MOLECULAR IDENTIFICATION AND TYPING

Identification of mutans and other oral streptococci by random amplified polymorphic DNA analysis

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The identification and classification of the non-haemolytic or viridans group of streptococci have long been recognised as difficult and unsatisfactory. Phenotypic and genotypic heterogeneity have resulted in ambiguous speciation, particularly with mutans streptococci and other oral streptococci. This study was done to determine whether random amplified polymorphic DNA (RAPD) analysis is useful to identify and even classify oral and other streptococci. DNA was prepared and purified from 25 strains of mutans streptococci including 11 reference strains of *Streptococcus mutans*, seven of *S. sobrinus*, three of *S. rattus* and one each of the four other species of the mutans group, together with 20 other reference species, mostly streptococci, and from 49 fresh isolates of mutans streptococci and of *S. mutans* from human saliva and dental plaque. DNA amplification was primed with each of three arbitrarily selected primers nine or 10 nucleotides in length. The amplified DNA fragments (amplicons) obtained were compared by agarose gel electrophoresis. Species- and strain-specific RAPD fingerprints were obtained not only from pure genomic DNA, but also from the supernates of crude cellular or colony extracts. Pending the analysis of numerous other strains, the data suggest that RAPD may be of value: (i) to distinguish the species *S. mutans* and *S. sobrinus* from each other and potentially from other species of oral streptococci, (ii) to differentiate and possibly classify oral streptococci and (iii) as a valuable tool in mutans streptococci epidemiology and transmission studies, by virtue of its rapidity, efficiency and reproducibility in generating genetic fingerprints of streptococcal isolates.

Introduction

The non-haemolytic or viridans group of streptococci make up a major component of the oral microbiota and may become opportunist pathogens. This group consists of several taxa and includes the group of mutans streptococci, formerly identified as *Streptococcus mutans*. Because *S. mutans* was so genetically heterogeneous it was divided into seven species [1, 2]. The most common member of mutans streptococci is *S. mutans*: most human beings harbour this streptococcus in dental plaque; a small percentage of people have *S. sobrinus* instead. *S. rattus* and *S. cricetus* can be found in some populations, but they are not common [3]. The other species – *S. ferus*, *S. macacae* and *S. downei* are

found only in animals. The identification and classification of viridans streptococci have long been recognised as difficult and unsatisfactory [4, 5] and have relied on batteries of morphological observations, biochemical and physiological tests and antigenic reactions [6–11]. Because of the phenotypic and genotypic heterogeneity of oral streptococci, new species are continually proposed [1, 5, 6, 12, 13]. As phenotyping and serotyping rely on gene expression and, consequently, may be unstable, DNA-based characterisation is now widely accepted. Several molecular typing methods are presently available and some of them have been applied to oral streptococci [14, 15]. They include DNA–DNA hybridisation [1, 12, 14], restriction endonuclease analysis (REA) [16–19], multilocus enzyme electrophoresis (MLEE) [20], ribotyping, PCR and colony hybridisation with DNA probes [21]. A PCR-based procedure of DNA fingerprinting, called arbitrarily primed PCR (AP-PCR) [22] or randomly amplified polymorphic DNA (RAPD) analysis [23], has been applied to strain identification

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and typing of oral bacteria [24–27], oral streptococci [28–30] and non-oral streptococci [31–34]. The present study used RAPD analysis for typing *S. mutans* and *S. sobrinus* strains and to explore the potential of this typing method to distinguish these from the other taxa that comprise the group of mutans streptococci.

Materials and methods

Bacterial strains, maintenance and growth conditions

The 45 bacterial strains used as reference in this study are listed in Table 1. They included 25 laboratory strains of mutans streptococci, 15 strains of other streptococci (mostly of oral origin) and 5 other unrelated species. A total of 49 clinical isolates was also studied, as shown in Table 1: 17 were dental plaque isolates (identified as mutans streptococci) from eight patients [17] provided by H. J. Sandham (University of Toronto) and 32 were isolated (characterised as *S. mutans*) in this laboratory from the saliva of 23 young adults [35]. The strains were kept both lyophilised and cryoconserved at -80°C . Bacterial cells were cultured at 37°C in 10 ml of glucose 0.5%-supplemented TYE medium containing (/L): trypticase (BBL Microbiology Systems) 17 g, yeast extract (Difco Laboratories) 3 g, NaCl 5 g and disodium phosphate 2.5 g. The purity and identity of the cultures used for the experiments were confirmed by Gram's stain, colony morphology on MSA (Difco) and on TYE agar containing glucose 0.5%, sugar fermentation patterns and with the API Rapid Strep test (API Laboratory Products, St Laurent, Canada). No further characterisation of the 45 reference strains was performed and the identity of the 49 clinical strains was established as described elsewhere [17, 35].

