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Rapid Lysostaphin Test to Differentiate *Staphylococcus* and *Micrococcus* Species

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A rapid, simple lysostaphin lysis susceptibility test to differentiate the genera *Staphylococcus* and *Micrococcus* was evaluated. Of 181 strains from culture collections, 95 of 95 *Staphylococcus* strains were lysed, and 79 of 79 *Micrococcus* strains were not lysed. The seven *Planococcus* strains were resistant. Clinical isolates (890) were tested with lysostaphin and for the ability to produce acid from glycerol in the presence of erythromycin. Overall agreement between the methods was 99.2%. All clinical *Micrococcus* strains (43) were resistant to lysostaphin, and all clinical *Staphylococcus* strains (847) were susceptible. Seven of the *Staphylococcus* strains did not produce acid from glycerol in the presence of erythromycin. This lysostaphin test provides results in 2 h. It is easier to perform than previously described lysostaphin lysis methods. It is also more rapid and accurate than the glycerol-erythromycin test.

In the eighth edition of *Bergey's Manual of Determinative Bacteriology* (2), the family *Micrococcaceae* consists of the genera *Staphylococcus*, *Micrococcus*, and *Planococcus*. Differentiation between the two former genera is important because of the pathogenic potential of staphylococci. Several differentiation tests have been used, including the ability of staphylococci to produce acid anaerobically from glucose (3).

The isolation of lysostaphin (9) provided a simple and rapid method for differentiating the two genera. "*Staphylococcus staphylolyticus*" produces an extracellular enzyme which lyses other species of staphylococci. The enzyme (endopeptidase) breaks the glycyl-glycine peptide linkages which occur in the cell walls of staphylococci. *S. aureus* is more susceptible to lysis than is *S. epidermidis*, but *Micrococcus* spp. are resistant (12, 13). Lachica et al. (7) found lysostaphin lysis to be more precise and convenient than anaerobic glucose fermentation in distinguishing between the genera.

Schleifer and Kloos (10) described a simple test system based on the ability of staphylococci to produce acid aerobically from glycerol in the presence of erythromycin and on their susceptibility to lysostaphin, a method previously described by Kloos et al. (6).

Gunn et al. (5) compared several methods for differentiating these genera and concluded that the most accurate, simple, and rapid was the lysostaphin test. Parisi (8) commented that it was doubtful that the test would be used routinely in clinical laboratories because of its many variables, including the influence of the culture medium on lysis and the cost of lysostaphin. Technical advances have now made this test very promising for routine use, as reported here.

MATERIALS AND METHODS

Organisms. A total of 181 strains obtained from culture collections and representing all the genera belonging to the family *Micrococcaceae* were tested. Many were used in the comprehensive taxonomic study of Feltham (4).

The strains were as follows (number tested): *S. aureus* (13), *S. epidermidis* (11), *S. intermedius* (5), *S. hominis* (5), *S. saprophyticus* (10), *S. haemolyticus* (8), *S. simulans* (6), *S. capitis* (5), *S. warneri* (4), *S. cohnii* (6), *S. xylosus* (6), *S. sciuri* subsp. *sciuri* (7), *S. sciuri* subsp. *lentus* (4), *S. hyicus* (1), *Staphylococcus* spp. (4), *Micrococcus sedentarius* (5), *M. nishinomiyaensis* (10), *M. lylae* (5), *M. kristinae* (5), *M. luteus* (15), *M. roseus* (10), "*M. mucilaginosus*" (7), *M. agilis* (4), "*M. radiodurans*" (2), "*M. radiophilus*" (1), "*M. radioproteolyticus*" (1), *M. halobius* (1), "*M. maripuniceus*" (1), *M. freudenreichii* (1), *M. varians* (9), *Planococcus* sp. (7), and *Micrococcus* sp. (2).

The strains were obtained from the following: American Type Culture Collection, Rockville, Md.; National Collection of Type Cultures, Central Public Health Laboratory, London, England; National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland; National Collection of Dairy Organisms, Shinfield, Berkshire, England; Czechoslovak Collection of Micro-organisms Brno, Czechoslovakia; Colworth Bacterial Culture Collection, Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford, England; W. E. Kloos, Department of Genetics, North Carolina State University, Raleigh; Deutsche Sammlung von Mikroorganismen, Gottingen, Federal Republic of Germany; and Department of Microbiology, University of Leicester, Leicester, England.

