RESOLUTION OF WHOLE PROTEINS OF SOME ORAL *Streptococci* BY POLYACRYLAMIDE GEL ELECTROPHORESIS ON SLAB AND DISC

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■ ABSTRACT: There has been much interest in the streptococci that are implicated in dental caries. Differences in the immunologic, morphologic and deoxiribonucleic acid (DNA) characteristics have been reported for this species. Acrylamide gel electrophoresis has been used as adjunct tool for the classification and identification of certain microorganisms. A disc electrophoresis and slab investigation of protein from oral *Streptococcus* strains were analysed by using intracellular proteins obtained from distinct methods. Cells were cultured for 48 hours centrifuged and the sedimented cells were submitted to the different methods. Method I - The sedimented cells were suspended in a small volume of Sodium Dodecyl Sulphate (SDS) solubilizing buffer; Method II - Sedimented cells were suspended in solution plus 0.15 g of glass beads. The cells were then broken by shaking for 3 minutes; Method III - The pellets were suspended in 0.5 M sucrose containing EDTA maintained in a low temperature before being solubilized. The resulting suspension were dissociated 3 minutes in a boiling water bath. Electrophoresis was carried out in a 20 mA direct current using bromophenol blue as tracking dye. The direct suspension in a buffered solubilizing solution and mechanic disintegration methods showed a better resolution of the protein profiles. Disc electrophoresis gels were not able to present a good resolution in comparison to the slab gel. In addition an important advantage of slab gels is also that a number of samples can be run side by side under identical conditions permitting that a large number of samples can be run and compared in a single gel.

■ KEYWORDS: *Streptococcus*; proteins; electrophoresis.

Introduction

The evidence that changes in biochemical and serological characteristics among *Streptococcus mutans* group are related to animal species from where were isolated

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is referred in the last edition of Bergey's manual (Hardie[5]), who describes the *Streptococcus mutans* group composed by the species *S. cricetus, S. rattus, S. mutans, S. sobrinus, S. ferus* and *S. macacae*,[3] inducing the researchers to study these species in a general way and particularly with emphasis in the identification and characterization of such microorganisms. Therein the whole cell proteins separation technique by electrophoresis in polyacrylamide gel (PAGE-SDS) has showed to be an important mean in the study of microorganisms isolated from several ecological niches [4, 7, 10, 13] and in special of the oral microbiology.[3, 5, 11] Aiming to contribute to the studies of identification and characterization of oral *Streptococci* culture samples of the *mutans* group isolated from oral cavity were analysed through disc and slab electrophoresis technique in polyacrylamide gel comparatively employing distinct methodologies to obtain total proteins.

Material and method

1. Bacteria strain

Samples of oral bacteria from the *Streptococcus* genus, kindly ceded by the Biomedical Sciences Institute from São Paulo University (sample V-5/*S. mutans* and I-4B/not identified biochemically used in the author's Master thesis) and by Lunds Universitet, Odontologiska Fakulteten, Malmö, Sweden (sample IB/*S. mutans* and P1/*S. sobrinus*) were used. The microbian strains were cultivated in test tubes containing 3.5 ml of Brain Heart Infusion (BHI – Difco) culture medium and incubated at 37°C during 48 hours. Following, the test tubes were kept under refrigeration (10° C) during 10 days where the samples were then renewed.

2. Samples preparation

Three methods were used to prepare the samples. The culture medium used was the Tryptone Yeast Extract (TYE). This medium was distributed in test tubes about 6.0 ml per tube, autoclaved in the following way:

2.1 Method I – The bacteria were incubated at 37°C for 48 hours. After the growth of culture, the cells were centrifuged at 3.000 rpm for 15 minutes, then washed twice in sterile 0.15 M NaCl solution and the final sediment was made up to a small volume (100 microliters) of SDS [8] according to what was described by Russel.[11]

2.2 Method II – Based on Cato et al.,[4] the test tubes containing TYE were incubated with the bacteria for 48 hours at 37°C. The cells were centrifuged (3,000 rpm/15 minutes) and washed in sterile 0.15 M NaCl solution. To the remaining precipitate, were added 0.15 g of glass beads (74 a 110 micrometers of diameter). The

cells were then submitted to agitation (tube agitator Phoenix – Mod. AT 56) for two periods of 3 minutes each.

