The Journal of Microbiology (2011) Vol. 49, No. 1, pp. 165-168
DOI 10.1007/s12275-011-1018-0

NOTE

Bacterial Model System for Screening and Determining Optimal Concentration of Anti-caries Natural Extracts

Min Jung Kim[1,2†], Chun Sung Kim[1,2†], Jae-Yoon Park[3†], Soon-Nang Park[1,2], So Young Yoo[1], Sook-Young Lee[4], and Joong-Ki Kook[1,2*]

[1]Department of Oral Biochemistry and [2]Oral Biology Research Institute, School of Dentistry, Chosun University, Gwangju 501-759, Republic of Korea
[3]Department of Biochemistry and Molecular Biology, Medical School, Chosun University, Gwangju 501-759, Republic of Korea
[4]MRC Center, School of Dentistry, Chosun University, Gwangju 501-759, Republic of Korea

(Received January 11, 2011 / Accepted January 26, 2011)

In general, an antimicrobial test for screening anti-caries natural extracts was performed by measuring the minimum bactericidal concentration (MBC) against the type strains of mutans streptococci. However, it is unclear if the antimicrobial efficiency of natural extracts on the type strains of mutans streptococci is the same on the clinical strains. In this study, we introduced a bacterial model system for the screening of anti-caries and determining the optimal concentration of them to develop oral hygiene products for Korean populations.

***Keywords*:** bacterial model system, screening, anti-caries, natural extract, mutans streptococci

Dental caries is the most common disease in the oral cavity and a chronic bacterial infectious disease. The major causative bacterium of dental caries is mutans streptococci (Loesche, 1986). Mutans streptococci are composed of *Streptococcus mutans*, *S. sobrinus*, *S. downei*, *S. rattus*, and *S. crecetus* (Kawamura *et al*., 1995). Among them, *S. mutans* and *S. sobrinus* inhabit the human oral cavity.

In recent years, natural extracts of plants have been used as antibacterial agents in traditional medicines, and have attracted considerable interest in the prevention of dental caries (Lee *et al*., 2003; Lim *et al*., 2003; Limsong *et al*., 2004; Song *et al*., 2007; Sampaio *et al*., 2009). Ethanol-extracted *Phellodendron amurense* (PA) and the powder of *Galla rhois* (GR) have strong antimicrobial activity against *S. mutans*. Methanol-extracted *Coptidis rhizome* (CR) exhibits antimicrobial activity against *S. mutans*, inhibits acid production, inhibits the glycosyltransferase activity, and has anti-adhesion activity for *S. mutans* (Jang *et al*., 2000; Choi *et al*., 2003; Kwak, 2004).

Generally, type strains of mutans streptococci are used to test the antimicrobial activity of natural extract against mutans streptococci (Xiao *et al*., 2006; Islam *et al*., 2008). However, it is unclear if the antimicrobial efficiency of natural extracts on the type strains of mutans streptococci is the same on the clinical strains. This laboratory isolated 77 clinical strains of *S. mutans* and 18 clinical strains of *S. sobrinus* from 88 Koreans (Yoo *et al*., 2007). This study examined whether the antimicrobial efficiencies of methanol-extracted PA, CR and GR against type strains (*S. mutans* ATCC 25175^T and *S. sobrinus* ATCC 33478^T) are the same as those on 55 clinical strains, 40 strains of *S. mutans*, and 15 strains of *S. sobrinus*. Based on the results of this study, a bacterial model system is suggested for screening anti-caries natural extracts and determining the optimal concentration of them for the development of oral hygiene products for Koreans.

Dried PA and GR were purchased from Donguihanbang (Korea) and dried CR was obtained from Borin Herb (Korea). Each dried plant was reduced to powder using a pulverizer (HMF-100; HANIL Electric Co., Korea). After adding 500 ml of methanol to 100 g of each plant powder, the solution was mixed in a shaking incubator at room temperature for 48 h. The extraction solution was filtered through No. 1 Whatman paper (UK) and concentrated with a rotator evaporator (80 rpm, IKA, RV 10, Korea) at 40°C. The extracts were lyophilized with a freeze dryer (LABCONCO, UK). The extracts were suspended in dimethyl sulfoxide (DMSO; Sigma, USA) to 200 μg/ml.

