V43, V49	Swine	<i>S. aureus</i> (biotype B)	4
M1, M4, M15, M21, M30, M44, M57, M61, M76, M86	Cow	<i>S. aureus</i> (biotype C)	4
R7, R12, R27, R38, R40, R53, R60, R74, R78, R82	Sheep	<i>S. aureus</i> (biotype C)	4
Z1, Z8, Z20	Hare	<i>S. aureus</i> (biotype D)	4
K2, K6, K7, K15, K18, K20, K25, K28, K31, K69	Horse	<i>S. intermedius</i> (biotype E)	2
L1, L5, L18, L19, L27, L28, L95, L99, L107, L108	Mink	<i>S. intermedius</i> (biotype E)	2
P5, P11, P12, P16, P55, P59, P67, P71, P75, P76	Dog	<i>S. intermedius</i> (biotype E)	2
H2, H4, H11, H13, H14, H17, H20, H27, H30, H55	Pigeon	<i>S. intermedius</i> (biotype F)	2
G1, G3, G5, G7, G8, G26, G29, G34, G132, G141	Fox	<i>S. intermedius</i> (biotype F)	4

allowed subdivision of human staphylococci into six lyogroups (11). Preparation of the bacteriolytic activity substrates, preparation and inoculation of the media, and evaluation of the bacteriolytic activity were carried out by the procedures described (11).

Bacteriolytic activity pattern analysis of the strains studied was performed without knowing strain origin and identification.

RESULTS

The bacteriolytic activity of the 103 strains studied was examined on the eight media of the assay system. The different patterns obtained and the number of strains exhibiting the same pattern are presented in Table 2. Based on pattern analysis and comparison, the strains tested could be subdivided into two different groups, designated *a* and *b*. Within group *a*, two subgroups (*a1* and *a2*) were further recognized.

Group *a*. Fifty-three strains were included in group *a* (41 in subgroup *a1* and 12 in subgroup *a2*). These strains showed a moderate to fairly large zone of transparency on TP2 medium with a rather indistinct outline. The zone of transparency on TP2P was absent or strongly decreased (subgroup *a1*) or identical (subgroup *a2*) as compared with that on TP2: this was the essential

difference between the two subgroups. A very large zone of transparency was exhibited on TP1, generally with a sharp outline. A moderate activity was shown on T0; a large and well defined zone of transparency occurred on T1; no or very modest activity was found on T3. A well-defined zone of transparency appeared on B15TP1, whereas there was no activity on a61TP2.

Group *a* included all the strains previously identified as *S. aureus* on the basis of other physiological characteristics (see Table 1) and none of the other strains tested. The bacteriolytic activity patterns of subgroup *a1* strains could not be differentiated from those of lyogroup 1 strains (11) which, within isolates from humans, proved to perfectly overlap with *S. aureus* (10). Therefore a precise overlap between group *a* and the species *S. aureus* can be stated.

The strains and the lytic activity type pattern of group *a* are shown in Table 3.

Group *b*. Fifty strains were included in group *b*. These strains produced a moderate zone of transparency on both TP2 and TP2P. Some, however, showed a slight activity decrease on the latter medium in comparison with the former. All strains showed a larger zone of transparency on TP1. Most strains exhibited a mod-

TABLE 2. *Bacteriolytic activity patterns of the 103 strains tested on the assay system media*

Pattern no.	No. of strains	Bacteriolytic activity on assay system medium:								Group and no. of strains
		TP1	TP2	TP2P	T0	T1	T3	B15TP1	a61TP2	
1	14	+++	+	—	±	++	—	+	—	a1 (41)
2	7	+++	+	±	±	++	—	+	—	
3	9	+++	+	—	+	++	—	+	—	
4	7	+++	+	—	±	++	±	+	—	
5	1	+++	+	—	+	++	±	+	—	
6	3	+++	+	—	±	++	—	+	—	
7	7	+++	+	+	+	++	±	+	—	a2 (12)
8	3	+++	+	+	+	++	—	+	—	
9	2	+++	+	+	±	++	—	+	—	
10	24	++	+	+	+	++	—	—	—	b (50)
11	12	++	+	+	±	++	—	—	—	
12	3	++	+	+	+	++	±	—	—	
13	5	++	+	±	+	++	—	—	—	
14	6	++	+	±	+	++	±	—	—	