2.3 Method III – The samples were harvested in TYE medium and incubated at 37ºC for 48 hours. Following, the bacteria cells grown into the test tubes was centrifuged (3,000 rpm/15 minutes) washed twice in sterile 0.15 M NaCl solution. The precipitate obtained was suspended again in 0.5 M sucrose and EDTA kept for 20 minutes in ice bath according to Heppel.[6] After centrifugation, the precipitate was solubilized in SDS.[8]

3. Electrophoresis

Whole proteins obtained according to the previous described methods were submitted to electrophoresis in polyacrylamide gel – sodium dodecyl sulphate (PAGE-SDS) [8] in disc and slab in discontinued system of buffer 0.025 M of Tris and 0.192 M of Glycine and 0.1 % of SDS in pH 8.3, with at 2.5% acrylamide stacking gel and at 7.5% acrylamide separating gel.

Approximately 100 micrograms of proteins[9] from the samples were solubilized in disrupting mixtures by immersion in water at 100ºC for 3 minutes. The electrophoresis was carried out in room temperature at 100 Volts constant current of 20 mA. After the flow, the gel was stained in 0.1% Coomassie Blue G-250. Destaining of the gel was done with a 7% acetic acid and 2.5% methanol then the slab gel was dehidrated, drained[12] and photographed on a low box. The disc gel was packed in tubes containing destaining solution and later photographed. The densitometric tracing was performed with a microdensitometry model Quich Scan-Flur-Vis and the relative mobility (Rm) expressed in diagram form was calculated in function of the distance run by the band until the bromophenol blue tracking dye was 1 cm from the botton of the gel.

Result

The results obtained by the technique of direct solubility (method I), showed the presence of 15 to 20 bands approximately for the slab gel and 5 to 9 bands for the disc gel, as can be seen in Figures 1-2 and 4-5. The electrophoretic profiles showed similar distributions in the gel, despite some differences where observed in the relatives mobility (Rm), among proteins of high molecular weight (Figure 2), i.e., Rm 0.17 – 0.30 – 0.31 – 0.39 – 0.58 and Rm 0.43 – 0.45 – 0.46 – 0.62 respectively for P1 and I-4B. Meantime, for the cylindric gel, it could be observed differences in Rm 0.30 – 0.31 – 0.39 for P1 and Rm 0.43 – 0.45 for I-B, as illustrated in the Figure 5. The Figures 3 and 6 showed these differences through the densitometric tracing.

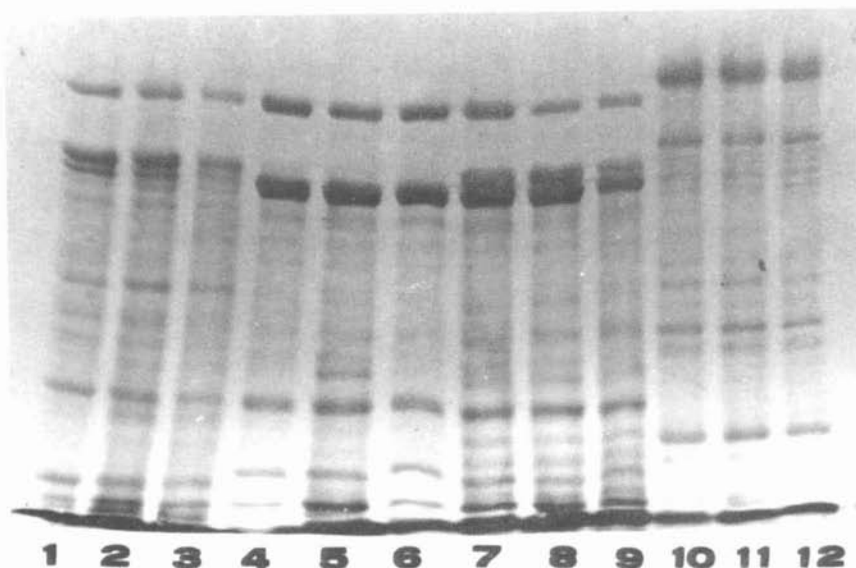

FIGURE 1 – Electrophoretic slab profile of the samples I-4B(A); V-5(B); IB(C); P1(D) of *Streptococcus*. Lines 1(A) - 4(B) - 7(C) - 10(D) - (Direct solubility method), lines 2(A) - 5(B) - 8(C) - 11(D) - (Mechanic disintegration method), lines 3(A) - 6(B) - 9(C) - 12(D) - (Osmotic shock method).