DNA isolation and purification

Bacteria were harvested by centrifugation and their DNA was prepared and purified according to Chassy and Giuffrida [36] with some modifications. Briefly, the cell lysate obtained by lysozyme and SDS treatments was extracted three times with saturated phenol (Amresco, Solon, OH, USA) and once with a 1:1 mixture of phenol:chloroform (1:1). The cellular RNA was eliminated by an RNAase treatment according to Smith et al. [37]. The total DNA was then collected by ethanol precipitation. The DNA content was calculated by measuring the absorbance at 260 nm and its quality was assessed by the 260:280 nm absorbance ratio and by electrophoresis on agarose gel.

Whole cell extraction

Cells from overnight broth cultures (50 μl) were harvested by centrifugation and 50 μl of sterile Tris buffer (10 mmol/L, pH 8.2) were used to suspend the cell pellet obtained. Alternatively, colonies of generally

<1 mm in diameter were individually transferred from agar plates into 50 μl of the same buffer. Whole-cell lysates were then prepared by incubating the cell suspension with lysozyme (Sigma) 1 mg/ml at 37°C for 30 min. The lysates were then extracted with an equal volume of saturated phenol at 55°C for 30 min. The extracts were centrifuged and 5 μl of supernate were used, without further purification, as a DNA template for the AP-PCR amplification reaction.

Working primers and random amplification

In preliminary experiments a total of 56 oligonucleotides (9–23 mer) were tested as primers for their capacity to amplify purified DNA of *S. mutans* strain NCTC 10449. This allowed the selection of three working primers responding to the following criteria: RAPD profile should be visible and clear under UV light and the genetic fingerprint should be composed of amplicons sufficient in number and located between a range of standard DNA ladder. These working primers were: 970-11 (5'GTAAGGCCG) from our bank, OPA-3 (5'AGTCAGCCAC) and OPA-18 (5'AGGTGACCGT) from Operon Technologies (Alameda, CA, USA). Amplification reactions were performed as described previously [24]. The 25- μl reaction mixtures contained Tris-HCl 20 mmol/L, pH 8.3, KCl 50 mmol/L, MgCl_2 4 mmol/L, gelatin 0.001%, 200 mmol/L each of dATP, dCTP, dGTP and dTTP, 30 pmoles of primer, 50 ng of purified DNA or 5 μl of supernate of whole cell or colony extract, and 1.25 U of Taq DNA polymerase (Pharmacia). The amplification was performed in a DNA thermal cycler (Perkin Elmer Cetus, Montréal, Canada) programmed for 30 cycles of 1 min at 94°C , 1 min at 32°C and 2 min at 72°C . A negative control without DNA was included in each AP-PCR run. All such controls were always negative. Amplicons obtained were compared after electrophoresis on an agarose 1.5% gel (Seakem, Rockland, MA, USA) in Tris-acetate buffer, detection with ethidium bromide and photography under UV transillumination as described elsewhere [24].

Results

RAPD fingerprinting of the reference strains of *S. mutans* and *S. sobrinus*

Each of the RAPD patterns obtained with *S. mutans* reference strains with primers 970-11, OPA-3 and OPA-18 contained a set of major amplicons that were shared by almost all the strains; the three predominant banding patterns thus obtained were clearly distinct from those of several related (Fig. 1) and unrelated (Fig. 2) species. However, the RAPD fingerprints contained only a limited subset of amplicons that are indicative of genetic polymorphism within the species.

