Evaluation of the Minitek Gram-Positive Set for Identification of Streptococci Isolated from Bovine Mammary Glands†

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A total of 127 isolates were used to evaluate the Minitek Gram-Positive Set for identification of streptococci cultured from bovine mammary glands. The overall accuracy of the Minitek Gram-Positive Set was 34.6%. Of 12 Streptococcus agalactiae strains, 4 (33.3%) were correctly identified. Of 43 Streptococcus dysgalactiae strains, 32 (74.4%) were identified correctly. Of 44 Streptococcus uberis strains, 42 (95.5%) were identified as Enterococcus spp. Poor performance was attributed to the limited number of veterinary strains in the data base. Incorporation of large numbers of veterinary isolates into the data base is needed for further development of this system.

Gram-positive, catalase-negative cocci belonging to the family Streptococcaceae are isolated frequently from bovine intramammary infections (IMI) (12, 13, 19). This group of organisms contains the contagious pathogen Streptococcus agalactiae, as well as the environmental streptococci Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus bovis, and enterococci (1, 13). Streptococci belonging to Lancefield group G are also isolated from bovine IMI (13, 18). Past methods (2, 13, 14) for the identification of streptococci isolated from bovine IMI separated these organisms into the aforementioned species.

Recent studies (3–5, 7, 9–11, 16, 17) of the physiochemical characteristics of the Streptococcaceae have resulted in a dramatic restructuring of this family. The enterococci and lactococci have been placed into the separate genera, Enterococcus and Lactococcus, respectively (16, 17). The description of S. dysgalactiae now encompasses strains previously designated Streptococcus equisimilis, group L streptococci, and human group G streptococci (9). Bovine and canine group G strains are now placed in a separate species, Streptococcus canis (7). Streptococcus equi and Streptococcus zooepidemicus were determined to be related at the species level as S. equi (9). However, since these two organisms can be differentiated by biochemical tests, subspecies status was retained (9). The group D nonenterococci, Streptococcus equinus and S. bovis, have been combined into the single species S. equinus (10). Additionally, two new species, Streptococcus alactolyticus and Streptococcus scharlatorycs, have been described (10).

Past methods (2, 13, 14) for identification of mastitis streptococci have relied upon conventional macrotube techniques. However, these methods are tedious and time-consuming. The Minitek Gram-Positive Set (MGPS) (BBL Microbiology Systems, Cockeysville, Md.) is a commercial system for the 24-h identification of staphylococci, micrococc, and streptococci. Although a recent evaluation (20) of the MGPS with staphylococci of bovine mammary gland origin yielded an accuracy level of 87.7%, accuracy with streptococci of bovine origin has not been established. The purpose of this study was to determine the accuracy of the MGPS for identification of streptococci isolated from bovine mammary glands.

MATERIALS AND METHODS

Cultures. A total of 127 streptococcal isolates of bovine mammary gland origin were used. The following reference strains were included in the study: S. agalactiae ATCC 27956, S. dysgalactiae ATCC 27957, S. uberis ATCC 19436, S. uberis ATCC 27958, S. uberis NCFB 2018, S. uberis NCFB 2038, S. bovis ATCC 27960, and S. bovis ATCC 33317. All isolates were identified by a conventional method described elsewhere (18) and the Rapid Strep system (Analytab Products, Plainview, N.Y.). Only isolates yielding the same identification with both systems were used in the study. Isolates were stored in full-strength Trypticase soy broth (BBL) at −20°C until activated. Each isolate was serially cultured twice on 5% bovine blood agar prior to testing.

MGPS. The MGPS consists of a rigid, transparent plastic plate with 20 wells into which substrate-impregnated paper disks are dispensed. The following substrate disks are included in the MGPS: arginine, arabinose, galactose, inulin, lactose, esculin, maltose, mannitol, mannose, raffinose, β-glucosidase, salicin, sorbitol, trehalose, glucose with nitrate, Voges-Proskauer, phosphatase, hippurate, pyrogallatmate (PYR), and leucine.

Procedures were performed as directed by the manufacturer. Briefly, test organisms were removed from a blood agar plate with a sterile cotton swab and suspended in 1.5 ml of MGPS broth until turbidity was equivalent to a McFarland standard of 1. An automatic pipette was used to dispense 0.05 ml of inoculum into each substrate well, with the exception of the arginine well. An inoculum volume of 0.1 ml was dispensed into the arginine well and overlaid with 0.1 ml of sterile mineral oil. Plates were covered, placed in a humidity chamber, and incubated at 37°C for 24 h. Appropriate reagents were added for determination of acetoin, nitrate reduction, hippurate hydrolysis, phosphatase, leucine aminopeptidase, and PYR arylamidase. Positive reactions were recorded and converted into a seven-digit profile number for species identification. The profile number was accessed in the MGPS profile index. The profile index provided an identification selection, confidence value, bio- type validity (frequency of occurrence), supplemental tests,
and atypical test results. All isolates yielding unlisted profile numbers were retested.

RESULTS

Overall, the MGPS identified 44 of 127 isolates (34.6%) correctly (Table 1). Of 12 S. agalactiae strains tested, 4 (33.3%) were identified correctly. A total of three PYR-positive strains were misidentified as Streptococcus pyogenes, and three were misidentified as group CFG streptococci. One trehalose-negative strain was identified as S. equi. The remaining strain was identified as Streptococcus intermedius (esculin positive, phosphatase negative).

