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Evaluation of the API 20 Strep System for Species Identification of Streptococci Isolated from Bovine Mastitis

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A new commercial system (API 20 Strep) for the species identification of streptococci associated with bovine mastitis was compared with the conventional biochemical methods. A total of 84 strains, including *Streptococcus agalactiae* (13), *S. dysgalactiae* (16), *S. uberis* (24), *S. faecalis* (12), *S. faecium* (5), and *S. bovis* (14) were tested. Using profile index and table data issued by the manufacturer, we identified 71.4% of strains by the API 20 Strep system. Our results indicate that improvement of the identification key is needed for accurate identification of streptococci isolated from bovine mastitis.

Identification of *Streptococcus* species involved in bovine udder infection has clinical and epidemiological importance for therapeutic decision and development of methods to control mastitis. Usually, serological typing is used in addition to biochemical methods. Recently, rapid and simple slide agglutination tests were introduced for serotyping (6), but conventional methods to obtain a complete biochemical profile required up to 72 h (4). A new 4-h commercial system for the identification of streptococci became available and was tested for human strains (5). The purpose of this report was to compare another system, API 20 Strep (API system, La Balme les Grottes, France), with conventional biochemical methods for the species identification of streptococci isolated from bovine mastitis. (This system is identical to the DMS Rapid Strep system by DMS Laboratories Inc., Flemington, N.J.)

MATERIALS AND METHODS

Bacterial strains. A total of 84 strains (in our collection) previously isolated from bovine milk samples collected at random were used in this evaluation. The 84 strains included 13 strains of *Streptococcus agalactiae*, 16 of *S. dysgalactiae*, 24 of *S. uberis*, 14 of *S. bovis*, 12 of *S. faecalis*, and 5 of *S. faecium*. Streptococci were identified by using tests and schemes previously described (3, 4). The following biochemical tests were performed: hippurate arginine and esculin hydrolysis, fermentation of mannitol, sorbitol, inulin raffinose, and trehalose, camp reaction, ability to grow at 10 and 45°C. All strains were serologically grouped with specific antisera after the Lancefield extraction procedure (2).

API test. All primary cultures in phosphate-buffered broth base with glucose (7) were streaked on sheep blood agar plate and examined for purity. The API 20 Strep was evaluated according to the instructions of the manufacturer. Briefly, a suspension was made from the culture grown on sheep blood agar plate by transferring a heavy inoculum of the culture to a tube containing 2 ml of distilled water. Three drops of suspension were placed into each microcapsule by using a Pasteur pipette. Strips were incubated at 37°C in a normal atmosphere. After 4 h of incubation, reagents were added and strips were exposed to a strong light (100 W, 10 s) for reading enzymatic activities. Test results were then recorded, and the strips were incubated again for 20 h. The interpretation of test results obtained at 4 and 24 h of incubation for identification of *Streptococcus* species was based on the profile index and table issued by the manufacturer.

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RESULTS AND DISCUSSION

A total of 60 strains (71.4%) were identified correctly by the API 20 Strep system (Table 1), using both the profile index and the table issued by the manufacturer. Most organisms (66.7%) were identified after 4 h of incubation, although occasionally incubation had to be extended to 24 h (by which time 69% were correctly identified) to achieve complete identification of one strain of *S. dysgalactiae* and two strains of *S. bovis*. Of 12 *S. faecalis* strains tested, the accordance with conventional biochemical procedures was 92%; this accordance was ca. 80% for *S. agalactiae*, *S. dysgalactiae*, *S. uberis*, and *S. faecium*. Similar findings were previously reported for *S. faecalis* (5). However, *S. bovis* gave less satisfactory results, recording only 21.4% identification. The inability of API 20 Strep to identify *S.*

TABLE 1. Identification of 84 bovine mastitis strains of streptococci by the API 20 Strep system after incubation for 4 and 24 h at 37°C

Organism	No. of strains tested	No. of strains identified correctly	
		Incubation for 4 h	Incubation for 24 h
S. agalactiae[a]	13	11	11
S. dysgalactiae[b]	16	12	13
S. uberis[c]	24	18	18
S. faecalis[d]	12	10	10
S. faecium[e]	5	4	3
S. bovis[f]	14	1	3

[a] Test results at variance with profile index and table data: one strain was arabinose positive and one strain was pyrrolidonylarylamidase positive.
[b] Three strains were ribose negative.
[c] Three strains were inuline negative; three strains were raffinose positive.
[d] One strain was sorbitol negative.
[e] One strain was sorbitol positive.
[f] Fourteen strains were α-galactisodase negative; 8 strains were arabinose positive.

TABLE 2. Comparative evaluation of profile index and table data for biochemical identification of bovine mastitis streptococci by the API 20 Strep system

Organism	No. of strains	No. of strains identified correctly	
		With profile index	With table
S. agalactiae	13	9	8
S. dysgalactiae	16	13	13
S. uberis	24	4	17
S. faecalis	12	11	8
S. faecium	5	3	4
S. bovis	14	3	0

bovis in our study differs somewhat from results reported by Waitkins et al. (8), who found correct identification for the six strains on the test.

The 24 strains unclassifiable by API 20 Strep would not have been mistaken with any other streptococcal species. For these strains, only one or two characteristics were not consistent with those given by the manufacturer in the profile index and table. However, some characteristics are of little value in differentiating streptococci recovered from bovine mammary glands, such as acid production from arabinose, sorbitol, and ribose (1, 4). On the other hand, variation in acid production from inulin and raffinose was previously reported for *S. uberis* (7). All *S. bovis* strains tested were α-galactosidase negative in contrast with data given by the profile index and table. If we preclude the possibility of a defective substrate, this suggests that negative reaction is distinctive for bovine strains.

The profile index was more critical in determining the final identification of streptococci isolated from bovine milk than was the table (Table 2). Thus, 51% of the streptococci tested in this study were consistent with the identification code in the API data base, and 60% were consistent with the table. Especially poor performance of the profile index was recorded in identifying *S. uberis*. Conversely, only the profile index allowed identification of 3 of 14 *S. bovis* strains.

Our results indicated on one hand that the profile index and table issued by the manufacturer had to be used in conjunction for identification and, on the other hand, that the data base needs to be improved.

We concluded that API 20 Strep is a simple and rapid method for species identification of streptococci involved in bovine mastitis, one which can replace the conventional biochemical methods. However, further refinement of the identification code data is needed, taking into account well-known variations of some biochemical tests, for accurate identification.

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