Use of primer 970-11 yielded the simplest fingerprints, composed of two major amplicons at 1585 and 977 bp

Table 1. Species, strains, origins and sources of the bacteria used in the study

Species*	Strains†	Obtained from	Source‡
Streptococcal reference bacteria			
<i>S. anginosus</i>	ATCC 33397	ATCC	—
<i>S. anginosus</i> (<i>S. constellatus</i>)	ATCC 27823	ATCC	Human patient
<i>S. anginosus</i> (<i>S. intermedius</i>)	ATCC 27335	ATCC	—
<i>S. bovis</i>	ATCC 35034	ATCC	—
<i>S. cricetus</i> (serotype a)	ATCC 19642	ATCC	Caries (hamster)
<i>S. downei</i> (serotype h) (<i>S. mutans</i>)	ATCC 33748	ATCC	Plaque (monkey)
<i>S. ferus</i> (serotype c)	ATCC 33477	ATCC	Mouth (wild rat)
<i>S. gordonii</i> (<i>S. sanguis</i>)	ATCC 10558	J. Rudney	Subacute bacterial endocarditis
<i>S. gordonii</i> (<i>S. sanguis</i>)	ATCC 10558	ATCC	Subacute bacterial endocarditis
<i>S. macacae</i> (serotype c)	ATCC 35911	ATCC	Plaque (monkey)
<i>S. mitis</i> biovar 2 (<i>S. mitior</i>)	ATCC 903	ATCC	Ulcerated sore throat
<i>S. mutans</i> (serotype c, e, f)	ATCC 25175	ATCC	Cariou dentine
	ATCC 31383	ATCC	Human mouth (Japan)
	ATCC 33534	I. R. Hamilton	Mutant of 6715-10
	ATCC 33535	ATCC	Human mouth (Switzerland)
	ATCC 35668	ATCC	—
	GS-5	C. Vadeboncoeur	Caries (human)
	OMZ175 (f)	M. Lavoie	Plaque (human)
	NTCC 10449 (c)	NCTC	Cariou dentine
	407-1 (c)	S. Alaluusua	—
	408-1 (e)	S. Alaluusua	—
	V4-91 (f)	S. Alaluusua	—
<i>S. oralis</i>	ATCC 35037	ATCC	Human mouth
<i>S. oralis</i> (<i>S. sanguis</i>)	ATCC 10557	ATCC	Subacute bacterial endocarditis
<i>S. parasanguis</i> (<i>S. mitis</i>)	ATCC 15909	ATCC	Human throat
<i>S. parasanguis</i> (<i>S. mitis</i>)	ATCC 15912	J. Rudney	Human mouth
<i>S. rattus</i> (serotype b)	ATCC 19645	ATCC	Caries (rat)
	BHT	ATCC	Plaque (human)
	FA-1	Van der Hoeven	Caries (rat)
<i>S. salivarius</i>	ATCC 25975	I. R. Hamilton	Saliva (human)
<i>S. sanguis</i>	ATCC 10556	ATCC	Subacute bacterial endocarditis
<i>S. sobrinus</i> (serotype d, g) (<i>S. mutans</i>)	ATCC 27352	ATCC	Rat
	(<i>S. mutans</i>)	ATCC	Human mouth
	(<i>S. mutans</i>)	ATCC	Plaque (human)
	C-4	C. Mouton	—
	F5-2 (d)	S. Alaluusua	—
	6715 (g)	H. J. Sandham	Plaque (human)
	8-1s (g)	S. Alaluusua	—
<i>S. thermophilus</i>	ATCC 19258	ATCC	Pasteurised milk
<i>S. vestibularis</i>	ATCC 49124	ATCC	Human oral cavity (England)
Non-streptococcal reference bacteria			
<i>Enterococcus faecalis</i> (<i>Strep. faecalis</i>)	ATCC 29212	ATCC	Urine
<i>Escherichia coli</i>	ATCC 15939	ATCC	—
<i>Lactococcus lactis</i> (<i>Strep. lactis</i>)	ATCC 11454	ATCC	—
<i>Porphyromonas gingivalis</i>	ATCC 49417	C. Mouton	Human periodontal lesion
<i>Staphylococcus aureus</i>	ATCC 6538	ATCC	Human lesion
Clinical isolates			
49 fresh isolates of mutans streptococci	17 strains (letter-labelled) [§]	H. J. Sandham [17]	Plaque (human)
	32 strain (number-labelled)	M. Lavoie [35]	Saliva (human)

*The current taxonomic position of the bacteria is indicated together with, when applicable, the name (in parentheses) they were given when first deposited at the ATCC.

†The serotype of the bacteria from the mutans streptococci group is indicated in parentheses when known.

‡The source of the bacteria is given when known.

