TYPING STREPTOCOCCUS MUTANS BY MULTILOCUS ENZYME ELECTROPHORESIS

Caracterização de Streptococcus mutans por eletroforese de isoenzimas

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Abstract

OBJECTIVES: It was evaluated the use of multilocus enzyme electrophoresis (MLEE) for the characterization of Streptococcus mutans. MATERIAL AND METHODS: Five strains of such microrganisms were grown in liquid culture medium and their cytoplasm proteins were extracted. After gel electrophoreses, the catalytic activity was explored for 23 isoenzymes. RESULTS: Six enzymes (mannitol-1-phosphate dehydrogenase, mannose-phosphate isomerase, nucleoside phosphorylase, phenylalanyl-leucine peptidase, leucine aminopeptidase, and glutamic-oxalacetic transaminase) furnished bands that allowed the strains characterization at intra-specific level. CONCLUSION: MLEE is a robust technique that may be used to explore diversity among clinical isolates of cariogenic S. mutans in epidemiological surveys.
Keywords: Streptococcus mutans; Dental caries; Isoenzymes.

Resumo

OBJETIVOS: Foi avaliado o uso da eletroforese de isoenzimas (MLEE) na caracterização de Streptococcus mutans. MATERIAL E MÉTODOS: Cinco cepas de S. mutans foram crescidas em caldo de cultura e tiveram suas proteínas citoplasmáticas extraídas. Apó s eletroforese em gel, a atividade catalítica foi testada para 23 isoenzimas. RESULTADOS: Seis enzimas (manitol-1-fosfato desidrogenase, manose-fosfato isomerase, nucleosídio fosforilase, fenilalanil-leucina peptidase, leucina aminopeptidase e transaminase glutâmicooxalacética) forneceram bandas eletroforéticas que permitiram a caracterização das cepas num nível intraespecífico. CONCLUSÃO : A MLEE é uma técnica robusta que pode ser usada para explorar a diversidade clonal de cepas clínicas de S. mutans em levantamentos epidemiológicos.
Palavras-chave: Streptococcus mutans; Cárie dental; Isoenzimas..

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Introduction

Streptococcus mutans is the putative cariogenic microrganism more associated to active lesions. Such organism has been intensely investigated in order to determine its central role in the cariogenic microbiota (1). In special for epidemiologic purposes, it is necessary to establish some criteria that may segregate two or more different genetic types, so-called clones, as distinct entities with their particularities.

Intra-species genetic heterogenecity can be ascertained by DNA-based methods, such as restriction enzyme analysis (2), ribotyping (3), arbitrary primed polymerase chain reaction (4) and pulsed field gel electrophoresis (5).

Multilocus enzyme electrophoresis (MLEE) is a robust resource that was largely employed for characterization of many groups of microorganisms as Gram-positive e negative bacteriae (6, 7), mycoplasms (8), filamentous fungi (9), yeasts (10) and protozoa (11). In parallel to its discrimination ability, MLEE has proven be a useful tool for establishing genetic diversity, even in small (12) or subdivided (13, 14) populations.

Gilmour et al. (15) proposed the use of MLEE to differentiate oral streptococci in some groups as mutans streptococci and sanguis streptococci. However, the potential of MLEE for S. mutans specimen differentiation was never evaluated before.

In this work, such potential was explored, as well as many electrophoresis/revelation systems were screened for the optimization of enzymatic bands detection.

Material and Methods

Five strains of S. mutans (ATCC25175TS, CD31, CD32, BP14, and DS02) and a strain of Staphylococcus aureus (LM003) were grown in brainhearth-infusion at 37ºC and pCO2 10%, for 24 hours. Cellular pellets were harvested by centrifugation, washed three times with 40mM PBS (pH7.5), and placed in microtubes in which an equal amount of 0.55mm glass beads and 1mL of PBS were added. Tubes were adapted in a Mini-Bead Beater (Biospec Inc., OK.) cell disruptor that was programmed for 4500rpm spins, in two cycles of one minute, each. After a centrifugation at 5000_g, supernatants were absorbed in 5_12mm Whatman-3 paper wicks that were placed at -700C, until use time.

Electrophoreses were carried out using 13% hydrolyzed starch supports in buffer solution
A [tris-citrate pH8.0 (tank) and 1:30 tris-citrate pH8.0 (gel)], solution B [tris-citrate pH6.3 (tank) and triscitrate pH6.7 (gel)], solution C [Borate pH8.2 (tank) and tris-citrate pH8.7 (gel)], and solution D [lithium hydroxide pH8.1 (tank) and 1:9 lithium hydroxide-tris-citrate pH8.3 (gel)].

After the running time, gels were sliced in 1.2mm thickness. Gel slices were revealed for enzyme active band detection of aconitase (ACO), alcohol dehydrogenase (ADH), a-amilase (a-AM), aspartate dehydrogenase (ASD), catalase (CAT), malic enzyme (ME), a-esterase (a-EST), b-esterase (b-EST), glucose dehydrogenase (GDH), glucose-6-phosphate dehydrogenase, glucosyltransferase (GTF), isocitrate dehydrogenase (IDH), 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). After appearing, the bands were scored according to their respective relative mobilities (16).

