Lactose-Sensitive and -Insensitive Cell Surface Interactions of Oral
Streptococcus milleri Strains and Actinomyces

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Of 158 oral Streptococcus milleri strains, 46 exhibited cellular coaggregation with the reagent strains of the
actinomyces coaggregation groups A, B, and/or E. All but 1 of the 33 serotype b, e, FF and k/G strains
belonged to streptococcus coaggregation group 2, and only 14 strains of limited seroclasses (g, l, Lancefield
group F, or untypeable) appeared to be members of group 5, 3, or 4 (10, 3, and 1 strain, respectively). Thus,
S. milleri infrequently exhibits lactose-inhibitable coaggregation with actinomyces.

Intergeneric bacterial coaggregation is thought to be one of the critical factors in the colonization and accretion
of human plaque bacteria on tooth surfaces (for a review, see
Cisar et al. [1, 2] and Kolenbrander [9, 10]). Coaggregation
of oral streptococci, mostly Streptococcus sanguis with
actinomyces, has been studied extensively (3, 4, 6-8, 11-14).

Our previous studies demonstrated that large numbers of
Streptococcus milleri isolates from human mouths (15, 17)
coaggregate with cells of actinomyces coaggregation groups
A and B and that all belong to serotype b, e, or f/F (5). The
coaaggregation reactions of these S. milleri strains are not
inhibited by lactose and are mediated by streptococcal
adhesins and actinomyces carbohydrate receptors, suggesting
that these strains belong to streptococcus coaggregation
group 2. It was also shown that a few strains of the other
serotypes produce different patterns of coaggregation with
the actinomyces cells (5), but their coaggregation reactions
have not been characterized in detail to date. Recently, we
have isolated many strains of serotypes other than b, e, and
f/F from the mouths of children (19). By employing these
strains, the present studies confirm and extend the previous
findings regarding the coaggregation of S. milleri with acti-
nomycoses (5, 14) which is not inhibited by lactose. Certain
oral S. milleri strains exhibit coaggregation which is inhib-
ited by lactose.

A total of 158 S. milleri strains were used. Of these, 70 and
85 strains were isolates from the mouths of young adults (15, 17)
and children (16), respectively, and 3 were laboratory
strains (S. milleri ATCC 10708 [b] and FW73 [i] and Strept-
occoccus intermedius ATCC 9895 [f/F]). Actinomyces viscos-
sus T14V, Actinomyces naeslundii W9VU45, and A. viscosus
T14AV were used as the reagent strains of actinomyces
coaggregation groups A, B, and E, respectively.

The conditions of bacterial growth have been described
previously (5). Briefly, cells were cultured anaerobically in
Todd-Hewitt broth (BBL Microbiology Systems, Cockeys-
ville, Md.) at 37°C for 18 h, harvested by centrifugation
(10,000 × g, 10 min, 4°C), and washed three times. They
were then suspended in coaggregation buffer (5 mM potas-
sium phosphate buffer [pH 7.0] containing 0.1 mM CaCl2,
50 mM NaCl, and 0.5 mM sodium deoxycholate) to a density
of 2.0 at an A550. In some experiments, streptococcal and
actinomyces cells were heated separately at 80°C for 20
min or at 100°C for 3 min or were digested with trypsin
(1.0 mg/ml, final concentration) (Difco Laboratories, De-
troit, Mich.) at 37°C for 3 h at pH 7.0 in the coaggregation
buffer as described previously (5). They were then washed
and standardized in the coaggregation buffer as described
above.

Coaggregation was determined by using the visual assay
method in a microtiter plate containing 96 round-bottomed
wells. Equal volumes (25 μl) of the dense cell suspensions
of each cell type (streptococcus and actinomyces) were
placed in a well of the microtiter plate (Falcon; Becton
Dickinson Laboratories, Lincoln Park, N.J.). The plates
were shaken for 2 min at the maximum setting on a micro-
titer shaker (Dynatech Corp., Tokyo, Japan) and then al-
lowed to stand for 30 min at room temperature. The degree
of coaggregation was visually assigned a score from 0, for
no visible coaggregation, to +3, for maximum coaggregation.
A typical appearance of the coaggregation degrees is shown
in Fig. 1. To determine the effects of lactose and EDTA,
coaggregation was assayed in the coaggregation buffer sup-
plemented with 60 mM lactose (100 mM in some experi-
ments) or 10 mM disodium EDTA and incubated as de-
scribed above.

The coaggregation assay was repeated to confirm the
reproducibility of the data.

Of the 158 strains tested, 46 (29.1%) coaggregated with at
least one of the reagent strains of actinomyces coaggregation
groups A, B, and E (Table 1). The cell-cell interactions were
completely inhibited by the presence of 20 mM EDTA (data
not shown).

