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Coaggregation Reaction between *Rothia dentocariosa* and *Streptococcus oralis*

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Summary

In the course of studies on assessment of coaggregation among oral indigenous bacteria, *Rothia dentocariosa* was found to coaggregate *Streptococcus oralis* and *Streptococcus mitis*. One heat-labile and one -stable binding sites on the bacterial surfaces may be responsible for coaggregation. The binding sites of *S. oralis* are destroyed by protease treatment. No inhibition of coaggregation activities was demonstrated in the presence of serum, saliva, sugars, and amino acids, however, EDTA inhibited the activities completely.

Introduction

Rothia dentocariosa is facultative anaerobic, gram-positive, and polymorphic rod. Even though it frequently is isolated from dental plaque and discussed its pathogenicity of periodontal disease and cariogenicity of dentin, very little is known about etiological properties of *R. dentocariosa*. We have demonstrated that this species elaborated trypsin-like proteinase (unpublished observations), which may function as the pathogenic factor of this microorganism in the oral cavity. Bacterial coaggregation is defined as the formation of a complex consists of different species. It is generally accepted that coaggregation is important to the initial development and the establishment of a pathogenic subgingival plaque[1-4]. The interactions in the coaggregation are highly specific, only limited species are able to be partners. Although mechanism of cell-to-cell binding is still controversial, surface components such as carbohydrates, fimbriae, and outer membrane proteins are thought to contribute to bacterial coaggregation[5]. We discuss in this report coaggregation between *Rothia dentocariosa* and *Streptococcus oralis*.

Materials and Methods

The cells were harvested by centrifugation at 10,000 xg for 10 min, washed with 50 mM phosphate buffer (pH 7.2) containing 150 mM NaCl (PBS), and finally suspended in coaggregation buffer[6], consisted of 1 mM Tris-HCl buffer (pH 8.0), 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 150 mM NaCl, and 0.02% NaN_3. Bacterial cells in these suspensions were confirmed not to aggregate spontaneously them-

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selves. Bacterial suspension and partner suspension were mixed (total 5 ml) and incubated at 37℃ for 1 h. Coaggregation was observed with the naked eye ; scored according to a visual rating scale of 0 through 2+ : 0, no visible aggregates in the cell suspension ; 1+, coaggregation with turbid supernatant ; 2+, coaggregation with clear supernatant (Fig. 1). Effects of heat treatment and protease treatment of bacterial cells on coaggregation were assessed as follows ; Cell suspension of *R. dentocariosa* and *Streptococcus oralis* ATCC 10557 were heated at 100℃ for 10 min and subjected to the coaggregation tests. Samples of these cell suspensions were also incubated at 37℃ for 60 min with trypsin, papain, and proteinase K separately at a concentration of 2 mg/ml in PBS. After protease treatment the cells were washed twice with the coaggregation buffer and the coaggregation assay was then carried out as described above.

Examinations of the effects of serum, saliva, sugars, amino acids, and EDTA on the coaggregation reaction between *R. dentocariosa* and *S. oralis* were carried out by the addition of each material to the reaction mixtures followed by the incubation.

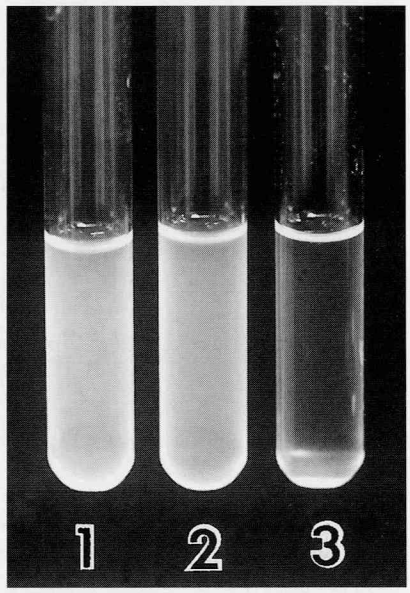

Fig 1 : The visual assay for coaggregation.

Tube 1 ; Cell suspension of *R. dentocariosa* No. 4 in coaggregation buffer. Tube 2 ; Cell suspension of *S. oralis* ATCC 10557 in coaggregation buffer. Tube 3 ; After mixing of half volume of the cell suspensions of tube 1 and 2 followed by incubation at 37° for 1 h.
Scores of aggregation of each tube are 0 (tube 1 and 2) and 2+ (tube 3).

Results and Discussion

Coaggregation of *R. dentocariosa* with many species of oral indigenous bacteria is demonstrated in Table 1. Two strains of *R. dentocariosa* (No. 4 and No. 8) exerted coaggregation only with *S. oralis* and *S. mitis*, partners of the coaggregationof *R. dentocariosa* are quite strictly limited. Therefore, the subsequent examinations to determine the properties of coaggregation of *R. dentocariosa* were performed using *S. oralis* as a partner species.

