Development of molecular methods for identification of *Streptococcus bovis* from human and ruminal origins

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Abstract

*Streptococcus bovis* has been identified as a causative agent in humans for a variety of diseases, including endocarditis, meningitis, and septicemia. Identification of *S. bovis* strains of human origin in clinical settings has been problematic due to variations in biochemical tests as compared to ruminal strains of *S. bovis*, and other streptococcal species. DNA-DNA hybridization with chromosomal DNA from various *S. bovis* strains indicates that strains of human origin are different from those of ruminal origin. Specific probes have been designed from *S. bovis* 16S rDNA gene sequences that differentiate strains of human and ruminal origin by direct hybridization and PCR analyses. These techniques now allow for rapid identification of *S. bovis* strains for clinical and other scientific investigations. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

*Streptococcus bovis* is a normal inhabitant of the rumen of ruminant animals such as cattle and sheep [1]. Although rarely isolated from the human intestinal tract, *S. bovis* can be the causative agent for a variety of diseases. These diseases include meningitis, endocarditis, and septicemia [2–5]. *S. bovis* bacteremia has also been recognized as a causative agent for bacteremia in HIV-infected patients [6]. More importantly, numerous reports have suggested a potential relationship between increased fecal carrier levels of *S. bovis* and human gastrointestinal disease, primarily colonic cancer [2,5,7–9]. However, identification of *S. bovis* in clinical settings has been difficult when compared to strains of rumen origin and other streptococci due to similarities in biochemical and phenotypic tests [10]. Several biochemical characteristics, such as the ability to ferment mannitol and melibiose, appear to vary among rumen and human *S. bovis* isolates [11]. While all strains of *S. bovis* exhibit the Lancefield group D surface antigen (as do enterococcal species), *S. bovis* strains are primarily identified by their ability to grow on starch and produce amylase activity [10,12,13]. However, several variants of *S. bovis* have been isolated that do not do hydrolyze starch [14]. In addition, DNA-DNA hybridization studies indicate that human and ruminal isolates are of different DNA homology groups [11,14,15].

Recent advances in bacterial systematics have allowed for the use of the 16S ribosomal RNA (rDNA) gene to be used as a target for detection and identification of various microorganisms (for examples, see [16,17]). Preliminary work suggested that the V1 region of the 16S gene from *S. bovis* may serve as such a target [11]. The present report describes the development of specific primers that target the 16S rDNA gene to rapidly identify and differentiate human and ruminal strains of *S. bovis*. 

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2. Materials and methods

2.1. Bacterial strains and media

Bacterial strains used in this report are indicated in Table 1. The sources of the strains have been previously identified [18], except strains RG [3] and FM [4]. All strains were grown anaerobically in routine growth medium (RGM, [19]), a complex Trypticase-yeast extract medium, containing 0.2% glucose. Purified DNA used for hybridizations and PCR analyses was isolated as described previously [11,20].

2.2. Development of PCR and hybridization primers

DNA sequences of reported 16S rDNA genes were recovered from the GenBank database. The 16S rDNA genes from strains of \textit{S. bovis} used in this study were isolated using PCR as previously described [11]. Sequencing of the 16S rDNA gene was carried out on purified PCR products using internal primers. For several strains, only the V1 region of the 16S rDNA gene was isolated for sequencing. The relevant GenBank accession numbers for the DNA sequences are given in Table 1. Alignment of the 16S rDNA sequences was carried out using the Lasergene MegAlign program (DNASTAR, Inc., Madison, WI, USA), and specific primers were designed based on the sequence similarity.

2.3. PCR amplification and analyses

The PCR reactions for identification of \textit{S. bovis} strains were carried out using a MJ Research MiniCycler. The PCR reaction mixture (50 µl) was composed of 5 µl Gene Amp 10×PCR buffer; 200 µM of each dNTP; 100 ng of purified DNA; 0.8 µM of each primer; and 0.5 U of Taq polymerase. All PCR components were purchased from Perkin Elmer, Foster City, CA, USA. The amplification program was: 94°C, 1 min, then 25 cycles of 94°C, 45 s, 46°C, 30 s, and 72°C, 2 min, followed by a final cycle of 72°C for 10 min. The PCR primer sets were JB1F1 (66-GACTTTAGCTTGCTAAAGTTGG-88) and JB1F2 (201-GAAAGGAGCAATTGCTTCAC-220), and 43143F1 (65-CTACTTTAGCTTGCTAGAGTAGA-88) and 43143R1 (201-GAAAGATGCAATTGCATCAC-220). Following amplification, 5 µl of the PCR mixtures were analyzed by gel electrophoresis in 1.5% agarose containing GelStar nucleic acid stain (FMC Bioproducts, Rockland, ME, USA).

