Identification of Clinically Relevant Viridans Group Streptococci to the Species Level by PCR

FABIEN GARNIER, GUY GERBAUD, PATRICE COURVALIN, AND MARC GALIMAND*

Unité des Agents Antibactériens and National Reference Center for Antibiotics, Institut Pasteur, 75724 Paris Cedex 15, France

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A PCR assay that allows identification of clinically relevant viridans group streptococci (*Streptococcus gordonii, S. mitis, S. mutans, S. oralis, S. salivarius*, and *S. sanguis*) to the species level and identification of milleri group streptococci (*S. anginosus, S. constellatus*, and *S. intermedius*) to the group level was developed. This assay was based on specific amplification of internal fragments of genes encoding D-alanine:D-alanine ligases which are species specific and ubiquitous in prokaryotes possessing peptidoglycan. The specificity of this assay was tested on 9 reference strains and 91 characterized clinical isolates. This assay offers a specific and rapid alternative to phenotypic or DNA-DNA hybridization methods for identification of clinically relevant viridans group streptococci.

Viridans group streptococci form an important part of the normal flora of the human oral cavity. They are responsible for several infections including purulent infections (14), endocarditis (7), septicemia (4), and meningitis (3). Viridans streptococci do not possess a specific group antigen and show variable reactions with Lancefield antisera (11). Their identification is based on different physiological and biochemical characteristics, but conventional phenotypic identification methods are sometimes unable to differentiate established species. First, not all strains in a species may be positive for a common trait (2, 19), and second, the same strain may give different results with repeated tests in the absence of changes in the corresponding genes (15, 25). Thus, rapid systems using standard phenotypic tests, such as API-20STREP or rapid ID 32 Strep, that are used in clinical laboratories are not totally satisfactory for accurate identification at the species level (16, 18). Species identification of viridans streptococci is useful in cases of infective endocarditis when the patient has relapsed and in cases of positive blood cultures and in assessing the involvement of a given strain in an infection. PCR has been extensively applied to species identification of infectious agents (8, 13, 21). PCR allows amplification of a preselected DNA region and is a highly specific and sensitive technique (20). In many instances, the target genes are involved in pathogenicity (13). In other cases, the target is a random cloned fragment from a genomic library selected by differential hybridization to the pathogen and its close relatives (23).

In this study, we have selected the gene encoding a D-alanine:D-alanine (D-Ala:D-Ala) ligase which is species specific and ubiquitous in prokaryotes possessing peptidoglycan. The D-Ala:D-Ala ligase catalyzes synthesis of the terminal dipeptide D-alanine–D-alanine of peptidoglycan precursors (26). We have developed a PCR assay which allows identification of six species (*Streptococcus gordonii*, *S. mitis*, *S. mutans*, *S. oralis*, *S. salivarius*, and *S. sanguis*) to the species level and identification of three species (*S. anginosus*, *S. constellatus*, and *S. intermedius*) to the group level.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. The reference strains of *Streptococcus* spp. used in this study were as follows: *S. anginosus* ATCC 33397, *S. bovis* NCTC 8177, *S. constellatus* ATCC 27823, *S. gordonii* ATCC 10558, *S.*

Streptococcal	Size of PCR product (bp)		Oligodeoxynucleotide	Positions ^a	GC content (%)
species		Pair	Sequence	Positions	
S. mitis S. oralis	372	А	5'-GTCGAAGGTGATGATATGAC-3' 3'-GACAGTACGCAGTCTTACGTC-5'	133–152 488–508	50 52
S. mutans	351	В	5'-ATTGAAGGCGAGCCTTTAGAAAG-3' 3'-CTAGGACAATAGCAAC-5'	133–155 472–487	43 43
S. salivarius	331	С	5'-GCAGCAGTAGCAGAGACGCT-3' 3'-CACGGACGTCTTCAGTACTG-5'	154–173 469–488	60 55
S. gordonii S. sanguis	208	D	5'-GTCGATGGCGAGGATCTAGAGC-3' 3'-TGCCGAGCGCTCTAACTCCA-5'	133–154 325–344	59 60

TABLE 1. Oligodeoxynucleotic	e primers for the first PCR
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^{*a*} Positions were derived from the alignment in Fig. 1.

* Corresponding author. Mailing address: Unité des Agents Antibactériens, Institut Pasteur, 28, rue du Docteur Roux, 75724 Paris Cedex 15, France. Phone: (33) 01 45 68 83 18. Fax: (33) 01 45 68 83 19. E-mail: galimand@pasteur.fr.

