

## Different Expression of mRNA of Lux-S in Low and High Caries Risk of Pre-School Children

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### Abstract

The *luxS* mRNA expressing autoinducer-2 (AI-2) is maintained among many species of bacteria, including *Streptococcus mutans*. AI-2 plays a decisive promoting role in biofilm formation and maturation. The aim of this study was to determine the *luxS* mRNA expression in relation to caries status and caries activity among pre-school children. The cross-sectional study included 76 pre-school children (31 boys and 45 girls) aged 3 to 5 years old, from a kindergarten in Jakarta, Indonesia. The children were screened for caries status using dmft index. Dental plaque samples were obtained for caries activity screening using Cariostat, and for *luxS* mRNA expression determination using quantification real time-PCR (qPCR). Out of 76 subjects examined *luxS* mRNA expression was determined in 43 subjects. We divided the subjects with *luxS* mRNA expression into two groups: group 1 consisted of children who are caries free (dmft zero) and had no caries activity (Cariostat score 0); group 2 was comprised of children with caries and caries activity were scored as 1 and above. There were 9 subjects in group 1 and 34 subjects in group 2, and the mean  $\pm$  SD of *luxS* mRNA expression was determined to be  $19.3 \pm 24.3$  ng/mL and  $41.7 \pm 29.1$  ng/mL, respectively, and statistically different ( $p = 0.03$ ). It can be concluded that the amount of *luxS* enzyme was lower in the caries free and no caries activity subjects compared to subjects with caries and caries active.

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### Introduction

Quorum sensing (QS) is a process by which bacteria communicate with each other by secreting chemical signals called auto inducers (AI). Among Gram-negative and Gram-positive bacteria, auto inducers -2 (AI-2) synthesized by the *luxS* enzyme is common.<sup>1</sup>

AI-2 is an important signal molecule in multi-species biofilms. AI-2 plays a decisive promoting role in biofilm formation and maturation. The *luxS* gene is found in many species of bacteria,<sup>2</sup> including *Streptococcus mutans*, *Streptococcus oralis*, *Porphyromonas gingivalis*, *Aggregatibacter actinomyces* *ceemcomitans* and other oral micro organisms.<sup>3</sup>

AI-2 can also mediate oral *Streptococci* and *Actinomycetes* to form mixed biofilm<sup>4</sup>, where the formation of biofilm depends on AI-2 produced by oral *streptococci*.<sup>5</sup>

In recent years, reports of interference of these bacterial QS systems and new ways of inhibiting biofilm formation, has opened new avenues for intractable infectious disease research.<sup>6,7</sup> To date the *luxS* gene expressed by oral *streptococci* in dental biofilm and its relation to caries activity has not been studied. The purpose of this study was to determine the expression level of *luxS* gene by oral *streptococci* in dental plaque, and its relation with caries activity in preschool children.

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### Methods

#### Caries status

Seventy-six children, aged 3 to 5 years old, from a kindergarten in Jakarta, Indonesia (39

boys and 37 girls) were recruited for this study. They were divided into two caries status groups, 15 caries free children and 61 children with caries whose decayed, missing, and filled tooth surface (dmfs) scores were  $9.3 \pm 5.3$ . Written informed consent was obtained from all parents, and the observational procedures were approved by the Institutional Ethical Committee of the Faculty of Dentistry, Universitas Indonesia. Pooled samples of dental plaque were taken with sterile cotton swabs from the buccal surfaces of the first mandibular primary molar.

The samples were immediately placed in sterilized tubes containing PBS with 2% sodium thioglycollate, stored on ice, and transferred to the laboratory within two hours.

### Cariostat

For caries activity testing, the cotton swabs with dental plaque samples were put into the Cariostat ampoule then incubated at 37°C for 48 hours. The CARIOSTAT test reagen contains sucrose and carbon source. The results were determined by color change of the Cariostat due to acid production by degradation of sucrose. The result were determined based on the change of pH values between 4.0 and 7.0, which was indicated with a color scale including Blue, Green, Yellowish Green, and Yellow.<sup>8</sup>

Scoring was done by comparing the test tubes against a white background to determine color change in pH. The Cariostat result shows the tendencies of bacterial information in the oral cavity environment through the variation of bacteria in the dental plaque, which is a result of differences in eating habits, oral care, and lifestyles.

The caries activity indicated by the Cariostat test shows the amount of dental caries and the speed of caries development. Therefore, it is necessary to refer to the previous test result in order to evaluate the risk of caries.

### Qualitative real time-PCR (qPCR)

Total RNA from growth in the THB media at 1, 5, 10, and 14 hours were extracted using the Trizol reagent (Invitrogen), according to the manufacturer's protocol. SYBR Premix Ex Taq (TAKARA) was used for analysis (ABI PRISM 7300). The 16S rRNA gene was a housekeeping internal control. Both qPCR primers for detecting *luxS* mRNA and 16 sRNA gene was as reported by SYBR Green was measured. The formula of

$2^{-\Delta\Delta C_t}$  was used to measure the level of *luxS* mRNA.<sup>9</sup>

### Results

Out of 26 subjects examined in the caries free group (dmft zero) using Cariostat, a caries inactive status (score 0) with a pH of 6.8 was shown in 13 subjects (50%), a caries active status (score 1) with a pH of 5.2 was shown in 11 subjects (42.3%), while 2 subjects had a pH of 4.5 (7.7%) (score 2), and none showed marked caries activity (score 3), with a pH of 3.6 (Table 1).

