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Modulations of circular RNAs on characteristics of *Streptococcus mutans* biofilm

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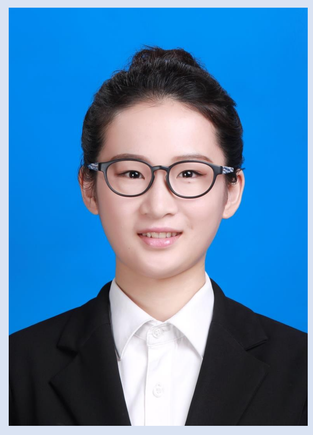

Acknowledgements: This study was conducted at State Key Laboratory of Oral Diseases, West China School of Stomatology, Sichuan University. This work was supported by grants from the National Natural Science Foundation of China (grant Nos. 81771068, 81670980, and 81800964), and Sichuan International Science and Technology Innovation Cooperation (Grant No. 2020YFH0010).

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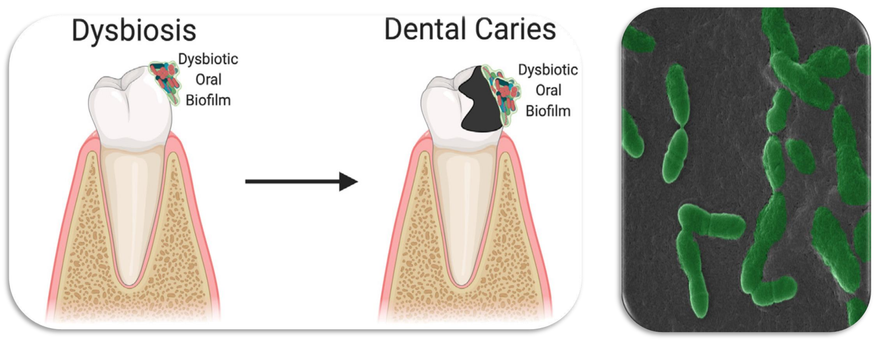
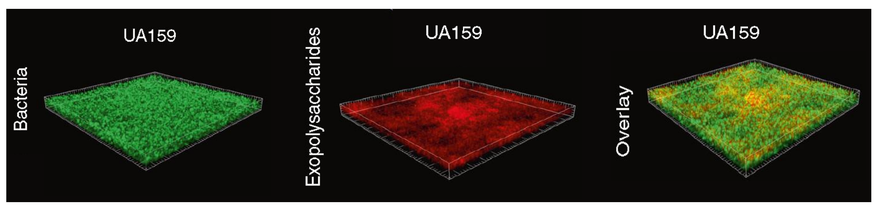
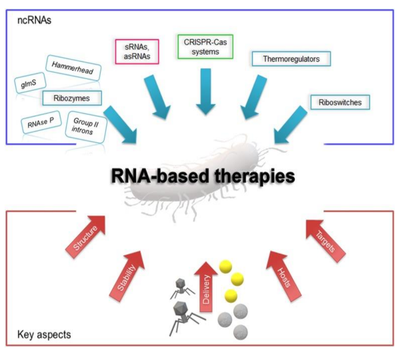
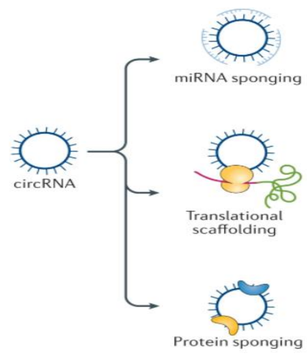
Introduction



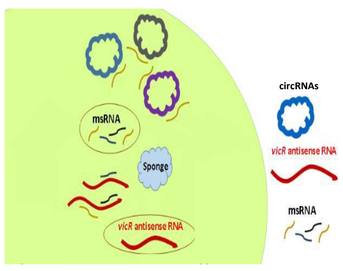


Streptococcus mutans is a major contributor to caries because of its synthesis of **Exopolysaccharides (EPS)** that aid in the formation of plaque biofilm.





Circular RNAs (circRNAs), a novel class of ncRNAs, contributes to diverse biological functions in eukaryotes.

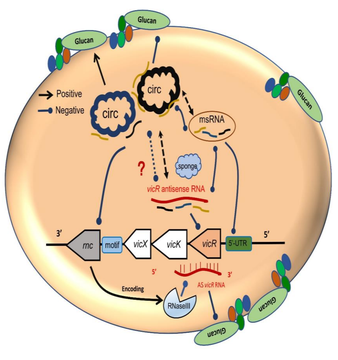


This study was to investigate the role of circRNAs playing in the post-transcriptional regulations of *Streptococcus mutans* on biofilm formation.

Materials and Methods

circRNA sequencing → circRNA expression profile analysis
circRNA secondary structure → RNAfold
circRNA splicing sites → sanger sequencing
quantification of circRNAs and genes → (q)RT-PCR
construction of overexpression strains → shuttle plasmid

Conclusion



circRNAs modulate EPS production and acidogenic capacity, which might induce the cariogenicity of *Streptococcus mutans*. This study brings us implications on an in-depth understanding of bacterial post-transcriptional regulation conducted by **regulatory RNAs**.