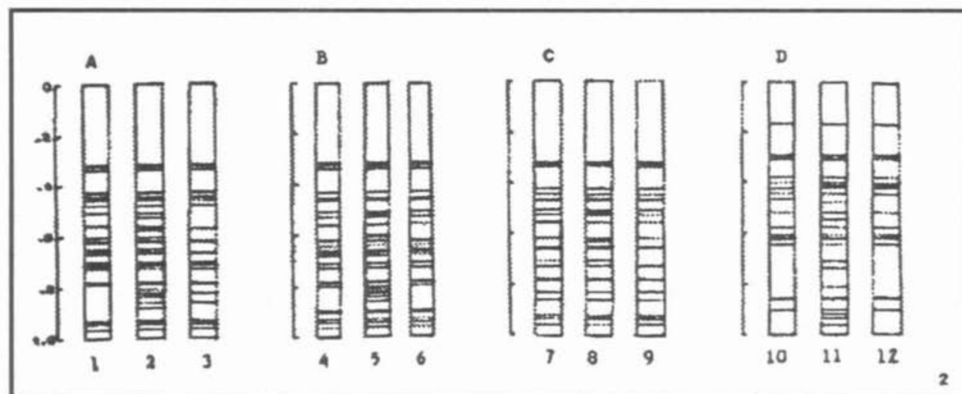

FIGURE 2 – Schematic slab diagram of the samples I-4B(A); V-5(B); IB(C); P1(D) of *Streptococcus*. Lines 1(A) - 4(B) - 7(C) - 10(D) - (Direct solubility method), lines 2(A) - 5(B) - 8(C) - 11(D) - (Mechanic disintegration method), lines 3(A) - 6(B) - 9(C) - 12(D) - (Osmotic shock method).

The results obtained by the technique developed by Cato et al.[4] (Method II), as showed in Figure 1 (lines 2, 5, 8 and 11), revealed a greater number of proteics bands, approximately 20 to 30 for the slab gel. Differences could be observed in the high molecular weight portion for the sample P1 (Figure 2) while for the strain V-5 (*S. mutans*), were detected in the Rm 0.60 – 0.82 – 0.84 – 0.90. For the sample I-4B, it was observed the presence of bands in the Rm 0.81 to 0.89. Similar results to the same methodology was observed for the cylindric gel, as showed in Figures 4 and 5.

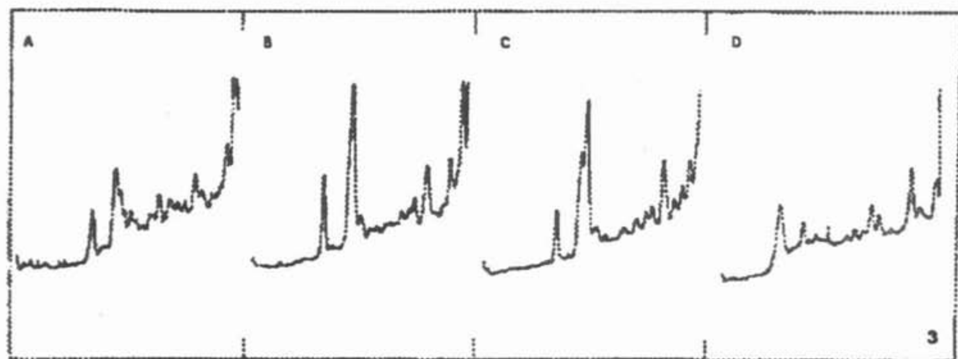

FIGURE 3 – Slab densitometry of the samples of *Streptococcus* I-4B(A); V-5(B); IB(C); P1(D). (Mechanic disintegration method).