Of the 43 S. dysgalactiae strains, 32 (74.4%) were identified as group CFG. A total of four β-glucosidase-negative strains were misidentified as Streptococcus mitis. Of six hippurate-positive strains, four were identified as S. intermedius and two were identified as S. agalactiae. A single sorbitol-positive strain was identified as a S. pyogenes.

A total of 44 S. uberis strains were included in the study. Of these, none was correctly identified as S. uberis. A total of 42 strains were identified as enterococci: 11 as Enterococcus faecalis, 30 as Enterococcus faecium, and 1 as Enterococcus durans.

Of 10 S. equinus strains, 2 (20.0%) were identified by the previous designation, S. bovis. Seven of the remaining eight strains were identified as S. salivarius. One strain was identified as Streptococcus sanguis. The single S. saccharolyticus strain included in the study was misidentified as an E. faecalis strain.

Of 17 E. faecalis strains, 6 (35.3%) were correctly identified by the MGPS. The remaining 11 E. faecalis strains (64.7%) were identified as E. faecium strains.

DISCUSSION

The poor performance of the MGPS in identifying streptococci of bovine mammary gland origin is probably due to the limited number of veterinary strains in the database. Previous evaluations (20–22) of commercial identification systems with staphylococci isolated from bovine mammary glands revealed similar deficiencies. Poutrel and Ryniewicz (15) evaluated the API 20 Strep system (marketed as the Rapid Strep system in the United States) with streptococci isolated from bovine mastitis and placed overall accuracy of the system at 71.4%. These workers concluded that an improved identification key was needed to enhance the accuracy of the system with bovine mastitis streptococci. It appears that veterinary isolates differ sufficiently from human isolates to reduce the accuracy of these systems.

Of 12 S. agalactiae strains tested, only 4 were correctly identified. Of the 5 misidentified strains, 3 were misidentified as S. pyogenes due to a positive PYR test. While human S. agalactiae strains are considered PYR negative (8), a recent study (18) determined that 34.0% of bovine S. agalactiae strains were PYR positive. Incorporation of bovine S. agalactiae strains would improve recognition of PYR-positive strains.

Species in Lancefield group C are involved in a variety of animal diseases, and veterinary diagnostic laboratories must provide species-level identification for this group of organisms (6). The MGPS database identifies S. equi and S. zooepidemicus to the species level rather than the currently accepted subspecies level (9). Furthermore, the MGPS correctly placed 74.4% of S. dysgalactiae strains in the group CFG category, but none was identified to the species level. Since S. dysgalactiae is the most frequently isolated group C streptococcus from bovine IMI (13, 18) and may be spread both during and between milkings (1), species-level identification is important for the development of accurate disease management programs.

The environmental streptococci include S. uberis, Enterococcus spp., and S. equinus (S. bovis). This group of organisms is not controlled by traditional mastitis control methods, and identification of these organisms is important for epidemiological purposes. Most strains of S. uberis, the most frequently isolated environmental streptococcus, were identified as Enterococcus spp. due to positive PYR hydrolysis. The MGPS biochemical chart lists S. uberis as PYR negative; however, a recent study (18) determined that 98.2% of S. uberis strains were PYR positive and that this characteristic was useful for the differentiation of S. uberis from other nongroupable streptococci. The inclusion of bovine S. uberis strains into the MGPS database would enhance the ability of the system to separate PYR-positive S. uberis strains from the enterococci. This would permit the correct identification of 95.5% of S. uberis strains in the present study.

The MGPS system identified only 2 of 10 S. equinus strains correctly by the previous designation, S. bovis. These strains were misidentified as S. salivarius due to failure to utilize trehalose. These strains also failed to use trehalose by conventional methods. Differences in trehalose utilization may be due to incubation conditions. The Rapid Strep system requires anaerobic incubation of the inoculum and the strip for all streptococcal isolates, whereas the MGPS recommends anaerobic incubation of the Minitest plate for viridans group streptococci. However, all strains possessed the group D antigen, and other biochemical characteristics were consistent with S. equinus.

S. saccharolyticus, previously included in S. bovis, is a newly described species which has been isolated from straw bedding material and teat skin (10). However, a recent study (18) demonstrated that this organism was isolated frequently from teat canals and IMI. Furthermore, this organism was probably misidentified as S. uberis by past identification.
methods (18). The single *S. saccharolyticus* strain included in the present study was misidentified as *E. faecalis*. Inclusion of strains of this species in the MGPS data base would further enhance the accuracy of this system.

The MGPS identified only 6 of 17 (35.3%) *E. faecalis* isolates correctly. The remaining 11 isolates were identified as *E. faecium*. Since the distributions of these two species in bovine mastitis are the same (19), genus-level identification for the enterococci is adequate.

In summary, the MGPS identified only 34.4% of streptococci isolated from bovine mammary glands. Poor performance may be attributed to the limited number of veterinary strains in the data base. Indeed, incorporation of additional veterinary isolates as suggested to permit identification of PYR-positive *S. agalactiae* strains, separation of *S. uberis* from enterococci, and acceptance of genus-level identification of enterococci as sufficient would increase the overall accuracy of the system to 78.7%. Thus, incorporation of veterinary isolates into the MGPS data base would enhance the utility of this system in veterinary bacteriology.

**LITERATURE CITED**