A total of 890 catalase-positive, gram-positive cocci were collected from clinical specimens. These were tested with lysostaphin and glycerol-erythromycin. All cultures were grown on Columbia agar (Oxoid Ltd., Basingstoke, England) supplemented with 7% defibrinated horse blood.

Lysostaphin test. Lysostaphin-coated paper disks (Roche Products Ltd., Welwyn Garden City, Hertfordshire, England) were used. Phosphate-buffered saline (2 ml) was pipetted into a tube. A suspension of the strain to be tested was prepared to match McFarland opacity standard number 1. One milliliter of this suspension was added to each of two tubes. A lysostaphin disk was added to one tube, which was then shaken vigorously. The second tube was a control. Both tubes were incubated at 37°C for 2 h in a water bath. A positive reaction was indicated by clearing of the turbidity in the tube with the lysostaphin disk in comparison with the control tube. A negative reaction was indicated by identical turbidity in both tubes.

Biochemical tests. Acid production from glycerol in the

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presence of erythromycin was tested by the method of Schleifer and Kloos (10), with readings taken at 24 and 48 h.

A total of 98 of the clinical isolates were tested by the APISTAPH identification system, used as recommended by the supplier (API Laboratories, Basingstoke, Hampshire, England; available in the United States as Staph Trac from Analytab Products, Plainview, N.Y.).

RESULTS

All 95 strains of staphylococci examined were susceptible to lysostaphin. The 79 micrococci and 7 planococci were resistant. Of 847 clinical *Staphylococcus* strains which were susceptible to lysostaphin, 840 produced acid from glycerol in the presence of erythromycin. It was necessary to incubate for 48 h to obtain a positive glycerol-erythromycin result in 69 of the strains. The seven strains which were susceptible to lysostaphin but negative in the glycerol-erythromycin test did not produce acid in glycerol-peptone water in the absence of erythromycin. They were identified by APISTAPH as staphylococci: three strains were *S. simulans*, one was *S. aureus*, and three gave a low discriminatory result between *S. aureus* and *S. simulans*. All 43 clinical *Micrococcus* strains which were resistant to lysostaphin were glycerol-erythromycin negative.

A total of 88 lysostaphin-susceptible and 10 lysostaphin-resistant clinical isolates were tested by the APISTAPH system. The 88 lysostaphin-susceptible strains were identified (number of strains) as *S. epidermidis* (28), *S. aureus* (9), *S. haemolyticus* (11), *S. hominis* (9), *S. simulans* (9), *S. warneri* (9), *S. cohnii* (3), *S. saprophyticus* (2), *S. capitis* (2), *S. hyicus* (1), and *S. xylosus* (1); APISTAPH was not able to identify to species level 4 strains which, however, had a test pattern consistent with that of the genus *Staphylococcus*.

The APISTAPH test pattern of the 10 lysostaphin-resistant strains indicated that these strains were *Micrococcus* spp.

DISCUSSION

DNA homology and cell wall composition are the characteristics most useful in separating *Micrococcus* and *Staphylococcus* species (8). The cell wall peptidoglycan of staphylococci contains large amounts of glycine, whereas micrococci have little glycine. Glycine is related to lysostaphin susceptibility. The spot lysostaphin susceptibility test (6) was demonstrated to be quite reliable (11). However, Baker (1) found that a commercially available lysostaphin test was less specific. He found that all *Micrococcus* strains tested were resistant but that 73% of lysostaphin-resistant organisms were *Staphylococcus* spp. In the present study, no *Staphylococcus* strain was resistant to lysostaphin.

The objections of Parisi (8) were not valid with respect to the test method described here. Our test was performed by a standard method with a phosphate-buffered saline solution, not a complex culture medium.

The test was easy to perform, with results in 2 h from initial isolation. This test was more rapid than previously described lysostaphin susceptibility tests. It should be useful in the food industry as well as in clinical applications.

We conclude that the lysostaphin test used in this study is an accurate method for differentiating staphylococci from micrococci and that results are obtained far more rapidly than with the glycerol-erythromycin test.

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