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The Effect of *LuxS* on the Biofilm Formation of *Streptococcus mutans*

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To construct *LuxS* deletion mutant of *Streptococcus mutans*, and study the effect of luxS mutation on the biofilm formation of streptococcus mutans under various conditions, and find out the differences between luxS mutant strain and streptococcus mutans. Long flanking homology polymerase chain reaction(LFH-PCR) was introduced to generate a gene disruption construct consisting of Emr cassette with long flanking homology regions to the target gene. The electroporation competence of *Streptococcus mutans* was then transformed with this PCR product. Then positive transformants were counted on selective agar which containing erythromycin and identified by PCR. The streptococcus mutans-luxS mutant and the standard strain were grown in three different conditions(BHI, 2% glucose-BHI, 2% saccharobiose-BHI), and the ability of S.mutans and LuxS mutant biofilm formation was examined in 24 h by scanning electron microscopy (SEM). Identification by PCR and sequencing confirmed the validity of the *LuxS* deletion mutant of *Streptococcus mutans*. Compared with S.mutans ,the LuxS mutant maintained with 2% sucrose displayed an apparent defect in biofilm formation. Conclusions: The successful construction of the LuxS deletion mutant, and the ability of sucrose-dependent biofilm formation will be down-regulated in Streptococcus mutans after LuxS gene was knocked out.

Key words: *Streptococcus mutans*; quorum sensing; *LuxS gene.*

Mature dental plaque is structurally and compositionally complex bacterial communities, which include more than 750 different species or phylotypes of bacteria. Streptococcus mutans(S. mutans) is a key etiological agent of caries, which acid end-products can cause dissolution of enamel. Quorum sensing(QS) means that the ability of bacteria to sense and respond to population density. Many bacterial physiological functions are regulated by QS systems, which include luminiescence, virulence,motility, biofilm formation, etc. LuxS-mediated signaling pathway is the enzyme that catalyzes the reactions leading to the production of the AI-2 signal molecule, and also affects biofilm formation and bacteriocin production in *S. mutans*[1-3].

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MATERIALS AND METHODS

Bacterial strains and growth conditions

S. mutans UA159 and its derivatives was maintained in Brain Heart Infusion. For selection of antibiotic-resistant colonies after genetic transformation, erythromycin (0.25 mg/L for S. mutans) was added to the medium. For bioûlm formation assays, *S. mutans* strains were grown in the Brain Heart Infusion supplemented with glucose or sucrose at a final concentration of 2%(*m*/*v*).

Construction of mutant strains and transformation assays

The schematic diagram of LuxS –deletion sees the Fig1.Primers used for deletion mutagenesis are listed in Table 1.

To construct LuxS -deletion mutant of S. mutans and get the capsule deficient strain which can be used in following research of LuxS genes of *S. mutans*, LFH-PCR was introduced to generate a gene disruption construct consisting of erm cassette with long flanking homology regions to the target gene. Then S. mutans strain UA159 was transformed directly with this PCR product. The LuxS-deletion mutant was obtained on the BHI agar containing erythromycin and identified by PCR and sequencing. The results implicated that LuxS gene was completely replaced with erm cassette.

Biofilm formation

S. mutans UA159 and its *LuxS* mutans was inoculated into 5-ml portions of BHI in 24-well polystyrene microtiter plates to form bioûlms. After 24h of incubation at 37°C under anaerobic conditions, the ability of biofilm formation was examined by scanning electron microscopy.

SEM

Biofilms grown on enamel for 24 h were rinsed by gently dipping the disks in PBS buffer and fixed with Trump fixative solution overnight. Fixed samples were then dehydrated through a graded series of ethanol concentrations, mounted, and sputter coated with gold-palladium. Samples were analyzed by SEM.

RESULTS

The *Streptococcus mutans* UA159 were grown in three different conditions, and the ability of its biofilm formation were examined in 24h by scanning electron microscopy (SEM x1000).