The results obtained by the method described in the item 2-3 are visualized in Figure 1, (lines 3, 6, 9 and 12) which are the electrophoretic profiles whole cell proteins of *S. mutans* group strain showed the presence of 15 to 19 bands as shown in Figure 2. The disc gel did not revealed differences in the electrophoretic profile, as can be observed by the former methodology (Figure 4).

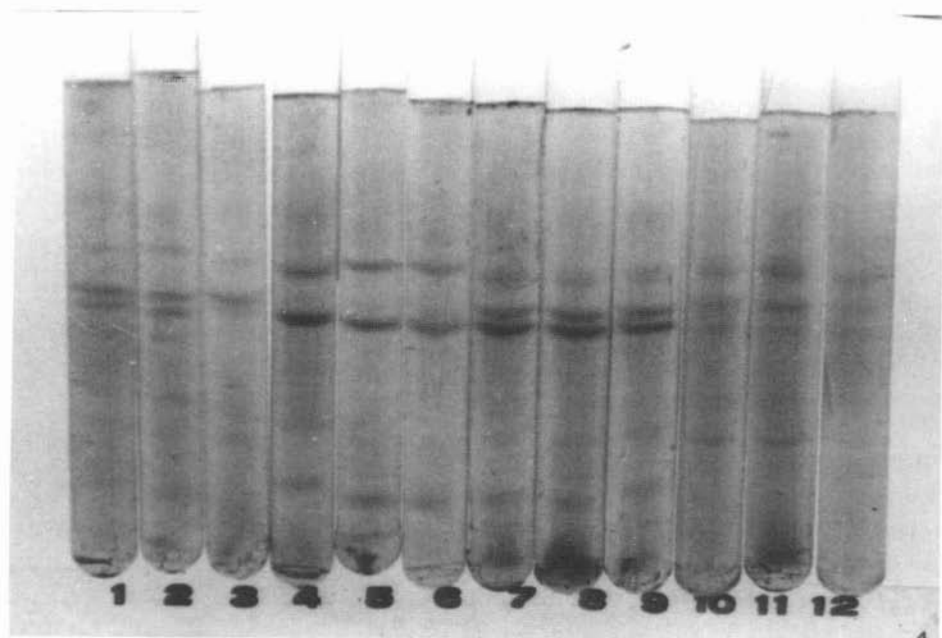

FIGURE 4 – Electrophoretic disc profile of the sample I-4B(A); V-5(B); IB(C); P1(D) of *Streptococcus*. Lines 1(A) - 4(B) - 7(C) - 10(D) - (Direct solubility method), lines 2(A) - 5(B) - 8(C) - 11(D) - (Mechanic disintegration method), lines 3(A) - 6(B) - 9(C) - 12(D) - (Osmotic shock method).

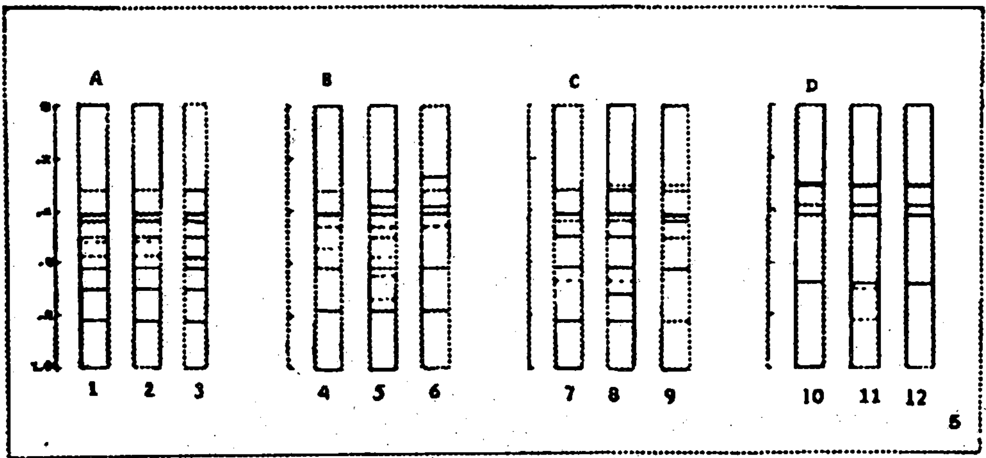

FIGURE 5 – Schematic disc diagram of the samples I-4B(A); V-5(B); IB(C); P1(D) of *Streptococcus*. Lines 1(A) - 4(B) - 7(C) - 10(D) - (Direct solubility method), lines 2(A) - 5(B) - 8(C) - 11(D) - (Mechanic disintegration method), lines 3(A) - 6(B) - 9(C) - 12(D) - (Osmotic shock method).

