Coaggregation of black-pigmented *Bacteroides* species with other oral bacteria

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Summary. Coaggregation of *Bacteroides gingivalis* and other black-pigmented bacteroides with several oral bacteria was studied with “reagent” strains specially prepared by methods that have been described previously. *B. gingivalis* coaggregated with *Veillonella*, *Capnocytophaga* and *Actinomyces* spp., but not with any *Streptococcus* spp. Coaggregation of *B. gingivalis* with other bacteria was inhibited and reversed by lactose. Of the asaccharolytic black-pigmented bacteroides, only *B. gingivalis* demonstrated any coaggregation with other bacteria, whereas within the saccharolytic species, *B. loescheii* showed a marked ability to coaggregate with several species of oral bacteria. This property of coaggregation by *B. gingivalis* may be an important factor in the pathogenesis of periodontal infections.

Introduction

The propensity of pathogenic bacteria to retain themselves in the oral cavity is an important prerequisite for initiating infection. This is usually achieved by direct attachment to the oral mucosa or to other bacteria already attached to the mucosa; thus inter-cellular adherence amongst oral bacterial species is an important factor in the pathogenesis of oral infections. Groups of bacteria of different species resulting from such associations may also derive mutual nutritional benefits and this is known to be important in the pathogenesis of experimental synergic anaerobic infections (Mayrand and McBride, 1980; Van Steenberg *et al.*, 1982; Van Winkelhoff *et al.*, 1983).

Oral pathogens such as *Streptococcus* and *Actinomyces* spp. are known to elaborate *in vitro* a network of highly specific cell-to-cell interactions in heterogeneous aggregates (Cisar *et al.*, 1979; Kolenbrander and Williams, 1981, 1983; Kolenbrander *et al.*, 1983).

Selective coaggregation among oral bacteria allows them to adhere and subsequently colonise surfaces. There is evidence which implicates *Bacteroides gingivalis*, a black pigmented, asaccharolytic, oral anaerobe, in the pathogenesis of adult periodontal infections (Slots, 1982), and coaggregation may be an important process in the patho-

genesis of periodontal disease. This study was designed to investigate the coaggregation of black-pigmented *Bacteroides* spp., especially *B. gingivalis*, with other oral bacteria *in vitro*.

Materials and methods

Bacterial strains

The following oral bacterial species including clinical and reference strains of oral *Bacteroides* spp. were used: *B. gingivalis* ATCC 33277, *B. asaccharolyticus* ATCC 25260, *B. endodontalis* ATCC 35406, *B. melaninogenicus* ATCC 33184, *B. intermedius* ATCC 33563, *B. corporis* ATCC 33547, *B. denticola* ATCC 33185 and *B. loescheii* ATCC 15930; clinical isolates of *B. gingivalis*, strains B5, C3, C7, C8, C9, and C43, and *B. asaccharolyticus*, strains BA12 and BA2, were kindly supplied by Dr F. Martin, Maryland University Dental School, Baltimore, MD. Other bacteria used were strains of *S. sanguis*, *S. morbillorum*, *S. mutans*, *Capnocytophaga* spp., *Fusobacterium* spp., *Veillonella* spp., *Actinomyces* spp., *Rothia denticariosa* and *Lactobacillus casei*. These were kindly supplied by Dr P. E. Kolenbrander, National Institute of Dental Research, Bethesda, MD. The black-pigmented *Bacteroides* spp. were maintained on pre-reduced Brain Heart Infusion Agar (Scott Laboratories Inc.) with supplement (BHIS) in an anaerobic chamber at 37°C.

Preparation of “reagent” strains

All strains were prepared as “reagent” strains by the method described by Kolenbrander *et al.* (1985). Briefly, bacterial strains were grown initially in suitable broths

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and harvested by centrifugation at 3000 *g* for 10 min during the logarithmic growth phase. Harvested cells were washed twice in phosphate-buffered saline (PBS, pH 7.2) and suspended in coaggregation buffer (sodium azide 0.02%, 0.15 M NaCl, 0.001 M CaCl₂, 0.0001 M MgCl₂ and 0.001 M Tris, pH 8.0).

