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Short communication

Detection of serotype *k* *Streptococcus mutans* in Thai subjects

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Introduction: *Streptococcus mutans*, known to be a pathogen of dental caries as well as bacteremia and infective endocarditis, is classified into four serotypes, *c*, *e*, *f* and *k*, based on the structures of serotype-specific polysaccharides. Serotype *k* was recently designated using blood isolates from Japanese subjects and such strains are considered to be virulent in the bloodstream. The purpose of the present study was to analyse the serotype distribution of strains isolated from Thai subjects and determine whether serotype *k* strains were present.

Methods: A total of 250 *S. mutans* strains were isolated from 50 Thai subjects, and serotypes of all strains were determined. Then, molecular and biological analyses were carried out for serotype *k* strains.

Results: Immunodiffusion and polymerase chain reaction analyses showed that serotype *c* was the most prevalent (70%), followed by serotypes *e* (22.8%), *f* (4.4%) and *k* (2.8%), which indicated that serotype *k S. mutans* strains occurred in Thai individuals at a similar rate to that previously reported for Japanese and Finnish populations. Molecular analyses of the seven serotype *k* strains showed extremely low expression of *rgpE*, which is related to glucose side-chain formation in serotype-specific rhamnose-glucose polymers, similar to previous reports for those other populations. In addition, analysis of the biological properties of the seven serotype *k* strains demonstrated low levels of sucrose-dependent adhesion, cellular hydrophobicity, dextran-binding activity and phagocytosis susceptibility by human polymorphonuclear leukocytes, which are characteristics similar to those of serotype *k* strains previously isolated in Japan.

Conclusion: Our results indicate the possibility of a worldwide prevalence of serotype *k* strains with properties in common with those of previously reported strains.

Key words: serotype *k; Streptococcus mutans;* Thai subjects

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Streptococcus mutans is widely accepted as the main pathogen of dental caries, while it is also known to be involved with bacteremia and infective endocarditis (9). In our previous study, *S. mutans* was frequently detected in cardiovascular specimens, such as heart valves and atheromatous plaques (17). Recently, certain *S. mutans* strains were reported that were capable of attaching to the surfaces of human coronary artery endothelial cells, then entering and surviving in those cells (1). Such data indicated the potential role of the bacterium in the pathogenesis of some types of cardiovascular disease.

S. mutans was previously classified into three serotypes, *c*, *e* and *f*, based on the structures of cell-wall-associated polysaccharides (14). However, some *S. mutans* strains isolated from patients with bacteremia or infective endocarditis could not be classified (6), which led to the designation of the novel serotype *k* (21). In general, the major serotype in the oral cavity is serotype *c* (approximately 70–80%), followed by serotype *e* (approximately 20%), while the prevalence of serotype *f* is low (<5%) (3, 4, 8, 10, 11, 29, 31). As for serotype *k*, its distribution in Japanese populations has been reported to range from 2 to 5% (21, 25). In addition, recent results indicate the possibility that *S. mutans* serotype *k* is virulent in blood (23). Serotype *k* has been found among strains isolated in Japan, Finland and the UK (18, 32), whereas there is no information available on the serotype distribution of strains isolated in Thailand. In the present study, we examined the serotype distribution of *S. mutans*

strains isolated from a Thai population to determine the presence of serotype *k*.

Stimulated saliva specimens were collected from 50 Thai individuals (25 male, 25 female; aged 13–62 years) with the approval of the Ethics Committee of Mahidol University. Serial dilutions of the specimens were cultured on mitis salivarius agar (Difco Laboratories, Detroit, MI, USA) containing bacitracin (0.2 U/ml; Sigma Chemical Co., St Louis, MO, USA), 0.001% (volume/volume) tellurite solution (Becton, Dickinson & Co., Sparks, MD) and 15% (weight/volume) sucrose. Five presumptive rough colonies of *S. mutans* were chosen from each subject, as our previous results showed that this number of isolates is adequate for whole-mouth *S. mutans* representation (13). All isolates were then confirmed to be *S. mutans* by biochemical analyses, such as sugar fermentation profiles for 1% mannitol, sorbitol, raffinose or melibiose as well as negative for dextran agglutination (7). For serotype determination, we used an immunodiffusion method with rabbit antisera specific for serotypes *c*, *e*, *f* and *k*, as well as polymerase chain reaction (PCR) with serotype-specific sets of primers, as described previously (25, 31).

Table 1 shows the serotype distribution for the samples from Thai subjects. Among the 250 *S. mutans* strains isolated, serotype *c* was the most frequently detected at a prevalence of 70%, followed by serotypes *e* (22.8%) and *f* (4.4%). These results are similar to those reported worldwide (3, 4, 8, 10, 11, 29, 31). In addition, seven serotype *k* strains (2.8%) were detected in samples from two subjects (4%). One of those was a 29-year-old man, from whom two serotype *k* strains were isolated and five serotype *k* strains were isolated from a 21-year-old woman. All serotype *k* strains in each subject were derived from a single clone (data not shown), which was confirmed using an arbitrary primed PCR technique. When the number of serotypes harbored by each subject was considered, 82% of the subjects had a single serotype, while the others had multiple serotypes, of which serotypes *c* and *e* were the most prevalent (Table 2). These findings are similar to those reported previously for Finnish and Japanese populations (2, 20).

