SHORT COMMUNICATION

Variation of phospholipid and fatty acid composition in *Streptococcus mutans*

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Introduction

Extensive investigations on *Streptococcus mutans* (*S. mutans*) as the etiological agent of human dental caries have been done during the past 20 years. Although *S. mutans* is a well defined species among oral streptococci and phenotypically homogenous, it had not been given an independent species position for a long time and even now, there are arguments against its taxonomic characterization. This is due to the heterogeneity in *S. mutans* when surveyed biologically, serologically, genetically and enzymatically. Pearch *et al.*¹⁾ revealed the presence of seven serotypes, and Coykendall²⁾ showed four genetic groups based on DNA base composition and DNA base sequence similarities of *S. mutans* strains.

It has been recently recognized that the bacterial lipid component and composition are effective indicators for bacterial chemotaxonomy, and are implicated in biological activities of bacteria³⁻⁵⁾. The authors⁶⁾ also reported that fatty acid composition of bacterial lipids were characteristic for individual oral streptococci.

The present paper describes the variation of phospholipids and their fatty acid composition in typical strains of *S. mutans*.

Materials and Methods

S. mutans : E-49 (serotype a), BHT (b), Ingbritt (c), OMZ-176 (d), LM-7 (e), OMZ-175 (f), and 6715 (g) were used. They were

incubated at 37 °C in Brain Heart Infusion (Difco) broth under anaerobic conditions. The cells were harvested, at a late logarithmic phase based on an individual growth curve, by centrifugation and washed twice with sterile saline. They were broken by sonication, and then lyophilized.

Lipids were extracted from lyophilized samples with a mixture of chloroform and methanol (2 : 1, v/v) according to the method of Bligh and Dyer⁷⁾. The extrated lipids were separated on a Silica-Gel 60 thin-layer chromatographic plate (Merck) with two-dimensional chromatography. The first solvent was chloroform/methanol/ammonia (65 : 35 : 8, v/v) and the second, chloroform/methanol/acetic acid/water (80 : 20 : 12 : 5, v/v). After development, the spots corresponding to phospholipids were detected with Dittmer's reagent. Phospholipids were identified by comparing their R_f values with standard ones (Serdary Research Laboratories). The spots were revealed again by coloration with iodine, and then scraped. Phospholipids were extracted from the scraped silica gel with 1.0 ml of a mixture of chloroform and methanol (6 : 1, v/v) twice. Fatty acids in phospholipids and total lipids (not treated by thin-layer chromatography) were methylated and their methyl esters were extracted with 3.0 ml of n-hexane. The esters were stored at -80 °C until gas chromatographic analysis. The solvent was evaporated to dryness, and then the residue was redissolved with 20 µl of n-hexane. An aliquot of the resulting so-

Table 1 Fatty acid composition of total lipid obtained from each strain.

Culture	% Total fatty acids									
	12 : 0 ^a	14 : 0	iso 16 : 0	16 : 0	16 : 1	18 : 0	18 : 1	20 : 0	20 : 1	Others
E-49	ND ^b	3.9	T ^c	41.1	1.8	12.9	22.8	T	14.7	2.3
BHT	1.5	8.3	T	47.5	4.9	10.6	17.7	T	9.2	T
Ingbritt	T	2.8	4.4	37.5	3.0	8.3	26.1	T	15.9	1.7
OMZ-176	ND	2.7	T	38.2	2.7	8.4	29.1	T	16.1	2.0
OMZ-175	T	3.5	T	38.4	2.5	10.8	26.2	1.1	15.4	1.8
LM-7	T	5.2	T	37.2	7.2	8.0	32.1	ND	8.0	2.0
6715	T	5.0	T	13.2	14.0	3.1	56.2	ND	5.8	2.3

^a Number of carbon atoms : unsaturated fatty acid

^b Not detectable

^c Trace quantity (< 1 % total peak area)

* Values are averages of three different determinations.

lution was subjected to gas chromatographic analysis. A model GC-8 gas chromatograph (Shimadzu Seisakusho) with a hydrogen flame ionization detector was used under the following operating conditions : column, 2 m glass column packed with 10 % diethylene glycol succinate polyester ; column temperature, 180 °C ; nitrogen flow rate, 30 ml/min. Fatty acids were identified by comparing their retention times with standard ones (Serdary Research Laboratories).

