Streptococci of the Mutans Group: Confirmation of Intrafamilial Transmission by Mutacin Typing

Rosa Vitória Palamin AZEVEDO¹
Flávio ZELANTE²

¹Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil
²Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brasil

We selected 22 families of different socioeconomic levels residing within the urban perimeter of Ribeirão Preto, State of São Paulo, and consisting of parents and at least two children, one of whom had to be edentulous on the occasion of the first collection of material. A total of 397 mutans strains isolated from 112 individuals were submitted to mutacin typing; 391 of them were from dental plaque and 6 from gingival mucosa, and only 95 (23.93%) were found to be mutacin producers. Seventy-one different bacteriogenic patterns were detected, as well as a greater proportion of individuals with multiple bacteriogenic types. In all families there were similarities between strains isolated from one or more family members. The mother was not considered to be the most likely source of infection and infrafamilial transmission of streptococci from the buccal cavity was confirmed.

Key Words: mutans streptococci, bacteriocin typing, mutacin, intrafamilial transmission.

Introduction

Information about the inter- and intraindividual implantation and dissemination of Streptococcus mutans suggests that buccal colonization by these microorganisms occurs by direct or indirect contact (Köhler and Brathall, 1978; Berkowitz and Jones, 1985). Its establishment appears to occur with the eruption of teeth; thus fathers and other persons with whom the children live constitute the primary source of infection (Köhler and Brathall, 1978; Berkowitz et al., 1981; Rogers, 1981, Köhler et al., 1984; Davey and Rogers, 1984; Masuda et al., 1985).

Studies carried out in countries with cultural and socioeconomic standards differing from those of Brazil have suggested that the mother is the most probable source of the mutans group for her children (Köhler et al., 1983).

Fitzgerald et al. (1983) observed that older individuals with natural teeth or artificial dentures may become a reservoir of microorganisms of the mutans group with varying cariogenic potentials. Thus, in the intimacy of family life, other members in addition to fathers and siblings may function as a source of transmission of cariogenic microorganisms to infants during the first months of life (Köhler et al., 1984; Masuda et al., 1985).
Massive and continuous transmission of viable \textit{S. mutans} cells favors their implantation, with the subsequent onset of infection. Thus, mothers with high levels of these bacteria can more easily transmit them to their children (Köhler and Brathall, 1978; Berkowitz et al., 1981; Brown et al., 1985). Conversely, mothers with low salivary concentrations of mutans bacteria tend to raise uninfected children (Köhler and Brathall, 1978). This observation was later confirmed by Berkowitz et al. (1981) and Köhler et al. (1984).

Epidemiologic studies carried out to elucidate the chain of transmission of the mutans group have demonstrated that analysis of bacteriocinogenic activity (mutacignogenesis) is appropriate for such purpose (Berkowitz and Jordan, 1975; Rogers, 1981), and have actually demonstrated intrafamilial transmission of these bacteria (Davey and Rogers, 1984; Berkowitz and Jones, 1985; Masuda et al., 1985).

The objective of the present investigation was to evaluate the prevalence of mutacin-producing strains among streptococci of the mutans group isolated from members of 22 families, and to determine the possible similarity of the intrafamilial bacteriocigenic pattern.

Material and Methods

We selected 22 families of different socioeconomic levels residing within the urban perimeter of Ribeirão Preto, State of São Paulo, and consisting of parents and at least two children, one of whom had to be edentulous on the occasion of the first collection of material, i.e., examined before the eruption of any tooth ("test infant").

A total of 397 strains identified as mutans streptococci were isolated from 135 samples (111 from dental plaque and 24 from gingival mucosa) obtained from 112 individuals and submitted to typing; 391 of the strains were from dental plaque and 6 from gingival mucosa (Azevedo et al., 1992).

The overlay technique was used for the detection of mutacin in the presence of 15 indicator strains. The results were read as recommended by Rogers (1972) and the diameters of the inhibition haloes (mm) were recorded. The corresponding strain was considered to be bac + (bacteriocin positive or bacteriocin producing).

The mnemonic digital system proposed by Tagg and Bannister (1979) and applied by Dametto (1987) was adopted for interpretation of the results. Groups of three indicator strains were used: a positive or moderately positive reaction for the first indicator in any group was scored as 4, a positive or moderately positive reaction for the second indicator in any group was scored as 2, a positive or moderately positive reaction for the first indicator was scored as 1, and a negative reaction was scored as zero. Thus, the following values were considered:

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>LM7</td>
<td>\textit{S. mutans}</td>
<td>4</td>
</tr>
<tr>
<td>14B6</td>
<td>\textit{S. sobrinus}</td>
<td>2</td>
</tr>
<tr>
<td>T14V</td>
<td>\textit{A. viscosus}</td>
<td>1</td>
</tr>
</tbody>
</table>

1st digit
14C3  \( \text{S. mutans} \)  4  
10L  \( \text{S. salivarius} \)  2  \( \text{2nd digit} \)  
27/85  \( \text{S. sanquis} \)  1  
JC5  \( \text{S. mutans} \)  4  
13L  \( \text{S. salivarius} \)  2  \( \text{3rd digit} \)  
69BI  \( \text{S. ratti} \)  1  
NY2665  \( \text{S. mutans} \)  4  
PBI  \( \text{S. ratti} \)  2  \( \text{4th digit} \)  
R5g  \( \text{S. mutans} \)  1  
AT10  \( \text{S. mutans} \)  4  
CIK  \( \text{S. ratti} \)  2  \( \text{5th digit} \)  
5  \( \text{S. aureus} \)  1  

The sum of the scores for each triad gave the “fingerprint” of each strain tested, expressed as a 5-digit number corresponding to its bacteriocinogenic pattern, its bacteriocin type and/or bacteriocin model.