Results and Discussion

Fig.1 Screening and expression of circRNAs in UA159 24h biofilm and planktonic cells. **A.** circRNA expression profile analysis. **B.** Functional categories and enrichment analysis for the originated genes of differentially expressed circRNAs.

20 circRNAs were up-regulated, whose length was 150-300 nt.
31 circRNAs were down-regulated, with length 150-200 nt.
Up-regulated circRNAs were involved in **glucose metabolic process** in GO (Gene Ontology) terms.

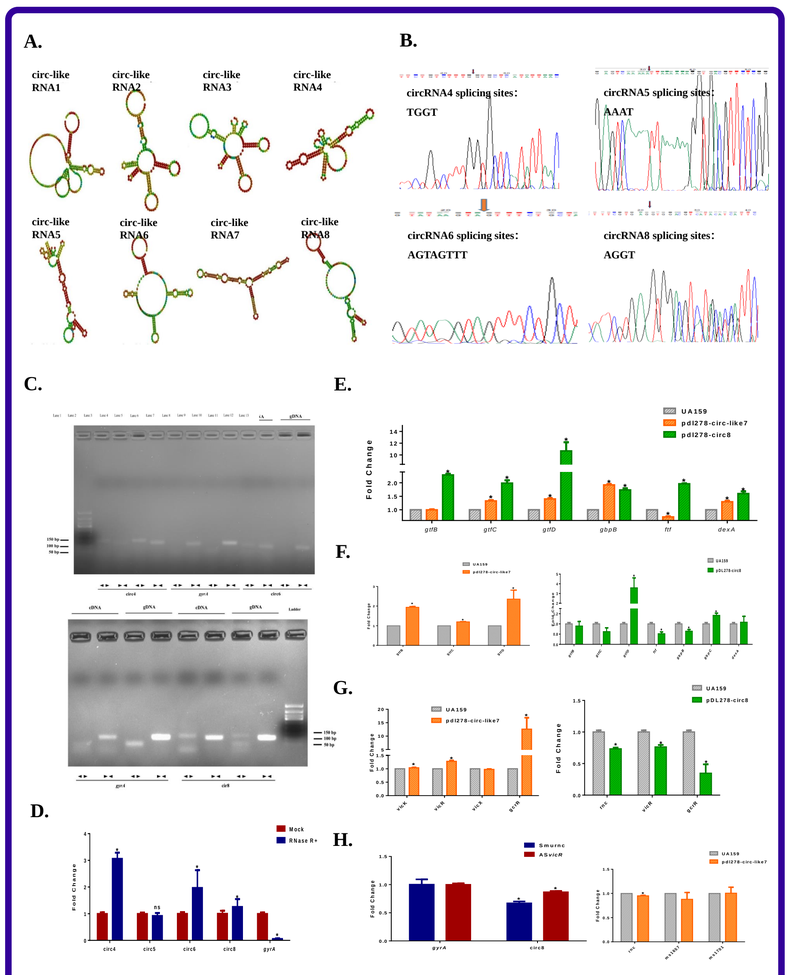


Fig.2 A-D. Confirmation of the circular characteristic of highly expressed circRNAs. **A.** Bioinformatics analysis of the circRNAs: secondary structure. **B.** The existence of circRNAs splicing sites was validated by sanger sequencing. **C.** The existence of circRNAs was validated by RT-PCR. Divergent primers amplified circRNAs in cDNA with *gyrA* serving as a negative control. **D.** RNA was treated with or without RNase R for qRT-PCR. The relative levels of circRNAs and *gyrA* mRNA were normalized to the values measured in the mock-treated group. Different expressional levels of EPS-associated genes in **E.** planktonic cells; **F.** biofilm cells detected by qRT-PCR. Effects of circRNAs overexpression on the transcription of genes involved in **G.** two-component signal transduction system; **H.** post-transcriptional signal system.

Fig.4 Biofilms of circ7 overexpression strain had increased adhesion force and lactic acid production. Overexpression of circ8 slightly enhanced biofilm formation. EPS/bacteria ratios of circ7 overexpression strain were dramatically higher.