Coaggregation assay

The coaggregation assay was performed according to the method of Kolenbrander *et al.* (1985). The turbidity of the harvested bacterial cells, suspended in coaggregation buffer, was adjusted in a photometer to an OD value (at 260 nm) which gave $c. 5 \times 10^9$ cells/ml. Each cell suspension was homogenised by shaking on a vortex mixer for 20 s. Equal volume of suspension (300 μ l) of the black-pigmented *Bacteroides* strains and the other test strains were mixed in labelled 3" $\times$ 1 $\frac{1}{4}$ " tubes and homogenised on a vortex mixer, after which the mixture was allowed to stand for 5 min. Two strains of *B. gingivalis* was similarly mixed and observed as above (as control) for auto-coaggregation. Coaggregation was assessed visually by gently rocking the tube against a dark background. Mixtures of bacterial species that showed no coaggregation were given a score of 0 on an arbitrary scale of 0–4. In such tubes a homogeneous suspension was still visible. A score of 4 was given when large aggregates were formed which settled at the bottom of the tube leaving a clear supernate. Scores of 1–3 represented various degrees of coaggregation observed in the tubes.

Lactose inhibition assay

Reversal or inhibition of aggregation between any two of the bacterial species was assessed by adding 300 μ l of 0.06 M lactose solution to tubes showing coaggregation, and vortex mixing for 5 s. Coaggregation was scored as described above.

Results

The extent of coaggregation between the asaccharolytic black-pigmented *Bacteroides* spp. and other oral bacterial species is shown in tables I and II. Strains of *B. gingivalis* coaggregated strongly with the *Veillonella* spp., *C. ochracea*, *C. gingivalis*, *A. viscosus*, *A. naeslundii* strains 19, TR 9 and RC 29, *A. wva* strain 963, *A. israelii* ATCC 12103 and *R. denticulariosa*, and weakly with *F. nucleatum* and some strains of *A. israelii* but not with streptococcal species. *B. asaccharolyticus* did not coaggregate with any of the oral species. *B. endodontalis* coaggregated in only one mixture—with *A. israelii* ATCC 12103. Coaggregation between *B. gingivalis* and other oral bacterial species was completely reversed or inhibited in the presence of 0.06 M lactose. This was a consistent phenomenon which

was also observed with *B. endodontalis* and *A. israelii* ATCC 12103.

Coaggregation of asaccharolytic and saccharolytic black-pigmented *Bacteroides* spp. with a selection of other oral bacterial species was compared (table II). There was no coaggregation between serotypes of *S. mutans* and *B. gingivalis* or *B. melaninogenicus*, but *B. corporis* and *B. intermedius* did so (scores 1–4) with *S. mutans* serotypes A and D, *B. denticola* with serotype D, and *B. loescheii* and *B. asaccharolyticus* with serotypes A, B, C and D. *B. loescheii* coaggregated consistently with all the other strains tested—*S. sanguis*, *S. mitis*, *L. casei*, *Actinomyces* spp. and *C. ochracea*. There was no auto-coaggregation between two strains of *B. gingivalis*.

Discussion

The results of this study demonstrated that *B. gingivalis* has a non-random specific affinity for coaggregation with oral bacterial species that include *Veillonella*, *Capnocytophaga*, *Actinomyces* and *Rothia* spp. Of note was the observation that *B. gingivalis* was the only member of the asaccharolytic black-pigmented *Bacteroides* group that coaggregated with several oral species, including those implicated in oro-dental infections. *B. asaccharolyticus* also coaggregated with some strains of *S. mutans* only. Among the saccharolytic *Bacteroides* spp. tested, *B. loescheii* was notable in its ability to coaggregate with several oral bacterial species.

Coaggregation between *B. gingivalis* and other species could be reversed by adding lactose, which suggests the involvement of a carbohydrate binding adhesin on the surface receptors of *B. gingivalis* (Cisar, 1982). In a previous similar study (Kolenbrander *et al.*, 1985), the single strain of *B. gingivalis* used did not coaggregate with *Actinomyces*, *Streptococcus* or *Capnocytophaga* spp. However, we found that *B. gingivalis* coaggregated with *Actinomyces* and *Capnocytophaga* spp., but not with *Streptococcus* spp. Our results with *B. asaccharolyticus* were in agreement with those of Kolenbrander *et al.* (1985). In our study, the ability of *B. loescheii*, from amongst the saccharolytic group of black-pigmented bacteroides, to coaggregate with a wide variety of oral bacterial species supports the findings of Kolenbrander *et al.* (1985).

