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Cariogenic properties of *Streptococcus mutans* clinical isolates with sortase defects

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ABSTRACT

Objective: In *Streptococcus mutans*, a Gram-positive pathogen of dental caries, several surface proteins are anchored by the activity of sortase enzyme. Although various reports have shown that constructed *S. mutans* mutants deficient of sortase as well as laboratory reference strains with a sortase gene mutation have low cariogenic potential, no known studies have investigated clinical isolates with sortase defects. Here, we examined the cariogenic properties of *S. mutans* clinical isolates with sortase defects as well as caries status in humans harboring such defective isolates.

Design: Sortase-defective clinical isolates were evaluated for biofilm formation, sucrose-dependent adhesion, stress-induced dextran-dependent aggregation, acid production, and acid tolerance. Additionally, caries indices of subjects possessing such defective isolates were determined.

Results: Our *in vitro* results indicated that biofilm with a lower quantity was formed by sortase-defective as compared to non-defective isolates. Moreover, impairments of sucrose-dependent adhesion and stress-induced dextran-dependent aggregation were found among the isolates with defects, whereas no alterations were seen in regard to acid production or tolerance. Furthermore, glucan-binding protein C, a surface protein anchored by sortase activity, was predominantly detected in culture supernatants of all sortase-defective *S. mutans* isolates. Although the sortase-defective isolates showed lower cariogenic potential because of a reduction in some cariogenic properties, deft/DMFT indices revealed that all subjects harboring those isolates had caries experience.

Conclusions: Our findings suggest the impairment of cariogenic properties in *S. mutans* clinical isolates with sortase defects, though the detection of these defective isolates seemed not to imply low caries risk in the subjects harboring them.

1. Introduction

Sortase is a transpeptidase enzyme important for attachment of proteins to the cell surface of Gram-positive bacteria (Spirig, Weiner, & Clubb, 2011). This enzyme functions to join proteins containing a cell-wall sorting signal, such as a C-terminal sorting signal with a LPXTG motif, to an amino group located on the cell surface (Fischetti, Pancholi, & Schneewind, 1990). Previous studies that used sortase-deficient mutants have shown that their surface proteins were produced and transferred across cell walls into culture supernatants, as the proteins are unable to bind to cell walls due to a lack of sortase processing (Mazmanian, Liu, Jensen, Lenoy, & Schneewind, 2000; Nobbs et al., 2007). The sortase enzyme in *Streptococcus mutans*, a well-known Gram-positive pathogen of dental caries, belongs to the SrtA subfamily (Ajdić et al., 2002; Comfort & Clubb, 2004), while various *S. mutans* surface proteins have been reported to contain an LPXTG motif (Ajdić et al., 2002; Nomura et al., 2012; Sato et al., 2004). In addition, some of these surface proteins, i.e., 190-kDa protein antigen (PA), dextranase, and glucan-binding protein C (GbpC), were previously demonstrated to be anchored by the action of sortase (Igarashi, Asaga, & Goto, 2003; Igarashi, Asaga, & Goto, 2004; Igarashi, Asaga, Sato, & Goto, 2004; Lee & Boran, 2003). As with other

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Gram-positive pathogens, sortase plays a crucial role in the pathogenesis of diseases related to *S. mutans*. Several studies have indicated the low cariogenicity of sortase-mutant strains (Igarashi, Asaga, Goto et al., 2004; Igarashi, Asaga, Sato et al., 2004; Lee & Boran, 2003; Lévesque et al., 2005), and some studies have suggested the use of sortase as a target for caries prevention (Hu, Huang, & Chen, 2013; Igarashi, Asaga, Goto et al., 2004).

In our recent study, we identified sortase-defective *S. mutans* among clinical isolates and those isolates were demonstrated to lose 190-kDa cell-wall-associated PA into culture medium (Lapirattanakul et al., 2015). In addition, all of the investigated sortase-defective clinical isolates showed markedly low hydrophobicity and decreased susceptibility to phagocytosis. In the present study, we investigated the cariogenic properties of these sortase-defective isolates, including adhesion, biofilm formation, acid production, and acid tolerance. Furthermore, characteristics and caries indices of human subjects harboring these sortase-defective isolates in the oral cavity are described.