The *S. mutans* ATCC 25175^T and *S. sobrinus* ATCC 33478^T were purchased from the American Type Culture Collection (ATCC, USA). The clinical strains of *S. mutans* (KCOM 1088, KCOM 1091, KCOM 1092, KCOM 1095, KCOM 1097, KCOM 1111, KCOM 1112, KCOM 1113, KCOM 1116, KCOM 1117, KCOM 1118, KCOM 1123, KCOM 1124, KCOM 1126, KCOM 1127, KCOM 1128, KCOM 2762, KCOM 1136, KCOM 1137, KCOM 1139, KCOM 1142, KCOM 1143, KCOM 1145, KCOM 1146, KCOM 1197, KCOM 1200, KCOM 1201, KCOM 1202, KCOM 1203, KCOM 1207, KCOM 1208, KCOM 1209, KCOM 1212, KCOM 1214, KCOM 1217, KCOM 1219, KCOM 1226) and *S. sobrinus* (KCOM 1061, KCOM 1150, KCOM 1151, KCOM 1152, KCOM 1153, KCOM 1157, KCOM 1158, KCOM 1159,

† These authors contributed equally to this work.
* For correspondence. E-mail: jkkook@chosun.ac.kr; Tel: +82-62-230-6877; Fax: +82-62-224-3706

Table 1. Minimum bactericidal concentration of *P. amurense*, *C. rhizome*, and *G. rhois* against mutans streptococci

Species & Strains	MBC (mg/ml)			Species & Strains	MBC (mg/ml)		
	PA	CR	GR		PA	CR	GR
ATCC[a] 25175[T]	1	0.25	0.5	KCOM 1200	0.25	0.5	1
KCOM[b] 1054	0.5	1	2	KCOM 1201	0.5	1	1
KCOM 1085	0.5	0.125	1	KCOM 1202	1	1	2
KCOM 1087	0.5	0.5	0.5	KCOM 1203	0.25	1	2
KCOM 1087	0.25	0.25	2	KCOM 1207	0.5	1	2
KCOM 1091	0.06	0.5	0.5	KCOM 1207	0.5	0.5	1
KCOM 1092	0.125	0.5	0.5	KCOM 1209	0.5	0.5	1
KCOM 1095	0.25	0.5	1	KCOM 1212	2	0.5	1
KCOM 1097	0.125	0.5	0.5	KCOM 1214	0.25	0.5	0.5
KCOM 1111	2	1	2	KCOM 1217	0.125	1	2
KCOM 1112	0.125	0.5	1	KCOM 1219	0.5	0.5	1
KCOM 1113	0.5	1	2	KCOM 1226	0.25	0.5	2
KCOM 1116	1	1	0.5	ATCC 33478[T]	0.125	0.5	0.25
KCOM 1117	0.125	0.125	0.5	KCOM 1061	0.5	0.5	0.25
KCOM 1118	0.125	0.5	0.5	KCOM 1150	0.25	0.5	0.5
KCOM 1123	0.125	0.5	2	KCOM 1151	0.25	0.25	0.25
KCOM 1124	0.25	0.5	0.5	KCOM 1152	0.25	0.5	0.5
KCOM 1126	1	1	2	KCOM 1153	0.5	0.5	0.5
KCOM 1127	0.5	0.5	2	KCOM 1157	1	1	1
KCOM 1128	2	0.5	2	KCOM 1158	0.5	0.5	0.5
KCOM 2762	0.5	0.25	1	KCOM 1159	0.5	0.5	0.5
KCOM 1136	1	0.5	2	KCOM 1185	0.25	0.5	0.25
KCOM 1137	0.25	0.5	0.5	KCOM 1191	1	0.5	0.25
KCOM 1139	0.25	1	0.5	KCOM 1193	1	0.5	0.5
KCOM 1142	0.5	0.5	2	KCOM 1196	1	0.5	1
KCOM 1143	0.25	0.5	1	KCOM 1221	1	1	0.25
KCOM 1145	0.25	0.5	2	KCOM 1228	0.5	0.5	0.5
KCOM 1146	0.25	0.5	1	KCOM 1218	0.125	0.5	0.125
KCOM 1197	0.5	1	2				

PA, *P. amurense*; CR, *C. rhizome*; GR, *G. rhois*; ATCC, America Type Culture Collection; KCOM, Korean Collection for Oral Microbiolgy.

KCOM 1185, KCOM 1191, KCOM 1193, KCOM 1196, KCOM 1221, KCOM 1228, KCOM 1218) were obtained from the Korean Collection for Oral Microbiology (KCOM, Korea). All strains were cultured in Todd Hewitt (TH, Difco, Lab., USA) broth or on agar plates in a 37℃ incubator in air containing 10% CO_2.