**Results**

Of the 397 strains submitted to bacteriocin typing in the presence of 15 indicators of different species, only 95 (23.93%) proved to be mutacin producers as detected in at least one dilution. In contrast, 302 strains (76.07%) did not produce mutacin. The strains were classified into 71 different bacteriocinogenic patterns, 39 of them in relation to \( \text{S. mutans} \), 11 in relation to \( \text{S. ratti} \), 17 in relation to \( \text{S. cricetus} \), and 1 in relation to \( \text{S. sobrinus} \) and \( \text{S. ferus} \) (Table 1).

**Table 1 - Behavior of the 397 strains of mutans streptococci submitted to bacteriocin typing.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Producer</th>
<th>Non-producer</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>( \text{S. mutans} )</td>
<td>55</td>
<td>28.50</td>
<td>138</td>
</tr>
<tr>
<td>( \text{S. cricetus} )</td>
<td>21</td>
<td>20.39</td>
<td>82</td>
</tr>
<tr>
<td>( \text{S. ratti} )</td>
<td>15</td>
<td>18.75</td>
<td>65</td>
</tr>
<tr>
<td>( \text{S. sobrinus} )</td>
<td>3</td>
<td>30.00</td>
<td>7</td>
</tr>
<tr>
<td>( \text{S. mutans (V)} )</td>
<td>0</td>
<td>0.00</td>
<td>10</td>
</tr>
<tr>
<td>( \text{S. ferus} )</td>
<td>1</td>
<td>100.00</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>95</strong></td>
<td><strong>23.93</strong></td>
<td><strong>302</strong></td>
</tr>
</tbody>
</table>

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In all families, the strains isolated from one or more family members were similar or identical (Table 2).

Table 3 shows the mutacinogenic patterns of the mutans strains isolated from members of one of the 22 families studied (family 8).

In all families but one, more than one bacteriocinogenic pattern was found. Family 8, consisting of 5 members, presented 3 types of bacteriocinogenic patterns shared by different members and 6 additional different types, in the same individual or not, with 3 members harboring multiple bacteriocin types.

Table 2 - Similar bacteriocinogenic models in members of the same family.

<table>
<thead>
<tr>
<th>Pairing</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother/child(children)</td>
<td>13/22*</td>
</tr>
<tr>
<td>Sib/sib(s)</td>
<td>11/22</td>
</tr>
<tr>
<td>Father/child(children)</td>
<td>6/15</td>
</tr>
<tr>
<td>Mother/father</td>
<td>5/15</td>
</tr>
<tr>
<td>Mother/father/child(children)</td>
<td>9/15</td>
</tr>
</tbody>
</table>

*Numerator = number of pairings; denominator = number of families.

Table 3 - Bacteriocinogenic pattern of mutans streptococcus species isolated from members of family 8.

<table>
<thead>
<tr>
<th>Species</th>
<th>Bact. pattern</th>
<th>Mother 30 years</th>
<th>Father 29 years</th>
<th>Sibs 5 years</th>
<th>1 year and 9 months</th>
<th>&quot;Test infant&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. mutans</td>
<td>00000</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20201</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>00001</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. rattus</td>
<td>00000</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>77777</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S. cricetus</td>
<td>00000</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>77777</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>00001</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>20001</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Mutans streptococci with a single bacteriocinogenic pattern were isolated from at least one or two members of 17 families, for a total of 32 individuals, whereas multiple bacteriocin types were always detected in the remaining individuals. Conversely, 76 individuals (67.86%) investigated (20 families) presented multiple types. Two different types of bacteriocinogenic patterns or bacteriocin type were detected in 39 members (51.32%) of 17 families, 3 types in 20 members (26.31%) of 14 families, 4 types in 12 members (15.79%) of 8 families, and 5 or more types in 5 members (6.85%) of 4 families.

In 7 of the 22 families, the mothers had a single bacteriocinogenic type. However, in 5 of these families (1, 14, 18, 19 and 22), the children had more than one type of bacteriocinogenic pattern differing from that of the mother. In the remaining families (13 and 20), some children harbored the single pattern detected in the buccal cavity of the mother.

