Routine Separation of Staphylococci from Micrococci Based on Bacteriolytic Activity Production

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A new method is described by which separation of staphylococci from micrococci can be achieved in routine laboratory use. The basis of this method is that bacteriolytic activity is produced by staphylococci but not by micrococci.

Methods for separation of staphylococci from micrococci, that are at the same time reliable, simple, and inexpensive, have not yet been developed. The classical monothetic method based on the ability of staphylococci, as opposed to micrococci, to produce acid from glucose under anaerobic conditions (1, 7, 8) is no longer acknowledged as satisfactory. It is now well established that some staphylococcal species fail to produce or produce only weak acid from glucose under anaerobic conditions, whereas some micrococci may anaerobically produce acid from this sugar (6, 9). Among the methods that have subsequently been proposed, the test system of Schleifer and Kloos (5) is being used most. However, though reliable, this method is still too complex to perform and read for an ideal routine test.

In a recent taxonomic report (G. Satta, P. E. Varaldo, M. Tenca, and L. Radin, *J. Gen. Microbiol.*, in press) we have shown that micrococci and planococci fail, as a rule, to produce bacteriolytic activity (BA), which is by contrast a general property of staphylococci (4, 11). Such a finding is exploited in the present study as the basis for a new and particularly reliable and simple method for routine separation of staphylococci from micrococci.

Most staphylococci and micrococci used in this work were isolated in our laboratory from clinical specimens or from the skin, nose, and throat of healthy volunteers. Several strains of various origins were obtained from personal collections (particularly large numbers were obtained from V. Hájek, Olomouc, Czechoslovakia, and P. B. Heczko, Krakow, Poland) and from official culture collections (Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia, and American Type Culture Collection, Rockville, Md.). Some of the strains used in this work have already been used and described in our previous studies (4, 10-12; G. Satta, G. Grazi, P. E. Varaldo, and R. Fontana, *J. Clin. Pathol.*, in press; G. Satta et al., *J. Gen. Microbiol.*, in

press). In total, 2,347 *Staphylococcus* and 328 *Micrococcus* isolates were tested, which included representatives of all recognized species of both genera. For the strains isolated in our laboratory, or those uncompletely identified by others, the *Staphylococcus/Micrococcus* separation was carefully determined by current methods (6) or by analysis of the DNA base composition whenever even a little doubt remained. Some of the strains tested for DNA base composition, as well as the assay procedure used, have already been reported (G. Satta et al., *J. Gen. Microbiol.*, in press).

All strains were tested for their ability to produce BA by using the test medium AH47TP2, i.e., TP2 base medium (11) containing heat-killed cells of *Micrococcus luteus* AH-47 (13). This strain was employed because some preliminary tests showed it to be more sensitive than other micrococci, including *M. luteus* ATCC 4698, to the BA of some staphylococcal strains (unpublished data). The substrate suspension was prepared and added to the melted base medium as described previously (4). Isolated colonies of strains to be examined were picked up by a needle and stabbed into the test medium. After 6 to 48 h of incubation, the isolates showing a zone of transparency around the spots of bacterial growth were recorded BA positive, whereas those exhibiting no zone of transparency were denoted BA negative.

BA was found to be produced by 2,340 staphylococci out of the 2,347 tested and 4 micrococci out of the 328 tested (Table 1). Therefore, virtually all staphylococci were BA positive, whereas virtually all micrococci were BA negative. Strain identification, performed according to current methods (2, 3), indicated that the seven BA-negative staphylococci all belonged to the same species (*S. xylosus*), and likewise did the four BA-positive micrococci (*M. luteus*). The strains of both groups represented a minority within their respective species, most *S. xylosus* strains being BA positive and belonging to lyo-

group IV (10), as well as most *M. luteus* strains being BA negative (G. Satta et al., J. Gen. Microbiol., in press).

Several features of these anomalous strains were examined, and two useful observations were made: (i) all of the seven BA-negative *S. xylosus* strains produced phosphatase, as opposed to all micrococci, which did not; (ii) all of the four BA-positive *M. luteus* strains produced an intense yellow pigment, whereas no *Staphylococcus* strain was found to produce a similar pigment. Therefore, a practical scheme to exploit the BA test in the routine separation of staphylococci from micrococci is shown in Fig. 1.

The BA test requires preparing one simple medium that does not involve the use of any sophisticated chemicals outside those normally employed in routine laboratories. Test performance requires only that a colony be picked up by a needle and stabbed into the test medium. Up to 15 isolates can be tested on a single plate. The BA test can most often be read within 6 to 18 h or, more rarely, within 24 or 48 h when the negative behavior of slow-growing micrococci has to be established. Test reading only requires checking for the presence of a zone of transparency around the spots of bacterial growth; when BA-positive strains are tested such a zone of transparency is quite evident, so that no problem arises in result evaluation or interpretation. Due to these characteristics, the BA test is an ideal test in its simplicity, rapidity, and inexpensiveness. The test is also very satisfactory for its reliability and correlates very well with DNA base composition. The BA test error (0.3% of

staphylococci and 1.2% of micrococci), though quite acceptable for a routine test, can be reduced virtually to zero by examining two features very easy and rapid to test for, such as pigment and phosphatase. The yellow pigment, when produced, is easily recognized on the same BA test medium. Phosphatase activity can be determined by several procedures, including two particularly simple methods recently developed in our laboratory: one allows the determination of phosphatase production within 2 to 4 h (L. Pippo, Ph. D. thesis, University of Genova, 1976; P. E. Varaldo et al., manuscript in preparation); the other allows phosphatase activity to be evaluated either on the same BA test medium or even on the isolating medium (G. Satta et al., J. Clin. Pathol., in press).

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TABLE 1. BA production by 2,347 *Staphylococcus* and 328 *Micrococcus* strains

Genus	BA positive		BA negative	
	No. of strains	% ^a	No. of strains	% ^a
<i>Staphylococcus</i>	2,340	99.7	7	0.3
<i>Micrococcus</i>	4	1.2	324	98.8

^a Referred to the total number of strains of either genus.

BA	+ → Yellow pigment	- → <i>Staphylococcus</i>
		+ → <i>Micrococcus</i> (a)
	- → Phosphatase	- → <i>Micrococcus</i>
		+ → <i>Staphylococcus</i> (b)

FIG. 1. Scheme for routine separation of staphylococci from micrococci based on BA production. (a) Minority of strains of the species *M. luteus*; (b) minority of strains of the species *S. xylosus*.