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Construction of a DNA Probe for the Specific Identification of *Streptococcus oralis*

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A DNA probe for the specific detection and identification of *Streptococcus oralis* was isolated from *Hin*dII-digested DNA of *S. oralis* NCTC 11427 and cloned in *Escherichia coli*. The plasmid pOS1 hybridized only to DNA of *S. oralis* and not to that of other viridans group streptococci.

Streptococcus oralis, a recently described species (1, 9), is a typical member of the viridans group streptococci and encompasses strains previously designated as *S. sanguis* II, "*S. mitior*," and "*S. viridans*." Viridans group streptococci are usually found in the upper respiratory tract of humans and animals (2, 3) and are also an important cause of endocarditis and bacteremia (3, 7). There exists no satisfactory system for the routine differentiation of viridans group streptococci (4). Conventional biochemical tests or rapid identification systems are not sufficient for an unequivocal classification and identification of these streptococci (13, 14). However, recent studies from our laboratory revealed that they can be clearly separated on the basis of DNA-DNA hybridization studies and cell wall and DNA base compositions (9, 14) into three major species: *S. mitis*, *S. oralis*, and *S. sanguis*. Since these properties cannot be easily determined in routine laboratories, we decided to develop specific DNA probes for their identification. In this report, we describe the construction of a DNA probe for the identification of *S. oralis*.

The bacterial strains used in this study are listed in Table 1. The *S. oralis* strains were mostly clinical isolates from different sources. Their physiological and chemotaxonomic characteristics, as well as their genetic relatedness, were described previously (9, 14). *Escherichia coli* JM83 and vector pTZ R19 (Pharmacia, Freiburg, Federal Republic of Germany) were applied for cloning experiments. Streptococci were cultivated anaerobically in a medium described previously (14); the incubation temperature was 37°C. The DNA probe was constructed in the following way. Chromosomal DNA from *S. oralis* NCTC 11427 and *S. pneumoniae* DSM 20556 was isolated by using the method of Marmur (10) with modifications given by Meyer and Schleifer (11); alternatively, the rapid method described by Heath et al. (6) was used. Plasmid DNA and chromosomal DNA were labeled by nick translation with [α-^{32}P]dATP or biotin-11-dUTP by using the reaction kit from Bethesda Research Laboratories, Eggenstein, Federal Republic of Germany. The conditions were those recommended by the manufacturer. Purified chromosomal DNA from *S. oralis* was digested with restriction endonuclease *Hin*dII. The selective enrichment of specific restriction fragments with biotinylated probe and avidin-agarose was accomplished by the method of Welcher et al. (16).

To find DNA fragments specific for *S. oralis* and not homologous to chromosomal DNA from closely related bacteria, hybridization in solution was done with *S. oralis* DNA restriction fragments and sheared biotinylated *S. pneumoniae* DNA fragments: 10 μg of *S. pneumoniae* DNA and 5 μg of *S. oralis* DNA fragments were heat denatured and incubated in 20 μl of aqueous solution for 4 h at 62°C. Then, 400 μl of buffer containing 0.1 M Tris hydrochloride (pH 7.5) and 0.15 M NaCl was added to the solution. Biotinylated probe and hybrids were incubated with avidin-agarose (160-μl suspension) (Sigma, Deisenhofen, Federal Republic of Germany) for 5 min at room temperature. Avidin-agarose with and without bound biotinylated DNA was pelleted by centrifugation (10,000 × *g*, 1 min). The free DNA fragments were collected from the supernatant by ethanol precipitation. The procedure described above was repeated twice. The remaining free DNA fragments were ligated with *Sma*I-digested vector pTZ R19 by using T4 ligase (Bethesda Research Laboratories). After transformation of *E. coli* JM83, plasmid DNAs were analyzed by agarose gel electrophoresis and transferred to Zeta Probe membranes (BioRad, Munich, Federal Republic of Germany). DNA was bound to the membrane by UV (256-nm) irradiation for 10 min (8).

The screening for recombinant plasmids specific for *S. oralis* strains was performed by Southern hybridizations (15) of membrane-bound plasmids to ^{32}P-labeled chromosomal DNAs of *S. oralis* ATCC 10557, *S. oralis* S603, *S. pneumoniae* DSM 20566, and *S. mitis* NCTC 3165. The membranes were incubated in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0]) containing 0.5% sodium dodecyl sulfate at 50°C for 1 h and then prehybridized in 3× SSC containing 0.1% sodium dodecyl sulfate overnight at 60°C. Heat-denatured labeled DNA was added to the mixture, and the hybridization was done for 16 h under prehybridization conditions. The membranes were washed four times with 3× SSC containing 0.1% sodium dodecyl sulfate for 15 min at 60°C. The membranes were air dried and exposed to an X-ray film. Of the tested plasmids, 23% hybridized only to DNAs of the two *S. oralis* strains. A selection of five plasmids (pOS1 to pOS15) which produced strong hybridization signals were tested in inverse fashion to check for specificity.