§Each of these 17 letter-labelled mutans streptococci isolates had a unique REA fingerprint [17].

||Each of these 32 number-labelled isolates was characterised as *S. mutans* [35].

and two minor ones (Fig. 1). This set of amplicons was common to all the reference strains of *S. mutans* tested, except for strains ATCC 31383 and 33534. Strain ATCC 31383 more closely resembled *S. rattus* than *S. mutans*, as three of the five amplicons generated by primer 970-11 were common to all *S. rattus* strains tested. This resemblance to *S. rattus* was also demonstrated with primers OPA-18 (Fig. 1) and OPA-9 (data not shown), although a perfect match with the typical *S. rattus* RAPD patterns was not obtained. Moreover, the API Rapid Strep[®] test does not clearly identify this strain as a member of the mutans streptococci.

The RAPD profiles of strain ATCC 33534 (also named DR 0001) were also quite distinct from the characteristic pattern of *S. mutans* (Fig. 1). A group of amplicons were ATCC 33534 strain-specific; other discrete amplicons were shared with other taxa of the mutans streptococci group, e.g., 990 bp (immediately above the 977-bp amplicon) obtained with primer 970-11, 955 bp with OPA-3 and 1660 bp with OPA-18. This genotypically uncharacteristic *S. mutans* ATCC 33534 showed a fermentation pattern similar to that of *S. mutans* strains, but failed to metabolise melibiose (data not shown).

Fig. 1. RAPD fingerprints obtained with the reference strains of the mutans streptococci group. When possible, more than one strain of each species was included in the study. Species names in labels are based on published results and phenotypic studies as described in Materials and methods. Purified DNA was amplified with the arbitrarily selected primers 970-11 (A), OPA-3 (B) and OPA-18 (C) as described in Materials and methods. Mol. wt markers (1-kb ladder) are in bp. Marked sizes indicate amplicons that constitute a distinctive set characteristic of the species *S. mutans* (left-hand side) and *S. sobrinus* (right-hand side).

Use of primers OPA-3 and OPA-18 also yielded a specific fingerprint for *S. mutans* strains so far tested. Each of these primers generated a short spectrum of amplicons, located between 661- and 2291-bp markers,

indicative of genetic polymorphism. A set of major amplicons at 2138, 1202 and 955 bp as generated by OPA-3, and at 2291 and 1995 bp as obtained with OPA-18 (Fig. 1), were shared by all strains of *S.*

Fig. 2. RAPD fingerprints of *S. mutans*, other streptococci and a few other unrelated species. Species names in labels and procedures were as indicated in Fig. 1 and Materials and methods. Mol. wt markers (1-kb ladder) are in bp. Marked sizes indicate amplicons that constitute a distinctive set characteristic of the species *S. mutans*.

mutans, except for strains ATCC 31383 and 33534, as for the set of amplicons generated by primer 970-11.

Overall, the fingerprints obtained from *S. mutans* with the selected primers were unique and species-specific. This unique signature was ascertained not only by comparison with other species within the mutans streptococci group (Fig. 1), but also with other streptococci and unrelated species (Fig. 2) within the limits of the small number of strains analysed. The fingerprints were thus helpful for strain identification and distinction.

The *S. sobrinus* RAPD fingerprints contained sets of major amplicons that were shared by all reference strains (Fig. 1). With primer 970-11, a quadruplet of bands located at 1122, 933, 588 and 501 bp characterised an *S. sobrinus* genotype which was clearly distinct from related (Fig. 1) and unrelated (Fig. 2) species, again within the limits of the number of strains tested. Use of primers OPA-3 or OPA-18 yielded a characteristic signature for *S. sobrinus* as a pattern located between 447 and 1884 bp (OPA-3) or between 1413 and 2113 bp (OPA-18).

Although the fingerprints of *S. mutans* and *S. sobrinus* were quite distinct pairwise, the observation of a shared amplicon in the RAPD profile was indicative of some degree of genetic relatedness, as revealed by the sharing of common amplicons by all strains of these two species tested. In Fig. 1, it can be seen that amplicons located at 501 bp and at 955 bp, obtained with primers 970-11 and OPA-3, respectively, were common to all reference strains of *S. mutans* and *S. sobrinus* tested in this study.