The LuxS gene mutans were grown in

Table 1. Primers used for construction of deletion mutants in this study

	Primer	bp
Primer1	5' -GCTTTTACCTCAAAATATTATTG-3'	144
Primer2	5' -GATACTTTGGATTATTTGCTTA-3'	129
Primer3	5' -ATCAAACAAATTTTGGGCCCGGAGTAAACTCCTTTTAGTTTT-3'	144
Primer4	5' -AATTCTATGAGTCGCTGCCGACTAATAAAAAGAGGATGGATACCC-3'	129
DAM212	5' -CCGGGCCCAAAATTTGTTTGAT-3'	780
DAM213	5' -AGTCGGCAGCGACTCATAGAAT-3'	780
L1	5' -ATGACAAAAGAAGTTACTGTTG-3'	483
L2	5' -TTACACTAGATGACGCTCAAAAGG-3'	483

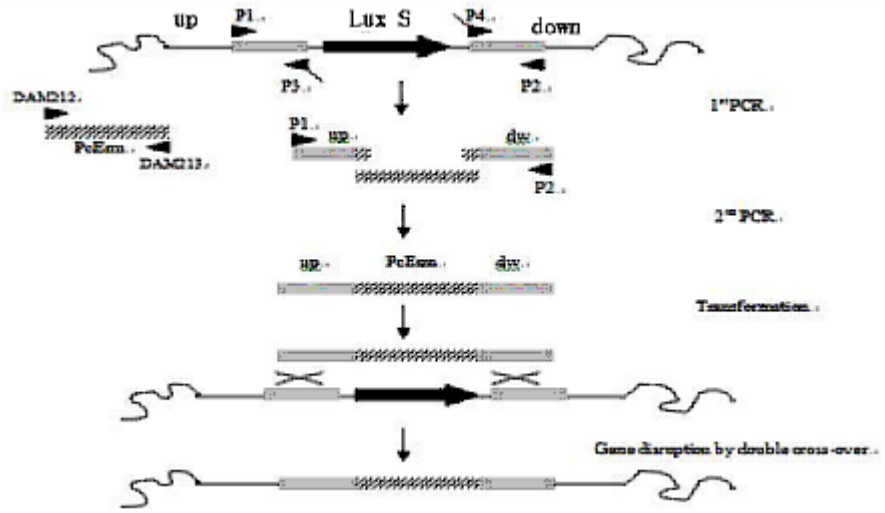
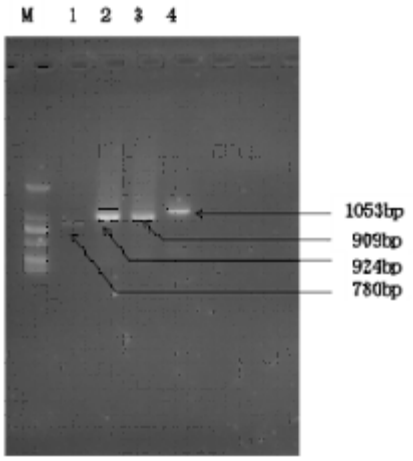


Fig. 1. The schematic diagram of LuxS –deletion



M:maker; 1:mutans:erm(780bp); 2: mutans:erm-UP(924bp); 3: mutans:erm-down(909bp); 4: mutans:UP-erm-DOWN(1053bp)

Fig 2. The PCR results of Lux *S mutans*

Fig. 4. 2% glucose-BHI

The LuxS gene mutans were grown in three different conditions,and the ability of its biofilm formation were examined in 24h by scanning electron microscopy (SEM x1000)

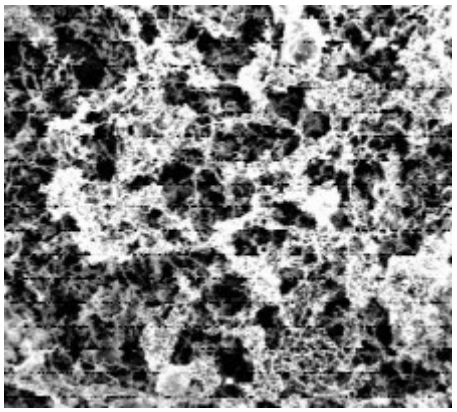


Fig. 6. BHI

The Streptococcus mutans UA159 were grown in three different conditions,and the ability of its biofilm formation were examined in 24h by scanning electron microscopy (SEM x1000)