J. CLIN. MICROBIOL.

S. gordonii S. sanguis S. mitis	TTCCTAGAAG	TGCTCAAAAT	GCCTTATGTC GCCTTATGTC GCCTTATGTT	GGCTGTAATA	TTTTATCTTC
S. oralis			GCCTTATGTC		
S. anginosus	TTTCTTGAAG	TGTTGAAAAT	GCCTTATGTG	GGTTGCAATA	TCCTTTCTTC
S. constellatus S. intermedius					
S. salivarius	TTCCTCGAAA				
S. mutans	TTTCTTGAAG	TTTTAAAAAT	GCCTTATGTG		
	** ** ***	* *** *	*** *****	** **	* * ** **
	51				100
S. gordonii S. sanguis			TTACGACCAA TCACGACCAA		
S. mitis			TCACGACCAA		
S. oralis			TTACGACCAA		
5. anginosus S. constellatus			TCACGACAAA TCACAACAAA		
5. intermedius			TCACAACTAA		
S. salivarius			TTATGACCAA		
S. mutans	TAGTGTAGCT ** * **		TTACAACAAA * * ** **		GAAAGTGCGA * *
	101				150
S. gordonii	CAGGTATTGC	TCAAGTACCT	TATGTAGCGG	TGGTTGATGG	
5. sanguis			TATGTGGCGC		
S. mitis S. oralis			TATGTGGCCA TATGTGGCTA		
S. anginosus	CTGGCATTCC	GCAAGTTCCC	TATGTAGCAG	TGATTGAAGG	GGAAAATATA
S. constellatus					
S. intermedius S. salivarius	CTGGTATTCC		TATGTAGCCG TATACAGTCT		
S. mutans			TATGTTGCTC		
	* *	** ** *	*** *	* * ** **	** * *
	151				200
S. gordonii			TGAAGAGAAA		CCGTCTTCAC
S. sanguis			CGAGGAGAAA		
S. mitis S. oralis			TGAAGAAAAA TGAAGAAAAA		
S. anginosus	GACGAAAAGA	TTGCAGCAGT	AGAAGCCAAT	CTGACTTATC	CAGTTTTTAC
S. constellatus					
S. intermedius S. salivarius			AGAAGTTAAT GCTTGAAAAA		
S. mutans			TGAAGAGAAA		
		* *	* **	* * * *	* ** **
	201				250
S. gordonii	201 CAAACCTTCT	AATATGGGCT	CCAGTGTCGG	CATTTCAAAA	250 TCTGATAATC
S. sanguis	CAAACCTTCT CAAACCTTCT	AATATGGGCT	CCAGTGTCGG	CATTTCTAAA	TCTGATAATC TCGGA.GACC
S. sanguis S. mitis	CAAACCTTCT CAAACCTTCT AAAACCATCT	AATATGGGCT AACATGGGTT	CCAGTGTCGG CTAGTGTCGG	CATTTCTAAA TATTTCTAAG	TCTGATAATC TCGGA.GACC TCTGAAAATC
S. sanguis	CAAACCTTCT CAAACCTTCT AAAACCATCT GAAGCCGTCA	AATATGGGCT AACATGGGTT AACATGGGTT	CCAGTGTCGG	CATTTCTAAA TATTTCTAAG TATTTCTAAG	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAACC
S. sanguis S. mitis S. oralis S. anginosus S. constellatus	CAAACCTTCT CAAACCTTCT AAAACCATCT GAAGCCGTCA AAAACCGTCA AAAACCGTCA	AATATGGGCT AACATGGGTT AACATGGGTT AATATGGGAT AATATGGGCT	CCAGTGTCGG CTAGTGTCGG CAAGTGTCGG CTAGTGTCGG CTAGTGTCGG	CATTTCTAAG TATTTCTAAG CATTTCTAAG CATTTCTAAG	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAACC TCTGAAAATC TCTGAAAATC
S. sanguis S. mitis S. oralis S. anginosus S. constellatus S. intermedius	CAAACCTTCT CAAACCTTCT AAAACCATCT GAAGCCGTCA AAAACCGTCA TAAACCGTCA	AATATGGGCT AACATGGGTT AACATGGGTT AATATGGGAT AATATGGGCT AATATGGGTT	CCAGTGTCGG CTAGTGTCGG CAAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG	САТТТСТААА ТАТТТСТААС ТАТТТСТААС САТТТСТААС САТТТСТААС САТТТСТААС	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAACA
S. sanguis S. mitis S. oralis S. anginosus S. constellatus	CAAACCTTCT CAAACCTTCT AAAACCATCT GAAGCGGTCA AAAACCGTCA AAAACCGTCA TAAACCATCA CAAACCTGCT TAAGCCAGCT	AATATGGGCT AACATGGGTT AACATGGGTT AATATGGGAT AATATGGGCT AATATGGGGT AATATGGGGT	CCAGTGTCGG CTAGTGTCGG CAAGTGTCGG CTAGTGTCGG CTAGTGTCGG CATCTGTTGG CTAGTGTTGG	САТТТСТААА ТАТТТСТААG САТТТСТААG САТТТСТААG САТТТСТААG САТТТСТААА GATTTCTAAA ТАТТТСТААА	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCTGAAAATG GCAGAAAATC
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. intermedius S. salivarius	CAAACCTTCT CAAACCATCT GAAGCCGTCA AAAACCGTCA AAAACCGTCA TAAACCGTCA CAAACCTGCT TAAGCCAGCT ** ** *	AATATGGGCT AACATGGGTT AACATGGGTT AATATGGGAT AATATGGGCT AATATGGGGT	CCAGTGTCGG CTAGTGTCGG CAAGTGTCGG CTAGTGTCGG CTAGTGTCGG CATCTGTTGG CTAGTGTTGG	САТТТСТААА ТАТТТСТААG ТАТТТСТААG САТТТСТААG САТТТСТААG САТТТСТААА GATTTCTAAA	TCTGATAATC TCGGAAGAC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCTGAAAATG GCAGAAAATC * ** *
S. sanguis S. mitis S. oralis S. anginosus S. constellatus S. intermedius S. salivarius S. mutans	CAAACCTTCT CAAACCTTCT AAAACCATCT GAAGCCGTCA AAAACCGTCA AAAACCGTCA CAAACCTGCT TAAGCCAGCT ** ** * 251	AATATGGGCT AACATGGGTT AATATGGGTT AATATGGGAT AATATGGGCT AATATGGGGT AATATGGGGT ** ***** *	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG CATCTGTTGG * *** **	САТТТСТААА ТАТТТСТААG САТТТСТААG САТТТСТААG САТТТСТААG САТТТСТААА GATTTCТААА ТАТТТСТААА **** * **	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCTGAAAATG GCGAAAAATC * ** *
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. intermedius S. salivarius	CAAACCTTCT CAAACCTTCT AAAACCATCT GAAGCCGTCA AAAACCGTCA TAAACCGTCA TAAACCATCA CAAACCGTCT TAAGCCAGCT ** ** * 251 AGGAAGAACT	AATATGGGCT AACATGGGTT AACATGGGTT AATATGGGAT AATATGGGAT AATATGGGT AATATGGGT AATATGGGT GCGTGCTTCT GCGTGCTTCT	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTGG CTAGTGTGG CTAGTGTTGG CTAGTGTTGG CTAGTGTTGG CTAGGACCTGG CTGGACCTGG	САТТТСТААА ТАТТТСТААG САТТТСТААG САТТТСТААG САТТТСТААG САТТТСТААА БАТТТСТААА АСТТТСТААА СТТТССАААТА СТТТССАААТА СТТТССАААТА	TCTGATAATC TCGGAAGACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCTGATAATG GCAGAAATC * ** * 300 CGATAGTCGG ACGACGCGGA
S. sanguis S. mitis S. oralis S. anginosus S. constellatus S. intermedius S. salivarius S. mutans S. gordonii S. sanguis S. mitis	CAAACCTTCT CAAACCATCT AAAACCATCT GAAGCCGTCA AAAACCGTCA TAAACCGTCA TAAACCATCA CAAACCATCA TAAGCCAGCT ** ** * 251 AGGAAGAACT AAGCAGAACT	AATATGGGCT AACATGGGTT AATATGGGTT AATATGGGT AATATGGGT AATATGGGT AATATGGGT CATATGGGT CCGTGCTTCT CGCTGAACC	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTGGG CTAGTGTTGG CTAGTGTTGG * *** ** CTGGACCTGG CTGGACCTGG TTGAAACTTG	САТТТСТАЛА ТАТТТСТАЛЯ САТТТСТАЛЯ САТТТСТАЛЯ САТТТСТАЛЯ САТТТСТАЛЯ ТАТТТСТАЛЯ ТАТТТСТАЛЯ **** * ** СТТТСАЛАТА ССТТССАЛТА	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATG GCAGAAAATC ***** 300 CGATAGTCGG ACGACGCCGA ACGACGCCGA
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. intermedius S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis	CAAACCTTCT CAAACCTTCT AAAACCATCT GAGCCGTCA AAAACCGTCA TAAACCGTCA TAAACCGTCA TAAGCCAGCT ***** 251 AGGAAGAACT AAGAGGAACT AAGAGGAACT	AATATGGGCT AACATGGGTT AACATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT CATATGGGTT CGGTGCTTCT TCGTCAAGCC CCGTCAAGCT	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG CTAGTGTTGG CTAGTGTTGG CTAGGACCTGG CTGGACCTGG TTGGAACCTG	САТТСТААА ТАТТСТААG САТТСТААG САТТСТААG САТТСТААG САТТСТААА АТТСТААА ТАТТСТААА ТАТТСТААА СТТССААТА СТТССААТА	TCTGATAATC TCTGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCTGAAAATC GCAGAAAATC * ** * 300 CGATAGTCGG ACGACGCCGA TGATAGCCGT TGACAGCCGT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. oralis S. constellatus	CAAACCTTCT CAAACCTTCT AAAACCATCT GAAGCCGTCA AAAACCGTCA TAAACCGTCA TAAACCATCA CAAACCTGCT ** ** * 251 AGGAGAACT AAGAGGAACT AAGAGGAACT AAGAGGAACT AAGAGGAACT	ANTATGGGT AACATGGTT AACATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT CATCAGGT CGGTGCTTCT GCGTGCTTCT GCGTCAGCT GCGTCTGCT	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG CTAGTGTTGG CTAGACTTG TTGGAACTTG TTGGAACTTG CTTGAATTGG CTTGAATTGG	САТТСТААА ТАТТСТААБ САТТСТААБ САТТСТААБ САТТСТААБ САТТСТААА АТТТСТААА СТТТСАААТА СТТТСАААТА СТТТСААТА ССТССААТА ССТССААТА СТТССААТА	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATG GCTGAAAATG GCAGAAAATC ***** 300 CGATAGTCGG ACGACGCGA TGATAGCCGT TGATAGCCGT
S. sanguis S. mitis S. oralis S. oralis S. anginosus S. intermedius S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. oralis S. constellatus S. intermedius	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAACCGTCA TAAACCATCA CAAACCTGCT TAAGCCAGCT ** ** * 251 AGGAAGAACT AAGCAGAACT AAGAGGAACT AAGAGGAACT AAGATGAATT	AATATGGGCT AACATGGGTT AACATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT CATCAAGCT GCGTGCTTCT GCGTCAAGCT GCGTCTGCT GCGTTCTGCT	CCAGGGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG CTAGTGTTGG CTGGACCTGG CTGGACCTGG CTGGAACTTG CTTGAATTGG CTTGAATTGG CTTAGATTAG	САТТТСТАЛА ТАТТТСТАЛА САТТТСТАЛА САТТТСТАЛА САТТТСТАЛА САТТТСТАЛА АТТТСТАЛА СТТТСАЛАТА СТТТСАЛАТА СТТТССАЛТА СТТТССАЛТА СТТТССАЛТА СТТТССАЛТА	TCTGATAATC TCGGAAGCC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCGATAATG GCGATAGCCGA TGATAGCCGT TGATAGCCGT TGATAGCCGT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. intermedius S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. oralis S. constellatus S. intermedius S. salivarius	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAACCGTCA TAAACCAGTC TAAGCCAGCT ** ** * 251 AGGAAGAACT AAGCAGGAACT AAGCAGGAACT AAGAGGAACT AAGAGGACT	AATATGGGCT AACATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT CATTCAAGCC GCGTGCTTCT GCGTGCTTCT GCGTTCAAGCT GCGTTCTGCT GCGTTCTGCT GCGTTCTGCT GCGTTCTGCT	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTGG CTGGACCTGG CTGGACCTGG CTGGACCTGG CTGGAACTGG CTTGAATTGG CTTAAGTTAG ATTGATCTGG	САТТТСТААА ТАТТТСТААG САТТТСТААG САТТТСТААG САТТТСТААА БАТТТСТААА САТТТСТААА СТТТСАААТА СТТТСААТА СТТТССААТА СТТТССААТА СТТТССААТА СТТТССААТА	TCTGATAATC TCGGAAGCC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCGAAAATG GCAGAAAATC **** 300 CGATAGTCGG TGATAGCCGT TGATAGCCGT TGATAGCCGT