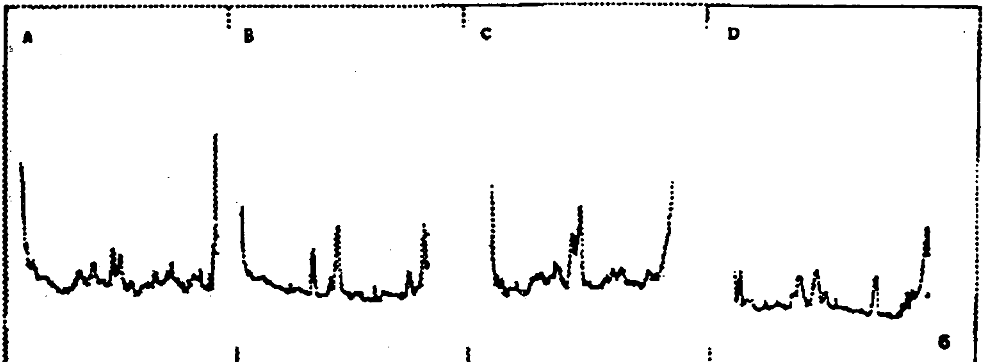

FIGURE 6 – Disc densitometry of the samples of *Streptococcus* I-4B(A); V-5(B); IB(C); P1(D). (Mechanic disintegration method).

Discussion

The determination of whole cell protein profiles of *Streptococcus*, analysed by Russel technique[11] demonstrated some differences among the samples. It was observed that only the strains codified as I-4B and P1 were different from the others in both techniques applied (slab and disc). The samples codified as V-5 and I-B characterized as *S. mutans*, showed a similar pattern when compared, while I-4B (not identified biochemically), revealed similarity with *S. mutans*, as can be verified in Figures 1 and 2. According to Ames[1] and Russel,[11] the advantage to use the

methodology of direct solubility, is due to factors such as the minimum manipulation of the material in a short period of time, associate to the possibility to eliminate the several steps of centrifuging and cellular disintegration proper of others methodologies with the same purpose.

The results obtained using the methodology of Cato et al.,[4] which purpose was to promove the mechanic disintegration of cells showed that the analysed samples of *S. mutans* group (V-5 and I-B) are similar except the samples P1 and I-4B, which presented differences when compared to the *S. mutans*, as can be verified in the Figures 1 and 2. It was possible to determine two distinct species (*S. mutans* and *S. sobrinus*). According to Moore et al.,[10] Seiter & Jay[13] and Simonsen & Shklair,[14] the advantage in employing this methodology is based upon the principle that the mechanic disintegration with glass beads make possible the breakage of the cellular membrane freeling all its intracellular contents, originating a greater number of bands which permits a better reproducibility in the electrophoretic profiles. In this way, it can be obtained sufficient quantities of cellular proteins to applicate in a reasonable and adequate space of time for the study of whole collections of bacteria growing into an important and efficient instrument in the routine characterization of microorganisms. Similar results were obtained by Heppel methodology,[6] where the samples were suspended again in sucrose and EDTA and maintained at low temperature before being dissolved demonstrating that the process of osmotic shock can be applied in the characterization of gram-positive microbial species *Streptococcus* (Figure 1), even though the manipulation of the sample is longer when compared to other technique. According to Heppel,[6] the effect of osmotic shock has been a proceeding very used for gram-negative bacteria in a general way, since unlike the gram-positive, these present a cell wall less thick which according to treating in hypotonic medium proper of the technique free more easily its cellular contents.