Discussion

Of the 397 isolated strains submitted to bacteriocin typing, 95 (23.93%) (Table 1) produced mutacin, inhibiting the growth of at least one of the 15 strains used as indicators. Kelstrup and Gibbons (1969) reported that 50.0% (6/13) of isolates obtained from human and animal samples corresponded to producer strains. Berkowitz and Jordan (1975) detected 99.16% (119/120) bacteriocinogenic strains among 120 S. mutans strains isolated from the buccal cavities of four mother/child pairs. Hamada and Ooshima (1975) observed that 74.33% (84/113) of mutans strains isolated from human dental plaque were producers of bacteriocin-like substances, and Rogers (1976a) isolated 70.0% (98/143) producers from strains supplied by various investigators.

When comparing the results of mutacin typing carried out by Hamada and Ooshima (1975) and by us with those for strains of different species obtained from clinical isolates, we noted a considerable difference, with the following frequencies of producer strains: 76.0% (65/85) and 28.50% (55/193) for S. mutans, 69.0% (9/13) and 30.0% (3/10) for S. sobrinus, and 67.0% (10/15) and 0.0% (bacteriocin-negative result) for S. mutans V, respectively.

Among the 95 strains producing bacteriocin-like substances, 71 different bacteriocinogenic patterns were characterized. The species that produced the largest number of different bacteriocin types in relation to the number of strains tested was S. sobrinus (3/3), followed by S. cricetus (21/17), S. rattus (15/11), and S. mutans (55/39), with respective ratios of 1.0, 1.3 and 1.4. These results disagree with those obtained by Rogers (1976a,b) and by Ikeda et al. (1982) who reported that S. mutans was the species with the largest number of models.

The bacteriocin-negative pattern 000000, although the most frequently detected (76.07%), permitted the detection of strains among members of the same family. At times, the lack of typability may represent a "marker" trait, especially in the case of a restricted cluster such as that represented by a family.
A factor that, in our opinion, facilitated interpretation of the results was the adoption of the scheme proposed by Tagg and Bannister (1979) and also utilized by Darnetto (1987). In 100.0% of the families studied, two or more of the members (one of them a parent) shared strains of the mutans group. This is not necessarily indicative, per se, of intrafamilial transmission. However, since in all families studied by us at least one of the children shared similar types with one or more of the other family members, we may argue that common types of these streptococci only occur within restricted family groups and that some strains may have been transmitted intrafamilially.

When analyzing the number of similarity pairings (Table 2) of the bacteriocinogenic models of the streptococci among the members of a family, we noted that none of them in particular can be characterized as the major source of dissemination of bacteria to the “test infant” or to the other children. In this respect, we observed that in several families the mothers worked outside the home and left the “test infants” in the care of other children, i.e., with siblings, or at day-care centers. These circumstances must have contributed to this dispersal of the probable source of primary infection. However, evaluation of the results obtained for the 13 families permits us to assume that maternal transfer was the predominant form of transmission. On this basis, we agree with Rogers (1981) with respect to the concept of intrafamilial transmission of mutans group, although it is not possible to characterize the primary source of infection in any family member. However, Berkowitz and Jordan (1975), Berkowitz et al. (1981) and Berkowitz and Jones (1985) reported that the mothers behaved as the major primary source of infection, possibly because these investigators only studied mother-child pairs.

The observation that 5 of the 24 “test infants” sampled harbored only one type of bacteriocinogenic pattern suggests that not all streptococci present in the remaining family members colonize the buccal cavity of the infant, confirming the observations of Berkowitz and Jones (1985).

On the other hand, the presence of only one type of bacteriocinogenic pattern in the samples collected from 32 (28.56%) individuals probably reflects the possible predominance of a type of microorganism of the mutans group in the colonization of a determined buccal niche. This is possibly related to the fact reported by Fukushima et al. (1985) that early inoculation of a given bacteriocinogenic strain permits its numerical predominance over all other strains of the same species or of closely related species.

We agree with Tagg and Bannister (1979) that bacteriocin typing is a simple and reliable method for the individualization of streptococcal strains which can be used as a complement of serotyping or biotyping in laboratories with no easy access to serologic classification. It can also be a useful means for the screening of strains not serologically typable, and can permit the individualization of strains of the same serotype or biotype.

In this respect, we intend to continue our investigations in this area by also serotyping strains of the mutans group in a family group, in an attempt to correlate biotyping, bacteriocin typing and serotyping data with the prevalence and chronology of colonization of “test infants” by different species of the mutans group.
Conclusions

Of the 397 mutans strains tested, only 95 (23.93%) proved to be mutacin producers. The frequency of a similar bacteriocinogenic pattern detected proved the occurrence of intrafamilial transmission of buccal streptococci.

The mother was not characterized as the sole source of infection, since similar bacteriocinogenic models were detected in the buccal cavity of the remaining members of the family group.

Bacteriocin typing (mutacin typing) is a reliable method of easy execution, although somewhat laborious, and is useful for the identification of strains of mutans streptococci.

References


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**Correspondence:** Dra. Rosa Vitória Azevedo, Departamento de Ciências da Saúde, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, 14040-903 Ribeirão Preto, SP, Brasil.

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