S. sanguis S. mitis S. oralis S. oralis S. anginosus S. intermedius S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. oralis S. constellatus S. intermedius	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAACCGTCA TAAACCATCA CAAACCTGCT TAAGCCAGCT ** ** * 251 AGGAAGAACT AAGCAGAACT AAGAGGAACT AAGAGGAACT AAGATGAATT	ANTATGGGCT AACATGGGTT AACATGGGTT ANTATGGGTT ANTATGGGTT ANTATGGGTT ANTATGGGTT ANTATGGGTT ANTATGGGTT GCGTGCTTCT GCGTGCTTCT GCGTCAAGCC GCGTCTGCT GTGTTCTGCT TCGTGCAGCG ANAACAAGCT	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTGGG CTAGTGTTGG CTAGTGTTGG CTAGGACCTGG CTGGACCTGG CTGGAACTTG CTTGAATTGG CTTGAATTGG ATTGCACCTG	САТТТСТААА ТАТТТСТААG САТТТСТААG САТТТСТААG САТТТСТААА БАТТТСТААА САТТТСТААА СТТТСАААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА	TCTGATAATC TCGGAAGCC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCGAAAATG GCAGAAAATC **** 300 CGATAGTCGG TGATAGCCGT TGATAGCCGT TGATAGCCGT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. intermedius S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. oralis S. constellatus S. salivarius S. salivarius S. mutans	CAAACCTTCT CAAACCTTCT AAAACCATCT GAAGCCGTCA AAAACCGTCA TAAACCGTCA TAAACCATCA CAAAACCGTCA TAAGCCAGCT ** ** * 251 AGGAAGCAGCT AAGAGGAACT AAGAGGAACT AAGAGGAACT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT	ANTATGGGT AACATGGTT AACATGGT AACATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT CATATGGGT CATATGGGT CGTGCTTCT GCGTGCTTCT GCGTCAGCC GCGTCTGCT GCGTCTGCT GCGTCTGCT GCGTCTGCT CGTGCAGCG AAAACAACCA	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTGG CTAGTGTTGG CTAGTGTTGG CTAGGACCTGG CTGGAACTGG CTGGAACTGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG	САТТСТААА ТАТТСТААG САТТСТААG САТТСТААG САТТСТААG САТТСТААА АТТТСТААА ТАТТСТААА ТАТТСТААА СТТТСАААТА СТТТСААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATG GCTGAAAATG GCAGAAAATC *** * 300 CGATAGTCGG ACGACGCGA TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. intermedius S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. anginosus S. constellatus S. salivarius S. mutans S. gordonii	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAAACCGTCA TAAACCATCA CAAACCTGGT TAAGCCAGCT ** ** * 251 AGGAAGAACT AAGCAGGACT AAGCAGGAACT AAGAGGAACT AAGAGGAACT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT	ANTATGGGCT AACATGGGTT ANTATGGGTT ANTATGGGTT ANTATGGGTT ANTATGGGTT ANTATGGGTT ANTATGGGTT ANTATGGGTT CGTGCTTCT CGTGCTTCT CGTGCAAGCT CCGTCAAGCT CCGTCCAAGCT CCGTCCACGC ANAACAAGCT	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG CTAGTGTTGG CTAGTGTTGG CTGGACCTGG CTGGACCTGG CTGGACCTGG CTGGAACTGG CTGGAATGG CTGAATGG CTGAATGG CTGAATGG CTGAATGG CTGAATGG CTGAATGG CTGAATGG CTGAATGG CTGAATGG CTGAATGG CTGAATGGC CTGAATGGCACTGG CTTAAGTAGCACTG CTGAACAGCT	САТТТСТААА ТАТТТСТААG САТТТСТААG САТТТСТААG САТТТСТААА АДТТСТААА АДТТСТААА САТТТСТААА **** * ** СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА СТТТСААТА	TCTGATAATC TCGGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC **** * 300 CGATAGTCGG CGATAGTCGG CGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. oralis S. anginosus S. constellatus S. salivarius S. mutans S. gordonii S. sanguis	CAAACCTTCT CAAACCTTCT CAAACCTTCT AAAACCATCT GAGCGTCA AAAACCGTCA AAAACCGTCA TAAACCATCT CAAACCGTCA TAAGCCACT AAAACCGTCA AAAACCGTCA TAAGCCACCT AAGACAGCACT AAGCAGAACT AAGCAGCACCT AAGCAGAACT AAGAGGAACT AAGAGGAACT AAGATGAATT AAGATGAATT AAGCAGCACT S011 GTACTAGTGG GTACTAGTGGTG	ANTATGGGT AACATGGGT AACATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT ** ***** GCGTGCTTCT GCGTGCTTCT GCGTCAAGCC CCGTCAAGCC GCGTCTGCT GCGTCTCTCT GTGTCAAGCC AAAACAAGCT * AGCAAGG AGCAAGGAA	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG * *** ** CTGGAACTGG CTGGAACTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATGGC TTGGAACGCT * *	САТТСТААА ТАТТСТААG САТТСТААG САТТСТААG САТТСТААG САТТСТААА АТТТСТААА СТТТСАААТА СТТТСАААТА СТТТСАААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА СТТССААТА	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCGAGAAAATC ***** 300 CGATAGTCGGA TGATAGCCGA TGATAGCCGA TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT
S. sanguis S. mitis S. oralis S. anginosus S. constellatus S. intermedius S. salivarius S. mutans S. gordonii S. anginosus S. intermedius S. salivarius S. salivarius S. sanguis S. gordonii S. sanguis S. mitis S. oralis	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAACCGTCA TAAACCAGTC TAAGCCAGCT ** ** * 251 AGGAAGAACT AAGCAGGAACT AAGCAGGAACT AAGAGGAACT AAGAGGAACT AAGAGGAACT GCACTGATCG GTACTAGTCG GTACTAGTCG GTACTAGTCG GTCTTCGTTGG	ANTATGGGT AACATGGTT AACATGGTT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT ** ***** * GCGTGCTTCT TCGTCAAGCT CCGTCAAGCT GCGTTCTGCT GCGTTCTGCT GCGTTCTGCT GCGTTCTGCT AATACAAGCT * AGCAAGGAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTGG CTAGTGTGG CTAGTGTGG CTAGTGTGG CTGGACCTGG TTGGAACTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATGGC TTGGAACGCT CGTGAACGCT CGGAACGGCT GGTGAATGCC	САТТСТАЛА ТАТТСТАЛА САТТСТАЛА САТТСТАЛА САТТСТАЛА САТТСТАЛА САТТСТАЛА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА СТТТСАЛАТА	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCGATAATC * ** * 300 CGATAGTCGG GGATAGCCGT TGATAGCCGT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. oralis S. constellatus S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. oralis S. mitis S. oralis S. oralis S. oralis S. oralis S. oralis S. oralis S. oralis S. oralis S. oralis	CAAACCTTCT CAAACCTTCT AAAACCATCT GAGCCGTCA AAAACCGTCA TAAACCGTCA TAAACCAGTCA CAAACCGTCA TAAGCCAGCT ** ** * 251 AGGAAGCAACT AAGCAGCAGT AAGCAGCAGT GCACTGATG GTACTAGTCG GTACTAGTCG GTCTTGGTTG GTCTTGGTTG GTCTTGGTTG	ANTATGGGT AACATGGTT AACATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT CGTGCTTCT GCGTGCTTCT GCGTCAGCT GCGTCTGCT GCGTCTGCT GCGTCTGCT GCGTCTGCT GCGTCTGCT GCGTCTGCT CGTCAAGCC AAAACAAGC AGCAAGG AGCAAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG * *** ** CTGGACCTGG CTGGACCTGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGC GGTGAATGCC GGTGAATGCC GGTGAATGCC	CATTICTAAA TATITICTAAG CATTICTAAG CATTICTAAG CATTICTAAA GATTICTAAA ATATITICTAAA CTTICAAATA CTTICAAATA CTTICCAATA CTTICCAATA CTTICCAATA CTTICCAATA CTTICCAATA CTTICCAATA CTTICCAATA CTTICCAATA CTTICCAATA CTTICCAATA CTTICCAATA CTCICAATA CTCICAATA CTCICAATA CTCICAATA CTCICAATA	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC ***** 300 CGATAGTCGG TGACAGCCGT TGATGGCTT AGGTTGGCTT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. constellatus S. salivarius S. mutans S. gordonii S. sanguis S. oralis S. oralis S. constellatus S. mutans S. gordonii S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. anginosus S. oralis S. anginosus S. constellatus	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAACCGTCA TAAACCGTCA TAAGCCGTCA TAAGCAGCTGCT TAAGCAGACT AAGCAGAACT AAGCAGAACT AAGCAGAACT AAGCAGAACT AAGCAGAACT AAGCAGAACT AAGCAGAACT AAGCAGACT TAAGCAGACT CTACTGGTG GTACTGGTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG	ANTATGGGT AACATGGGT AACATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT CATCAGG CGGTGCTTCT GCGTGCTTCT GCGTCAGCC CCGTCAAGCC CCGTCAAGCC CCGTCAGCT GCGTTCTGCT GCGTTCTGCT GCGTTCTGCT TCGTCCAGCG ANAACAAGC AGCAAGG AGCAAGG AGCAAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG CTAGTGTTGG CTAGTGTTGG CTAGTGTTGG CTGGACCTGG CTGGACCTGG CTGGAACTGG CTTGAATTGG CTTGAATGG CTTGAATGG CTTGAATGGC TGGAACGCT TCGAACGCT GGTAATGCC GGTAATGCC TGCAATGCC	CATTICTAAG TATITCTAAG CATTICTAAG CATTICTAAG CATTICTAAA GATTICTAAA GATTICTAAA CATTICTAAA CATTICAAATA CTTICAAATA CTTICAATA CTTICAATA CTTICAATA CTTICAAATA CTTICAAATA CTTICAAATA CTTICAAATA CTTICAAATA CTTICAAATA CTTICAAATA CTTICAAATA CTTICAAATA CTTICAAATA CTTICAAATA CTTICAAATA CTTICAAATA	TCTGATAATC TCGGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAACGCGA GCATAGTCGG ACGATGCCGA TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATGGCTT AGGTTGGTTT AAGTTGGTTT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. constellatus S. salivarius S. mutans S. gordonii S. anginosus S. constellatus S. mutans S. gordonii S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. oralis S. constellatus S. mitis S. oralis S. anginosus S. constellatus S. anginosus S. constellatus S. anginosus S. constellatus S. salivarius	CAAACCTTCT CAAACCTTCT CAAACCTTCT AAAACCATCT GAGCCGTCA AAAACCGTCA AAAACCGTCA TAAACCATCT CAAACCGTCA TAAGCCACT AAAACCGTCA AAAACCGTCA TAAGCCAGCT ** ** * 251 AGGAGAGCAT AAGCAGCAGCA AAGCAGCAGCA AAGAGGAACT AAGAGGAACT AAGAGGAACT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGAATT AAGATGATT AGGATGATT AGGATGATT AGGATGATT AGGATGATT AGGATGATT AGGATGATT AGGATGATT AGGATGATT GACTGACTGATG GTCTTGGTGG GTCTTGATTG ATCTTGATG	ANTATGGGT AACATGGGT AACATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT CGTGCTTCT GCGTGCTTCT GCGTCAAGC CCGTCAAGC CCGTCAAGC GCGTCTGCT GCGTCTCTC GCGTCTCTC GCGTCTCTC GCGTCTCTC GCGTCTCCC TCGTCAAGC AAAACAAGC AGCAAGG AGCAAGG AGCAAGG AGCAAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG CTAGTGTTGG CTAGTGTTGG CTAGACCTGG CTGGAACTGG CTGGAACTGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTGGAACGCT GGTAAACGCT GGTAAACGGGT GGTAAATGCG GGTGAATGCC GGTGAATGCC GGTGAATGCC GGTCAATGCG TGTCAATGCG TGTCAATGCG	CATTICTANA TATTICTANG CATTICTANG CATTICTANG CATTICTANG CATTICTANA ATTITCTANA TATTICTANA TATTICTANA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATG GCTGAAAATG GCTGAAAATG ***** 300 CGATAGTCGG TGATAGCCGT AGGTTGGTTT AAGTTGGTTT AAGTTGGTTT AAGTTGGTTT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. constellatus S. salivarius S. mutans S. gordonii S. anguis S. anginosus S. salivarius S. sanguis S. salivarius S. sanguis S. sanguis S. mitis S. oralis S. anguis S. mitis S. constellatus S. constellatus S. constellatus S. constellatus S. constellatus S. constellatus S. constellatus	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAAACCGTCA TAAACCGTCA TAAACCATCA CAAACCTGCT TAAGCAGGTC TAAGCAGGACT AAGCAGGACT AAGCAGGACT AAGCAGGACT AAGCAGGACT AAGCAGGACT AAGCAGGACT AAGCAGGACT GCATCAGTG GTCTTGGTG GTCTTGGTG GTCTTGGTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG	ANTATGGGCT AACATGGGTT AACATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT CGCGTGCTTCT CGCGTGCATCT CCGTCAAGCT CCGTCCAAGCT CCGTCCAAGCT CGCGTCTCGCT CCGTCCAAGCG ANAACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTGGG CTAGTGTGGG CTAGTGTGGG CTAGTGTGGG CTAGTGTGGG CTGGACCTGG CTGGACCTGG CTGGACTGG CTGGACTGG CTGGACTGG CTGAATGG CTGAATGGC CTGAACGCCT CGGGAATGCC GGTGAATGCC GGTGAATGCG GGTGATGCG CGGGATGCG	CATTCCTAAG TATTTCCTAAG CATTTCCTAAG CATTTCCTAAG CATTTCCTAAA GATTTCCTAAA GATTTCCTAAA CATTCCTAAA CATTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTCCAATA	TCTGATAATC TCGGAAGCC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC ***** 300 CGATAGTCGG CGATAGTCGG CGATAGCCGT TGATAGCCGT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. constellatus S. salivarius S. mutans S. gordonii S. anginosus S. constellatus S. mutans S. gordonii S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. oralis S. constellatus S. mitis S. oralis S. anginosus S. constellatus S. anginosus S. constellatus S. anginosus S. constellatus S. salivarius	CAAACCTTCT CAAACCTTCT AAAACCATCT GAGCCGTCA AAAACCGTCA TAAACCGTCA TAAACCAGTCA CAAACCGTCA TAAGCCAGCT ** ** * 251 AGGAAGAACT AAGCAGCAGCT AAGCAGCAGCT AAGCAGCAGCT GCACTGGTG GTCTTGGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG	ANTATGGGCT AACATGGGTT AACATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT AATATGGGTT CGCGTGCTTCT CGCGTGCATCT CCGTCAAGCT CCGTCCAAGCT CCGTCCAAGCT CGCGTCTCGCT CCGTCCAAGCG ANAACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTGGG CTAGTGTGGG CTAGTGTGGG CTAGTGTGGG CTAGTGTGGG CTGGACCTGG CTGGACCTGG CTGGACTGG CTGGACTGG CTGGACTGG CTGAATGG CTGAATGGC CTGAACGCCT CGGGAATGCC GGTGAATGCC GGTGAATGCG GGTGATGCG CGGGATGCG	CATTICTANA TATTICTANG CATTICTANG CATTICTANG CATTICTANG CATTICTANA ATTITCTANA TATTICTANA TATTICTANA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA CTTICANATA	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC ***** 300 CGATAGTCGGA TGATAGCCGA TGATAGCCGA TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATGGTTT AGGTTGGTTT AAGTTGGTTT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT AGGTTGGTAT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. constellatus S. salivarius S. mutans S. gordonii S. mitis S. oralis S. constellatus S. mutans S. gordonii S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. oralis S. anginosus S. constellatus S. constellatus S. intermedius S. salivarius S. salivarius S. salivarius S. salivarius S. mutans	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAACCGTCA TAAACCATCA CAAACCTGCT TAGGCAGCT ***** 251 AGGAAGAACT AAGCAGGACT AAGCAGGACT AAGCAGGACT AAGCAGGACT AAGCAGGACT AGGAGGAGCT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGACT AGGAGGAGC ACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGC ACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGC ACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGC ACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGC ACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGACT AGGAGGAGGAGGACT AGGAGGAGGAGGAGGAGGACT AGGAGGGAGGAGGAGGACT AGGAGGGGAGG	ANTATGGGT AACATGGGT AACATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT CGTGCTTCT CGTGCAGCC CCGTCAGCT CCGTCAGCC CCGTCAGCC GCGTCTCTC GCGTCTCTC GCGTCTCTC GCGTCTCGCT TCGTCAAGCC AAAACAGC AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG ACCAAGG ACCAAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTGGG CTAGTGTGGG CTAGTGTGGG CTAGTGTGGG CTAGTGTGGG CTAGTGTGG CTGGACCTGG CTGGACCTGG CTGGACTGG CTGGACTGG CTGGACTGG CTGGACTGG CTGGACCGCT TCGACGCT CGGGAATGCC GGGAATGCC GGCGATGCC CGCGATGCC TGCCATCGG TGCGATGCC TGTGGTGCCC CGTGGATGCC CGGGGATGCC CGGGGATGCC CGGGGATGCC	CATTCCTAAA TATTTCCTAAG CATTTCCTAAG CATTTCCTAAG CATTTCCTAAA GATTTCCTAAA GATTTCCTAAA CATTCCTAAA CATTCCTAAA CTTTCCAAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTCCAATA	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC ***** 300 CGATAGTCGG TGATAGCCGT T
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S. sanguis S. mitis S. oralis S. oralis S. anginosus S. constellatus S. intermedius S. mutans S. gordonii S. anginosus S. anginosus S. salivarius S. mitis S. anginosus S. salivarius S. mitis S. oralis S. anginosus S. constellatus S. oralis S. anginosus S. constellatus S. salivarius S. salivarius S. mitis S. salivarius S. mutans	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAACCGTCA TAAACCGTCA TAAACCATCT CAACCTGCT TAGGCAGGTACT AAGCAGGAACT AAGCAGGAACT AAGCAGGAACT AAGCAGGAACT AAGCAGGAACT AAGCAGGAACT AAGAGGAACT AAGATGATT AGGCAGGACT AAGATGATT AGGCAGGACT CTTCGTTAG GTCTTGGTAG GTCTTGGTAG GTCTTGGTAG GTCTTGGTAG GTCTTGGTAAC GTCTTGGTAAC TTCTGGTAAC	ANTATGGGT AACATGGTT AACATGGTT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT CGCTGCTTCT CGCCAGCCTTCT CGCCAGCC CCGTCAGCT CCGTCAGCC GCGTTCTGCT GCGTTCTGCT GCGTTCTGCT GCGTTCTGCT GCGTTCTGCT AGCAAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG TTGGACCTGG TTGGACCTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG CTTGAATTGG ATTGCACTTG GTAATTGC CTTGAACGGCT GGTGAATGCG TGCCAATGCG TGCCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG	CATTICTANA TATITICTANG CATTICTANG CATTICTANG CATTICTANG CATTICTANA CATTICTANA CTTICANT	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATG GCGATAGTCG * *** * 300 CGATAGTCGG TGATAGCCGT AGGTTGGTTT AGGTTGGTTT AGGTTGGTTT AGGTTGGTT
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S. sanguis S. mitis S. oralis S. oralis S. constellatus S. constellatus S. salivarius S. mutans S. gordonii S. anguis S. anginosus S. constellatus S. mitis S. anginosus S. sanguis S. mitis S. mitis S. mutans S. gordonii S. anginosus S. constellatus S. salivarius S. salivarius S. salivarius S. salivarius S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. anginosus S. constellatus S. constellatus	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAACCAGTCA TAAACCAGTCA TAAACCAGCA CAAACCAGCT ** ** * 251 AGGAAGAACT AAGCAGGAACT AAGCAGGAACT AAGCAGGAACT AAGAGGAACT AAGAGGAACT AAGAGGAACT GCACTGATGG GTACTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAAC TTCTGGGCAAC	ANTATGGGT AACATGGGT AACATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT ******* GCGTGCTTCT GGCTGCTTCT GGCTGCTTCT GGCTCAGCT GCGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCAAGG AACAAGG AGCAAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTGGACCTGG CTGGACCTGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTGGACTGG ATGGCACCTG GTGAATGC GGTGAATGCG TGCCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTGATGCCC TGTGGTGCCCT GGTGATGCCC TGTGGTGCCCT AAAGCAGTTC AAAGCAGTTG	CATTCCTAAA TATTTCCTAAG CATTTCCTAAG CATTTCCTAAG CATTTCCTAAG CATTTCCTAAA GATTTCCTAAA CATTCCTAAA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CCTGGGGAA CCCAGGGGAA	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCGATAGTGG GCAGAAAATC ***** 300 CGATAGCCGA TGATAGCCGA TGATAGCCGA TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT AGGTTGGACT AGGTTGGACT AGGTTGGTTT AAGTTGGTTT AGGTTGGTTT AGGTTGGTT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. constellatus S. salivarius S. mutans S. gordonii S. anguis S. anginosus S. constellatus S. mitis S. anginosus S. sanguis S. mitis S. mitis S. mutans S. gordonii S. anginosus S. constellatus S. salivarius S. salivarius S. salivarius S. salivarius S. salivarius S. mutans S. gordonii S. sanguis S. mitis S. anginosus S. constellatus S. constellatus	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAACCAGTCA TAAACCAGTCA TAAACCAGCA CAAACCAGCT ** ** * 251 AGGAAGAACT AAGCAGGAACT AAGCAGGAACT AAGCAGGAACT AAGAGGAACT AAGAGGAACT AAGAGGAACT GCACTGATGG GTACTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAAC TTCTGGGCAAC	ANTATGGGT AACATGGGT AACATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT ******* GCGTGCTTCT GGCTGCTTCT GGCTGCTTCT GGCTCAGCT GCGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCTGCT GGGTCAAGG AACAAGG AGCAAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTGGACCTGG CTGGACCTGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTTGAATGG CTGGACTGG ATGGCACCTG GTGAATGC GGTGAATGCG TGCCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTGATGCCC TGTGGTGCCCT GGTGATGCCC TGTGGTGCCCT AAAGCAGTTC AAAGCAGTTG	CATTCCTAAA TATTTCCTAAG CATTTCCTAAG CATTTCCTAAG CATTTCCTAAG CATTTCCTAAA GATTTCCTAAA CATTCCTAAA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CTTCCAATA CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CGTGAAATG CCTGGGGAA CCCAGGGGAA	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC GCGATAGTGG GCAGAAAATC ***** 300 CGATAGCCGA TGATAGCCGA TGATAGCCGA TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT AGGTTGGACT AGGTTGGACT AGGTTGGTTT AAGTTGGTTT AGGTTGGTTT AGGTTGGTT
S. sanguis S. mitis S. oralis S. oralis S. constellatus S. constellatus S. salivarius S. mutans S. mutans S. mitis S. oralis S. constellatus S. mitis S. anginosus S. mutans S. gordonii S. sanguis S. anginosus S. constellatus S. constellatus S. anginosus S. constellatus S. salivarius S. mutans S. gordonii S. sanguis S. mutans S. gordonii S. sanguis S. mutans	CAAACCTTCT CAAACCTTCT AAAACCATCT AAAACCATCT AAAACCATCT AAAACCGTCA AAAACCGTCA TAAACCAGTCA TAAACCAGTCA TAAACCAGCA CAAACCAGCT ** ** * 251 AGGAAGAACT AAGCAGGAACT AAGCAGGAACT AAGCAGGAACT AAGAGGAACT AAGAGGAACT AAGAGGAACT GCACTGATGG GTACTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAGTGG GTCTTGGTAAC TTCTGGGCAAC	ANTATGGGT AACATGGGT AACATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT AATATGGGT CGTGCTTCT GCGTGCTTCT GCGTCAGCC CCGTCAAGCC CCGTCAAGCC CCGTCAAGCC GCGTCTCTC GCGTCTCGT GCGTCTCGC GCGTCTCGC GCGTCTCGC AAAACAAGC AGCAAGG AGCAGG	CCAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTCGG CTAGTGTTGG CTAGTGTTGG CTGGACCTGG CTGGAACTGG CTGGAACTGG CTTGAATGG CTTGAATGG CTTGAATGG CTGGAATGCC GGTGAATGCC GGTGAATGCC GGTGAATGCC GGTGAATGCC TGTCAATGCG TGTCGATGCC TAGGAGCCCT AAAGCACGTTT AAAGCAGTTT AAACGCACTAA	CATTICTANA TATTICTANG CATTICTANG CATTICTANG CATTICTANA GATTICTANA GATTICTANA CATTICTANA CTTICANATA CTTICANATA CTTICANTA CCTGGAGATTG CGTGANTG CGTGANTG CGTGANTG CGTGANTG CCTGGAGAT CCCGGGAN CCAGGTGAN CCCAGGTGAN CCCAGGTGAN	TCTGATAATC TCGGA.GACC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAATC TCTGAAAACA GCGATAGTCGG GCAGAAATG GCAGACGCGA GCGATAGCCGA TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT TGATAGCCGT AGGTTGGTTT AGGTTGGTTT AGGTTGGTAT *****