The antimicrobial activities of the methanol-extracted PA, GR and CR were evaluated by determining the minimum bactericidal concentration (MBC). The bacterial strains were cultured in TH broth at 37℃ in an incubator for 24 h and added to a 96-well plate to a final concentration of 1×10^6 CFU/ml. The extract was added to each well to a final concentration of 1, 2, 4, 8, and 16 μg/ml. The final concentration of DMSO in each well was 1%. One percent concentration of DMSO in the medium and medium only group was used as the double negative groups. Ampicillin (final concentration, 100 μg/ml) was used as a positive control. After 24 h incubation under the appropriate conditions, aliquots (10 μl) of the

Table 2. Summary of MBC values of *P. amurense*, *C. rhizome*, and *G. rhois* of against the clinical strains of *S. mutans* and *S. sobrinus*

Concentration of extracts (mg/ml)	*S. mutans* (no, %)			*S. sobrinus* (no, %)			Total (no, %)		
	PA	CR	GR	PA	CR	GR	PA	CR	GR
0.063	1 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)
0.125	7 (17.5)	2 (5.0)	0 (0.0)	1 (6.7)	0 (0.0)	1 (6.7)	8 (14.5)	2 (3.6)	1 (1.8)
0.25	12 (30.0)	2 (5.0)	0 (0.0)	4 (26.7)	1 (6.7)	5 (33.3)	16 (29.1)	3 (5.5)	5 (9.1)
0.50	13 (32.5)	24 (60.0)	11 (27.5)	5 (33.3)	12 (80.0)	7 (46.7)	18 (32.7)	36 (65.5)	18 (32.7)
1.00	4 (10.0)	12 (30.0)	12 (30.0)	5 (33.3)	2 (13.3)	2 (13.3)	9 (16.4)	14 (25.5)	14 (25.5)
2.00	3 (7.5)	0 (0.0)	17 (42.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (5.5)	0 (0.0)	17 (30.9)
Total	40 (100)	40 (100)	40 (100)	15 (100)	15 (100)	15 (100)	55 (100)	55 (100)	55 (100)

PA, *P. amurense*; CR, *C. rhizome*; GR, *G. rhois*

Table 3. Bactericidal effect of *P. amurense*, *C. rhizome*, and *G. rhois* against mutans streptococci isolated from Korea

Species	PA			CR			GR		
	MBC_{50}	MBC_{90}	MBC_{100}	MBC_{50}	MBC_{90}	MBC_{100}	MBC_{50}	MBC_{90}	MBC100
S. mutans (n=40)	0.25	1	2	0.5	1	1	1	2	2
S. sobrinus (n=15)	0.5	1	1	0.5	1	1	0.5	1	1
Total (n=55)	0.5	1	2	0.5	1	1	1	2	2

PA, *P. amurense*; CR, *C. rhizome*; GR, *G. rhois*; MBC_{50}, MBC_{90}, and MBC_{100} minimal bactericidal concentration required to suppress of the growth of 50, 90, and 100% of mutans streptococci, respectively.

broth from wells were plated onto BHI agar and incubated overnight at 37°C. The lowest concentration that allowed no visible growth on agar (99.9% killed) was considered to be the MBC.

The MBC value of three natural extracts against the clinical strains of *S. mutans* and *S. sobrinus* was higher than that against the type strains ranging from 7.5% to 90% and from 60% to 93.3%, respectively (Tables 1 and 2). The MBC_{90} values of the three natural extract used in this study against the clinical strains of *S. mutans* and *S. sobrinus* were 1 mg/ml except for CR against *S. sobrinus*, 2 mg/ml. These results showed that the type strains of mutans streptococci are not sufficient to evaluate the anti-cariogenicity or determine the optimal concentration of the natural extract for developing oral hygiene products.

Interestingly, 11 clinical strains (KCOM 1054, KCOM 1111, KCOM 1113, KCOM 1116, KCOM 1126, KCOM 1228, KCOM 1136, KCOM 1197, KCOM 1202, KCOM 1207, KCOM 1217) of *S. mutans* and 3 clinical strains (KCOM 1157, KCOM 1196, KCOM 1221) of *S. sobrinus* had an above MBC_{90} value of two or three natural extracts. Therefore, these 14 clinical strains and 2 type strains of mutans streptococci are recommended for screening anti-caries natural extracts and determining the optimal concentration of them for the development of oral hygiene products. This system was named "bacterial model system for screening and determining the optimal concentration of anti-caries natural extracts"

In a previous study, the minimum inhibitory concentration (MIC) of oleanolic acid against 2 type strains and 55 clinical strains of mutans streptococci was similar (Kim *et al*., 2010). The discrepancy of these results is unclear but might be due to the existence of many types of compound in the crude extracts of plants. This means that the antimicrobial effect of the combined action of several compounds in the crude extract against each strain might be different. The other reason is the different methods used in these studies. Generally, the MBC value is higher than the MIC, and the MBC value is more variable among strains than the MIC. This result suggests that the bacterial model system developed in this study can be recommended if the crude extracts are used for the test antimicrobial activity against mutans streptococci. On the other hand, the type strains and/or a few clinical strains of mutans streptococci can be used if a single compound is used. The half maximal inhibitory concentration (IC_{50}) of methanol-extracts of PA, GR, and CR on the normal human gingival fibroblast was 0.125, 0.5, 02.5 mg/ml, respectively, and had cell toxicity >1 mg/ml (MBC_{90} value) (data not shown). Therefore, to use these extracts for the development of oral hygiene products, compounds with no cell toxicity to human oral cells but only an antimicrobial effect on mutans streptococci need to be purified from these natural extracts.