Results and Discussion

As bacteria grow to suit different environments, they have characteristic forms and functions. Thus, components of and composition in bacterial lipids, which play important roles to maintain the membrane functions at the bacterial cell-outer environment interface, should become specific to individual bacteria ⁹⁾. Characteristic phospholipids and their fatty acid composition have been used as useful indicators for bacterial chemotaxonomy in recent years ¹⁰⁾. In addition to their chemotaxonomic usefulness, they have been implicated in bacterial metabolism. Drucker¹¹⁾ reported that fatty acid fingerprints of *S. mutans* varied with carbohydrate sources in the media. Markevics *et al.* ⁵⁾ described that extracellular glucosyltransferase production by *Streptococcus salivarius* was enhanced along with potassium ion concentrations in media, and its secretion was directly related to the altered ratio of stearic

(18 : 0) and oleic (18 : 1) acids in the bacterial membrane. It was also shown that the change of fatty acid composition was due to the altered composition of phospholipids, in which the fatty acid composition of cardiolipin was altered ¹²⁾.

Chemotaxonomic characterization of *S. mutans* based on fatty acid composition of its lipids led to its distinction from the other oral streptococci ⁶⁾. However, difference among the heterogeneous species has not been studied well.

Phosphatidylglycerol and cardiolipin were the major components in phospholipids of all strains of *S. mutans* employed. OMZ-175 and OML-176 strains offered another minor spot corresponding to phospholipid on a thin-layer chromatographic plate. The other five minor spots were obtained from LM-7 strain. These minor spots were not coloured with either alpha-naphthol or ninhydrin reagents but they were coloured with Dittmer's reagent.

Fatty acid composition of total lipids is shown in Table 1. Straight chain fatty acids with even carbon numbers were the major components in all strains. Palmitic (16 : 0) acid showed the highest value of all fatty acids, and oleic (18 : 1) and eicosenoic (20 : 1) acids followed in that order except for the 6715 strain. The 6715 strain contained oleic (18 : 1) acid with an overwhelmingly high composition ratio of 56.2 %. The values of palmitic (16 : 0) and palmitoleic (16 : 1) acids

Table 2 Fatty acid composition of phospholipids obtained from each strain.

Culture	% Total fatty acids									
	12 : 0 ^a	14 : 0	iso 16 : 0	16 : 0	16 : 1	18 : 0	18 : 1	20 : 0	20 : 1	Others
Phosphatidylglycerol										
E-49	1.9	6.6	2.4	41.4	3.0	11.0	21.4	1.1	10.0	1.2
BHT	5.3	13.1	ND ^b	42.4	5.8	10.3	16.4	T ^c	4.6	1.9
Ingbritt	T	6.4	5.6	36.0	4.2	6.3	29.2	T	9.3	1.9
OMZ-176	T	T	1.5	28.2	2.8	10.4	30.2	1.7	22.5	1.8
OMZ-135	1.5	5.9	1.3	43.0	3.5	7.0	26.1	T	9.9	1.4
LM-7	ND	4.8	T	41.6	6.6	2.1	31.5	T	7.0	5.0
6715	T	4.1	T	12.8	19.9	1.2	46.2	T	6.1	8.3
Cardiolipin										
E-49	T	3.3	3.9	37.7	2.8	11.0	21.4	T	16.1	1.3
BHT	2.5	6.2	ND	41.0	4.2	10.3	22.9	T	10.4	2.0
Ingbritt	T	2.2	23.4	22.6	1.9	6.3	15.3	1.2	13.5	13.0
OMZ-176	T	T	1.5	28.2	2.8	10.4	30.2	1.7	22.5	1.8
OMZ-175	1.5	5.9	1.3	43.0	3.5	7.0	26.1	T	9.9	1.4
LM-7	T	6.2	T	36.2	7.3	3.9	24.3	2.2	9.9	9.1
6715	T	3.4	T	12.4	16.5	3.3	52.8	ND	9.9	T