**Table 1.** The distribution of cariostat scores based on caries status

Cares status	Cariostat scores	N (%)
Caries free	0	13 (50%)
	1	11 (42.3%)
	2	2 (7.7%)
	3	0
Caries	0	11 (22%)
	1	16 (32%)
	2	16 (32%)
	3	7 (16%)

Of the 50 children in the caries group, 11 had a caries inactive status (22%) (score 0), 16 had a caries active score with a pH of 5.2 (32%) (score 1), 16 had a pH of 4.5 (32%) (score 2) and seven had a pH of 3.6 (16%) (score 3). The expression of the *luxS* mRNA enzyme was determined in 15 subjects out of 26 caries free children and in 28 subjects out of 50 children with caries.

Finally, we divided the subjects with *luxS* mRNA expression into two groups: group 1 (no caries) consisted of caries free (dmft zero) and no caries activity (Cariostat score 0) children; and group 2 (caries) consist of children with caries and caries active (Cariostat scores 1 and above). Of the 9 subjects in the group 1 and 34 subjects in group 2 the mean  $\pm$ SD of *luxS* mRNA expression determined were  $19.3 \pm 24.3$  ng/mL and  $41.7 \pm 29.1$  ng/mL respectively. After statistically analysis using Student's t-test, the *luxS* mRNA expression in group 1 was significantly lower than in group 2 ( $p = 0.03$ ).

### Discussion

*Streptococcus mutans* is recognised as a major aetiological agent of dental caries. One of

its important virulence factors is its ability to form biofilms on tooth surfaces. Previous studies of the *S. mutans luxS* gene has demonstrated that *luxS* is required for a variety of cellular processes ranging from biofilm formation to acid tolerance.<sup>10</sup> Previously, it has been reported that *S. mutans* interacts with the other oral flora of the dental plaque to mediate interspecies communication known as *luxS*-mediated QS.<sup>11</sup>

The *luxS*-mediated QS is well characterized to elicit interspecies communication and modulate multiple traits crucial to the establishment of *S. mutans* pathogenesis. Hence, the *S. mutans* flourishes in the buccal cavity via, the activation of the *luxS* gene which leads to the production of AI-2 and ensures its survival and virulent expression in multispecies environment. Researchers have shown that *luxS* deficient strains affects the expression of the virulence determinant to a greater extent (>50 %) and in parallel up regulate their acid-adaptive behaviors to increase their survival rate.<sup>12</sup> Additionally, the *luxS* gene is highly conserved among the Gram positive and Gram negative bacteria and may operate as a global regulator and so be an essential factor for a drug target.<sup>11</sup>

In this study, the subjects were divided into two groups, the first group was “no caries risk” due to dmft zero as well as the Cariostat scored of zero. The second group was “caries risk”, consisting of subjects with caries and Cariostat scored one and above. After the PCR procedure, the expression level of the *luxS* gene was determined in 43(56.6%) subjects and undetermined in 33 (43.4%) subjects. Out of the 43 subjects with determined *luxS* expression, 9 children were allocated as “no caries risk” while 34 children were “caries risk”. The results of this study showed that the level of *luxS* mRNA expression in subjects with “no caries risk”, were significantly lower compare to “caries risk” subjects.

These findings support the theory that *S. mutans luxS* enzyme is required for biofilm formation as well as acid tolerance.<sup>10</sup> The result of this present study showed relationship between low-pH and higher amount of *luxS* seems consistent with previous findings that found a direct linkage between low-pH survival and dental caries. Cariogenic *streptococci*, such as *S. mutans*, need to survive within acid environment as a result of lactic acid production

through fermentable sugars in dental biofilm.<sup>13</sup>

The formation of biofilm depends on AI-2 produced by oral *Streptococci* mediated by the *luxS* enzyme. It is already known that bacteria expressing a mutation in the *luxS* gene in oral *Streptococci* cannot express AI-2, resulting in a failure of communication between oral *Streptococci* and Actinomycetes. This failure leads to loose biofilm formation and significantly decreased bacterial density.<sup>5</sup> In addition, there is enormous functional diversity in poly microbial biofilm development in the oral cavity. For instance, AI-2 of *Aggregatibacter actinomy cetemcomitans* inhibits biofilm formation by *Candida albicans*.<sup>14</sup>

Finally, the findings of this study may provide important understanding into the utilization of Cariostat as an uncomplicated diagnostic tool for predicting the dental caries risk based on the molecular *luxS* functionality in the development of dental biofilm.

## Conclusions

Based on our results it can be concluded that the amount of the *luxS* enzyme was lower in caries free and no caries activity subjects by comparing to subjects with caries and caries active.

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