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A Simple Test System for the Separation of Staphylococci from Micrococci

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A simple test system for the separation of staphylococci from micrococci is described, which is based on the ability of staphylococci to produce acid aerobically from glycerol in the presence of 0.4 μg of erythromycin per ml and on their sensitivity to lysostaphin.

The most widely accepted routine test for identifying staphylococci is based on their ability to produce acid from glucose under anaerobic conditions, while micrococci lack this ability (1, 9, 10). The separation of micrococci and staphylococci by the classical oxidation-fermentation test, however, raises several problems, since recent systematic studies have identified certain species of staphylococci that may fail to produce or that produce only small amounts of acid from glucose under anaerobic conditions and micrococci that may anaerobically produce small to moderate amounts of acid from glucose (3, 4, 5, 6, 8). In the present study, we shall describe some properties of these organisms which can be determined by simple techniques and can be used for the accurate separation of staphylococci from micrococci in the routine laboratory.

Staphylococci and micrococci used in this study were isolated from human skin (4, 5, 8), dust, food (enterotoxin-producing *Staphylococcus aureus* strains obtained from H. J. Sinell, Berlin), animals (*S. aureus* strains of biotypes A-F obtained from V. Hajek, Olomouc, Czechoslovakia, and coagulase-negative cocci obtained from M. Roguinsky, Nouzilly, France), and wounds and abscesses from hospitalized patients (coagulase-negative, deoxyribonuclease-positive strains of staphylococci obtained from T. Waldström, Stockholm, Sweden). In addition, several strains used were obtained from different culture collections (American Type Culture Collection, Rockville, Md.; Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia; National Collection of Type Cultures, London, England). In total, 425 randomly selected strains were tested, including representatives of all presently recognized species of *Micrococcus* (5) and *Staphylococcus* (4, 8).

The ability of strains to produce acid aerobically from glycerol in the presence of 0.4 μg of erythromycin per ml was studied by using the following medium (pH 7.0): $NH_4H_2PO_4$, 1 g; KCl, 0.2 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; yeast extract, 2 g; glycerol, 10 ml; bromocresol purple, 0.04 g; agar (Oxoid), 9 g; and distilled water, 1,000 ml. Purple agar base (Difco) can also be used in place of the above medium. A 4-mg sample of erythromycin was dissolved in 0.5 ml of 95% ethanol, filled up to 10 ml with distilled water, and sterilized by filtration. The basal medium was autoclave sterilized and then was cooled down to 45 to 50 C before adding 1 ml of the sterile erythromycin solution to 1,000 ml of basal medium. Six to eight culture streaks were radially applied to each prepared, dried agar plate. Cultures were incubated at 35 C for 27 to 48 h. The sensitivity of strains to lysostaphin and lysozyme was tested by an agar overlay technique as previously described (5, 8).

Studies on gram-positive, catalase-positive cocci isolated from human skin have shown that all 939 strains of staphylococci tested produced acid aerobically from glycerol (4, 8), while from 650 strains of micrococci only 38 strains were able to produce acid from glycerol (5). In the course of the same studies it also became evident that the growth of most micrococci were inhibited by erythromycin at low levels (0.1 to 0.2 μg/ml), whereas all of the staphylococci were resistant to at least 0.4 μg/ml. From these observations and the results of lysostaphin and lysozyme sensitivity tests (4, 5, 8), we concluded that a test system combining the lytic action of lysostaphin and lysozyme and the fermentation of glycerol in the presence of erythromycin should form the best practical scheme to separate staphylococci from micrococci in the routine laboratory. The results obtained from this test system are compiled in Table 1. The staphylococci were sensitive or slightly resistant

TABLE 1. *A simple test system for the separation of staphylococci from micrococci*

Genus	No. of strains tested	Resistance to		Acid (aerobically) from glycerol-erythromycin medium (0.4 μg of erythromycin/ml)
		Lysozyme (25 μg/ml)	Lysostaphin (200 μg/ml)	
Staphylococcus	313	+	−, (±)[a]	+
	6	+	−	−
Micrococcus	89	+, (±)	+	−
	12	−	+, ±, −	−
	5	+	+	+

