Phenotypic Characterization of CO$_2$-Requiring Strains of *Streptococcus bovis* from Koalas

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We examined phenotypic characteristics of six mannitol-fermenting strains of *Streptococcus bovis*, including two unusual CO$_2$-requiring strains isolated from koala feces. These strains did not grow in air, but grew in air supplemented with CO$_2$ and under reduced oxygen conditions. All six strains had the same biochemical characteristics, except that the CO$_2$-requiring strains did not produce β-N-acetylgalactosaminidase.

*Streptococcus bovis* is commonly found in the normal gastrointestinal floras of many animals (2, 3, 8, 16) and is often isolated in clinical situations, such as cases of human bacteremia (9, 10, 13), endocarditis (7, 17), and bovine mastitis and udder infections (5, 12). Although *S. bovis* is usually considered to be a facultative anaerobe (6), Latham et al. (11) reported that this species includes strains which are strictly anaerobic when they are first isolated. Recently, Osawa (14) and Osawa and Mitsuoka (15) investigated the phenotypic characteristics of facultatively anaerobic strains of *S. bovis* obtained from various sources. We isolated apparently anaerobic strains from the feces of two healthy koalas (*Phascolarctos cinereus* (Goldfuss)), and in this study we characterized these strains.

Six strains of *S. bovis*, including two that are not able to grow in air, were obtained from our collections at the Lone Pine Koala Sanctuary (strains LPKS 1, LPKS 6, LPKS 31, and LPKS 32) and the Department of Microbiology, University of Queensland, Queensland, Brisbane, Australia (strains UQM 3549 and UQM 3552). The sources of the strains are indicated in Table 1. Cultures of these strains, which were stored as lyophiles, were revived and subcultured anaerobically at least three times on plates containing Columbia blood agar (Oxoid, Ltd., Basingstoke, Hampshire, United Kingdom) in an atmosphere enriched with 8 to 10% CO$_2$ by using Bio-bags (Becton Dickinson and Co., Cockeysville, Md.) before use.

The oxygen requirements of the strains were determined by incubating inoculated Columbia blood agar plates aerobiocally in the air, microaerophilically (5 to 15% CO$_2$) in an anaerobic jar equipped with a BBL Campy-Pak apparatus (Becton Dickinson and Co.) or in a candle jar (strains LPKS 31 and LPKS 32 only), and anaerobically in an anaerobic jar equipped with a BBL GasPak apparatus (Becton Dickinson and Co.) and in Bio-bags (Becton Dickinson and Co.) at 37°C for up to 5 days. The ability to grow in an atmosphere containing 3 to 12% CO$_2$ in air was determined by enriching the air in a jar with CO$_2$ generated by using a BBL CO$_2$ GasPak apparatus (Becton Dickinson and Co.). The biochemical characteristics of the strains were determined by following the three commercially available identification kits: API 20 STREP (API System, Montalieu, Verscieu, France), ATB 32A (API System), and RapID ANA II system (Innovative Diagnostic Systems, Inc., Atlanta, Ga.). The colonial morphology, cellular morphology, Gram staining, and catalase and hemolytic activities of the strains were also determined. Growth and clear zone formation on tannin-treated brain heart infusion agar were observed in order to determine tannin-protein complex degradation, as described previously (14, 15).

The strains were all catalase negative, gram-positive coccii which produced smooth, white, alpha-hemolytic colonies on Columbia blood agar and larger white flat colonies with surrounding clear zones on tannin-treated brain heart infusion agar. The anaerobiosis and biochemical characteristics of the strains are summarized in Table 1. Strains LPKS 1, LPKS 6, UQM 3549, and UQM 3552 grew on Columbia blood agar aerobically and anaerobically, whereas strains LPKS 31 and LPKS 32 did not grow in the presence of 100% air but grew under anaerobic or reduced oxygen conditions in a candle jar or in a jar equipped with a Campy-Pak apparatus. This observation suggested that strains LPKS 31 and LPKS 32 are aerotolerant. However, further testing showed that these strains had a requirement for CO$_2$ and grew well in air supplemented with CO$_2$.

Most anaerobic atmospheres currently used include CO$_2$ at concentrations ranging from 3 to 15%. Our observations demonstrate the need to include a CO$_2$-air control to accurately interpret requirements for anaerobic growth. In addition, the possibility of the presence of CO$_2$-requiring strains must be taken into account when the total streptococci in koala feces are determined.

A CO$_2$ requirement has been determined for some strains of other species of streptococci. For example, the inability of some strains of *"Streptococcus milleri"* to grow in air alone has been misinterpreted as a dependence on anaerobic conditions (1, 6) which could be overcome by CO$_2$ supplementation of an aerobic atmosphere.

Strains of *S. bovis* normally grow aerobically. However, Latham et al. (11) described the isolation of anaerobic strains from rumina and ceca of cows and calves. The growth of these strains was enhanced by the addition of CO$_2$, and after repeated subculturing in air containing 10% CO$_2$ these strains grew on plates incubated aerobically. In contrast, we did not observe any tendency for the koala isolates to grow in air alone after repeated subculturing.

Devries et al. (4) described *Streptococcus cecorum* as a species which includes strains that are similar to *S. bovis* strains obtained from the ceca of chickens, the growth of which is enhanced by, but not dependent on, the addition of CO$_2$. However, *S. cecorum* is only distantly related to *S.
bovis as determined by DNA-DNA homology experiments and may be distinguished by positive reactions for ribose, melezitose, β-glucuronidase, and alkaline phosphatase. In this study aerobic strains and CO₂-requiring strains were found to have the same biochemical characteristics, as determined by API 20 STREP tests, and were identified as S. bovis biotype I (mannitol fermenting). On the other hand, subsequent tests in which we used ATB 32A and RapID ANA II tests on the six strains of S. bovis revealed that these strains share the same biochemical characteristics, except that CO₂-requiring strains LPKs 31 and LPKs 32 lack β-N-acetylglucosaminidase. Studies with more strains are needed to determine whether the observed absence of this enzyme is specific to the CO₂-requiring strains of S. bovis or is a variable characteristic within the species.

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REFERENCES


