Isolation, identification and characterization of lactic acid bacteria from preschool children and its correlation with dental caries

Ayumi Shimada [1], Masafumi Noda [2], Yasuyuki Matoba [2], Takanori Kumagai [2], Masanori Sugiyama [2], Katsuyuki Kozai [1]

1 Pediaric Dentistry, Hiroshima University, Hiroshima, Japan
2 Molecular Microbiology and Biotechnology, Hiroshima University, Hiroshima, Japan

Introduction

Lactic acid bacteria (LAB), especially genus *Lactobacilllus*, have been associated with the presence or progression of dental caries in preschool children in many previous reports. Both the abilities to live in a low-pH environment and to produce organic acids contribute to the caries initiation and progression. Nevertheless, what kinds of species of LAB are related to and what characteristics are associated with dental caries have not been clarified.

1. **Is there a correlation between the dental caries contraction situation and the detected bacterial strain?**
2. **What kinds of characteristics should bring about caries in isolates?**

Study population

The subjects are patients who consulted the Hiroshima University Hospital of Pediatric dentistry. Prior to starting this study, we obtained informed consent from the guardians of all study participants. Additionally, the clinical study was approved by the ethics committee of Hiroshima University and performed according to the guidelines of the Declaration of Helsinki.

Table 1. Characteristics of subjects

	Male (n =39)	Female (n = 35)	Total (n = 74)	*P*-values§
Age† (months)	42.0 ± 13.8	39.8 ± 12.8	41.0 ± 13.3	0.484
dmft‡ (%)	39.1 ± 4.9	31.9 ± 4.8	35.7 ± 3.5	0.296
dt‡ (%)	24.8 ± 4.5	22.1 ± 4.0	23.5 ± 3.0	0.670

†mean ± S.D. , ‡mean ± S.E. , § Welch's *t*-test (Male vs Female)

Isolation and identification of oral LAB

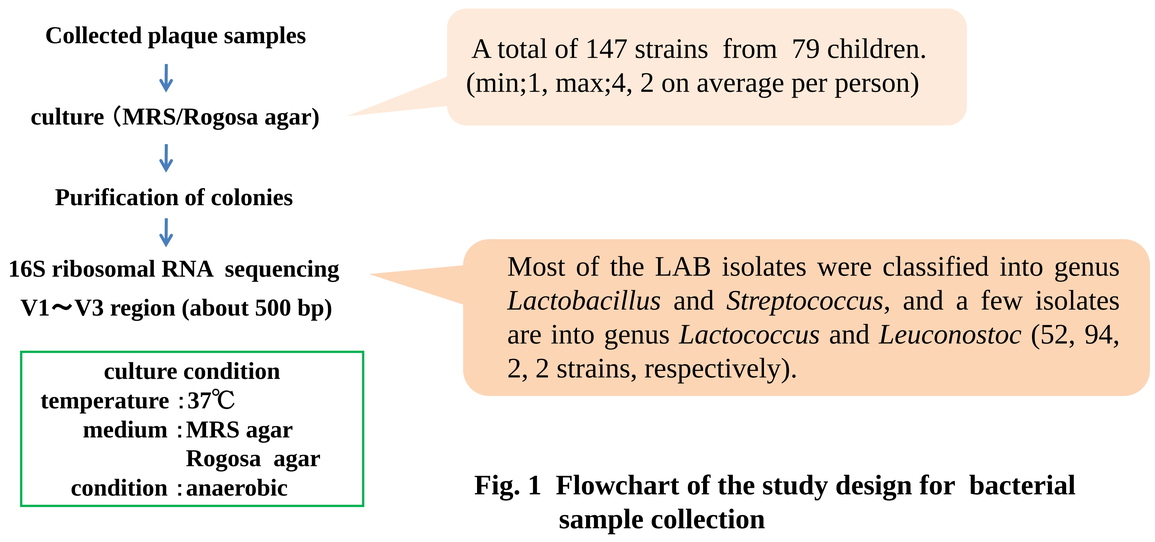


Fig. 1 Flowchart of the study design for bacterial sample collection

Correlation between dental caries and LAB

Table2 shows that the mean dt scores were significantly higher in the presence of *L. gasseri*, *L. fermentum*, or *L. salivarius* than in the absence of these species. Compared as the dmft score, although significant differences were observed between the presence and absence of *L. gasseri* or *L. salivarius*, the difference was not significant but showed a positive trend in the presence of *L. fermentum*.