[a] Parentheses around a symbol denote a frequency of <20%. Symbols: +, resistant (no visible growth inhibition) or acid production (yellow indicator color under culture streak or zone of yellow indicator color surrounding culture streak); ±, slightly resistant (partial growth inhibition); and −, sensitive (complete growth inhibition) or no acid production.

to lysostaphin and were resistant to a low concentration of lysozyme. Most of the strains which were slightly resistant to lysostaphin produced acid from glycerol. In contrast, the slightly lysostaphin-resistant or lysostaphin-sensitive micrococci did not produce acid from glycerol and were sensitive to lysozyme. Only six out of 319 strains of staphylococci did not produce acid from glycerol. These strains, however, were easily distinguishable from micrococci since they were sensitive to lysostaphin and resistant to lysozyme. Most strains of micrococci tested were resistant to both lysostaphin and lysozyme and did not ferment glycerol. Only five strains were able to produce acid from glycerol in the presence of erythromycin. These strains, however, could be easily separated from staphylococci by their resistance to lysostaphin.

The proposed test system provides several advantages in comparison to the standard oxidation-fermentation test (7, 9). First, the separation of staphylococci from micrococci is more satisfactory and correlated with deoxyribonucleic acid base composition (4, 5, 8). Second, it is easier to perform than tests used to detect the anaerobic fermentation of glucose. Third, the results are obtained much quicker than with the classical methods. Usually the staphylococci can be recognized within 6 to 18 h and even the micrococci, which grow slower, can be identified after 24 to 48 h. With regard to the lysostaphin test, it should be mentioned that the composition of the growth medium is important. The growth of staphylococci in a serine-rich medium results in an increase in serine content of the cell wall (7) and this renders the cells more resistant to lysostaphin (2). In complex media, the source of peptone will influence the amino acid concentration. Peptone prepared from meat (e.g., Bacto-peptone) exhibits a high glycine and a relatively low serine content, whereas peptone prepared from casein is much lower in glycine and relatively high in serine content (7). Thus, if one uses media which contain peptone from casein, one has to supplement them with 0.2 to 0.3% of glycine for the lysostaphin test, whereas in media prepared with peptone from meat no additions have to be made.

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LITERATURE CITED

1. Baird-Parker, A. C. 1963. A classification of micrococci and staphylococci based on physiological and biochemical tests. J. Gen. Microbiol. **30**:409-427.
2. Browder, H. P., P. A. Tavormina, and W. A. Zygmunt. 1968. Optical configuration of staphylococcal cell wall serine. J. Bacteriol. **96**:1452-1453.
3. Evans, J. B., and W. E. Kloos. 1972. Use of shake cultures in a semisolid thioglycolate medium for differentiating staphylococci from micrococci. Appl. Microbiol. **23**:326-331.
4. 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.
5. Kloos, W. E., T. G. Tornabene, and K. H. Schleifer. 1974. Isolation and characterization of micrococci from human skin, including two new species: *Micrococcus lylae* and *Micrococcus kristinae*. Int. J. Syst. Bacteriol. **24**:79-101.
6. Schleifer, K. H. 1973. Chemical composition of staphylococcal cell walls, p. 13-23. *In* Contributions to microbiology and immunology, vol. 1. S. Karger, Basel.
7. Schleifer, K. H., W. P. Hammes, and O. Kandler. 1975. Effect of endogenous and exogenous factors on the primary structure of the peptidoglycan. Adv. Microb. Physiol., vol. 13.
8. 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.
9. Subcommittee on Taxonomy of Staphylococci and Micrococci. 1965. Recommendations. Int. J. Syst. Bacteriol. **15**:109-110.
10. Subcommittee on Taxonomy of Staphylococci and Micrococci. 1971. Minutes of meeting. Int. J. Syst. Bacteriol. **21**:161-163.