In summary, these 14 clinical strains and 2 type strains of mutans streptococci are strongly recommended for screening and determining the optimal concentration of anti-caries natural extracts for the development of oral hygiene products for Koreans.

This study was supported by a grant from the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A091074).

References

Choi, I.W., C.H. Jung, and Y.K. Park. 2003. Anticariogenic activities of various plant extracts. *Korean J. Food Sci. Technol.* 35, 1221-1225.

Islam, B., S.N. Khan, I. Haque, M. Alam, M. Mushfiq, and A.U. Khan. 2008. Novel anti-adherence activity of mulberry leaves: inhibition of *Streptococcus mutans* biofilm by 1-deoxynojirimycin isolated from *Morus alba*. *J. Antimicrob. Chemother.* 62, 751-757.

Jang, G.H., B.Y. Ahn, and S.H. Oh. 2000. Anticariogenic effects of *Coptis chinensis* Franch extract. *Korean J. Food Sci. Technol.* 34, 1396-1402.

Kawamura, Y., X.G. Hou, F. Sultana, S. Liu, H. Yamamoto, and T. Ezaki. 1995. Determination of 16S rRNA sequences of *Streptococcus mitis* and *Streptococcus gordonii* and phylogenetic relationships among members of the genus *Streptococcus*. *Int. J. Syst. Bacteriol.* 45, 406-408.

Kim, M.J., C.S. Kim, W.H. Ha, B.H. Kim, Y.K. Lim, S.N. Park, Y.J. Cho, and *et al*. 2010. Antimicrobial effects of oleanolic acid against *Streptococcus mutans* and *Streptococcus sobrinus* isolated from a Korean population. *Int. J. Oral Biol.* 35, 191-195.

Kwak, D.J. 2004. Antibacterial activities of *Phellodendri Cortex* on the *Streptococcus mutans*. *J. Korean Soc. Hygienic Sciences.* 10, 99-107.

Lee, E.S., T.Y. Ahn, J.J. Yoon, J.K. Kook, B.R. Lee, and D.K. Kim. 2003. Restraint effect on leaf-extract from *Camellia sinensis* and seed-extract from *Casia tora* against periodontopathogens. *J. Korean Acad. Dent. Health* 27, 569-579.

Lim, S.H., J.S. Seo, Y.J. Yoon, K.W. Kim, S.O. Yoo, H.S. Kim, J.K. Kook, B.R. Lee, J.H. Cha, and J.Y. Park. 2003. Effect of leaf-extract from *Camellia sinensis* and seed-extract from *Casia tora* on viability of mutans streptococci isolated from the interface between orthodontic brackets and tooth surfaces. *Korea. J. Orthod.* 33, 381-389.

Limsong, J., E. Benjavongkulchai, and J. Kuvatanasuchati. 2004. Inhibitory effect of some herbal extracts on adherence of *Streptococcus mutans*. *J. Ethnopharmacol*. 92, 281-289.

Loesche, W.J. 1986. Role of *Streptococcus mutans* in human dental decay. *Microbiol. Rev*. 50, 353-380.

Sampaio, F.C., S. Pereira Mdo, C.S. Dias, V.C. Costa, N.C. Conde, and M.A. Buzalaf. 2009. *In vitro* antimicrobial activity of *Caesalpinia ferrea* Martius fruits against oral pathogens. *J. Ethnopharmacol*. 124, 289-294.

Song, J.H., T.C. Yang, K.W. Chang, S.K. Han, H.K. Yi, and J.G.

Jeon. 2007. *In vitro* effects of a fraction separated from *Polygonum cuspidatum* root on the viability, in suspension and biofilms, and biofilm formation of mutans streptococci. *J. Ethnopharmacol*. 112, 419-425.

Xiao, J., Y. Liu, Y.L. Zuo, J.Y. Li, L. Ye, and X.D. Zhou. 2006. Effects of *Nidus vespae* extract and chemical fractions on the growth and acidogenicity of oral microorganisms. *Arch. Oral Biol*. 51, 804-813.

Yoo, S.Y., S.J. Park, D.K. Jeong, K.W. Kim, S.H. Lim, S.H. Lee, S.J. Choe, Y.H. Chang, I. Park, and J.K. Kook. 2007. Isolation and characterization of the mutans streptococci from the dental plaques in Koreans. *J. Microbiol.* 45, 246-255.