Effect of heat treatment of cells on the coaggregation is quite clear. Between *R. dentocariosa* and *S. oralis*, coaggregation occurred, if either of the two was not heated, even though the partner was heated (Table 2). Then the following hypothesis concerning this event may be possible ; two pairs of binding sites responsible for coaggregation, one heat–labile and one heat–stable, exist on both bacterial surfaces. However, if both species are heated, coaggregation is impossible, because the binding sites of the two species are destroyed. In the case of heating only one species, coaggregation takes place since heat–stable binding sites endure. The similar observations were reported also in the coaggregation reaction between *Porphyromonas gingivalis* and *Treponema denticola*[7].

Effect of protease treatment on the coaggregation is summarized in Table 3. The binding activity of *R. dentocariosa* was not reduced after treatment with trypsin, papain, and proteinase K, indicating components of the binding sites of this species are not proteinaceous. Whereas, trypsin–or papain–treated *S. oralis* could coaggregate with *R. dentocariosa* no longer and proteinase K significantly abrogated the binding activity.

Finally, effects of body fluids (serum and saliva), sugars, amino acids, and a chelator (EDTA) on

Table 1 : Coaggregation reaction among *Rothia dentocariosa* and oral bacterial species

Bacterial species	*Rothia dentocariosa* No. 4	No. 7
Actinomyces viscosus ATCC 15987	0	0
Actinomyces viscosus T 14 V	0	0
Actinomyces naeslundii ATCC 12104	0	0
Corynebacterium matruchotii ATCC 14266	0	0
Streptococcus mutans Ingbritt	0	0
Streptococcus sanguis ATCC 10556	0	0
Streptococcus oralis ATCC 10557	2+	2+
Streptococcus mitis ATCC 9811	2+	2+
Streptococcus salivarius ATCC 9759	0	0
Propionibacterium acnes ATCC 6919	0	0
Capnocytophaga gingivalis ATCC 33624	0	0
Capnocytophaga sputigena ATCC 33612	0	0
Fusobacterium nucleatum MD 6	0	0
Actinobacillus actinomycetemcomitans ATCC 29523	0	0
Porphyromonas gingivalis ATCC 33277	0	0
Porphyromonas gingivalis FDC 381	0	0
Prevotella intermedia ATCC 25611	0	0
Prevotella heparinolyticus ATCC 35895	0	0

Table 2 : Effect of heat treatment on the coaggregation reaction between *R. dentocariosa* and *S. oralis*

Bacteria		
R. dentocariosa No. 4	*S. oralis* ATCC 10552	Coaggregation
unheated	unheated	2+
unheated	heated	0
heated	unheated	2+
heated	heated	0

Table 3 : Effect of protease treatment of the bacterial cells on the coaggregation

Enzyme	Enzyme-treated *R. dentocariosa* No. 4 + Untreated *S. oralis* ATCC 10557	Enzyme-treated *S. oralis* ATCC 10557 + Untreated *R. dentocariosa* No. 4
none	2+	2+
trypsin	2+	0
papain	2+	0
proteinase K	2+	1+

Table 4 : Effect of treatments of *R. dentocariosa* No. 4 and *S. oralis* ATCC 10557 by serum, saliva, sugars, amino acids, and EDTA on the coaggregation reaction

	Coaggregation
none	2+
human serum (10%)	2+
human saliva (10%)	2+
human saliva (20%)	2+
sugars (100 mM)	
mannose	2+
galactose	2+
raffinose	2+
lactose	2+
milibose	2+
amino acids (100 mM)	
L-arginine	2+
L-alanine	2+
L-leucine	2+
L-lysine	2+
EDTA (10 mM)	0
EDTA (50 mM)	0

the coaggregation were examined (Table 4). Serum, two different concentration of saliva, sugars and amino acids tested exhibited no effect. Some bacterial coaggregation and hemagglutination were found to be inhibited by basic amino acids, arginine and/or lysine[8-12], however, no inhibition was observed in the coaggregation between *R. dentocariosa* and *S. oralis*. The reason of these findings is still obscure, despite treatment by trypsin and papain to *S. oralis* caused apparent loss of aggregation activity.

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抄録：*Rothia dentocariosa* と *Streptococcus oralis* との共凝集反応

中村　武，藤村節夫　（松本歯大・口腔細菌）

口腔常在菌間での共凝集を調べる過程で *Rothia dentocariosa* が *Streptococcus oralis* および *Streptococcus mitis* と共凝集反応をおこすことが分かった．細菌表層の凝集部位は熱に対して安定なものと不安定のものの2種類が存在すると考えられる．*S. oralis* の凝集部位はプロテアーゼ処理で破壊される．血清，唾液，糖，アミノ酸では共凝集活性は阻害されないが，EDTA では完全に阻害される．