The Relevance of Bacterial Lytic Activity in the Taxonomy of the Micrococcaceae: Failure of its Production by *Micrococcus* and *Planococcus* as Opposed to *Staphylococcus*

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INTRODUCTION

We have previously shown that almost all staphylococci, both coagulase-positive and coagulase-negative, produce a detectable lytic activity, and that there are significant differences in the lytic activities of *Staphylococcus aureus* and other staphylococci (Satta *et al.*, 1977). Subsequent studies have shown that the determination of the type of lytic activity is a valid method for species separation and identification among both coagulase-positive and coagulase-negative staphylococci (Varaldo *et al.*, 1978*a*; Varaldo & Satta, 1978; Varaldo *et al.*, 1978*b*). In this paper we analyse the production of this activity among micrococci and planococci and show that these micro-organisms do not generally produce any detectable lytic activity.

METHODS

Bacterial strains. The Micrococcaceae isolated in our laboratory were of human origin and were obtained from the healthy skins of 33 adults and 49 children. Preliminary strain discrimination was performed as suggested by Kloos *et al.* (1974). The Gram-positive and catalase-positive cocci were characterized and identified as to genus and species according to current criteria (Kloos *et al.*, 1974; Kloos & Schleifer, 1975*a, b*; Schleifer & Kloos, 1975*a, b*). Thirty-eight Micrococcaceae of various origins were supplied by the Czechoslovak Collection of Microorganisms (CCM): 27 strains were of *Micrococcus* (CCM nos 169, 268, 314, 337, 351, 410, 418, 559, 633, 679, 837, 839, 884, 2131, 2140, 2390, 2539, 2591, 2669, 2670, 2671, 2690, 2691, 2692, 2693, 2694, 2697), and 11 of *Planococcus* (CCM nos 316, 1849, 2069, 2104, 2387, 2388, 2389, 2414, 2415, 2416, 2706).

Determination of the ability to produce lytic activity. Heat-killed cells of *M. luteus* ATCC 4698 and of *M. luteus* AH-47 were used as substrates for lytic activity. Strain AH-47 was used because in some preliminary tests it proved to be more sensitive than strain ATCC 4698 to the lytic activity of some staphylococcal strains (unpublished data). Substrate suspensions were prepared and added to the molten media as described previously (Satta *et al.*, 1977). To test for the production of lytic activity, TP1-base and TP2-base media (Varaldo *et al.*, 1978*a*) were used; the former was supplemented with heat-killed cells of *M. luteus* ATCC 4698 (medium TP1) and the latter with heat-killed cells of *M. luteus* AH-47 (medium AH47TP2). The test media were poured into Petri dishes and allowed to solidify. Isolated colonies of strains to be tested for lytic activity production were picked up by a needle and stabbed into the two test media. Isolates showing a zone of transparency around the spots of bacterial growth on either of the two test media were recorded as lytic-activity positive, while those showing no zone of transparency were recorded as lytic-activity negative.

Determination of DNA base composition. The DNA, isolated from the various bacterial strains by the method of Marmur (1961), was dissolved in 0.1 × SSC buffer (SSC buffer: 0.15 M-NaCl plus 0.015 M-sodium citrate, pH 7.0) and checked for purity by ultraviolet absorbance scanning in a Beckman DB spectrophotometer. The DNA buoyant density was determined by equilibrium density gradient centrifugation in a Beckman model E analytical ultracentrifuge. The mol % guanine plus cytosine content of the DNA was calculated from the DNA buoyant density (ρ) by the formula: % (G + C) = $(\rho - 1.660)/0.00098$ (Schildkraut *et al.*, 1962).

Table 1. *Lytic activity production of the 282 strains studied*

No. of strains		Genus	Species	Lytic activity production
Freshly isolated strains	CCM strains			
121	4	<i>Micrococcus</i>	<i>M. luteus</i>	—
75	3	<i>Micrococcus</i>	<i>M. varians</i>	—
11	2	<i>Micrococcus</i>	<i>M. lylae</i>	—
6	2	<i>Micrococcus</i>	<i>M. sedentarius</i>	—
3	4	<i>Micrococcus</i>	<i>M. roseus</i>	—
3	3	<i>Micrococcus</i>	<i>M. kristinae</i>	—
0	4	<i>Micrococcus</i>	<i>M. nishinomiyaensis</i>	—
0	3	<i>Micrococcus</i>	<i>M. agilis</i>	—
0	1	<i>Micrococcus</i>	<i>M. halobius</i>	— *
7	0	<i>Micrococcus</i>	U	—
3	1	<i>Micrococcus</i>	<i>M. luteus</i>	+
3	0	<i>Staphylococcus</i>	<i>S. xylosus</i>	—
0	4	<i>Planococcus</i>	<i>P. citreus</i>	—
0	1	<i>Planococcus</i>	<i>P. halophylus</i>	—
0	6	<i>Planococcus</i>	U	—
8	0	U	U	—
4	0	U	U	+

U, Unidentified.

* The lytic activity of this strain (CCM 2591), which requires at least 5 % NaCl in the medium, was tested on modified media containing 5.5 % NaCl.