Of the 73 isolates from adults, 47.9% (35 strains) were
coaggregation positive, but only 12.9% (11 strains) of the 85
isolates from children were coaggregation positive. This may
be largely due to the fact that the strains of serotypes b, e,
f/F, and k/G, of which all strains except one were coaggre-
gative (see below), comprised 44.1 to 43.8% of the adult
isolates tested (17) but only 1.2% of the child isolates
examined (16). Thus, overall frequency of coaggregation
of the oral S. milleri with the actinomyces was 29.1% (Table 1),
which is not as high as the 61% frequency of other oral
streptococci, including S. sanguis (14).

All of the serotype b, e, and f/F strains (4, 2, and 24
strains, respectively), which have been shown to coaggre-
gate with the reagent strains of actinomyces coaggregation
groups A and B (5), were also reactive with group E reagent strain T14AV (Table 1). In addition, all except one of the serotype k/G strains (2 strains) coaggregated with all 3 actinomyces strains. The coaggregation reactions were lactose insensitive (data not shown), and heat or trypsin treatments abolished the coaggregation ability of the S. milleri cells but not that of the actinomyces cells (Table 2). These results have confirmed our earlier conclusions (5) and have shown that all S. milleri strains of serotypes b, e, f/F, and k/G, except one (k/G), are representative members of streptococcus coaggregation group 2 which possess adhesin(s) uninhibited by lactose on their cell surfaces.

Thus, so far as we have examined, most (69.6%) of the coaggregation between oral S. milleri and actinomyces was not inhibited by lactose. This is in marked contrast to the very frequent inhibition (92%) by lactose of coaggregation between other oral streptococci and actinomyces (14). S. milleri appears to be unique among oral streptococci with regard to intergeneric cellular coaggregation.

In contrast, of the 125 strains of the other serotypes, including untypeable strains, only 14 (3 serotype g, 5 i, 4 untypeable/Lancefield group F [designated −/F], and 2 untypeable/ungroupable −/−) showed reactivity with the actinomyces reagent strains (Tables 1 and 2). All the coaggregations were inhibited by 60 mM (100 mM in certain cases) lactose (data not shown).

The two g, four i, and four −/F strains coaggregated with the actinomyces group B reagent strain only (Table 2). Coaggregation factors on these streptococcal strains were heat and trypsin resistant. These findings indicate that the 10 serotype g, i, and −/F strains belong to coaggregation group 5.

The other g strain and the two −/− strains reacted with actinomyces group A and B cells but not with group E cells (Table 2). Cells of strain 1106 (−/−) retained their reactivity with both group A and group B actinomyces cells, even after heat and trypsin treatments. However, strain K214-1K (g) cells lost their reactivity after trypsin treatment, though not after heat treatment. Strain K213K (−/−) also became unreactive with actinomyces group A cells but not with group B cells after these treatments. In contrast, strain K119-1K (i) coaggregated with cells of actinomyces group E as well as with groups A and B (Table 2). However, its reactivity with cells of all the actinomyces reagent strains was inhibited by lactose. Heat or trypsin treatment of K119-1K cells did not affect their reactivity with group A and B cells at all but clearly did so with group E cells. Thus, although all strains except one (−/−) showed somewhat inconsistent reactivities after heat or trypsin treatment of paired cells and although the interactions of the i strain with group A and E cells were also inhibited by lactose atypically, these three g or −/− strains and the i strain appear to be members of groups 3 and 4, respectively.

The coaggregation factors on these S. milleri cells which mediated the lactose-sensitive interaction with actinomyces were generally like carbohydrates in nature (Table 2). Cells of most coaggregative strains of serotypes g, i, and −/F carried carbohydrate receptors which reacted only with actinomyces group B adhesins (group 5 factors). Strains 1106 (−/−) and K214-1K (g; determined on the basis of its heat susceptibility) possessed receptors for adhesins on cells of actinomyces groups A and B (group 3 factors). K214-1K (g) cells appeared, as suggested by their trypsin susceptibility, to possess adhesins for receptors on group A and B cells also. K213K (−/−) cells which also coaggregated with both

![FIG. 1. Visual assay for streptococcus-actinomyces coaggregation in the microtiter plate. Equal volumes of dense cell suspensions of each cell type were placed in a round-bottomed well and mixed for 2 min on a microtiter shaker. After the suspensions were allowed to stand for 30 min at room temperature, the degree of coaggregation was visually assigned a score from 0 to +3.](http://iai.asm.org/)