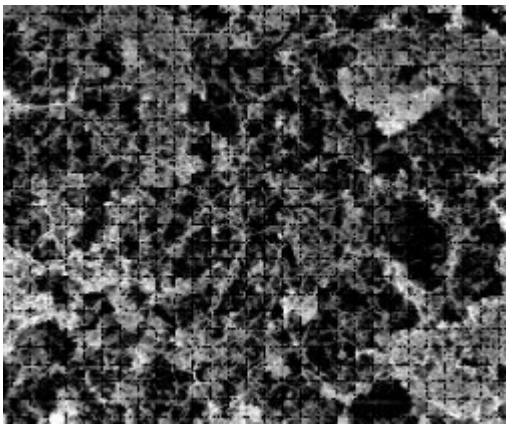


Fig. 3. BHI

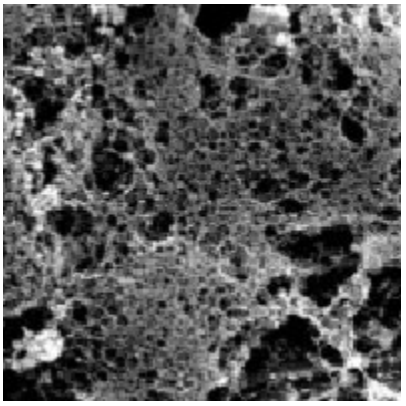


Fig. 5.2% sucrose-BHI

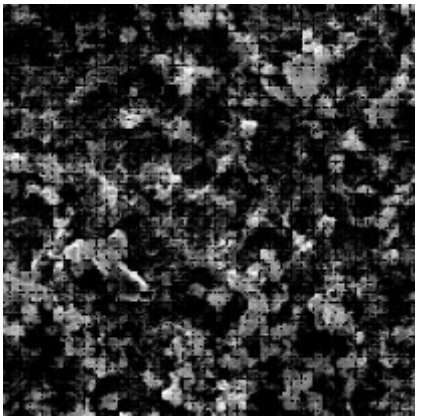


Fig. 7. 2% glucose-BHI

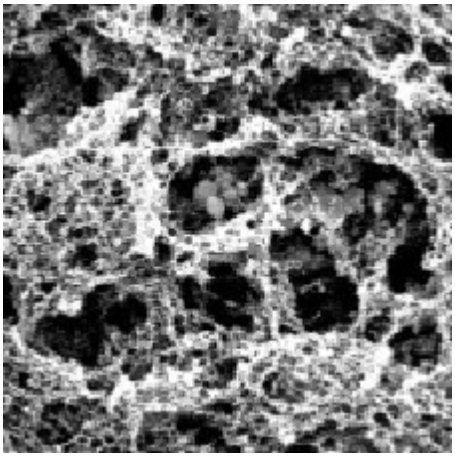


Fig. 8. 2% sucrose -BHI

The strains were grown in 2% sucrose-BHI,and the ability of *S.mutans* and LuxS mutant biofilm formation were examined in 24h by scanning electron microscopy (SEM x40000).