There were no positive correlations between dental caries and detection of *S. salivarius*, *S. mitis*, and *S. anginosus*.

Table 2. Caries statuses in subjects with or without detection of frequently isolated

Species			dmft† (%)	P-values‡	dt† (%)	P-values‡
L. gasseri	detected	(n = 15)	54.7 ± 6.4	] 0.004**	39.7 ± 7.0	] 0.016*
	no detected	(n = 59)	30.9 ± 3.8		19.4 ± 3.2	
L. fermentum	detected	(n = 14)	48.6 ± 7.1	] 0.064	35.7 ± 6.4	] 0.026*
	no detected	(n = 60)	32.7 ± 3.8		20.2 ± 3.3	
L. salivarius	detected	(n = 11)	58.6 ± 8.8	] 0.021*	46.8 ± 10.1	] 0.023*
	no detected	(n = 63)	32.0 ± 3.6		19.5 ± 2.8	
S. salivarius	detected	(n = 39)	30.2 ± 5.0	] 0.094	18.4 ± 3.7	] 0.078
	no detected	(n = 35)	41.7 ± 4.5		29.2 ± 4.7	
S. mitis	detected	(n = 14)	26.0 ± 6.8	] 0.145	16.8 ± 4.7	] 0.165
	no detected	(n = 60)	38.0 ± 3.9		25.1 ± 3.5	
S. anginosus	detected	(n = 8)	38.1 ± 8.6	] 0.771	20.0 ± 7.3	] 0.664
	no detected	(n = 66)	35.4 ± 3.7		24.0 ± 3.3	
S. mutans	detected	(n = 8)	68.8 ± 7.6	] 0.001**	39.4 ± 12.7	] 0.211
	no detected	(n = 66)	31.7 ± 3.5		21.6 ± 3.0	

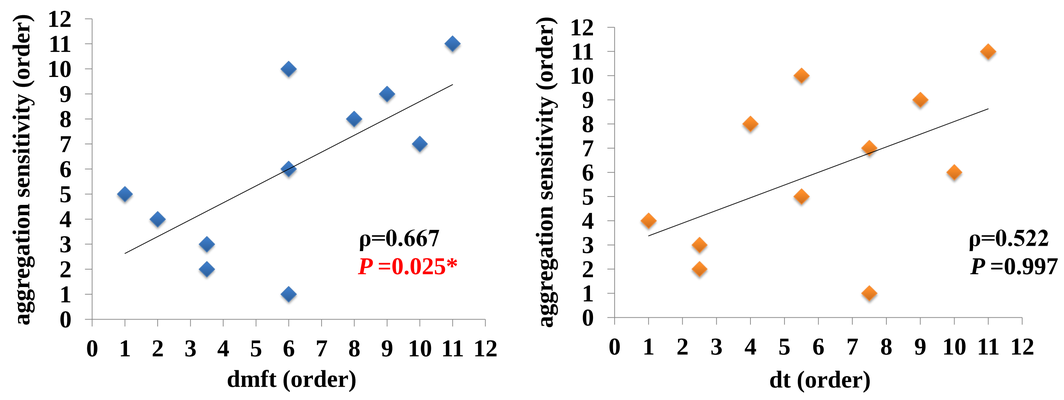
†mean ± S.D. , ‡Welch's t-test (detected vs non-detected, $P < 0.05$ *, $P < 0.01$ **)

Characteristics of isolated oral LAB

1) the sensitivity to saliva-induced aggregation

As shown in Fig. 2, the saliva-induced aggregation is correlated with dental caries level in *L. gasseri* and *L. salivarius*, but not related to *L. fermentum* (dmft ; ρ = 0.496 and *P* = 0.175, dt ; ρ = 0.277 and *P* = 0.470, data not shown).