Chromosomal DNAs from 33 different streptococci (Table 1) were prepared by using the rapid isolation protocol described by Heath et al. (6). For dot hybridization, 5-μg samples of each DNA were denatured in 200 μl of 0.4 N NaOH–1.5 M NaCl for 15 min at room temperature, spotted onto a Zeta Probe membrane by using a minifold filtration unit (Schleicher & Schuell, Dassel, Federal Republic of Germany), and immobilized by UV irradiation. Hybridiza-

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TABLE 1. Strains studied and their reactions with pOS1

Organism (source of filter-bound DNA)	Strain no. and/or origin[a]	Hybridization with radiolabeled DNA of pOS1	Location in Fig. 1[b]
S. oralis	DSM 20066	+	f5
S. oralis	Kiel 61162	+	b3
S. oralis	Kiel 50493	+	b1
S. oralis	Gehring S229	+	b4, b5
S. oralis	Gehring S426	+	c2, c3
S. oralis	Gehring S410	+	c1
S. oralis	Gehring S438	+	c4, c5
S. oralis	Gehring S440	+	d1, d2
S. oralis	Gehring S444	+	d3, d4
S. oralis	Gehring S447	+	d5
S. oralis	Gehring S526	+	e1, e2
S. oralis	Gehring S662	+	e3, e4
S. oralis	Gehring S603	+	d6
S. oralis	ATCC 10557	+	g1
S. pneumoniae	DSM 20566	−	e5
S. pneumoniae	Hakenbeck R6	−	f2
S. pneumoniae	Hakenbeck RC-1	−	f1
S. pneumoniae	Hakenbeck Curi	−	f3
"*S. viridans* III"	Kiel 45527	−	a1
S. suis	NCTC 10237	−	g3
Streptococcus sp. group O	Kiel 119/48	−	g4
S. anginosus	DSM 20563	−	g5
S. constellatus	DSM 20575	−	h1
"*S. viridans* IV"	Kiel 42841	−	a2, a3
S. sanguis	Kiel 12700	−	g2
S. sanguis	ATCC 10556	−	h2
S. sanguis	Gehring S432F	−	h4
S. sanguis	Moore D79H-19	−	b6
S. sanguis	Moore E7R-30	−	e6
S. mitis	NCTC 3165	−	h3
S. mitis	Gehring S497	−	h5
S. mitis	Gehring S225	−	f4
Streptococcus sp.	Moore D79B-19	−	a6

[a] ATCC, American Type Culture Collection, Rockville, Md.; DSM, Deutsche Sammlung von Mikroorganismen, Göttingen, Federal Republic of Germany; Gehring, W. Gehring, Lehrstuhl für Experimentelle Zahnheilkunde, Würzburg, Federal Republic of Germany; Hakenbeck, R. Hakenbeck, Max-Planck-Institut für Molekulare Genetik, Berlin, Federal Republic of Germany; Kiel, Streptokokkenzentrale, Kiel, Federal Republic of Germany; Moore, W. E. C. Moore, Virginia Polytechnic Institute and State University, Blacksburg; NCTC, National Collection of Type Cultures, London, United Kingdom.

[b] Designation of the location refers to both Fig. 1A and B.

tions to the labeled specific probes were done as described above.

To avoid false-negative results, the DNA content of any spot was checked by hybridization to a universal 23S rDNA (genes coding for rRNA) probe which hybridizes to any procaryotic total DNA (A. Regensburger, W. Ludwig, and K. H. Schleifer, J. Gen. Microbiol., in press); the results of these experiments are shown in Fig. 1A. The corresponding results for pOS1 are shown in Fig. 1B. The probe reacted with 14 of 14 *S. oralis*, none of 4 *S. pneumoniae*, none of 4 *S. sanguis*, and none of 4 *S. mitis* strains and with none of 7 other streptococci. Hybridization of the specific probe pOS1 to chromosomal DNA from streptococci allowed a rapid and confident identification of *S. oralis* strains (Table 1). The probe hybridized to DNA of any *S. oralis* strain but not to DNAs from other viridans group streptococci, proving that pOS1 is a useful DNA probe for the specific identification and detection of *S. oralis*.

Further studies are under way to construct DNA probes for the other two difficult-to-identify viridans group streptococci, namely *S. mitis* and *S. sanguis*. The probes may be useful for identifying the different species from a large collection of clinical isolates of viridans group streptococci. The genetically identical strains could then be tested with a variety of substrates to devise a conventional or rapid system for the correct identification of viridans group streptococci.

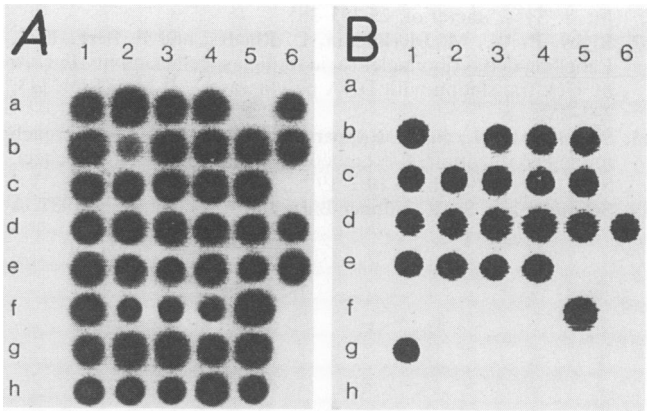

FIG. 1. Dot hybridization of various streptococcal DNAs with universal 23S rDNA probe (A) and *S. oralis*-specific pOS1 DNA probe (B).

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