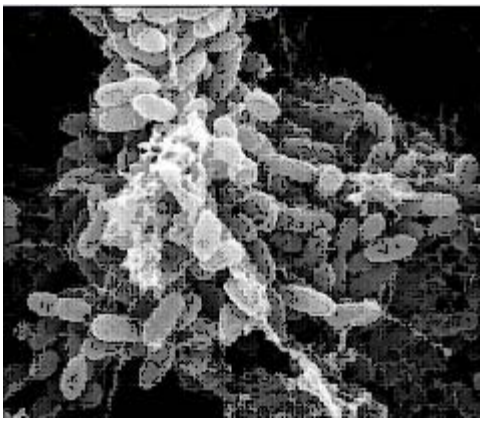


Fig. 9. *Streptococcus mutans* UA159

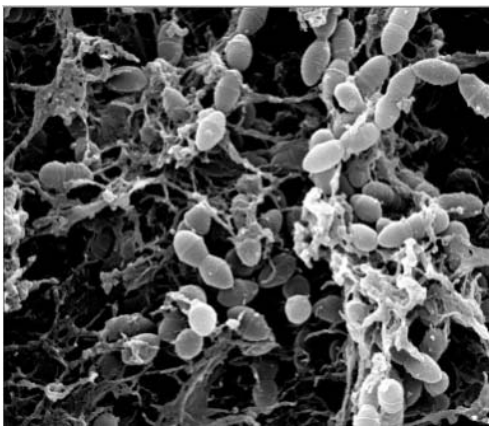


Fig. 10. *LuxS* gene mutans

DISCUSSION

Bacteria in the biofilm, cells have unique phenotypic characteristics, which can exhibit increased to resist different unfavourable factors, which are different from their planktonic counterparts, such as environmental stresses, resistance to antimicrobial compounds and host immune defense mechanisms. unfavourable factors. The dental plaque is the net result of structurally and compositionally complex bacterial communities, which form well-differentiated structures with distinct thickness by bacteria cooperating to.

By interfering with quorum sensing signaling mechanism, *S. mutans* which use cell-cell to control virulence could potentially be attenuated. LuxS-mediated signaling pathway is affects biofilm formation, acid tolerance, bacteriocin production, and the transformation of S. mutans have been demonstrated in many studies[4-6].
Firstly, LuxS gene fragments were amplified from the S. mutans DNA 5'- and 3'- region, in this way that the ET marker which has the base pairs extensions homologous to add to one of their end. Secondly, long primer which is from the one strand of each of these molecules by a ET as template in a PCR. Finally, the PCR-made disruption cassette flanked by short homology regions was replaced by LFH-PCR-generated disruption cassette, transformation into the *S. mutans*.

In this study,the biofilms of *S. mutans* UA159 and its mutans were grown for 24 h in different conditions(BHI,2% glucose-BHI,2% sucrose-BHI). Compared with S.mutans UA159, the luxS mutant maintained in BHI medium containing 2% sucrose displayed an defect in biofilm formation,while they showed no significant deviation in BHI medium containing 2% glucose by scanning electron microscopy at 1000.Huang ZW *et al* report that LuxS mutant started to form a biofilm from the 6th hour, the wild-type strain start to form biofilm from the 10 th hour.The biofilm were no difference after 12 hours, and a luxS-dependent signal may play an important role in the early biofilm formation of *Streptococcus mutans.* May be this is reason that there were no difference in biofilm formation between wild-type strain and mutans in BHI, 2% glucose-BHI in our study.

From the SEM results,there were no

difference in bacteria shape between wild-type strain and mutans,however,the biofilms of the luxS mutant formed loose and rare in sucrose medium compared to the wild-type strain.Therefore, luxS gene in *S.mutans* can affect biofilm formation in dental plaque, and the luxS gene is possible to regulate sucrose –dependent biofilm formation. Mi Y report that biofilm formation by the luxS mutant was easier desquamation than wild-type strain. Scanning electron microscopy (X40000)also revealed that biofilms of the wild-type strain formed larger clumps and the exocellular polysaccharide secretion dense in sucrose medium compared to the luxS mutant in our study. We guess that it may explain the mutans was easier desquamation in Mi Y's study.

In a word, luxS-dependent interspecies quorum sensing can play a role in biofilm formation.

CONCLUSIONS

1. The successful construction of the LuxS deletion mutant can be used in further functional genome research.
2. The *Lux*S quorum sensing was blocked,the growth of *Streptococcus mutans* will be change. While the environment is full with abundant nutrition, the quantity of bacterial was increase.
3. *Lux*S gene in S.mutans can play a role in dental plaque biofilm formation, and the *Lux*S gene is possible to regulate sucrose – dependent biofilm formation.
4. There were no significant difference in biofilm cell soluble protein expression between Streptococcus mutans and its mutans at early mature stage.

ACKNOWLEDGMENTS

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