Table 1. Serotype distribution of 250 *Streptococcus mutans* isolates obtained from 50 subjects

Serotype	No. of isolates (n = 250)	No. of subjects (n = 50)
c	175 (70.0%)	38 (76.0%)
e	57 (22.8%)	16 (32.0%)
f	11 (4.4%)	3 (6.0%)
k	7 (2.8%)	2 (4.0%)

Table 2. Individual serotype distribution in 50 subjects

Serotype	No. of subjects	Serotype	No. of subjects
Single	41 (82.0%)	*c*	30 (60.0%)
		e	8 (16.0%)
		f	2 (4.0%)
		k	1 (2.0%)
Multiple	9 (18.0%)	*c* and *e*	7 (14.0%)
		c and *f*	1 (2.0%)
		e and *k*	1 (2.0%)

Serotype-specific polysaccharides of *S. mutans* are composed of rhamnose-glucose polymers, with a backbone of rhamnose and side chains of α-linked or β-linked glucosidic residues (14). The unique characteristic of serotype *k* compared with the other serotypes is a drastic reduction of glucose side chains (6, 21), which is considered to be caused by a lack of glucosyltransferase activity from *rgpE* gene, leading to inhibition of glucose polymer production by serotype-specific polysaccharides (27). This is the first report of serotype *k* strains isolated in Thailand so we decided to analyse the etiology of the loss of glucose side chains in the present serotype *k* strains. Reverse transcriptase-mediated PCR analyses identified no transcription of *rgpE* in any of the seven isolates (data not shown). This result showed that the lack of *rgpE* gene expression in serotype *k* strains isolated in Japan was also present in the strains from Thai individuals.

We previously reported that serotype *k* strains commonly possess defects of major surface proteins, such as protein antigen (PA) and glucan-binding proteins (Gbps) (19, 24, 26). These defects bring about low levels of cellular hydrophobicity, sucrose-dependent adhesion and dextran-binding ability, as well as low susceptibility to phagocytosis (19, 21, 24, 26). Moreover, a glucose side-chain-defect mutant (MT8148GD), which produced a precipitation band with a serotype *k* antibody, also showed greatly decreasing levels of all those properties compared with its serotype *c* parent strain (21, 22). Therefore, we performed experiments to evaluate sucrose-dependent adhesion, cellular hydrophobicity, dextran-binding ability and phagocytosis susceptibility using the seven serotype *k* strains isolated from the Thai subjects using previously described methods (12, 15, 21, 30).

Compared with the serotype *c S. mutans* strain MT8148 (28), nearly all of the serotype *k* strains isolated from our Thai subjects showed significantly low levels of sucrose-dependent adhesion, cellular hydrophobicity, dextran-binding ability, and phagocytosis susceptibility (Table 3). Exceptions were seen in the sucrose-dependent adhesion rates of strains TLJ26-2, TLJ26-3 and TLJ26-5, and the cellular hydrophobicity rate of TLJ11-5. To clarify these results, we performed Western blot analyses to detect the expression of major surface proteins, including PA and GbpA, using our previously reported methods (5, 26). Interestingly, none of the serotype *k* strains from the Thai subjects expressed GbpA, and the expression of PA was only found in the strains from one subject (TLJ26-1, TLJ26-2, TLJ26-3, TLJ26-4 and TLJ26-5) (data not shown). GbpA is known to play a role in the glucan-binding properties of *S. mutans* in the presence of sucrose (16). Hence, the attenuation of GbpA expression could partially explain the decreases in both sucrose-dependent adhesion and dextran-binding ability found in most of the serotype *k* strains isolated in the present study. However, the sucrose-dependent adhesion rates of some of the present serotype *k* strains (TLJ26-2, TLJ26-3 and

Table 3. Biological properties of serotype *k* strains isolated in Thailand

Strains	Sucrose-dependent adhesion (%)	Cellular hydrophobicity (%)	Dextran-binding (A405)	Phagocytosis rate (%)
MT8148	91.2 ± 1.0	84.5 ± 0.9	0.117 ± 0.002	87.2 ± 5.9
TLJ11-2	64.6 ± 1.7**	70.9 ± 5.6*	0.061 ± 0.005**	62.0 ± 3.4**
TLJ11-5	56.4 ± 3.8**	78.9 ± 6.6	0.056 ± 0.002**	59.2 ± 6.2**
TLJ26-1	86.5 ± 0.8**	63.6 ± 7.7*	0.062 ± 0.003**	55.6 ± 7.2**
TLJ26-2	90.6 ± 0.9	74.4 ± 2.0**	0.061 ± 0.004**	58.0 ± 5.1**
TLJ26-3	90.1 ± 1.1	71.0 ± 3.7**	0.071 ± 0.006**	59.6 ± 5.1**
TLJ26-4	88.3 ± 0.8*	69.4 ± 2.4**	0.071 ± 0.004**	60.0 ± 4.6**
TLJ26-5	90.5 ± 1.0	73.1 ± 1.8**	0.063 ± 0.002**	58.4 ± 6.3**

Statistically significant differences between MT8148 and other strains (by Fisher's post-test for least significant differences analysis).
*$P < 0.01$.
**$P < 0.001$.

TLJ26-5) were compatible with that of MT8148 (Table 3). Hence, other protein antigens may have influenced these results.

In summary, this is the first report to describe the presence of serotype *k S. mutans* strains in a Thai population and it adds the detection of serotype *k* in another country besides Japan, Finland and the UK. In addition, the biological properties of the serotype *k* strains isolated in Thailand were similar to those of the serotype *k* strains isolated in Japan. Additional experiments as well as clinical studies are necessary to provide conclusive evidence of an association between cardiovascular disease and the presence of *S. mutans* serotype *k*.

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