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	gordonii			CCAGTGTCGG		
s.	sanguis			CCAGTGTCGG	*****	
	mitis			CTAGTGTCGG		
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	intermedius	TAAACCATCA	AATATGGGTT	CTAGTGTTGG	CATTTCTAAA	TCTGAAAACA
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	gordonii	GTACTAGTCG	AGCAAGG		CGTGAGATTG	AGGTTGGACT
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s. s.	sanguis mitis	GTACTAGTCG GTACTGGTTG GTCTTGGTTG	AGCAAGGAGA AACAAGG	TCGAACGGCT GGTAAATGCC	CGCGAGATTG CGTGAAATCG	AGGTTGGACT AGGTTGGGCT AGGTTGGCCT
s. s. s.	sanguis mitis oralis	GTACTAGTCG GTACTGGTTG GTCTTGGTTG GTCTTGGTAG	AGCAAGGAGA AACAAGG AGCAAGG	TCGAACGGCT GGTAAATGCC GGTGAATGCC	CGCGAGATTG CGTGAAATCG CGTGAAATCG	AGGTTGGACT AGGTTGGGCT AGGTTGGCCT AGGTTGGTCT
s. s. s. s.	sanguis mitis oralis anginosus	GTACTAGTCG GTACTGGTTG GTCTTGGTTG GTCTTGGTAG GTCTTGATTG	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG	AGGTTGGACT AGGTTGGGCT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT
s. s. s. s.	sanguis mitis oralis anginosus constellatus	GTACTAGTCG GTACTGGTTG GTCTTGGTTG GTCTTGGTAG GTCTTGATTG GTCTTGATTG	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG	AGGTTGGACT AGGTTGGGCT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT
s. s. s. s. s.	sanguis mitis oralis anginosus constellatus intermedius	GTACTAGTCG GTACTGGTTG GTCTTGGTTG GTCTTGGTAG GTCTTGATTG GTCTTGATTG	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG	AGGTTGGACT AGGTTGGGCT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AAGTTGGTTT
s. s. s. s. s. s.	sanguis mitis oralis anginosus constellatus intermedius salivarius	GTACTAGTCG GTACTGGTTG GTCTTGGTTG GTCTTGGTAG GTCTTGATTG GTCTTGATTG ATCTTGATTG	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTGGTTGCC	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG	AGGTTGGACT AGGTTGGGCT AGGTTGGCCT AAGTTGGTCT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT
s. s. s. s. s. s.	sanguis mitis oralis anginosus constellatus intermedius	GTACTAGTCG GTACTGGTTG GTCTTGGTTG GTCTTGGTAG GTCTTGATTG GTCTTGATTG ATCTTGATTG GTCTTGATTG GTCTTGATTG	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTGGTTGCC TGTGGATGCG	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATCG CGTGAGATTG	AGGTTGGACT AGGTTGGGCT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT AGGTTGGTAT
s. s. s. s. s. s.	sanguis mitis oralis anginosus constellatus intermedius salivarius	GTACTAGTCG GTACTGGTTG GTCTTGGTTG GTCTTGGTAG GTCTTGATTG GTCTTGATTG ATCTTGATTG	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTGGTTGCC	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG	AGGTTGGACT AGGTTGGGCT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT AGGTTGGTAT
s. s. s. s. s. s.	sanguis mitis oralis anginosus constellatus intermedius salivarius	GTACTAGTCG GTACTGGTTG GTCTTGGTTG GTCTTGGTAG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTTTTAATTG * * * *	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTGGTTGCC TGTGGATGCG	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATCG CGTGAGATTG	AGGTTGGACT AGGTTGGGCT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AGGTTGGTTT AGGTTGGTAT * ****** *
s. s. s. s. s. s.	sanguis mitis oralis anginosus constellatus intermedius salivarius mutans	GTACTAGTCG GTACTGGTTG GTCTTGGTTG GTCTTGGTAG GTCTTGATTG GTCTTGATTG GTCTTGATTG ATCTTGATTG ATCTTGATTG 3TTTAATTG 351	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AACAAGG * *****	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTGGTTGCC TGTGGATGCC **	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAGATTG ** ** ** *	AGGTTGGACT AGGTTGGCCT AGGTTGGCCT AAGTTGGTCT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT AGGTTGGTAT * ***** *
s. s. s. s. s. s.	sanguis mitis oralis anginosus constellatus intermedius salivarius mutans gordonii	GTACTAGTCG GTACTGGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTTTTAATTG * * * * 351 TCTTGGTAAC	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AACAAGG * *****	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG TGTGGTATGCC TGTGGATGCC ** AAAGCAGTCT	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATCG CGTGAAATCG CGTGAGATTG ** ** ** * TCCAGGGGAA	AGGTTGGACT AGGTTGGCCT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT AGGTTGGTAT * ***** * 400 GTTGTTAAGG
s. s. s. s. s. s. s.	sanguis mitis oralis anginosus constellatus intermedius salivarius mutans gordonii sanguis	GTACTAGTCG GTACTAGTTG GTCTTGGTG GTCTTGGTG GTCTTGATG GTCTTGATTG ATCTTGATTG ATCTTGATTG ATCTTGATTG STI SI SI CTCTGGTAAC TTTGGGCAAC	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG ****** ACCAGG * *****	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTGGTTGCC TGTGGATGCG ** AAAGCAGTCT AGAGTACCCT	CGCGAAATCG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATCG CGTGAGAATCG ** ** ** ** TCCAGGGGAA ACCTGGAGAA	AGGTTGGACT AGGTTGGCCT AGGTTGGCCT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT AGGTTGGTAT AGGTTGGTAT AGGTTGGTAA 4000 GTTGTTAAGG GTGGTCAAGG
s. s. s. s. s. s. s. s. s. s.	sanguis mitis oralis anginosus constellatus intermedius salivarius mutans gordonii sanguis mitis	GTACTAGTCG GTACTAGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG GTCTTGATTG ATCTTGATTG STTTTAATTG * * * * 351 TCTTGGTAAC TTTGGGCAAT ACTGGGTAAC	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG * ***** ACTGATGTCA GTTGACGGTCA TACGATGTTA	ТСGААСGGCT GGTAAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTGATGCC TGTGGATGCC ** АААGCAGTCT АААGCAGCCT	CGCGAGATTG CGTGAAATCG CGTGAAATGG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATCG CGTGAGATG ** ** ** ** * TCCAGGGGAA ACCTGGGGAA TCCAGGAGAA	AGGTTGGACT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT ********* 400 GTTGTTAAGG GTGGTCAAGG GTGGTCAAGG
5. 5. 5. 5. 5. 5. 5. 5. 5. 5. 5.	sanguis mitis oralis anginosus constellatus intermedius salivarius mutans gordonii sanguis mitis oralis	GTACTAGTCG GTACTAGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTTTTAATTG GTTTTAATTG STTTTAATTG GTTTTAATTG GTTTTAATTG CTTGGGCAAC TTTGGGCAAC CTTGGGCAAC	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AACAAGG * ***** ACTGATGTCA GTTGACGTCA TACGATGTTA TACGATGTGA	TCGAACGGCT GGTAAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTGGATGCC TGTGGATGCC ** AAAGCAGCGCT AGAGCACGCCT	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAGATTG ** ** ** ** TCCAGGGGAA ACCTCGAGAA TCCTGGGGAA	AGGTTGGACT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT ****** * 400 GTGGTCAAGG GTGGTCAAGG GTGGTCAAGG
5. 5. 5. 5. 5. 5. 5. 5. 5. 5. 5. 5. 5. 5	sanguis mitis oralis anginosus constellatus intermedius salivarius mutans gordonii sanguis mitis oralis anginosus	GTACTAGTCG GTACTAGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG STCTTGATTG STTTTGGCAAT ACTGGGTAAC CTTGGGCAAT ACTGGGAAAT	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG ACCAAGG ACCAAGG ACCAATGTCA TACGATGTCA TACGATGTCA TACGATGTCA	TCGAACGCT GGTAAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCGATGCC TGTGGATGCC TGTGGATCCC AAAGCAGCTCT AGAGTACCCT AAAGCACGCT	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAGATTG ** ** ** ** TCCAGGGGAA ACCTGGAGAA TCCTGGAGAA TCCTGGTGAA	AGGTTGGACT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT AGGTTGGTAT ********* 400 GTGGTCAAGG GTGGTCAAGG GTAGTCAAGG GTAGTCAAGA
s. s. s. s. s. s. s. s. s. s. s. s. s. s	sanguis mitis oralis anginosus constellatus intermedius salivarius mutans gordonii sanguis mitis oralis anginosus constellatus	GTACTAGTCG GTACTAGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG GTCTTGATTG ATCTTGGTAAC TTTGGCAAT ACTGGGCAAC ACTGGGAAAT	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGGAGCA TACGATGTCA GTTGACGTCA GAAGGAGCCA	TCGAACGCT GGTAAATGCC GGTGAATGCG TGTCAATGCG TGTCAATGCG TGTCGATGCC TGTGGTTGCC TGTGGATGCC ** AAAGCACGCT AGAGCACGCT AAAGCACGTT	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATCG CGTGAAATCG CGTGAGATG CCTGGGGAA ACCTCGGGGAA ACCTCGGGGAA CCCGGGTGAG GCCAGGTGAG	AGGTTGGACT AGGTTGGCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT ********* 400 GTTGTTAAGG GTGGTCAAGG GTGGTCAAGG GTAGTCAAGG GTAGTCAAAG GTAGTCAAAG
s. s. s. s. s. s. s. s. s. s. s. s. s. s	sanguis mitis oralis anginosus constellatus intermedius salivarius mutans gordonii sanguis mitis oralis constellatus intermedius	GTACTAGTCG GTACTAGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTTTTAATTG GTTTTAATTG GTTTTAATTG GTTTTAATTG GTTTTAATTG GTTTGGGCAAT ACTGGGCAAT ACTGGGCAAT ACTTGGAAAT	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG ACTGATGTCA GTTGACGTCA TACGATGTCA GAAGGAGCCA GAAGGAGCCTA	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG TGTCGATGCC TGTGGATGCC TGTGGATGCC TGTGGATGCC TGTGGATGCC TGTGGATGCC AAAGCACGCT AAAGCACGTT AAAGCAGTTT AAAGCAGTTT	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATCG CGTGAAATCG CGTGAAATCG CCGGGGAA TCCCGGGGAA TCCCGGGGAA CCCAGGTGAG GCCAGGTGAG	AGGTTGGACT AGGTTGGCT AGGTTGGTCT AAGTTGGTT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT AGGTGGTAA AGGTGGTAAGG GTGGTCAAGG GTGGTGAAAG GTAGTGAAAG GTGGTGAAAG
S. S. <td< td=""><td>sanguis mitis oralis anginosus constellatus intermedius salivarius mutans gordonii sanguis mitis oralis anginosus constellatus intermedius salivarius</td><td>GTACTAGTCG GTACTAGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG STCTTGATG ATCTGGTATG STTTTGGGCAAT ACTGGGCAAT ACTTGGAAT ATTTGGGAAC CCTTGGCAAT</td><td>AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AACAAGG ACTGATGTCA GTGACGTCA TACGATGTCA TACGATGTCA GAAGGAGCCA GAAGGAGCCA AACGAGCTA</td><td>TCGAACGCT GGTAAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCGATGCC TGTGGATGCC TGTGGATGCC TGTGGATCCC AAAGCAGTCT AAAGCACGCT AAAGCACGTT AAAGCACTTG AAAGCACTAA</td><td>CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATCG CGTGAGATTG ** ** ** ** TCCAGGGGAA TCCAGGGGAA TCCAGGGGAA CCCAGGTGAG CCCAGGTGAG ACCAGGTGAA</td><td>AGGTTGGACT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT * ***** * 400 GTGGTCAAGG GTGGTCAAGG GTGGTCAAGG GTAGTCAAGG GTAGTCAAAG GTAGTCAAAG GTAGTCAAAG</td></td<>	sanguis mitis oralis anginosus constellatus intermedius salivarius mutans gordonii sanguis mitis oralis anginosus constellatus intermedius salivarius	GTACTAGTCG GTACTAGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG STCTTGATG ATCTGGTATG STTTTGGGCAAT ACTGGGCAAT ACTTGGAAT ATTTGGGAAC CCTTGGCAAT	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AACAAGG ACTGATGTCA GTGACGTCA TACGATGTCA TACGATGTCA GAAGGAGCCA GAAGGAGCCA AACGAGCTA	TCGAACGCT GGTAAATGCC TGTCAATGCG TGTCAATGCG TGTCAATGCG TGTCGATGCC TGTGGATGCC TGTGGATGCC TGTGGATCCC AAAGCAGTCT AAAGCACGCT AAAGCACGTT AAAGCACTTG AAAGCACTAA	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATCG CGTGAGATTG ** ** ** ** TCCAGGGGAA TCCAGGGGAA TCCAGGGGAA CCCAGGTGAG CCCAGGTGAG ACCAGGTGAA	AGGTTGGACT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT * ***** * 400 GTGGTCAAGG GTGGTCAAGG GTGGTCAAGG GTAGTCAAGG GTAGTCAAAG GTAGTCAAAG GTAGTCAAAG
s. s. s. s. s. s. s. s. s. s. s. s. s. s	sanguis mitis oralis anginosus constellatus intermedius salivarius mutans gordonii sanguis mitis oralis constellatus intermedius	GTACTAGTCG GTACTAGTTG GTCTTGGTTG GTCTTGGTTG GTCTTGATTG GTCTTGATTG GTCTTGATTG GTCTTGATTG STCTTGATG ATCTGGTATG STTTTGGGCAAT ACTGGGCAAT ACTTGGAAT ATTTGGGAAC CCTTGGCAAT	AGCAAGGAGA AACAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AGCAAGG AACAAGG AACAAGG AACAAGG TACGATGTCA TACGATGTCA GAAGGAGCCA AACGACTGTCA ACTGATGTCA	TCGAACGGCT GGTAAATGCC GGTGAATGCC TGTCAATGCG TGTCAATGCG TGTCGATGCC TGTGGATGCC TGTGGATGCC TGTGGATGCC TGTGGATGCC TGTGGATGCC AAAGCACGCT AAAGCACGTT AAAGCAGTTT AAAGCAGTTT	CGCGAGATTG CGTGAAATCG CGTGAAATCG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATTG CGTGAAATCG CGTGAGATTG ** ** ** ** TCCAGGGGAA TCCAGGGGAA TCCAGGGGAA CCCAGGTGAG CCCAGGTGAG ACCAGGTGAA	AGGTTGGACT AGGTTGGCCT AGGTTGGTCT AAGTTGGTTT AAGTTGGTTT AAGTTGGTTT AGGTTGGTAT * ***** * 400 GTGGTCAAGG GTGGTCAAGG GTGGTCAAGG GTAGTCAAGG GTAGTCAAAG GTAGTCAAAG GTAGTCAAAG