^a Number of carbon atoms : unsaturated fatty acid, ^b Not detectable^c Trace quantity (<1% total peak area), * Values are averages of three different determinations.

were significantly different from those of the other strains. The total unsaturated fatty acids accounted for 76 % and this value characterized the 6715 strain. Branched chain fatty acid (iso 16 : 0) of 4.4 % was specific for the Ingbritt strain.

Fatty acid composition of two major phospholipids, phosphatidylglycerol and cardiolipin, is shown in Table 2. Although all strains contained fatty acids with even carbon numbers similar to total lipids, there were some differences in their composition ratios. Eicosenoic (20 : 1) acid of more than 20% in both phospholipids characterized the OMZ-176 strain. The OMZ-176 strain showed a similarity to the OMZ-175 strain in both phospholipid composition and fatty acid composition of the total lipids. However, there were considerable differences among these strains concerning composition ratios of myristic (14 : 0), palmitic (16 : 0) and eicosenoic (20 : 1) acids. The 6715 strain contained a large amount of oleic (18 : 1) acid in both phospholipids similarly to total lipids. The total unsaturated fatty acids in its phosphatidylglycerol and cardiolipin accounted for 72.2 and 79.2

%, respectively. The cardiolipin of the Ingbritt strain specifically showed a high value of branched chain fatty acid.

Fatty acid composition of total and phospholipids from the E-49 strain was considerably different from our previous report ⁶⁾. Such difference might be due to differences in the liberation methods used to obtain the fatty acids from bacterial lipid extracts, i. e. acid-liberation for gas chromatographic and alkaliliberation for high-performance liquid chromatographic analysis, or in the methods used to calculate the composition, i. e. the expression in weight unit for gas chromatographic (according to a conventional method) and in molar unit for high-performance liquid chromatographic analysis. Anyhow, this discrepancy needs to be resolved.

Individual strains of *S. mutans* showed variation in their phospholipid and fatty acid composition. Especially, the differences in the fatty acid composition of phospholipids characterized the Ingbritt, OMZ-176 and 6715 strains concerning the composition ratios of branched chain fatty, eicosenoic (20 : 1) and unsaturated fatty acids, respectively. The fat-

ty acid composition did not show a relationship with serotypes. Lipid components, mainly contained in the cell-membranes, may not directly reflect the differences among the polysaccharide antigens in cell-wall.

Phospholipid and fatty acid composition vary with the age of the bacterial cells and cultural conditions. Since the harvesting points were set on a late logarithmic phase based on the growth curve of the individual strain and cultured under the same conditions, it is considered that the variation of the lipid composition which might be related to age was avoided. Fatty acids in phospholipids and glycolipids, and proteins in the cell-membrane play important roles in the condition of the membrane, such as the fluidity. It is also known that fatty acid compositions in phospholipids vary based on the differences of the proteins and/or glycolipids in order to keep the fluidity of cell-membrane constant¹³⁾. Thus, the variations observed among strains may reflect both the qualitative and quantitative differences of the constituents in cell-membrane. However, the results described above should provide a basis for discussion on the differences between various strains of *S. mutans*, although the precise classification of the species might not be easy using only the fatty acid composition of the bacterial lipids.

Lambert and Moss¹⁴⁾ described the classification of oral streptococci based on differences in the fatty acid composition of their total lipids. The present study shows the importance of the fatty acid composition of the phospholipids for such a classification. This is apparent from the specific composition of phospholipids of the Ingbritt and OMZ-176 strains. Whether the variation of phospholipid and fatty acid composition is widely found in other clinical isolates and how such variation are related to bacterial metabolism still remain to be solved.

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