a) *Lb. gasseri*



b) *Lb. salivarius*

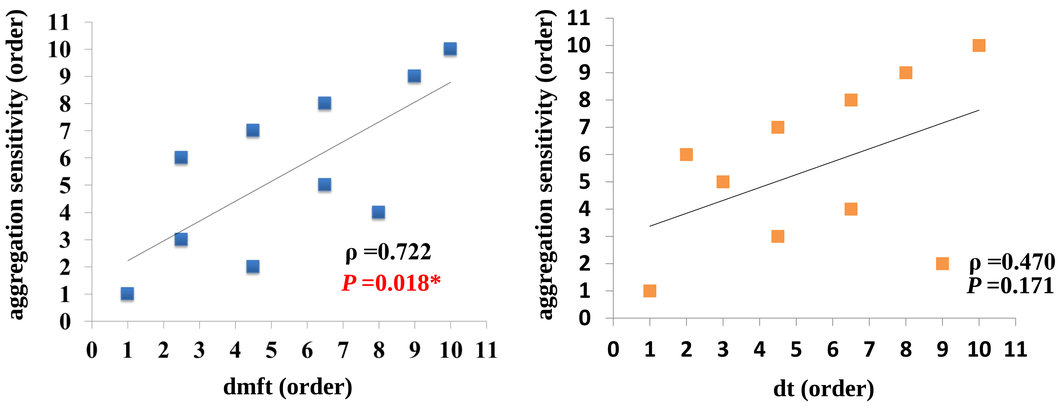


Fig. 2. The relationship between aggregation sensitivity of two *Lactobacillus* species and the caries status of their carriers (dmft or dt). (a) *L. gasseri*, (b) *L. salivarius*, Spearman's rank correlation, P < 0.05*, P < 0.01**

2) acid resistance ability

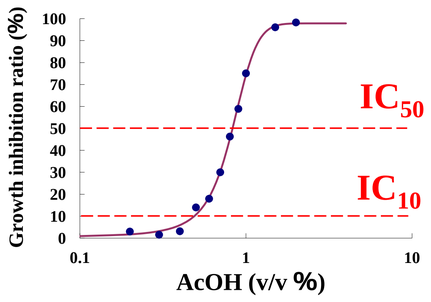
To verify the correlation between the LAB isolates and dental caries, we evaluated the acid resistance of *Lactobacillus* isolates that cause dental caries

Logistic curve equation fitting:

$$Y = \frac{\alpha}{1 + \beta e^{-\gamma X}}$$

X: AcOH concentration

Y: Inhibition rate



It shows that the resistance of *L. fermentum* and *L. salivarius* to organic acids seemed to be strain specific and positively correlated with caries severity, but no correlations are observed between caries scores and the acid resistance of each strain in *L. gasseri* (Table. 3).

Table 3. The relationship between IC_{50} and IC_{10} values of three *Lactobacillus* species and the caries status of their carriers (dmft or dt).

Strain	dmft		dt	
	I.C.$_{50}$	I.C.$_{10}$	I.C.$_{50}$	I.C.$_{10}$
Lb. salivarius	ρ=0.483	ρ=0.612	ρ=0.488	ρ=0.720
	P=0.157	*P*=0.060	*P*=0.153	*P*=0.019*
Lb. gasseri	ρ=-0.133	ρ=-0.179	ρ=-0.124	ρ=-0.165
	P=0.696	*P*=0.598	*P*=0.717	*P*=0.628
Lb. fermentum	ρ=0.849	ρ=0.790	ρ=0.628	ρ=0.561
	P=0.004**	*P*=0.011*	*P*=0.070	*P*=0.116

, Spearman's rank correlation , $P < 0.05$* , $P < 0.01$**

Conclusion

Statistical analysis shows that the preschool children carrying *L. gasseri*, *L. fermentum*, or *L. salivarius* have significantly higher prevalence of dental caries than do those without detection of those species. When comparing the acid tolerance against organic acid among these isolates. it was indicated that growth ability in acidic environments correlated with the caries status of individuals, but in *L. gasseri*, it was not related since the bacterium exhibits the ability to induce adaptation of the acid tolerance and general stress response. Our results suggest that specific *Lactobacillus* species may be a risk factor in dental caries, and the differences in the ability to tolerate acid may be related to caries activity.