The results obtained with the employement of distinct technique in order to get the electrophoresis patterns of total proteins suggest that Russel methodology,[11] based on the direct solubility associated to polyacrylamide gel electrophoresis in slab has some advantage over other technique proposed by Heppel [6] and Cato et al.[4] The straight use of SDS in addition to recuperate some cellular proteins and to modify their electric loads seams to permit a better visualization and separation of the diverses substances in the gel plus a short cut in the preparation of the different samples. Comparatively, the technique employed by Cato et al.,[4] although requires a longer preparation of the material to be analysed, can be considered also an eficient method of study once it was obtained a better resolution of the electrophoretic profile with the increase of the number of visualized bands. This certainly would contribute positively to the studies of relations among the microbian species. Although, both (slab and disc) have been performed under the same conditions (buffer, gel porosity, electric current and quantity of applied protein), the decisive factor for the differences on the detected electrophoretic profiles could be due to small differences in the polymerization lenght or diameter of the gel which when the same sample is applied demonstrate that the disc electrophoresis was not able to present a good resolution

comparing to the slab gel which suggests an option for it as rotine.[2] An important advantage yet, of slab gels is that a number of sample slots can be formed within a single slab so that several samples can be runs side by side under identical conditions and compared without ambiguity. This is particulary useful in screening procedures where a large number of samples must be examined and compared as in some clinical investigation or when multiple application of a single sample are made to different slots and the gel subsequently sliced and examined in different ways (e.g. one slice stained for protein, another for enzyme activity, a third for autoradiography, etc). When a gel rod apparatus is used each sample is run a separate rod so that for an accurate comparison of different samples conditions must be identical in all rods throughout the experiment. This can be difficult to ensure.

From the informations obtained in this research, it is possible to asseverate that the employment of polyacrylamide gel electrophoresis technique can be a complementary tool in the characterization of microorganisms, particularly of the oral cavity, confirming some studies referred to in this investigation.[3, 14]

Conclusion

The direct suspension in a buffered solubilizing solution and mechanic disintegration methods revealed a better resolution protein profiles. Disc electrophoresis gels were not able to present a good resolution in comparison to the slab gel. In addition an important advantage of slab gels, is also that a number of samples can be run side by side under identical conditions permitting that a large number of samples can be run and compared in a single gel.

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▪ *RESUMO: Com o propósito de se aplicar as técnicas de eletroforese em gel de poliacrilamida em placa vertical e disco como uma contribuição aos estudos de identificação e da caracterização de* Streptococcus *orais e ainda ressaltar a capacidade de resolução de cada técnica, amostras de culturas do grupo* mutans *foram analisadas empregando-se três metodologias distintas na obtenção de proteínas intracelulares. Amostras de microorganismos foram semeadas em tubos contendo meio de cultura TYE. Em seguida, incubados a 37°C em jarra de anaerobiose durante 48 horas. Após o crescimento bacteriano, as células foram centrifugadas (3.000 rpm – 13 minutos) e lavadas duas vezes em solução estéril de NaCl 0,15 M. Aos precipitados resultantes, foram aplicadas as seguintes metodologias: Método I - o precipitado final foi ressuspendido em pequeno volume de SDS (solubilização direta). Método II - ao precipitado resultante, adicionou-se 0,15 grama de pérolas de vidro (74 a 110 µm de diâmetro), o qual foi submetido em seguida à agitação por dois períodos de 3 minutos cada (mod. Phoenix AT 56) – choque mecânico. Método III - o precipitado foi ressuspendido em sacarose 0,5 M e EDTA e mantido em banho de gelo por 20 minutos (choque osmótico). As suspensões finais obtidas foram dissociadas por imersão em água a 100°C por 3 minutos. A eletroforese (placa e disco) processou-se em temperatura ambiente a 100 V em corrente constante de 20 mA. De modo geral, os resultados obtidos demonstraram, em primeiro lugar, que as metodologias de solubilização direta e choque mecânico permitem melhor visualização e separação das diversas proteínas nos géis. Em segundo, verificou-se que o PAGE em placa permite uma melhor resolução dos perfis protéicos, além de possibilitar que várias amostras possam ser analisadas em um único gel.*

▪ *PALAVRAS-CHAVE:* Streptococcus; *proteínas; eletroforese.*

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