2.4. DNA hybridization analyses

Oligonucleotide primers JB1F1 and 43143F1 were 3’-end-labeled with digoxigenin using the Genius 5 kit (Boehringer-Mannheim, Indianapolis, IN, USA) according to manufacturer’s instructions. Hybridization analyses of specific primers with purified DNA were carried out using a Minifold II slot-blot apparatus (Schleicher and Schuell, Keene, NH, USA) according to manufacturer’s instructions. Following overnight hybridization with the specific labeled primer, chemiluminescence detection of hybridization was carried out by using the Genius 1 kit (Boehringer Mannheim, Indianapolis, IN, USA) according to the manufacturer’s instructions and exposure to X-ray film.

3. Results and discussion

Previous analyses of total DNA hybridizations indicated that ruminal and human strains of \textit{S. bovis} produced three distinct hybridization groups, two within the ruminal strains and one containing the human isolates [11]. In order to further determine the phylogenetic relationship between these microorganisms, the 16S rDNA genes for selective representatives of each hybridization group were determined. The full sequence was determined for ruminal strain JB1 and human strain ATCC 43143, while the sequence for the ruminal strain ATCC 33317 (type strain) had already been determined (GenBank Accession Number AB0022482). Partial sequences were determined for other strains within each group (see Table 1). Overall, the 16S rDNA sequence similarities reflected the DNA hybridization analyses. The ruminal strains had at least
99% similarity between strains, as did the human strains. However, the similarity between human and ruminal strains was below 97% (data not shown), suggesting the strains represent different species. Most of the differences in sequences were found within the first 350 bases, which encompass the V1 region of the 16S rDNA gene. These data correlate with previous work indicating the sequence of the V1 regions of these strains may be different [11].

Potential primer target sequences were identified within the first 300 bases of the 16S rDNA genes, based on sequence similarity (see Section 2). The forward primers were used for hybridization analyses with purified DNA from bacterial strains, and the results are shown in Table 1. The oligonucleotide primers were found to be specific for the respective ruminal and human strains of *S. bovis*, with one exception. Ruminal strain 7H4 did not hybridize with either primer, which supports previous data indicating this strain is not *S. bovis* [13,18]. Worth noting is the hybridization of the JB1 primer with *Streptococcus equinus* ATCC 9812, the type strain of the species. Analysis of the V1 region of this strain demonstrated that the DNA sequence is 100% identical with several of the ruminal strains and 99% with JB1. Farrow et al. [15] previously reported that *S. equinus* 9812 and *S. bovis* 33317, the type strain, had greater than 70% DNA-DNA homology and therefore represented a single species. The DNA hybridization, PCR results (see below), and 16S rDNA sequence data would appear to indicate that these are indeed the same species. No hybridization was observed with DNA from *Streptococcus salivarius*, *Streptococcus suis*, *Streptococcus uberis*, as well as *Enterococcus faecalis* (data not shown).

The primers were next used for PCR identification of *S. bovis* strains, and the results are shown in Table 1 and Fig. 1. The PCR results mirrored those of the hybridizations, indicating that the PCR test can be applied as a rapid method for identification of *S. bovis* strains from different origins. Both techniques produced negative results with related *Streptococcus* and *Enterococcus* species, as well as the ruminal bacterium *Butyrivibrio fibrisolvens*. It is of interest to note the positive identification of *S. bovis* strains RG and FM. These strains were recently identified
as the causative agents for human septic arthritis [3] and infant meningitis [4], respectively. These results demonstrate the potential clinical application for the PCR test for rapid identification of S. bovis.

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References