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Conventional Identification of *Streptococcus uberis* Isolated from Bovine Mastitis in Argentinean Dairy Herds

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ABSTRACT

The objective of this study was to evaluate a conventional scheme for identifying *Streptococcus uberis* strains isolated from bovine mastitis. Seventy-five gram-positive, catalase-negative cocci were collected from cows with mastitis from 19 dairy herds located in the east-central region of Argentina. Five American Type Culture Collection strains and bovine isolates were identified by the API 20 Strep system and by restriction fragment length polymorphism analysis of 16S rDNA. A conventional scheme based on 11 biochemical tests was selected for identification of *Strep. uberis* strains: the Christie–Atkins–Munch-Petersen reaction; hydrolysis of Arg, esculin, and sodium hippurate; growth in inulin, mannitol, raffinose, salicin, and sorbitol; and growth at 45°C and in 6.5% NaCl. Reference strains and 25 bovine isolates were classified accurately to the species level by the conventional scheme in a blind assay. Each reference strain and each bovine isolate were identified as belonging to the same species following these 3 methods. The remaining 50 isolates identified as *Strep. uberis* by the API 20 Strep system and 16S rDNA RFLP were assayed by the conventional scheme. This scheme correctly identified 47 (94%) of 50 isolates as *Strep. uberis* by comparing their biochemical profile with that of the reference strain. Three (6%) of the 50 isolates were classified as *Strep. uberis* by the API 20 Strep system and by 16S rDNA RFLP and were identified as *Enterococcus faecalis* by the conventional scheme. Thirty percent of the *Strep. uberis* strains showed biochemical profiles identical to the *Strep. uberis* American Type Culture Collection 27958 strain. Seventy percent of the *Strep. uberis* strains demonstrated variability compared with the reference strain, resulting in 19 different biochemical profiles. The conventional scheme proposed in this study resulted in a relatively low number of misidentifications and could biochemically identify not only typical, but also atypical *Strep. uberis* strains. This conventional scheme can be considered an adequate method for identifying *Strep. uberis* strains isolated from bovine mastitis because of its affordable cost in developing countries, and it may contribute to determining the frequency of isolation of *Strep. uberis* strains in Argentinean dairy herds.
Key words: *Streptococcus uberis*, bovine mastitis, conventional identification

INTRODUCTION

Bovine mastitis is the most costly disease among dairy cows. Estimates of losses caused by mastitis range from US$35 to $295 per cow per year (DeGraves and Fetrow, 1993). In Argentina milk production losses have been estimated at US$221 million a year (Asociación Argentina de lucha contra la mastitis, 1983).

Streptococcus uberis is known worldwide as an environmental pathogen responsible for a high proportion of cases of clinical and subclinical mastitis in lactating cows and is also the predominant organism isolated from mammary glands during the nonlactating period (Bradley, 2002; Khan et al., 2003). Accurate and cost-effective methods of identifying mastitis pathogens are important for the diagnosis, surveillance, and control of this economically important disease among dairy cows (McDonald, 1984; Leigh, 1999).

Gram-positive, catalase-negative, esculin-hydrolyzing cocci isolated from cases of clinical and subclinical mastitis are commonly categorized as *Strep. uberis* in routine diagnostic laboratories. The heavy workload of mastitis diagnostic services does not allow more accurate identification. Upon closer examination, however, many of these bacteria may in fact be very different from this major streptococcal pathogen. The differential scheme for mastitis streptococci and enterococci of the US National Mastitis Council (National Mastitis Council, 1990) lists *Strep. uberis*, *Streptococcus bovis*, *Streptococcus equinus*, *Enterococcus faecalis*, *Enterococcus faecium*, and *Enterococcus saccharolyticus* as esculin-hydrolyzing species and *Streptococcus dysgalactiae* and

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Streptococcus equi as esculin-variable species. More recently, 2 new esculin-hydrolyzing streptococcal species, *Streptococcus parauberis* (Williams and Collins, 1990) and *Streptococcus plurianimalium* (Devriese et al., 1999), were isolated from bovine mastitis. However, according to different authors (King, 1981; Calvinho et al., 1991; Khan et al., 2003), *Strep. uberis*, *Strep. dysgalactiae*, *Strep. bovis*, *Strep. equinus*, and *E. faecalis* represent the esculin-positive or esculin-variable cocci most frequently isolated from mastitic cows. Farrow and Collins (1984) examined a collection of strains, most of which were nonhuman isolates, and reported that the phenotypically described *Strep. bovis* and *Strep. equinus* type strains belonged to a single DNA group and corresponded to the same species.