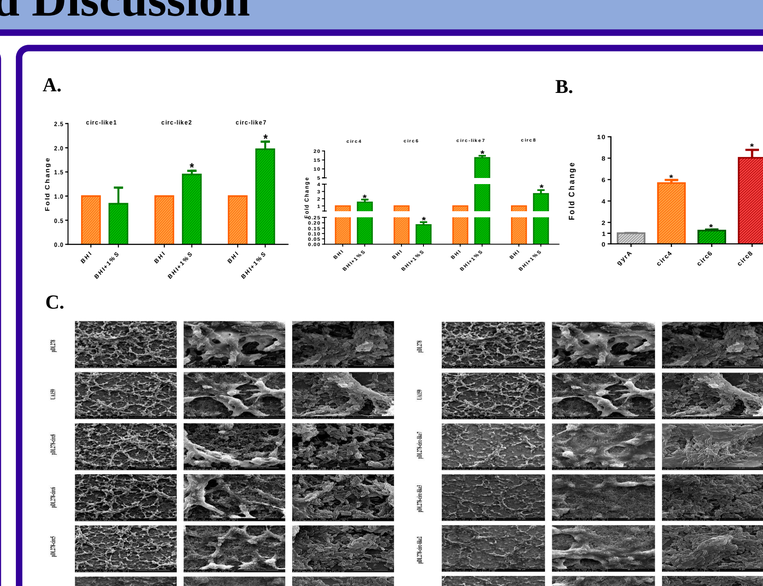


Fig.3 Detection of functional circRNAs. **A.** Different expressional levels of circRNA with added sucrose detected by qRT-PCR. **B.** Different expressional levels of circRNA in biofilm cells detected by qRT-PCR. Representative images in **C.** Scanning electron microscopy (SEM); **D.** Atomic force microscopy (AFM) observation of biofilms grown by the mutants.

we found **circular-structure RNA (circ8) and circular-like RNA (circ7)** that might account for sucrose degradation and biofilm formation of *Streptococcus mutans*.

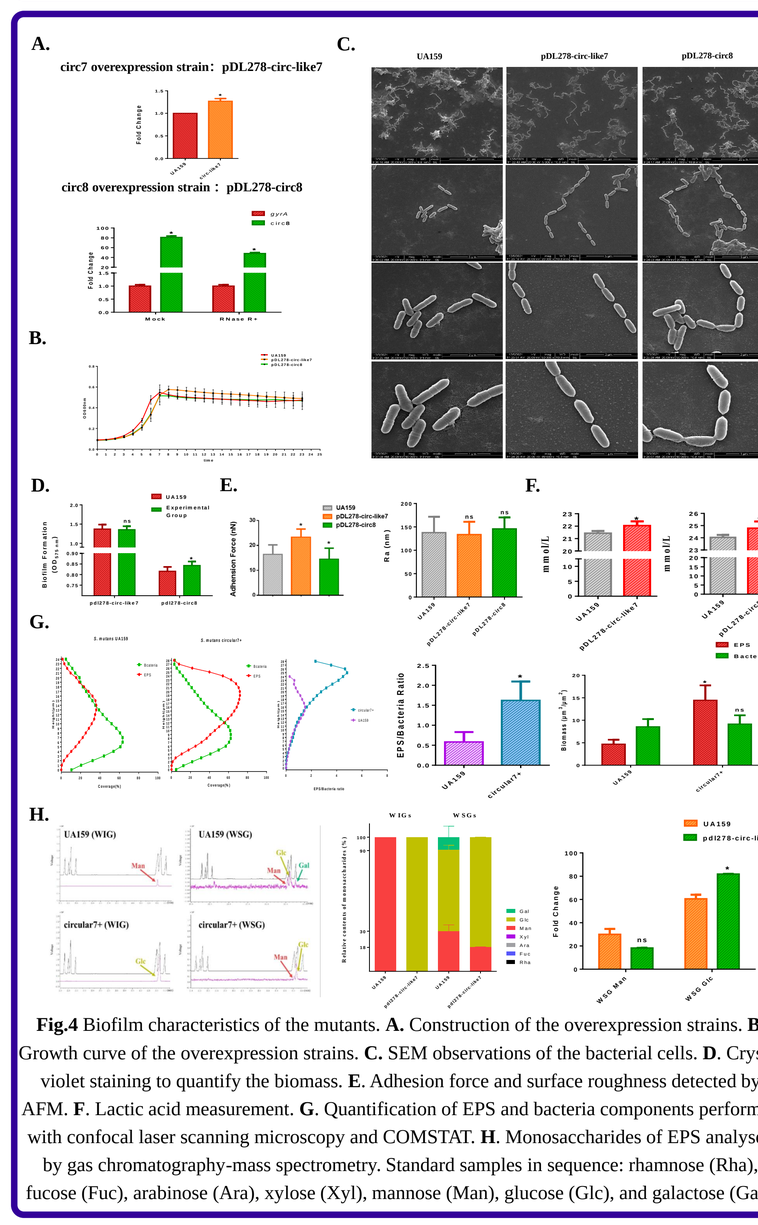


Fig.4 Biofilm characteristics of the mutants. **A.** Construction of the overexpression strains. **B.** Growth curve of the overexpression strains. **C.** SEM observations of the bacterial cells. **D**. Crystal violet staining to quantify the biomass. **E**. Adhesion force and surface roughness detected by AFM. **F**. Lactic acid measurement. **G**. Quantification of EPS and bacteria components performed with confocal laser scanning microscopy and COMSTAT. **H**. Monosaccharides of EPS analysed by gas chromatography-mass spectrometry. Standard samples in sequence: rhamnose (Rha), fucose (Fuc), arabinose (Ara), xylose (Xyl), mannose (Man), glucose (Glc), and galactose (Gal).

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