FIG. 1. Comparison of the sequences of fragments within genes coding for D-Ala:D-Ala ligases from *S. anginosus* (ATCC 33397), *S. constellatus* (ATCC 27823), *S. gordonii* (ATCC 10558), *S. intermedius* (ATCC 27335), *S. mitis* (NCTC 12261), *S. mutans* (NCTC 10449), *S. oralis* (NCTC 7864), *S. salivarius* (ATCC 9758), and *S. sanguis* (NCTC 7863). Asterisks indicate nucleotides that were identical in all sequences. Dots indicate gaps introduced to optimize alignment.

intermedius ATCC 27335, S. mitis NCTC 12261, S. mutans NCTC 10449, S. oralis NCTC 7864, S. salivarius ATCC 9758, and S. sanguis NCTC 7863. A total of 91 Streptococcus strains from the bioMérieux collection (La Balme-les-Grottes, France) were also tested. The species and number of the 91 strains tested were as follows: S. anginosus, 9; S. constellatus, 10; S. gordonii, 10; S. intermedius, 6; S. mitis, 10; S. mutans, 10; S. oralis, 10; S. salivarius, 10; S. sanguis, 10; and S. vestibularis, 6. Plasmid pCRII (Invitrogen, San Diego, Calif.) was used for the cloning of the PCR products. Escherichia coli INVcF' One Shot (Invitrogen) was the host strain for recombinant plasmids. The recombinant plasmids were pAT437 (ddl gene from S. bovis [ddl_{S. bovis}]), pAT443 (ddl_{S. gardonii}), pAT442 (ddl_{S. salivarius}), and pAT446 (ddl_{S. intermedius}). All strains were grown at 37°C in brain heart infusion broth and on agar (Difco Laboratories, Detroit, Mich.) not supplemented or supplemented with horse blood (5% [vol/vol]). For milleri group streptococci, the incubation was under anaerobic conditions.

