Studies on the lytic enzyme from *Streptococcus mutans*

VI The correlation between the lytic ability and bacteriocin activity against *Streptococcus sanguis*, and artificial plaque forming ability of oral bacteria *Streptococcus mutans*

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Abstract : Lytic ability and bacteriocin activity against *Streptococcus* (*S.*) *sanguis* ATCC 10556, 10557 and 10558 and artificial plaque forming ability by 119 strains of *S. mutans* were examined. Previously we have shown that 104 strains were serotype c/e/f and 15 were d/g.

Serotype c/e/f strains showed a fairly good lytic ability against *S. sanguis* ATCC 10558, but not against ATCC 10556 and 10557. They showed a higher bacteriocin activity against *S. sanguis* ATCC 10556 and 10557 than against ATCC 10558. In serotype d/g strains, a slight lytic ability against *S. sanguis* ATCC 10558 but not two others was seen, and an extremely slight bacteriocin activity against all *S. sanguis* strains was noted. The artificial plaque forming ability by d/g type strains was clearly higher than c/e/f type strains. These facts seem to show that these factors from *S. mutans* compensate for the defects of each other. From these results, it is probable that not only bacteriocin activity, but the lytic ability and artificial plaque forming ability of *S. mutans* also take part in the decrease of *S. sanguis* in the dental plaque.

Introduction

In the dental plaque on sound tooth surface, *Streptococcus* (*S.*) *sanguis* is predominant in the streptococcal flora, but in the dental plaque on the tooth where the onset of dental caries is seen, or in the presence of a carious lesion, *S. mutans* shows a marked increase while *S. sanguis* decreases¹⁻⁸⁾. As antagonistic agents from *S. mutans* against *S. sanguis*, bacteriocin or bacteriocin-like substances are known⁹⁻¹²⁾. We have reported that *S. mutans* strains freshly isolated from the dental plaque of an infant lysed heated cells of *S. sanguis* ATCC 10558 strain⁸⁾.

The present report describes the correlation between the lytic ability and bacteriocin activity against *S. sanguis* strains, and the

artificial plaque forming ability of *S. mutans* strains isolated from the dental plaques of infants.

Materials and Methods

Organisms

S. sanguis ATCC 10556 (serotype : I), 10557 (serotype : II) and 10558 (serotype : III) strains were kindly donated by Prof. K. Kaneko, Iwate Medical University, Iwate, Japan. One hundred and nineteen strains of *S. mutans* (Table 1) were freshly isolated from the dental plaques of 20 Japanese infants¹⁷⁾.

Lytic ability assay

Stab culture method was used for the measurement of the lytic abilities of *S. mutans* against *S. sanguis*. Cells of 48 hr-culture on Mitis-Salivarius agar (MS agar, Difco)

plates were stab inoculated TSL agar plate¹⁷⁾ containing heat-killed cells of *S. sanguis*, and incubated at 37 °C for 5 days at an atmosphere of 95 % nitrogen and 5 % carbon dioxide. The TSL agar plate consisted of Trypticase soy broth (BBL): 21 g, Heart infusion broth (Difco): 3 g, yeast extract (Difco): 3 g, L-cysteine · HCl: 0.3 g, sodium thioglycollate: 0.3 g, hemin: 5 mg, menadione: 0.5 mg, K₂HPO₄: 2.5 g, agar: 17 g, deionized water: 1,000 ml, and heat-killed cells of *S. sanguis* strains: 10–15 g (wet weight), and was adjusted to pH 7.3.

Lytic abilities were determined by the widths of the lytic zones formed around the colonies of stab strains.

Bacteriocin activity assay

In a modification of the methods of Weerkamp *et al.*¹¹⁾, cells of 48 hr-culture on MS agar plates were stab inoculated into Trypticase soy agar plate containing 0.5 % yeast extract (TS-Y agar plate), and incubated at 37 °C for 48 hr anaerobically. The plates were then exposed to chloroform vapor 30 min and aired. After this, over-night cultures of indicator strain, diluted ten-fold into TS soft agar (0.5 % agar), were sprayed over the plates, which were incubated for another 24 hr.

Bacteriocin activities were measured by the widths of clear inhibition zones appearing around the colonies of producer strains.

Artificial plaque forming ability

A modification of the methods of Fitzgerald *et al.*¹⁸⁾ was used as the test system. Sun cobalt clasp wires (diameter: 1.2 mm, Sankin Industry Co., Ltd) were inserted in rubber stoppers and suspended in tubes containing 0.2 ml of an over-night culture of the test organism and 10 ml of the TYS medium, and incubated at 37 °C for 24 hr anaerobically. The TYS medium composed of Trypticase peptone (BBL): 17 g, yeast extract: 5 g, K₂HPO₄: 5 g, sucrose: 100 g and deionized water: 1,000 ml, and was adjusted to pH 7.3.

Each day a tube of fresh medium (10 ml) was incubated with 0.2 ml of an over-night culture of the test organisms and the wires were transferred to it. After 8 days, the artificial plaques on the wires were dried

Table 1 Lytic ability and bacteriocin activity against *S. sanguis* ATCC 10556, 10557 and 10558 strains and artificial plaque forming ability of *S. mutans* isolated from the dental plaques of 20 infants

