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CLONAL CHARACTERIZATION OF *STREPTOCOCCUS MUTANS* STRAINS BY MULTILOCUS ENZYME ELECTROPHORESIS

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SHORT COMMUNICATION

ABSTRACT

Twenty-one *Streptococcus mutans* strains were clustered by Multilocus Enzyme Electrophoresis (MLEE). Six isoenzymes showed strong infra-specific discriminatory power (M1P, MPI, PLP, NSP, GOT, and LAP). MLEE is a robust technique that may be used to explore clonal diversity of *S. mutans* isolates in epidemiological surveys.

Key words: *Streptococcus mutans*, isoenzymes, clonal variability

Streptococcus mutans is an organism intensely investigated in order to determine its role in the cariogenic microbiota (5). For ecological purposes, it is necessary to establish some criteria that may segregate two different genetic types, so-called clones, as two distinct entities.

Multilocus enzyme electrophoresis (MLEE) is a robust resource largely employed for the characterization of microorganisms, such as gram-positive e gram-negative bacteria (2,3), mycoplasms (11), filamentous fungi (1), yeasts (7), and protozoa (6).

Gilmour *et al.* (4) carried out a pioneer study using MLEE for clustering related species of oral streptococci in groups such as mutans streptococci and sanguinis streptococci. Although, this methodology may segregate not related strains within a certain species, it was never used before for *S. mutans* specimen differentiation.

In this study, one *S. mutans* type-strain (ATCC-25175), other twenty-one strains obtained from three patients (patient #1: CD03, CD30, CD31, CD32, CD33, CP04, CP07, CP10, CP13, CS02, and CS05; patient #2: BP14, BP15, BP17, and BS01; patient #3: DD03, DD04, DS02, DS04, DS07, and DS08), and a *Staphylococcus aureus* strain (LM-003) were grown in Brain-Hearth-Infusion at 37ºC and pCO_2 10%, for 24 hours. Cellular pellets were harvested by centrifugation, washed three times with 40 mM PBS (pH7.5), and placed in microtubes in which an equal amount of 0.55 mm glass beads and 1 mL of PBS were added. Tubes were adapted in a Mini-Bead Beater cell disruptor (Biospec Inc., OK.) that was programmed for 4500 rpm spins, in two cycles of 1 minute, each. After a centrifugation of 5000 x g, supernatants were absorbed in 5 x 12 mm Whatman-3 paper wicks that were kept at -70ºC until use. Electrophoresis were carried out in 13% hydrolyzed starch supports in buffer systems A, B, C, and D (12).

After the running time, gels were sliced (1.2 mm thickness) and revealed for enzyme active band detection of aconitase (ACO), alcohol dehydrogenase (ADH), α-amilase (α-AM), aspartate dehydrogenase (ASD), catalase (CAT), malic enzyme (ME), α-esterase (α-EST), β-esterase (β-EST), glucose dehydrogenase (GDH), glucose-6-phosphate dehydrogenase, glucosyltransferase (GTF), isocitrate dehydrogenase (IDH),

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lactate dehydrogenase (LDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), mannitol dehydrogenase (MADH), mannitol 1-phosphate dehydrogenase (M1P), mannose phosphate isomerase (MPI), nucleoside phosphorylase (NSP), peroxidase (PER), phenylalanyl leucine peptidase (PLP), sorbitol dehydrogenase (SDH), superoxide dismutase (SOD), and glutamic-oxalacetic transaminase (GOT) (12). The bands were scored according to their relative mobilities.

Among the enzyme systems, the major part of dehydrogenases (ACO, ADH, ASD, GDH, G6PD, IDH, LDH, MDH, MADH, ME, SDH), as well as α-AM, CAT, α-EST, β-EST and PO showed no activity for any *S. mutans* strain. The possibility of reagent failure was discarded, since enzymatic bands for *S. aureus* were detected for many of these systems. Such enzymes may be either produced in lower level than the method can detect or not produced by the *S. mutans* strains. Superoxide dismutase (SOD) showed the same thin band in all *S. mutans* isolates. The same observation was previously reported by Gilmour *et al.* (4), who also evaluated the activity of this enzyme (also called indophenol oxidase – IPO) and found a minimum grade of variability within *S. mutans* isolates. Due to this lack of variability, SOD was not taken in account for further parts of the experiment.

For glucosyltransferase (GTF), a typical enzyme produced by *S. mutans*, the expected electrophoretic bands did not appear. According to Wunder and Bowen (13), glucosyltransferases are enzymes that act at extracellular environment. This may, at least in part, explain why glucosyltransferase activity was not observed. After cell harvesting, pellets were washed to remove traces of culture medium and metabolic products, among them, probably were the glucosyltransferases.

Table 01 shows the electrophoretic profiles of LAP, M1P, NSP, PLP, and MPI in buffer system A. Two loci for GOT (GOT1 and GOT2) were visualized when buffer system D (12) was employed for electrophoresis.

Genetic diversity for a single locus was calculated (4) as $h = [1-\Sigma x_i^2][n/(n-1)]$, where x_i is the frequency of the ith allele at the locus, n is the number of isolates, and $n/(n-1)$ is a correction for bias in small samples (10). Mean diversity per locus is the arithmetic average of h over all loci assayed. In this study, genetic diversities for the seven loci evaluated were 0.785 (GOT1), 0.416 (GOT2), 0.487 (LAP), 0.789 (M1P), 0.855 (NSP), 0.715 (PLP), and 0.520 (MPI). The mean diversity for the loci was 0.652.

In this study, the discrimination ability MLEE for cariogenic organisms was verified. Strains CD30, CD31, CS02, CD03, CS5, and CP04 differed from strains CD32, CD33, CP07, CP10, and CP13. These two clusters of strains were isolated from a same individual (patient C), and their enzymatic patterns suggest that they belong to different clones. Indeed, average genetic diversity between both clones was determined as 0.468. The remaining strains were isolated from two other non-related subjects, without a high degree of concordance among electrophoretic patterns. In parallel to the discrimination ability, MLEE has proven to be a useful tool for establishing genetic diversity, even in small (10) or subdivided (8,9) populations. This method detects allelic frequencies prompter than other methodologies, such as RAPD and RFLP. These facts support the premise that MLEE may be used in surveys in which intra-species determination of *S. mutans* is required.

RESUMO

Caracterização clonal de cepas de *Streptococcus mutans* por eletroforese de enzimas codificadas por multilocus

Vinte e uma cepas de *Streptococcus mutans* foram agrupadas pela eletroforese de enzimas codificadas por multilocus (MLEE). Seis isoenzimas apresentaram forte poder discriminatório (M1P, MPI, PLP, NSP, GOT e LAP). A MLEE é uma técnica robusta que pode ser empregada no estudo da diversidade clonal de cepas de *S. mutans*, em estudos epidemiológicos.

Palavras-chave: *Streptococcus mutans*, isoenzimas, variabilidade clonal

Table 1. Electromorphotypes of *Streptococcus mutans*.

Patient	Strains	Allele at enzyme locus*						
		GOT1	GOT2	LAP	M1P	NSP	PLP	MPI
#1	CD30, CD31, CS02, CD03, CS05, CP04	2	1	3	1	1	4	2
#1	CD32, CD33, CP07, CP10, CP13	2	2	2	4	2	3	1
#2	BP14, BP15, BP17, BS01	1	1	3	2	1	3	2
#3	DS02, DS04, DS07, DS08, DD03, DD04	1	1	2	3	1	2	1
	ATCC-25175	1	2	1	2	3	1	1

* According to the anodal migration

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