CLONAL DIVERSITY OF STREPTOCOCCUS MUTANS CLARKE (1924) IN CARIES-FREE ADULTS

Diversidade clonal de Streptococcus mutans Clarke (1924) em adultos livres-de-cárie

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Abstract

Very few information about the clonal profile of Streptococcus mutans in caries-free adults is available. Microbial samples of saliva, tongue dorsum, and dental biofilm were taken from eight caries-free volunteers
(DMFT = 0). Presumptive colonies of mutans streptococci were identified until species level. After confirmation, S. mutans strains were grown in liquid culture media and harvested for whole-cell protein extraction. Multilocus Enzyme Electrophoresis (MLEE) separated protein extracts and the bands' patterns were assessed for six enzymes. Results showed that two or more distinct clonal types (avg = 4.75) were found cocolonizing all the enrolled subjects, confirming the premise that caries-free subjects are prone to be colonized by more than one strain of S. mutans.
Keywords: Streptococcus mutans; Dental caries; MLEE; Clonal diversity; Isoenzymes.

Resumo

Pouca informação acerca do padrão de colonização de Streptococcus mutans em adultos livresde-cáries está disponível. Amostras microbianas de saliva, dorso de língua e biofilme dental foram coletadas de oito voluntários livres-de-cáries (CPOD = 0). Colônias com aspecto de estreptococos do grupo mutans foram identificadas até o nível de espécie. Após a confirmação, as cepas de S. mutans foram cultivadas em caldo e colhidas para extração das proteínas totais. A Eletroforese de Enzimas Multilocus (MLEE) separou as proteínas e os padrões de bandeamento foram determinados para seis enzimas. Os resultados mostraram que dois ou mais clones distintos (média de 4,75 clones) foram encontrados co-colonizando todos os indivíduos, confirmando a premissa de que indivíduos livres-de-cáries são propensos a serem colonizados por mais de uma cepa de S. mutans.
Palavras-chave: Streptococcus mutans; Cárie dental; MLEE; Diversidade clonal; Isoenzimas.

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Among the mutans streptococci, Streptococcus mutans Clarke (1924) is the putative cariogenic organism more routinely associated to active lesions (HAMADA; SLADE, 1980). Despite the great number of articles concerning to the S. mutans involvement in caries development, Kreulen et al. (1997) pointed out that very few information about this bacterial population structure in caries-free adults is available. Bowden (1997) pointed out the necessity of comprehension about the clonality patterns of S. mutans in caries-free subjects. According to this author, it is important to ascertain whether S. mutans populations in subjects free of caries exhibit or not the same clonal diversity in caries-active groups. In the present paper we propose the using of multilocus enzyme electrophor esis (MLEE) for clone determination of S. mutans obtained from adult caries-free subjects.

Eight caries-free, 17-21 year-old volunteers
(DMFT = 0) were recruited, after expressed concordance with the Local Ethical Committee's statements. From each subject, stimulated saliva, coronal dental biofilm, and tongue dorsum swab were sampled. All samples were dispersed, plated on Mitis-Salivarius-Bacitracin-Sucrose-Agar, and incubated (37ºC/pCO2 10%/24-48h). Twenty characteristic colonies per plate were biochemically tested to identify the S. mutans species. Confirmed strains were grown in BHI broth (37ºC/pCO2 10%/24h). Cells were harvested, washed with 40mM PBS (pH7.5), and added to microtubes with 0.55mm glass beads plus 1mL of PBS. Cells were disrupted in a Mini-Bead Beater (Biospec Inc., OK.) at 4500rpm/ 1min. Supernatants were absorbed in 5x12mm Whatman-3 paper wicks and stored at -700C. Electrophoreses were carried out in 13% hydrolyzed starch supports in buffer solution A (GILMOUR et al., 1987). After the running time, gels were rapidly revealed for leucine aminopeptidase, mannitol-1-phosphate dehydrogenase, mannose-phosphate isomerase, nucleoside phosphorylase, phenylalanyl-leucine peptidase, and glutamic-oxalacetic transaminase prospecting.

Bands were scored according to their respective relative mobilities and arranged in order to generate the bands' pattern of each isolate. In a same individual, different band patterns received alphanumerical codes.
In this survey, two or more distinct clonal types of S. mutans were found colonizing all the subjects enrolled (avg = 4.75 clones). In tongue dorsa an average of 2.00 clones was found. For dental biofilm an average of 2.5 clones was observed. Saliva displayed an average of 2.75 clonal types. No differences in number or clonal types were detected (t test: $p > 0.05$) among the intraoral sites. Table 1 shows the clonal distribution of S. mutans in each prospected subject.

Table 1. Clonal distribution of Streptococcus mutans among the subjects

	Clones per site		
Subject	Tongue dorsum	Dental biofilm	Saliva
#1	1A*(7**), 1B(3)	1A(6), 1B(1), 1C(3)	1A(9), 1D(1)
#2	2A(3), 2D(1), 2E(1)	2A(1)	2A(2), 2B(2), 2C(2)
#3	3A(10)	3A(10)	3A(1), 3B(6), 3C(3)
#4	4A(8), 4D(1), 4E(1)	4A(3), 4D(1)	4B(5), 4C(4), 4F(1)
#5	5A(5), 5E(1), 5F(1)	5A(2), 5B(2), 5C(2)	5A(9), 5D(1)
#6	6A(10)	6A(7), 6B(2), 6C(1)	6A(10
#7	7A(10)	7A(5), 7C(2), 7D(1), 7E(1), 7F(1)	7A(7), 7B(2), 7G(1)
#8	8B(1), 8C(4)	8A(4), 8B(3)	8A(5), 8B(1), 8D(2), 8E(1), 8F(1)

*Majuscule letters indicate the different clonal types found in a same individual; **Numerals in parentheses are the number of isolates.

Contrasting to our results, Alaluusua et al. (1996) related that six caries-free children only harboured one ribotype of S. mutans. According to them, it was possible that from plaque samples they found only primary strains, and other strains, if they existed were below the detection level or their proportion in the sample was low. In the Alaluusua-study, they evaluated a smaller number (3 to 7, avg = 4.5 strains per volunteer) of strains than we did (12 to 30, avg = 26.3 strains per volunteer). Enforcing our findings, Kreulen et al. (1997) also have evaluated a great number of strains (more than 30 strains per volunteer) and obtained two to five RAPD profiles of S. mutans in caries-free children. According to these last authors, the randomly selection of thirty or more colonies per subject assures that more than ninety percent of the strain types of S. mutans present in the sample will be obtained with ninety-five percent confidence. For half of our volunteers thirty colonies were taken.

Other consideration that must be taken in account is that our volunteers had already passed by the two "windows of infectivity" (CAUFIELD et al., 1993) and may have aggregated new S. mutans clones to those primary strains. Among the niches studied, we observed that the numerical dominant strains (designed by majuscule letters A) on dental biofilm were also found in higher rates in other niches. In a recent study, Redmo-Emanuelsson et al. (2003) detected identical genotypes occurring dispersedly in multiple dental surfaces in cariesactive patients. In our research, the exceptions were found just for saliva of volunteer #4 and tongue dorsum of volunteer #8. Such findings enforce the thesis that some clones tend to be prevalent on mutans streptococcal communities formed on hard surfaces.

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