TABLE 3. *Strains and bacteriolytic activity type patterns of the groups identified*

Group	Strains	Species	Bacteriolytic activity type pattern on assay system medium:							
			TP1	TP2	TP2P	T0	T1	T3	B15TP1	a61TP2
a1	Cowan I Wood 46 AT12, BD16, BE8, BZ20, O10 B30, B61, B84 S18, S26 V1, V12, V18, V22, V26, V29, V32, V36, V43, V49 M1, M4, M15, M21, M30, M44, M57, M61, M76, M86 R7, R12, R27, R38, R40 R53, R60, R74, R78	<i>Staphylococcus aureus</i>	+++	+	—	±	++	—	+	—
a2	S24, S31, S36, S42, S45, S50, S70, S83 R82 Z1, Z8, Z20	<i>S. aureus</i>	+++	+	+	+	++	±	+	—
b	K2, K6, K7, K15, K18, K20, K25, K28, K31, K69 L1, L5, L18, L19, L27, L28 L95, L99, L107, L108 P5, P11, P12, P16, P55, P59, P67, P71, P75, P76 H2, H4, H11, H13, H14, H17, H20, H27, H30, H55 G1, G3, G5, G7, G8, G26, G29, G34, G132, G141	<i>S. intermedius</i>	++	+	+	+	++	—	—	—

erate activity on T0; a well defined zone of transparency was exhibited on T1; activity on T3 was either none or very modest. No zone of transparency appeared on B15TP1 and a61TP2. The bacteriolytic activity patterns of these strains were, on the whole, very similar to those of human strains included in lyogroup 2 (11).

Group *b* included all the strains previously identified as *S. intermedius* on the basis of other physiological characteristics (see Table 1) and

none of the other strains tested. Therefore a precise overlap between group *b* and the species *S. intermedius* can be stated.

The strains and the bacteriolytic activity type pattern of group *b* are shown in Table 3.

DISCUSSION

The present study, as compared with previous findings on bacteriolytic activity patterns of

staphylococci of human origin (10, 11), shows that coagulase-positive staphylococci do not represent a homogeneous group of strains, when isolates from various animal sources other than humans are also considered. Two different type patterns of bacteriolytic activity have in fact been recognized, and two respective groups of coagulase-positive staphylococci (*a* and *b*) have been identified.

A perfect overlap between group *a* and the species *S. aureus* has been demonstrated, as well as between group *b* and the species *S. intermedius*. This fact significantly confirms the separation of *S. intermedius* from *S. aureus* (2), and also enhances the value of bacteriolytic activity pattern analysis in species separation.

The subdivision of group *a* into the two subgroups *a1* and *a2*—essentially based on the occurrence of a lytic activity decrease on TP2P with respect to TP2—probably has minor relevance to bacteriolytic activity. In fact TP2P is a phenolphthalein diphosphate-containing medium, and we have already observed inhibition of bacteriolytic enzymes owing to phosphatase activity in phenolphthalein diphosphate-containing media (10, 11). Therefore the difference between subgroups *a1* and *a2* seems more likely to be linked to quantitative and/or qualitative differences in phosphatase production (6; Grazi et al., manuscript in preparation).

The similarity in bacteriolytic activity pattern between group *b* and lyogroup 2—the former corresponding to *S. intermedius* and the latter previously proven to overlap with Kloos and Schleifer's *S. simulans* (10)—suggests close physiological and taxonomic relations between these two groups of strains. Both have similarities to *S. aureus* and share several of its specific properties; both however, lack some of the most common characteristics of *S. aureus* and most other staphylococci: acetoin and pigment are not produced, and in peptone-containing media acid production from maltose is not detected (2, 5, 10). Moreover, differences in the reported cell wall compositions of *S. simulans* (5) and *S. intermedius* (9) do not seem particularly sharp, when considering certain source-dependent variations in the serine content of *S. intermedius* peptidoglycan (9). Both group *b* (or *S. intermedius*) in animals and lyogroup 2 (or *S. simulans*) in humans seem to occupy an intermediate position between *S. aureus* and *S. epidermidis*. Phylogenetic and ecological relations between

these two groups of strains seem worthy of further investigation.

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REPRINT REQUESTS

Address reprint requests to: Dr. Pietro E. Varaldo, Istituto di Microbiologia dell'Università di Genova, Viale Benedetto XV, 10, 16132 Genova, Italy.

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