RAPD fingerprinting of clinical isolates of mutans streptococci and of *S. mutans*

A total of 49 isolates, together with *S. mutans* reference strain NCTC 10449, were compared in RAPD with primers 970-11, OPA-3 and OPA-18, and typical RAPD patterns are presented in Fig. 3. The characteristic RAPD profiles unique to *S. mutans*, as defined with the reference strains (Figs. 1 and 2), were found in 46 of the 49 isolates tested. All three isolates which showed patterns quite different from the typical RAPD profiles of *S. mutans* were from the 17 letter-labelled samples identified as mutans streptococci: B smooth (Bs), H and O. Each of the 32 number-labelled isolates characterised as *S. mutans* showed the RAPD profiles unique to *S. mutans*. Strain Bs showed a close resemblance to the *S. sobrinus* reference strains based on a comparison of the banding patterns obtained with primers 970-11 and OPA-3. A re-examination of this strain Bs revealed that it was morphologically different from strain B, from which it had been isolated, and biochemical tests confirmed this strain as *S. sobrinus*. Strains H and O had distinct patterns that did not correspond to any of the reference strains tested. The

API Rapid Strep[®] test identified isolates Bs and H as mutans streptococci and isolate O as *S. sanguis*.

The white (Jw) and yellow (Jy) variants of isolate J showed the amplicons generated by all three primers that characterise the *S. mutans* species. However, they were distinguished from one another by extra amplicons generated by primer OPA-3 and by a preferential amplification of the 1660-bp amplicon generated by primer OPA-18. It can also be seen in Fig. 3 that the presence or absence of some of the amplicons generated by the three primers or the intensity of the fluorescence of other amplicons distinguishes some *S. mutans* strains from the others. This indicates that sets of amplicons can be used to identify the *S. mutans* species and others may possibly detect the clonal type.

AP-PCR on broth cultures and colonies

Whole-cell extracts from broth cultures and colonies were used as DNA templates in the AP-PCR procedure with primers 970-11, OPA-3 and OPA-18 (results not shown). Of all the amplicons obtained with the corresponding purified DNA preparations, only those >2.9 kb in size, when primer OPA-3 was used, were not found when crude cellular or colony extracts were used as DNA templates. The RAPD patterns characteristic of *S. mutans* species obtained with pure DNA were otherwise generated. Comparison of the patterns obtained from corresponding broth culture and colony extracts shows differences in the 'cleanness' and the quantity of the amplification products. These could possibly be explained by variations in the size of the colonies used for the extractions as compared with the more constant cell number found in the overnight cultures used for the broth extracts, as suggested by others [38].

Discussion

It has been shown that the polymorphism in length of the amplified sequences (amplicons) of RAPD fingerprints obtained by AP-PCR can be used to compare bacterial strains [22–24], including the groups of mutans streptococci [28, 29] and mitis streptococci [30]. In the present study, this application of RAPD analysis to genotyping of oral streptococci confirmed that the RAPD assay can distinguish the species *S. mutans* and *S. sobrinus* from each other. Moreover, despite the small number of reference strains tested, the data suggest that the RAPD assay can distinguish these species from other oral streptococci, as fingerprints unique to *S. mutans* or *S. sobrinus* strains could be generated with several primers. As previously demonstrated by Saarela et al. [28] for cell lysates the study also showed that RAPD fingerprints similar to those obtained with pure genomic DNA could be generated from crude broth culture and colony extracts.

Fig. 3. RAPD fingerprints of clinical isolates of mutans streptococci. The procedure was as indicated in Fig. 1 and Materials and methods. Reference strain *S. mutans* NCTC 10449 was included as a positive control. Mol. wt markers and indicated sizes are as in Fig. 2. Letter-labelled isolates identified as mutans streptococci were from dental plaque [17] and number-labelled isolates characterised as *S. mutans* were from saliva [35].

RAPD performed on *S. mutans* reference strains and on 49 clinical isolates (Figs. 1, 2 and 3) showed, with very few exceptions, that a fingerprint unique for the species could be obtained. This RAPD fingerprint was highly specific to *S. mutans* and its reproducibility was excellent, not only between DNA extractions of a given strain, but also from one experiment to the next – as can be seen with strain 104449 in Figs. 1, 2 and 3. Divergence from the typical *S. mutans* profile of strain ATCC 31383 suggests that this strain should no longer be considered as *S. mutans*. As the banding pattern of ATCC 31383 showed a closer resemblance to all three *S. rattus* strains so far tested – although a perfect match with the typical *S. rattus* RAPD patterns was not obtained – it may be that strain ATCC 31383 represents a clonal group within the *S. rattus* taxon. With regard to strain ATCC 33534, it has been suggested recently that melibiose-negative strains would represent a cryptic species in the *S. mutans* taxon [21].