Identification of *Strep. uberis* is currently based on observation of the cultural and morphological characteristics, determination using biochemical tests, and enzyme activity (Leigh, 1999; Khan et al., 2003). On the other hand, several commercial microbial identification systems have also been used to differentiate *Strep. uberis* from the other streptococci and enterococci isolated from bovine mastitis (Watts, 1989; Freney et al., 1992), and more recently, molecular tools such as PCR-based protocols have been proposed to provide an accurate identification of *Strep. uberis* isolates (Hassan et al., 2001; Schlegel et al., 2003; Kawata et al., 2004). Among these, RFLP analysis of 16S rDNA was proposed as a general method for bacterial identification and typing (Jayarao et al., 1992). Perhaps the greatest disadvantage of identification techniques based on commercial rapid systems and molecular tools is that these methods can be expensive for most laboratories that wish to offer analyses at an affordable cost. The objective of this study was to evaluate a conventional scheme based on 11 biochemical tests for identification of *Strep. uberis* strains collected from the mammary glands of cows with mastitis from 19 dairy herds located in the east-central region of Argentina.

MATERIALS AND METHODS

Bacteria

Five American Type Culture Collection (**ATCC**) strains, sent by M. Gottschalk and B. Jayarao, including *Streptococcus agalactiae* ATCC 27956, *Strep. dysgalactiae* ATCC 27957, *Strep. uberis* ATCC 27958, *Strep. equinus* ATCC 27960, and *E. faecalis* ATCC 19433, were used in this study. In addition, over a period of 12 mo, 75 bacterial cultures were collected and presumptively identified as streptococci or enterococci by colony appearance, gram stain reaction, and catalase test (Hogan et al., 1999). The collected isolates were obtained from mammary glands of cows with mastitis from 19 dairy herds located in the east-central region of Argentina. Four isolates per dairy herd were stored at –20°C in Todd–Hewitt broth (Sigma-Aldrich Co., St. Louis, MO) with 20% glycerol. All ATCC strains and bovine isolates were subcultured from storage media onto 5% sheep blood agar plates and were identified by the API 20 Strep system (bioMérieux, Inc., Durham, NC; Poutrel and Ryniewicz, 1984) and by 16S rDNA RFLP, as previously described (Jayarao et al., 1992).

Biochemical Tests

Eleven biochemical tests were selected for identification of *Strep. uberis* strains: the Christie–Atkins–Munch-Petersen (**CAMP**) reaction (Hogan et al., 1999); esculin hydrolysis (Hogan et al., 1999); sodium hippurate hydrolysis (Baron et al., 1994a); Arg hydrolysis (McDonald and McDonald, 1976); growth in inulin, mannitol, raffinose, salicin, and sorbitol (McDonald and McDonald, 1976); and growth at 45°C and in 6.5% NaCl (Baron et al., 1994b). The Todd–Hewitt broth was used as a basal medium for temperature- and salt-tolerance tests. Five reference strains and 25 bovine isolates previously identified by the API 20 Strep system and by 16S rDNA RFLP, were classified to the species level by biochemical tests in a blind assay. Five representative colonies, obtained by streaking 5 μL of Todd–Hewitt broth-glycerol onto 5% sheep blood agar plates, were picked from each plate and used in each biochemical test. The remaining 50 isolates of bovine origin, identified by the API 20 Strep system and by 16S rDNA RFLP, were subjected to the biochemical tests, and the results were compared with biochemical profiles obtained from reference strains. Each isolate was identified to the species level when 8 of the 11 biochemical tests were identical to one of the reference strains and at least 2 were different from each of the other reference strains.

RESULTS

Reference strains were identified to the species level by the API 20 Strep system and by 16S rDNA RFLP. Biochemical tests of reference strains were determined by using the conventional scheme proposed for *Strep. uberis* identification. Reference strains were classified accurately to the species level in a blind assay by comparing their biochemical profiles with previously reported results (McDonald and McDonald, 1976; Garvie and Bramley, 1979; Watts, 1988; Lämmler, 1991; Devriese et al., 1999; Facklam, 2002; National Mastitis Council, 2002; Fortin et al., 2003; Khan et al., 2003). Table 1 shows the identification of the reference strains by the conventional scheme obtained after each biochemical test was repeated 3 times for each isolate.