Some oral bacterial species included in this study, e.g., *Streptococcus*, *Veillonella* and *Actinomyces* spp., colonise the oral cavity at an early stage, and the attachment of *B. gingivalis* to some of these early colonisers may be responsible for its retention in

Table I. Coaggregation of asaccharolytic black-pigmented bacteroides with other oral bacteria

Oral bacterial species	Coaggregation score with <i>Bacteroides</i> strains								
	C5	C43	C3	C7	C8	C9	BA12	BA	BE
<i>V. parvula</i> PK 1914	4	4	4	4	4	4	0	0	1
<i>V. atypica</i> PK 1911	4	4	4	4	4	4	0	0	0
<i>C. gingivalis</i> PC1000	0	0	0	0	0	0	0	0	0
<i>C. gingivalis</i> 27	2	3	4	4	4	4	0	0	0
<i>C. sputigena</i> 4	1	2	3	3	3	3	0	0	0
<i>C. ochracea</i> 25	3	4	4	4	4	4	0	0	0
<i>F. nucleatum</i> PK1904	0	0	2	1	2	1	0	0	0
<i>F. nucleatum</i> PK1909	4	1	2	2	2	2	0	0	0
<i>S. sanguis</i> PK488	0	0	0	0	0	0	0	0	0
<i>S. sanguis</i> J22	0	0	0	0	0	0	0	0	0
<i>S. sanguis</i> SS34	0	0	0	0	0	0	0	0	0
<i>S. sanguis</i> H1	0	0	0	0	0	0	0	0	0
<i>S. sanguis</i> DL1	0	0	0	0	0	0	0	0	0
<i>S. morbillorum</i> SB86	0	0	0	0	0	0	0	0	0
<i>A. viscosus</i> 35	3	4	4	4	4	4	0	0	0
<i>A. naeslundii</i> 19	3	4	4	4	4	4	0	0	0
<i>A. naeslundii</i> TR9	3	4	4	4	4	4	0	0	0
<i>A. naeslundii</i> RC29	2	3	4	4	4	4	0	0	0
<i>A. naeslundii</i> TR60	0	0	0	0	0	0	0	0	0
<i>A. naeslundii</i> PK2056	0	2	2	3	3	2	0	0	0
<i>A. wva</i> 963	2	3	3	3	3	3	0	0	0
<i>A. israelii</i> PK13	2	2	2	2	2	2	0	0	0
<i>A. israelii</i> PK14	0	2	2	2	2	2	0	0	0
<i>A. israelii</i> PK16	0	2	2	2	2	2	0	0	0
<i>A. israelii</i> ATCC12103	4	4	4	4	4	4	0	0	0
<i>R. dentocariosa</i> PK44	0	2	4	4	4	3	0	0	0

C = *B. gingivalis* strains; BE = *B. endodontalis*; BA = *B. asaccharolyticus* ATCC25611; BA12 = *B. asaccharolyticus* strain 12.

Table II. Coaggregation between black-pigmented *Bacteroides* spp. and selected oral bacteria

Test strains of oral species	Coaggregation score with						
	BG	BA	BM	BL	BC	BD	BI
<i>S. mutans</i> serotype A	0	4	0	3	1	0	3
<i>S. mutans</i> serotype B	0	4	0	4	0	0	0
<i>S. mutans</i> serotype C	0	1	0	3	0	0	0
<i>S. mutans</i> serotype D	0	4	0	2	4	1	4
<i>S. sanguis</i> ATCC 10556	0	0	0	2	0	0	0
<i>S. sanguis</i> ATCC 10557	0	0	0	1	0	0	0
<i>S. sanguis</i> 167	0	0	0	1	0	0	0
<i>S. mitis</i> 903	0	0	0	1	0	0	0
<i>L. casei</i> 393	1	0	0	4	0	0	0
<i>L. casei</i> 64H	0	0	0	4	0	0	0
<i>L. casei</i> ATCC 4646	0	0	0	4	0	0	0
<i>A. viscosus</i> T14 AV	2	0	0	2	0	0	0
<i>A. viscosus</i> T14 V	2	0	0	4	0	0	0
<i>C. ochracea</i>	3	0	0	3	0	0	0

BG = *B. gingivalis*; BA = *B. asaccharolyticus*; BM = *B. melaninogenicus*; BL = *B. loescheii*; BC = *B. corporis*; BD = *B. denticola*; BI = *B. intermedius*.

the oral cavity. It has been suggested that *B. loeschei* may act as a bridge in coaggregating multiple bacterial species (Kolenbrander *et al.*, 1985). The specific importance of coaggregation *in vivo* may be related to the enhancement of pathogenicity by adherence and the aggregation of synergically

compatible oral bacterial species. This may also partly explain the polymicrobial nature of many oro-dental infections.

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