<table>
<thead>
<tr>
<th>Table 1. Coaggregation of S. milleri with actinomyces*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>S. milleri type and serotype</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Uninhibited by lactose</td>
</tr>
<tr>
<td>b</td>
</tr>
<tr>
<td>e</td>
</tr>
<tr>
<td>f/F</td>
</tr>
<tr>
<td>k/G</td>
</tr>
<tr>
<td>Inhibited by lactose</td>
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<tr>
<td>g</td>
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<tr>
<td>i</td>
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<tr>
<td>−/F</td>
</tr>
<tr>
<td>−/−</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

*None of the 36 strains of seroclasses a/A (1 strain), c/C (7 strains), d (3 strains), h (11 strains), j (3 strains), −/g− (4 strains), and −/g, j− (7 strains) coaggregated with any of the actinomyces reagent strains used.

*The designations +1, +2, and +3 are coaggregation scores (see text).
TABLE 2. Effects of heat and protease treatments on *S. milleri*-actinomyces coaggregation

<table>
<thead>
<tr>
<th><em>S. milleri</em> strain</th>
<th>Coaggregation score after heat or trypsin treatment of <em>S. milleri</em> and actinomyces group(^a):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (T14V)</td>
</tr>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>K110K (b)(^b)</td>
<td>+1</td>
</tr>
<tr>
<td>K226K (e)(^b)</td>
<td>+1</td>
</tr>
<tr>
<td>K99K (EB)(^b)</td>
<td>+2</td>
</tr>
<tr>
<td>K214-2K (k/G)</td>
<td>+2</td>
</tr>
<tr>
<td>K218K (k/G)</td>
<td>+2</td>
</tr>
<tr>
<td>1208-1 (g)</td>
<td>+2</td>
</tr>
<tr>
<td>1208-2 (g)</td>
<td>+2</td>
</tr>
<tr>
<td>1302-1 (−/F)</td>
<td>0</td>
</tr>
<tr>
<td>1302-2 (−/F)</td>
<td>+3</td>
</tr>
<tr>
<td>1308 (−/F)</td>
<td>+3</td>
</tr>
<tr>
<td>1311 (−/F)</td>
<td>+2</td>
</tr>
<tr>
<td>0401-1 (i)</td>
<td>+2</td>
</tr>
<tr>
<td>0403-1 (i)</td>
<td>+2</td>
</tr>
<tr>
<td>0919-2 (i)</td>
<td>+2</td>
</tr>
<tr>
<td>0905 (i)</td>
<td>+2</td>
</tr>
<tr>
<td>1106 (−/−)</td>
<td>+2</td>
</tr>
<tr>
<td>K214-1K (g)</td>
<td>+2</td>
</tr>
<tr>
<td>K213K (−/−)</td>
<td>+1</td>
</tr>
<tr>
<td>K119-2K (i)</td>
<td>+2</td>
</tr>
</tbody>
</table>

\(^a\) None, Neither streptococci nor actinomyces were treated; S, only streptococci were treated; A, only actinomyces were treated; SA, both streptococci and actinomyces were treated with heat (100°C for 3 min and/or 80°C for 20 min) or trypsin (1.0 mg/ml, 37°C, 3 h).

\(^b\) Basically similar results were obtained for all of the other 3 serotype b strains, the 1 e strain, and the 23 EF strains. See also Eifuku et al. (5).

actinomyces reagent strains carried receptors for lectins on group B cells and lectins for receptors on group A cells (group 3 variant factors). K119-2K (i) cells possessed receptors for group A and B cells in addition to lectins for group E cells (group 4 variant factors).

Conversely, these findings indicate that the reagent strains of actinomyces groups A, B, and E possessed carbohydrate receptors for the lactose-sensitive lectins on some of the group 3 and 4 *S. milleri* strains. Presence of the carbohydrate receptors for lactose-sensitive streptococcal adhesins on these actinomyces cells has not been reported in previous studies (see reference 10).

From the assay in the microtiter plates, the majority (70.9%) of 158 oral *S. milleri* strains, including all 36 strains of serotypes a/A, c/C, d, h, j, untypable/g—cross-reactive (designated −/g−), and untypeable/g—and j—cross-reactive (−/gj−), did not exhibit coaggregation with the reagent strains of actinomyces groups A, B, and E (Table 1). When the coaggregation pattern between actinomyces and streptococci described by Kolenbrander and colleagues (3, 11, 13, 14) is applied indiscriminarily, the findings only indicate the possibility that some of these *S. milleri* strains may possess the ability to coaggregate with cells of actinomyces coaggregation group D and may belong to streptococcus coaggregation group 6. This possibility needs to be examined in further studies.

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**REFERENCES**


