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The Sec secretion system of *Streptococcus mutans* regulates the formation of persisters by affecting EPS production

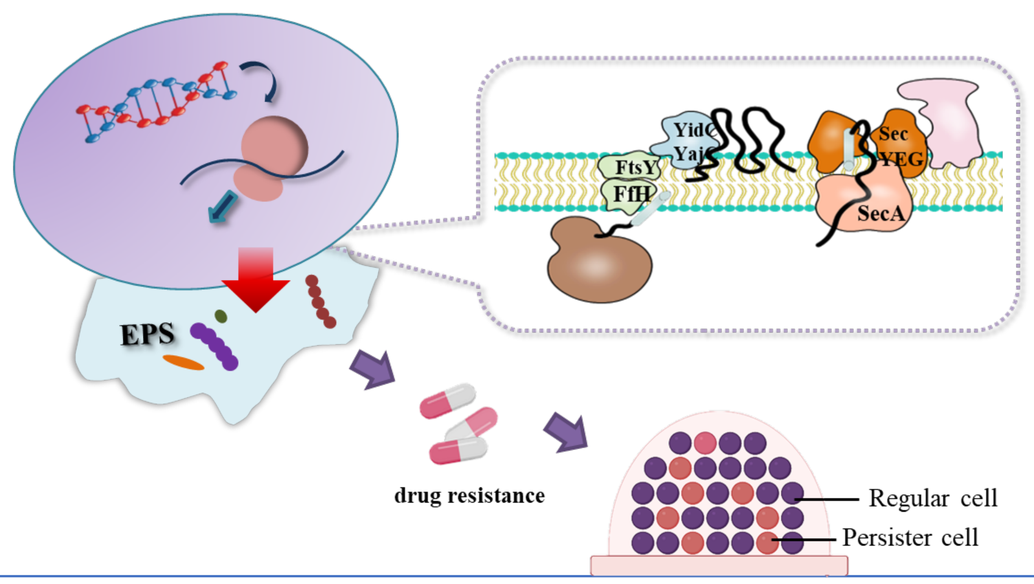
Yuyao Huang [1,2], Yaling Jiang[1,2], Hao Li [1,2], Biao Ren [1*], Lei Cheng [1,2,*]

[1] State Key Laboratory of Oral Diseases &West China Hospital of Stomatology & National Clinical Research Center for Oral Diseases, Sichuan University, Chengdu, 610041, China

[2] Department of Operative Dentistry and Endodontics, West China Hospital of Stomatology, Sichuan University, Chengdu, 610041, China

INTRODUCTION

Biofilms are crucial for bacterial resistance and refractory infections, which contain a high proportion of persister cells. *Streptococcus mutans* acts as the main cariogenic bacterial species. Persisters of S. mutans in biofilms significantly increased their ability to produce exopolysaccharides (EPS). However, the specific mechanism is still unclear. In this study, we investigated the mechanism of *Streptococcus mutans* drug-tolerant persister cells induced by novel quaternary ammonium: dimethylaminododecyl methacrylate (DMADDM).



MATEIALS & METHODS

The antibacterial quaternary ammonium DMADDM was synthesized. The following strains were used in this study: sec secretion system mutant strain and complement strain, wild type of *Streptococcus mutans*. The high concentration of DMADDM was used to induce the formation of persistent cells in biofilms, and the persistence and heritability assay were measured by CFU count, dead and alive staining and MIC detection. The production of extracellular polysaccharides(EPS) was measured by anthrone sulfuric acid method and crystal violet staining. The transcriptome sequencing of persister cells in biofilm was tested.

RESULTS

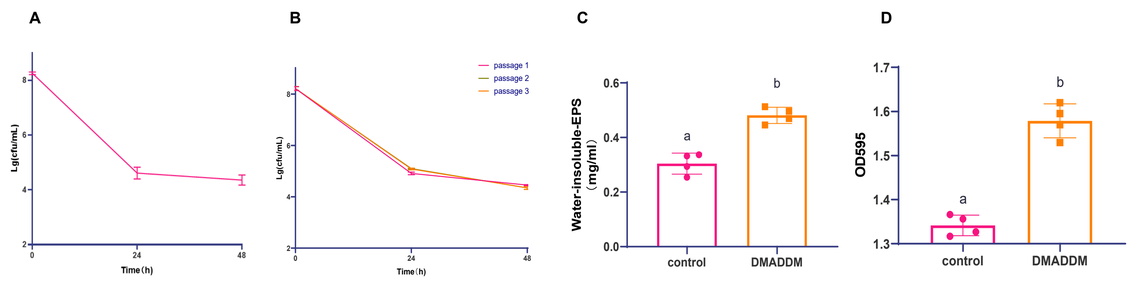


Figure 1 Formation of non-heritable DMADDM-tolerant S. mutans persisters. A, Time-dependent killing curves of S. mutans biofilm cultures (n = 3). **B,** Non-heritable nature of S. mutans biofilm persisters.(n = 3). **C,** The water-insoluble glucans of persister and untreated seed control biofilms (n = 4). **D,** Crystal Violet Staining of persister and untreated seed control biofilms (n = 4). each values is mean ± SD (The different letters indicate the significant difference between the bars)