2. Materials and methods

2.1. Subjects and sample collection

Three subjects harboring sortase-defective *S. mutans* were found among a total of 150 subjects examined as part of our recent work (Lapirattanakul et al., 2015) and enrolled in the present study. Their characteristics as well as caries indices in terms of deft and DMFT (decayed, extracted/missing, filled teeth) during their first visit to the dental hospital of the Faculty of Dentistry, Mahidol University, are summarized in Table 1. All sortase-defective isolates in this study were collected at this dental visit. Information regarding caries indices in subsequent dental visits is also presented for 2 of the 3 subjects (Table S1). Furthermore, 6 years after the first visit, we collected a stimulated saliva specimen from Subject A and the persistence of harbored sortase-defective isolates was determined. All study procedures were approved by the Ethics Committee of Mahidol University (MU-IRB 2008/084.1206), and all subjects as well as the parents of child subjects approved their participation.

2.2. Bacterial isolates

Fifteen *S. mutans* isolates from 3 subjects examined in our previous work (Lapirattanakul et al., 2011, 2015) were used in this study (5 isolates per subject) (Table 1). To isolate these organisms, we cultured stimulated saliva samples on mitis salivarius agar plates (Difco Laboratories, Detroit, MI, USA) containing bacitracin (0.2 U/ml; Sigma, St. Louis, MO, USA), 0.001% (v/v) tellurite solution (Becton, Dickinson and Company (BDC), Franklin Lakes, NJ, USA), and 15% (w/v) sucrose. After 2 days of incubation, presumptive rough colonies of *S. mutans* were selected and confirmed to be *S. mutans* by results of biochemical tests, including fermentation of 1% mannitol, sorbitol, raffinose, or melibiose in a phenol red broth base (Difco), and negative findings for dextran aggregation. In addition, the same method was also used to identify 10 *S. mutans* isolates in a saliva sample obtained from Subject A. Serotype determination of these 10 isolates was done by PCR, as previously described (Lapirattanakul et al., 2011). The complete genome sequence of *S. mutans* strain UA159 (Ajdić et al., 2002), which does not have any sortase defect, was used as the control for all of the following experiments.

2.3. Sucrose-dependent adhesion assay

Assays of sucrose-dependent adhesion to a glass surface were performed as previously described (Kawabata & Hamada, 1999). Briefly, test strains were grown at 37 °C for 18 h at a 30° angle in brain heart infusion broth (BHI; Difco) containing 1% sucrose. Following incubation, the culture tubes were subjected to vigorous vortexing for 3 s, after which non-adhesive cells were transferred to fresh tubes.

Table 1
Characteristics and caries indices at the first dental visit of 3 subjects harboring sortase-defective *S. mutans*.

Subject	Sex	Age	Presented teeth[a]	Caries index[b]		Salivary *S. mutans* count (CFU/ml)	*S. mutans* isolates[c]
				deft	DMFT		
A	Female	7y 11m	6 DC21 | 12CD 6 6EDC21 | 12CDE6	6 (2, 2, 2)	3 (0, 0, 3)	1.4×10^3	**TLJ127-1**, TLJ127-2, TLJ127-3, **TLJ127-4**, TLJ127-5
B	Female	6y 4m	6EDCBA | ABCD 6 6EDC2 1 | 1 2 CD 6	13 (5, 2, 6)	0 (0, 0, 0)	5.3×10^5	TLJ74-1, **TLJ74-2**, TLJ74-3, TLJ74-4, TLJ74-5
C	Female	40y 0m	7654321 | 1234567 7654321 | 1234567	N/A	4 (0, 0, 4)	4.0×10^3	**TLJ10-3, TLJ10-4, TLJ10-5,** TLJ10-6, **TLJ10-7**

[a]Total teeth in oral cavity, as indicated by Palmer notation system.
[b]Numbers in parentheses indicate d, e, and f for deft index, or D, M, and F for DMFT index.
[c]Bold letters indicate sortase-defective isolates.
y = years; m = months.
N/A = not applicable.