RESULTS AND DISCUSSION

Several hundred isolates obtained from healthy humans were identified at the genus level and tested for lytic activity production. *Staphylococcus* strains which produced lytic activity were not studied further. The remaining strains totalled 244; 27 additional representative strains of known micrococcal species and 11 planococci were also tested for lytic activity production. Out of the total 282 strains studied (Table 1), 252 were micrococci and did not produce lytic activity. Four micrococci, belonging to *M. luteus*, produced lytic activity. Three strains were lytic-activity negative staphylococci and identified as *S. xylosus*. For another 12 isolates (four producers and eight non-producers of lytic activity) the separation between *Staphylococcus* and *Micrococcus* was uncertain. None of the 11 *Planococcus* strains produced any lytic activity.

As DNA base composition is useful in separating micrococci from staphylococci (Silvestri & Hill, 1965; Kocur *et al.*, 1971), it was determined for the lytic-activity positive micrococci, the lytic-activity negative staphylococci and the strains of uncertain identity. The % G+C values of the three lytic-activity negative strains previously identified as *S. xylosus* were within the range for staphylococci. Likewise, the % G+C values of the four lytic-activity positive strains previously identified as *M. luteus* were within the range for micrococci. Of the 12 strains of uncertain classification, the eight lytic-activity negative strains showed G+C values ranging from 65.3 to 72.9 % which are consistent with that of *Micrococcus*, whereas the four lytic-activity positive strains showed G+C values ranging from 30.2 to 36.7 % which are consistent with that of *Staphylococcus*. The DNA base composition and other major characters of these strains are summarized in Table 2.

Thus, out of 264 *Micrococcus* and 11 *Planococcus* strains tested, all of the planococci and 98.5 % of the micrococci produced no lytic activity. Considering both our previous findings (Satta *et al.*, 1977; Valardo *et al.*, 1978a) and unpublished data from this laboratory, the incidence of lytic-activity negative *Staphylococcus* strains can be estimated as less than 0.5 %.

Table 2. DNA base compositions and major characteristics of 19 *Micrococcaceae*

Strain	Lytic activity production	Gly-cerol-erythro-mycin test*	Susceptibility to		Production of				Acid from†			Mol % G+C	Identification
			Lyso-zyne*	Lyso-staphin*	Pig-ment†	Oxidase	Acetoin	Phos-phatase	Glucose	Man-nose	Xylose		
K1-1	+	—	—	—	y	+	—	—	—	—	—	74.5	<i>M. luteus</i>
K1-6	+	—	—	—	y	+	—	—	—	—	—	73.4	<i>M. luteus</i>
Mi-8	+	—	—	—	y	+	—	—	—	—	—	72.4	<i>M. luteus</i>
CCM 559	+	—	—	—	y	NT	NT	NT	NT	NT	NT	73.1	<i>M. luteus</i>
Mi-15	+	—	±	±	c	—	±	—	—	+	—	32.6	<i>Staphylococcus</i> sp.
K1-17	+	—	±	±	gw	—	—	+	—	+	±	30.2	<i>Staphylococcus</i> sp.
K3-3	+	—	±	±	gw	—	—	—	—	+	±	34.6	<i>Staphylococcus</i> sp.
K3-11	+	+	+	+	gw	—	—	—	—	+	—	36.7	<i>Staphylococcus</i> sp.
K2-12	—	+	—	—	c	—	±	—	+	+	±	38.7	<i>S. xylosus</i>
K3-4	—	+	—	—	wc	—	—	—	+	+	+	33.5	<i>S. xylosus</i>
K3-12	—	—	+	+	gw	±	—	—	+	+	+	34.6	<i>S. xylosus</i>
Mb-19	—	±	+	+	yw	—	—	+	—	—	—	69.4	<i>Micrococcus</i> sp.
Md-13	—	±	+	+	c	±	—	—	—	±	—	65.3	<i>Micrococcus</i> sp.
Ma-7	—	—	±	±	gw	—	—	—	—	±	±	71.4	<i>Micrococcus</i> sp.
Ge-8	—	+	±	±	yw	—	—	—	—	+	—	66.3	<i>Micrococcus</i> sp.
Gr-9	—	—	±	±	c	±	±	—	—	+	—	68.8	<i>Micrococcus</i> sp.
Gs-18	—	±	—	—	wc	±	—	—	—	—	—	72.9	<i>Micrococcus</i> sp.
Ef-10	—	+	±	±	gw	—	—	—	—	±	—	68.2	<i>Micrococcus</i> sp.
Co-11	—	±	+	+	wc	—	±	—	—	±	—	71.0	<i>Micrococcus</i> sp.

+, Positive reaction; ±, weak reaction; —, negative reaction; NT, not tested.

* According to the test system of Schleifer & Kloss (1975a).

† c, Cream; gw, grey-white; wc, white-cream; y, yellow; yw, yellow-white.

‡ Tested under aerobic conditions.

The present study shows that planococci and most micrococci do not produce lytic activity, thus differing from staphylococci. This supports our original proposal of exploiting lytic activity as a new tool in bacterial taxonomy. Our studies on the lytic activity of the Micrococcaceae demonstrate the possibility of an initial separation of staphylococci from the other Micrococcaceae, followed by screening of staphylococci into the different species on the basis of their lytic-activity patterns (Varaldo *et al.*, 1978*a*; Varaldo & Satta, 1978; Varaldo *et al.*, 1978*b*). It is evident that these findings not only give taxonomists a new tool for systematic research, but also raise new possibilities in clinical bacteriology.

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