<i>S. mutans</i> serotype# No of strain	<i>S. sanguis</i> strain	Lytic ability†					Bacteriocin activity†					Artificial plaque forming ability			
		+ 4	+ 3	+ 2	+ 1	±	ave.	+ 6	+ 5	+ 4	+ 3	+ 2	+ 1	±	ave.
c / e / f 104	ATCC 10556	—	—	—	—	—	104	15	1	9	29	30	20	—	3.15
	ATCC 10557	—	—	—	—	—	104	18	11	12	13	31	19	—	3.24
	ATCC 10558	3	36	30	29	6	—	2.15	—	5	25	41	34	—	2.00
d / g 15	ATCC 10556	—	—	—	—	—	15	—	—	—	—	—	—	15	0.7
	ATCC 10557	—	—	—	—	—	15	—	—	—	—	—	—	15	0.7
	ATCC 10558	—	—	—	—	15	—	0.8	—	—	—	—	—	15	0.6
18.5±11.2§															
67.2±22.6															

: with reference to the report described by Hamada & Slade (1980)

† : Widths of lytic or clear inhibition zones; — : no zone, ± : < 1 mm, 1 mm ≤ + < 2 mm, 2 mm ≤ + < 3 mm, 3 mm ≤ + < 4 mm, 4 mm ≤ + < 5 mm, 5 mm ≤ + < 6 mm, 6 mm ≤ + 6

§ : Mean and standard deviation

ave. : Average ** : p < 0.01

and the weight were determined.

Results and Discussion

In Table 1, lytic ability and bacteriocin activity against *S. sanguis* ATCC 10556, 10557 and 10558 strains, and artificial plaque forming ability of 119 strains of *S. mutans* isolated from 20 infants were summarized. Previously we have shown that 104 strains from 19 infants were serotype c/e/f group and 15 strains from one infant were d/g with reference to the report described by Hamada & Slade¹⁹⁾ on the basis of their biological characteristics¹⁷⁾.

Based on the biotype, *S. sanguis* ATCC 10556 strain is similar to ATCC 10558 strain²⁰⁾. But on the DNA base sequence, ATCC 10556 strain belongs to genetic group 3, while ATCC 10558 strain belongs to group 1²¹⁾. Rosan²²⁻²⁴⁾ showed that ATCC 10558 strain had a and b antigens in the cell walls, but ATCC 10556 strain had only a antigen on the immunoelectrophoretic test. Moreover, it was reported that *S. sanguis* ATCC 10557 strain did not possess lipoteichoic acids in the cell walls while ATCC 10556 strain did²⁵⁾. Thus, the structural materials of cell walls differed among these three strains. This seems to be because only *S. sanguis* ATCC 10558 strain was lysed by *S. mutans* strains, but the other two ATCC strains were not sensitive to them. Furthermore, a large difference between serotype c/e/f and d/g groups was observed in lysis of *S. sanguis* ATCC 10558 strain by *S. mutans* strains ($p < 0.01$).

On the other hand, it is described that

bacteriocins (or mutacins) produced from *S. mutans* are present in molecular weight from low M.W. : 1,000 to 3,500¹²⁾ to high (M.W. : 973,00)²⁶⁾, and hinder the growth of numerous gram positive organisms including *S. sanguis*⁹⁻¹²⁾. In this experiment using the stab culture method, all 119 strains of *S. mutans* showed bacteriocin activities against three ATCC strains of *S. sanguis* (Table 1). However, the activities of serotype c/e/f strains were stronger than those of serotype d/g strains. And serotype c/e/f strains showed stronger bacteriocin activities against *S. sanguis* ATCC 10556 and 10557 strains which were not lysed by all *S. mutans* strains, but weaker activities against ATCC 10558 strain which was fairly lysed ($p < 0.01$).

Since dental plaque contains large quantities of microorganisms, their accumulation results in the lowering of oxidation-reduction potential by the growth of oxygen dependent organisms and lowering of pH by the growth of acidogenic bacteria. It is suggested that these lowerings of oxidation-reduction potential^{13,14)} and of pH^{15,16)} are favorable to growth of *S. mutans* in opposition to the reduction of metabolic activities of *S. sanguis*. In this study, the artificial plaque forming ability of *S. mutans* was clearly higher in serotype d/g strains with showed very weak lytic ability and bacteriocin activity, than in serotype c/e/f strains ($p < 0.01$).

These results seem to show that the main inhibiting factors of serotype c/e/f strains to the growth of *S. sanguis* strains are the bacteriocin activity and lytic ability, and that of serotype d/g strains is the artificial plaque forming ability.

抄録: *Streptococcus (S.) mutans* の *S. sanguis* に対する溶菌作用活性, bacteriocin 活性および人工歯垢形成能を測定し, これらの因子間の関連性について検討した。*S. mutans* は20名の幼児の歯垢から分離した104株 (serotype c/e/f: 104株と d/g: 15株) を用いた。c/e/f 型の *S. mutans* は, *S. sanguis* ATCC 10558 株に対して良好な溶菌作用を示したが, ATCC 10556 株と 10557 株に対してはまったく溶菌作用を示さなかった。また, これらの *S. mutans* 株は, *S. sanguis* ATCC 10556 と 10557 の2株に対して良好な bacteriocin 活性を示したが, ATCC 10558 株に対しては比較的弱い活性を示した。また, *S. mutans* の菌株の違いによってもその強さは異なった。

一方, d/g 型の *S. mutans* は *S. sanguis* ATCC 10558 株に対してのみ, 弱い溶菌活性を示し, さらに bacteriocin 活性もいずれの *S. sanguis* 株に対しても非常に弱い活性しか示さなかった。しかしながら, 人工歯垢形成能は, c/e/f 型の菌株に比べて明らかに高い形成能を示した。これらのことから, こ

これらの3因子は *S. sanguis* の増殖の抑制という点で互いに相補的關係を示す傾向があることがわかった。

以上の結果から、生体中においても *S. mutans* が *S. sanguis* に対して溶菌作用を示すものとするれば、溶菌作用や人工菌垢形成能もまた、bacteriocin 活性とともに菌垢中の *S. sanguis* の減少に少なからず関与する可能性があることが示唆された。

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