A dendrogram was generated after the overall gel analysis, using Euclidean Distance coefficient (17) calculated by NTSYS 1.70 (Applied Biostatistics, Inc.). The tree was generated by the unweighted pair-group arithmetic average (UPGMA) clustering method.

Results and Discussion

Among the analyzed enzyme systems, the major part of dehydrogenases (ACO, ADH, ASD, GDH, G6PD, IDH, LDH, MDH, MADH, ME, SDH), as well as a-AM, CAT, a-EST, b-EST, and PO showed no activity for any S. mutans strain. The possibility of reagent failure was non-considered once enzymatic bands for S. aureus were detected for many revelation systems. Perhaps, such enzymes are either produced in smaller quantities than the method can detect or they are not produced by S. mutans. Superoxide dismutase (SOD) showed just a thin band zone that did not vary among the S. mutans and S. aureus isolates; thus, it was not taken in account for further parts of the experiment. Such observation was previously reported by Gilmour, Whittam, Kilian and Selander (15), that also had evaluated the activity of such enzyme (also called indophenol oxidase IPO) and found a minimum degree of variability within S. mutans isolates.

In the specific case of glucosyltransferase (GTF), a typical enzyme produced by S. mutans, it would be reasonable to expect the occurrence of some electrophoretic bands that did not appear. According to Wunder and Bowen (18), glucosyltransferases are enzymes that act in the extracellular environment. This may, at least partially, to explain why glucosyltransferase activity was not observed. After cell harvesting, pellets were washed in order to remove traces of culture medium and metabolic products, probably including the glucosyltransferases.

Figure 1 - represents an electrophoretic gel of S. mutans isolates resolved for MPI activity. Consistent enzymatic bands can be visualized.

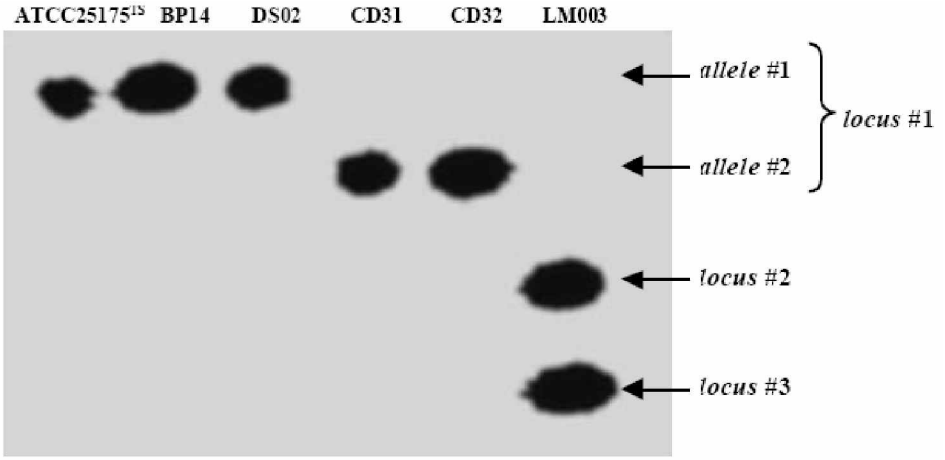

Fig. 1 -Electrophore tic variation profiles for mannose-phosphat isomerase produced by Streptoc occus mutans.

Table 1 shows the electrophoretic profiles of LAP, M1P, NSP, PLP and MPI resolved in buffer system A. Two loci for GOT (GOT1 and GOT2) were visualized when buffer D was employed for electrophoresis.

Table 1 - Electromorphotypes of Streptococcus mutans strain

	allele at enzyme locus *						
	GOT1	GOT2	LAP	M1P	NSP	PLP	MPI
CD31	2	1	3	1	1	4	2
CD32	2	1	3	1	1	4	2
BP14	1	2	2	4	2	3	1
DS02	1	1	2	3	1	2	1
ATCC25175TS	1	2	1	2	3	1	1

* According to the anodal migration TS Type strain

The genetic diversity for a locus (16) was calculated as h = 1-Sxi2[n/(n-1)], where xi is the frequency of the ith allele at the locus, n is the number of electromorphotypes (ET) in the sample, and n/(n-1) is a correction for bias in small samples (12). Mean diversity per locus ( ) is the arithmetic average of h over all loci assayed. For our study, genetic diversities for the seven evaluated loci were 0.35, 0.35, 0.55, 0.35, 0.45, 0.65, and 0.35, for GOT1, GOT2, LAP, M1P, NSP, PLP, and MPI, respectively; the mean diversity for the loci was 0.435.

In order to determine the genetic distance among the strains, a non-rooted dendrogram was built (Fig. 2). Such tree shows that strains CD31 and CD32 could not be separated in two different clusters. Also the MLEE could segregate the remaining strains in different individual clusters.