DNA manipulation. Total DNA from streptococci was prepared by the cetyldimethylethyl-ammonium bromide method (1). Degenerate oligode-oxynucleotides V1 [GGIGA(A/G)GA(T/C)GGI(T/A)(C/G)I(T/C/A)TICA(A/ G)GG] and V2 [TT(A/G)TGI(T/A/G)AIGGICCIAA(A/G)TG] (10), where I stands for inosine, are complementary to sequences encoding conserved amino acid motifs in D-Ala:D-Ala ligases of E. coli (22, 28) and the related glycopeptide resistance enzyme VanA (9). Amplification of DNA fragments by PCR using ca. 50 ng of template DNA and primers V1 and V2 at the concentration of 0.1 μ M each in a total volume of 100 μ l was performed with a DNA thermal cycler (model 2400; Perkin-Elmer Cetus, Emeryville, Calif.) as described previously (10). The Taq DNA polymerase was purchased from Amersham Life Science (Cleveland, Ohio). The PCR conditions were as follows: 2 min at 94°C for the first step; 30 cycles, with 1 cycle consisting of 1 min at 94°C, 1 min at 54°C, and 1 min at 72°C; and 10 min at 72°C for the last step. The PCR products were purified by agarose gel electrophoresis followed by extraction from the cut-out low-melting-point agarose block with the Sephaglas Kit (Pharmacia, Uppsala, Sweden). Recombinant plasmids were prepared by the Wizard Miniprep proce-

Streptococcal group and	Size of PCR product (bp)		Oligodeoxynucleotide		GC content (%)
species		Pair	Sequence	Positions ^a	
Group A or milleri group	217	Е	5'-TGCAGAAGTAGAGGCAAATC-3'	162–181	45
			3'-TTCCTCGGTTTTCGTCAACCG-5'	362-382	52
Group B					
S. mitis	259	F	5'-TGAAATCGAGGTTGGCCTAC-3'	333-352	50
			3'-TTCCC(G/T)CTCTAAAAGGATTTGC-5'	571-592	45
S. oralis	563	G	5'-CTTATGTCGGCTGCAATATCC-3'	23-43	47
			3'-TTCCC(G/T)CTCTAAAAGGATTTGC-5'	571-592	45
Group C					
S. gordonii	260	Н	5'-GTCGATGGCGAGGATCTAGAGC-3'	133-154	59
0			3'-CAGAAGGTCCCCTTCAACAA-5'	377-396	50
S. sanguis	374	Ι	5'-GTCGATGGCGAGGATCTAGAGC-3'	133-154	59
			3'-GACTACGCAGTTTTACGTCTC-5'	490-510	47

TABLE 2. Oligodeoxynucleotide primers for the second PCR

^{*a*} Positions were derived from the alignment in Fig. 1.

dure (Promega, Madison, Wis.). The DNA sequences of PCR products were determined by the dideoxynucleotide chain terminator technique (24) by using universal or specific oligodeoxynucleotides (Unité de Chimie Organique, Institut Pasteur, Paris, France) as the primers, $[\alpha^{-33}S]dATP$ (Amersham Radiochemical Centre, Amersham, England), and T7 DNA polymerase (T7 Sequencing Kit; Pharmacia) according to the manufacturer's recommendations. For Southern hybridization, DNA was transferred by vacuum onto Nytran membranes (Schleicher and Schuell, Dassel, Germany). Prehybridization and hybridization were performed under stringent conditions at 68°C in 0.1% sodium dodecyl sulfate-0.05% nonfat dry milk-6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) for 3 and 18 h, respectively. Probes were labeled with $[\alpha^{-32}P]dCTP$ (Amersham Radiochemical Centre) by the nick translation procedure (Nick Translation Kit; Amersham International, Little Chalfont, England).