Although only one strain each of *S. cricetus*, *S. ferus*, *S. macacae* and *S. downei* were tested in the present study, it can already be seen that a specific characteristic RAPD profile could be constructed for each of the seven species within the group of mutans streptococci (Fig. 1). Such profiles, if corroborated by the analysis of numerous other strains, would then correspond to and confirm the diversity of this group of oral streptococci, as well described by Coykendall [1]. Likewise, Rudney and Larson [30] have shown recently that RAPD allows rapid identification of genotypic groups corresponding closely to mitis group species established by phenotype.

Furthermore, the polymorphism in length of amplicons of such RAPD profiles allowed not only for species identification and distinction between *S. mutans* and *S. sobrinus*, but also possibly indicated relatedness between species of the mutans streptococci group. For instance, with primer 970-11, it can be seen that the 977-bp amplicon is common to all *S. mutans* strains tested, an indication that it could be used as a species-specific marker. On the other hand, amplicons are shared with other species: the 1585-bp amplicon shared with *S. rattus* and the discrete 501-bp amplicon shared with *S. rattus* and *S. sobrinus*. This relatedness between taxa within the viridans streptococci family and divergence in taxonomic structures merits a more complete and sophisticated approach than the visual analysis used here. In addition to analysis of a much greater number of strains, a cluster assisted analysis with Lance and Williams genetic distance algorithm to construct the dendrogram (phenogram) for all groups of viridans streptococci could be applied, as in a previous work to construct a biotyping scheme for *Porphyromonas gingivalis* [39].

It was possible to distinguish the white (Jw) and yellow (Jy) variants of isolate J from one another (Fig. 3). Such

data suggest that sets of amplicons can be used to discriminate between clonal types. Like other studies [28], the present study could not differentiate between serotypes of *S. mutans* as, irrespective of the primer used, the unique RAPD patterns obtained with serotype c, e, or f of *S. mutans* so far tested were similar. Thus it would appear that the division of strains of mutans streptococci into serogroups has little relation to the underlying genetic structure of the species. This lack of relationship has also been shown for *P. gingivalis* [40, 41].

The 17 letter-labelled clinical isolates of mutans streptococci from eight patients, each of which had a unique REA fingerprint [17], were also identified by a single genetic pattern with the three working primers. As the RAPD profiles have only a small number of bands and, consequently, are much easier to read than the corresponding REA patterns, they should be considered helpful for epidemiological and transmission studies of mutans and other oral streptococci, as already shown with *P. gingivalis* [27] and suggested for mutans streptococci [28, 29].

In this study, three working primers were selected from among 56 tested for their capacity to differentiate mutans streptococci. It should be noted that if these primers showed useful similarities between the RAPD profiles of almost all 15 reference and 49 clinical *S. mutans* strains tested (Figs. 1 and 3), they also showed dissimilarities between strains of some of the non-mutans streptococci species tested, as shown in Fig. 2 for *S. oralis*, for example. Moreover, they showed similar profiles for *S. oralis* 10557 and *S. sanguis* 10556. This highlights the limitations of the selected primers for certain species. However, it must also be recognised that the identity of these strains of *S. oralis* and *S. sanguis* have always been problematic – as demonstrated by their reclassification as shown in Table 1. The RAPD profiles obtained in this study suggest that *S. oralis* 10557 might indeed be an *S. sanguis* strain as previously established. This also stresses the need for confirmation of identifications by other methods.

In this study, fingerprints were compared for similarity by eye and the molecular sizes of the major amplicons were assigned by hand. It is clear that the handling of large numbers of polymorphic banding patterns for molecular systematics would benefit from improvements, for the detection of and discrimination between amplicons, resulting from the use of computer-integrated densitometry for band sizing and computer-assisted image capture, data storage and analysis methods, as suggested previously [41].

In summary, the data indicate that AP-PCR can generate characteristic and unique RAPD fingerprints that are highly specific for *S. mutans* and *S. sobrinus* from both purified DNA and crude cellular or colony extracts. From these data it can be anticipated that a

working RAPD protocol could be used: (i) to distinguish the species *S. mutans* and *S. sobrinus* from other species of oral streptococci, and (ii) to possibly identify and sub-classify all oral streptococci. The data also confirm that RAPD fingerprinting is a potentially valuable tool in bacterial epidemiology and transmission studies of oral streptococci by virtue of its rapidity, efficiency and reproducibility in exploring the genomic polymorphism of these organisms.

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