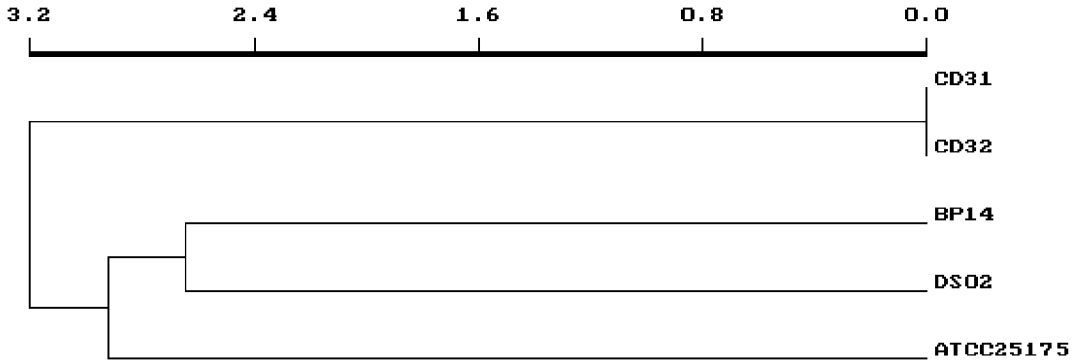

Fig. 2 - Euclidian genetic distances of Streptococcus mutans strains generated

In our study, it was intended to verify if MLEE could be applied as a discriminating tool for the cariogenic bacterium S. mutans. Strains CD31 and CD32 were isolated from tongue dorsum of the same individual, and both displayed enzymatic patterns that may ascertain their condition as belonging to the same clone. The remaining strains were isolated from nonrelated subjects, neither establishing a considerable degree of concordance among electrophoretic patterns, nor been grouped as a unique entity by the Euclidian Distance criterion

Conclusions

The results here presented support the proposition of MLEE employment for clonal differentiation of Streptococcus mutans.

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References

1. Hamada S, Slade HD. Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiol Rev 1980; 44: 331-384.

2. Caulfield PW, Walker TM. Genetic diversity within Streptococcus mutans evident from chromosomal DNA restriction fragment polymorphism. J Clin Microbiol 1989; 27: 274-278.

3. Alaluusua S, Mä ttö J, Grö nroos L, Innilä S, Torkko H, Asikainen S et al.. Oral colonization by more than one clonal type of mutans streptococcus in children with nursing-bottle dental

caries. Archs Oral Biol 1996; 41: 167-173.

4. Gronroos L, Alaluusua S. Site-specific oral colonization of mutans streptococci detected by arbitrarily primed PCR fingerprinting. Caries Res 2000; 34: 474-480

5. Jordan C, LeBlanc DJ. Influences of orthodontic appliances on oral populations of mutans streptococci. Oral Microbiol Immunol 2002; 17:65-71.

6. Baptist JN, Shaw CR, Mandel M. Comparative zone electrophoresis of enzymes of Pseudomonas solanacearum and Pseudomonas cepacia. J Bacteriol 1971; 108: 799-803.

7. Caugant DA, BfVre K, Gaustad P, Bryn K, Holten E, HfIby EA et al. Multilocus genotypes determined by enzyme electrophoresis of Neisseria meningitidis isolated from patients with systemic disease and from healthy carriers. J Gen Microbiol 1986; 132: 641-652.

8. O'Brien SJ, Simonson JM, Grabowski MW, Barile MF. Analysis of multiple isoenzyme expression among twenty-two species of Mycoplasma and Acholeplasma. J Bacteriol 1981; 146: 222-232.

9. Araú jo JV, Junghans TG, Alfenas AC, Gomes AP. Isoenzyme analysis of Arthrobotrys a nematode-trapping fungus. Braz J Med Biol Res 1997, 30: 1149-1152.

10. Naumov GI, Naumova ES, Sniegowiski PD. Differentiation of European and Far East Asian populations of Saccharomyces paradoxus by allozyme analysis. Int J Syst Bact 1997; 47: 341-344.

11. Meloni BP, Lymberi AJ, Thompson RCA. Isoenzyme electrophoresis of 30 isolates of Giardia from humans and felines. Am J Trop Med Hyg 1988; 38: 65-73.

12. Nei M. Estimation of average heterozygosity and genetic distance from a small sample of individuals. Genetics 1978; 89: 583-590.

13. Nei M. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 1973; 70:3321-3323.

14 Nei M. F-statistics and analysis of gene diversity in subdivided populations. Ann Hum Genet 1977; 41: 225-233.

15. Gilmour MN, Whittam TS, Kilian M, Selander RK. Genetic relationships among the oral streptococci. J Bacteriol 1987; 169: 5247-5257.

16. Selander RK, Caugant DA, Ochman H, Musser JM, Gilmour MN, Whittam TS. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. Appl Environ Microbiol 1986; 51: 873-884.

17. Sneath PHA, Sokal RQ. Numerical taxonomy. San Francisco: Freeman, 1973, 482p.

18. Wunder D, Bowen WH. Effects of antibodies to glucosyltransferase on soluble and insolubilized enzymes. Oral Dis 2000; 6: 289-296.

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