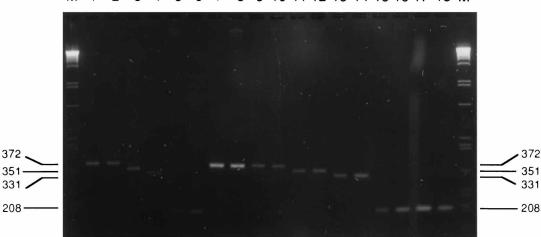
Sequence analysis. Nucleotide sequences were analyzed with Genetics Computer Group software (6), and the phylogenetic analysis was performed with the PHYLIP program package (12).

Nucleotide sequence accession numbers. The sequences were submitted to GenBank and were assigned the following accession numbers: U69162 ($ddl_{S. obvis}$), U69163 ($ddl_{S. gordonii}$), U69164 ($ddl_{S. mitis}$), U69165 ($ddl_{S. mutans}$), U69166 ($ddl_{S. oralis}$), U69167 ($ddl_{S. salivarius}$), U69168 ($ddl_{S. sanguis}$), U91912 ($ddl_{S. anginosus}$), U91913 ($ddl_{S. intermedius}$), and U91914 ($ddl_{S. constellatus}$).

RESULTS AND DISCUSSION

Design of oligodeoxynucleotides. Internal portions (ca. 600 bp) of the genes coding for D-Ala:D-Ala ligases in nine species of streptococci (*S. anginosus, S. constellatus, S. gordonii, S. intermedius, S. mitis, S. mutans, S. oralis, S. salivarius,* and *S. sanguis*) were amplified by PCR with oligodeoxynucleotides V1 and V2 and cloned into the pCRII vector. Southern hybridization with total DNA of each strain was carried out to confirm the origins of the PCR products (data not shown) that were subsequently sequenced on both strands. Sequence comparison indicated that the inserts corresponded to internal portions of *ddl* genes.

The partial sequences of the nine *ddl* genes were aligned (Fig. 1) and showed high degrees of identity. Pairs of oligode-oxynucleotides, each intended to prime amplification of a fragment within a *ddl* gene, were selected in nonconserved regions.



A 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M

FIG. 2. First-PCR analysis of total DNA from reference and wild strains of viridans group streptococci. Lanes: 1, *S. mitis* NCTC 12261; 2, *S. oralis* NCTC 7864; 3, *S. mutans* NCTC 10449; 4, *S. salivarius* ATCC 9758; 5, *S. gordonii* ATCC 10558; 6, *S. sanguis* NCTC 7863; 7 and 8, *S. mitis*; 9 and 10, *S. oralis*; 11 and 12, *S. mutans*; 13 and 14, *S. salivarius*; 15 and 16, *S. gordonii*; 17 and 18, *S. sanguis*; M, bacteriophage λ DNA (Pharmacia) digested with *PstI* used as size standards. PCR products were resolved by electrophoresis on a 2% agarose–Tris–borate–EDTA gel containing 0.5 µg of ethidium bromide per ml. The sizes of the PCR products (in base pairs) are indicated to the sides of the gel.

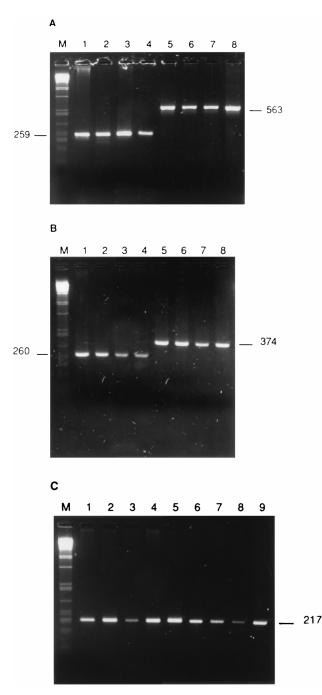


FIG. 3. Second-PCR analysis of total DNA from wild and reference strains of viridans group streptococci. (A) PCR with oligonucleotide pairs F and G. Lanes: 1, *S. mitis* NCTC 12261; 2 to 4, *S. mitis*; 5, *S. oralis* NCTC 7864; 6 to 8, *S. oralis*. (B) PCR with oligonucleotide pairs H and I. Lanes: 1, *S. gordonii* ATCC 10558; 2 to 4, *S. gordonii*; 5, *S. sanguis* NCTC 7863; 6 to 8, *S. sanguis*. (C) PCR with oligonucleotide pair E. Lanes: 1, *S. anginosus* ATCC 33397; 2, *S. constellatus* ATCC 27823; 3, *S. intermedius* ATCC 27335; 4 and 5, *S. anginosus*; 6 and 7, *S. constellatus*; 8 and 9, *S. intermedius*. In all panels, lanes M contained bacteriophage λ DNA (Pharmacia) digested with *PstI* used as size standards. PCR products were resolved by electrophoresis on a 2% agarose–Tris–borate–EDTA gel containing 0.5 μg of ethidium bromide per ml. The sizes of the PCR products (in base pairs) are indicated to the sides of the gels.

Primers of similar sizes and with a GC content ranging from 43 to 60% were designed to avoid variations in annealing temperature and to allow their simultaneous use in a single reaction mixture. However, due to the small sizes of the internal frag-

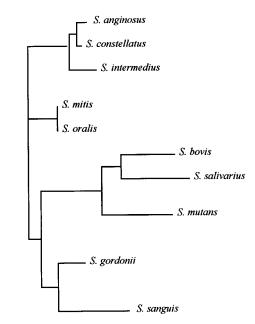


FIG. 4. Phylogenetic relationship among streptococci. The tree was constructed by the neighbor-joining method and slightly modified taking into account the results of maximum-parsimony and bootstrapping analysis.

ments sequenced (600 bp), the high degrees of identity for the nine sequences, and the fact that each amplification product should be assigned to a species on the basis of its size, a two-stage PCR appeared necessary. In the first PCR with oligodeoxynucleotide pairs A to D, the amplification products obtained with pairs B and C could be assigned to a single species (Table 1). Pair A amplified *S. oralis* and *S. mitis*, and pair D amplified both *S. gordonii* and *S. sanguis*. A second PCR using primers F and G or H and I (Table 2) allowed the differentiation of *S. oralis* from *S. mitis* or *S. gordonii* from *S. sanguis*, respectively, whereas pair E (Table 2), used alone, allowed identification of the species from the milleri group.

PCR experiments. PCRs were performed with DNA from every reference strain as a template. Occurrence of nonspecific bands led us to modify the PCR conditions as follows: (i) for the first PCR and for the second PCR with oligonucleotide pairs F and G or H and I, 2 min at 94°C for the first step; 20 cycles, with 1 cycle consisting of 1 min at 94°C, 1 min at 56°C, and 1 min at 72°C; and 10 min at 72°C for the last step; and (ii) for the second PCR with oligonucleotide pair E, 2 min at 94°C for the first step; 30 cycles, with 1 cycle consisting of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C; and 10 min at 72°C for the last step.

The sizes of the amplification products obtained under these conditions differed sufficiently to allow identification of the reference strains (data not shown).

We finally investigated our strategy by testing 91 characterized strains of viridans group streptococci. The PCR results confirmed the identification of 60 strains (10 *S. gordonii*, 10 *S. mitis*, 10 *S. mutans*, 10 *S. oralis*, 10 *S. salivarius*, and 10 *S. sanguis* strains) to the species level (Fig. 2 and 3A and B and data not shown) and the assignment of 25 strains (10 *S. anginosus*, 9 *S. constellatus*, and 6 *S. intermedius* strains) to the milleri group (Fig. 3C and data not shown), whereas the 6 strains of *S. vestibularis* were identified as *S. salivarius* (data not shown). A relatively high degree of relatedness has been observed by DNA-DNA hybridization between strains of *S. vestibularis* and *S. salivarius* (5, 27). These data are consistent with the observation that the oligonucleotides designed for *S. sali-varius* also amplified a fragment of total DNA from *S. vestibularis*. However, to the best of our knowledge, *S. vestibularis* has not been reported to be responsible for purulent infections, endocarditis, septicemia, or meningitis and is thus unlikely to be isolated from foci of infection.

Phylogenetic analysis. The amino acid sequence deduced from the DNA region between oligonucleotides V1 and V2 of 10 species (*S. anginosus, S. bovis, S. constellatus, S. gordonii, S. intermedius, S. mitis, S. mutans, S. oralis, S. salivarius,* and *S. sanguis*) was used for phylogenetic analysis (Fig. 4). The phylogeny obtained was compared with that derived from 16S rRNA sequences (17). The topologies of the two trees obtained with the neighbor-joining method (12) were superimposable except for *S. gordonii* and *S. constellatus.* The difference concerns the position of nodes of these two species.

Identification of viridans group streptococci to the species level is required for certain infections. DNA-DNA hybridization with the type strain is the "gold standard" technique for identification to the species level. However, this method requires radioisotopes and involves complex procedures, and its application is thus limited to research or reference laboratories. Our PCR assay provides a specific and rapid alternative to phenotypic or DNA-DNA hybridization methods for identification of clinically relevant viridans group streptococci to the species or group level within 48 h from the time of isolation of the microorganism.

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