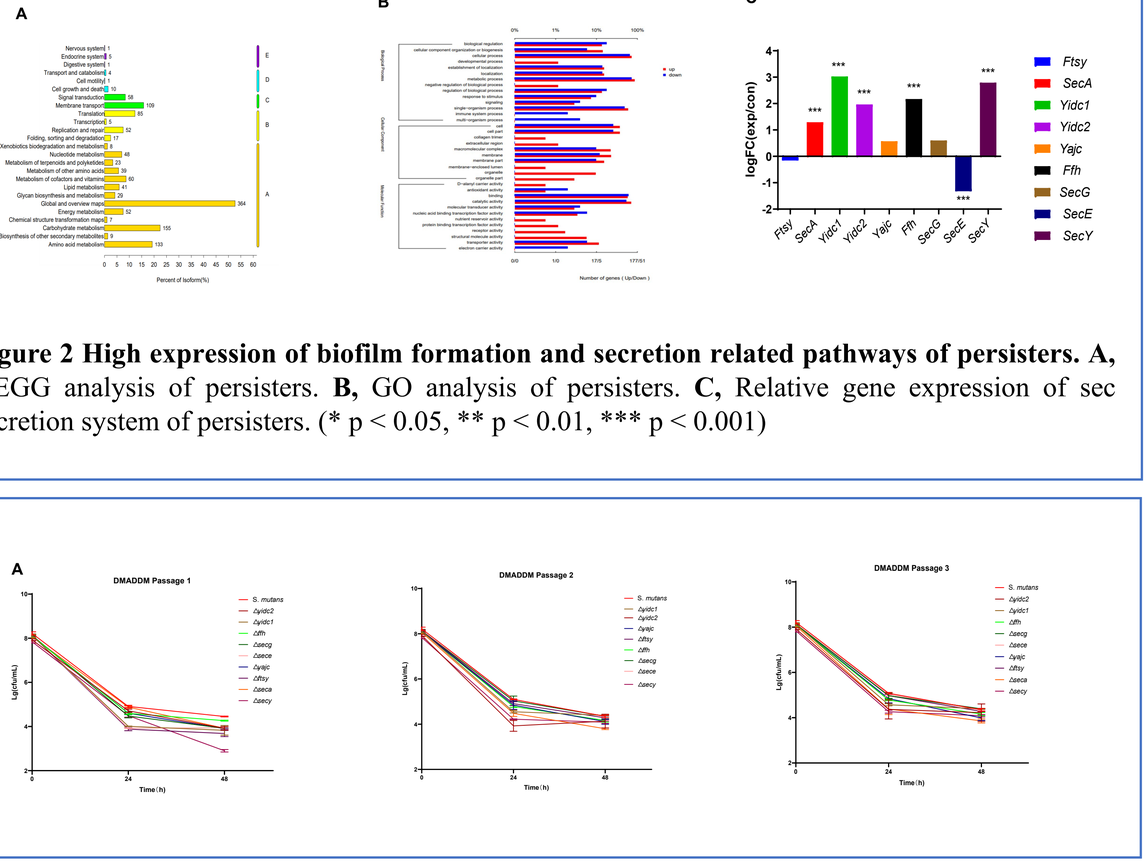


Figure 2 High expression of biofilm formation and secretion related pathways of persisters. A, KEGG analysis of persisters. **B,** GO analysis of persisters. **C,** Relative gene expression of sec secretion system of persisters. (* p < 0.05, ** p < 0.01, *** p < 0.001)

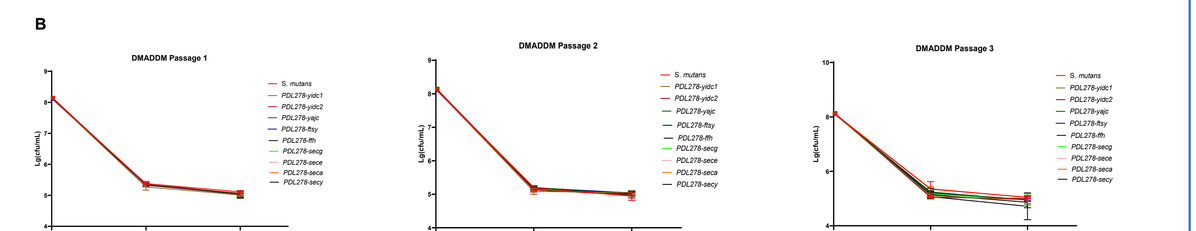


Figure 3 Formation of non-heritable DMADDM-tolerant persisters of the sec secretion system gene knockout and replacement strain. A, Non-heritable nature of sec secretion system gene knockout strain biofilm persisters.(n = 3). **B,** Non-heritable nature of sec secretion system gene replacement strain biofilm persisters.(n = 3). Data are presented as mean ± standard deviation. (The different letters indicate the significant difference between the bars)

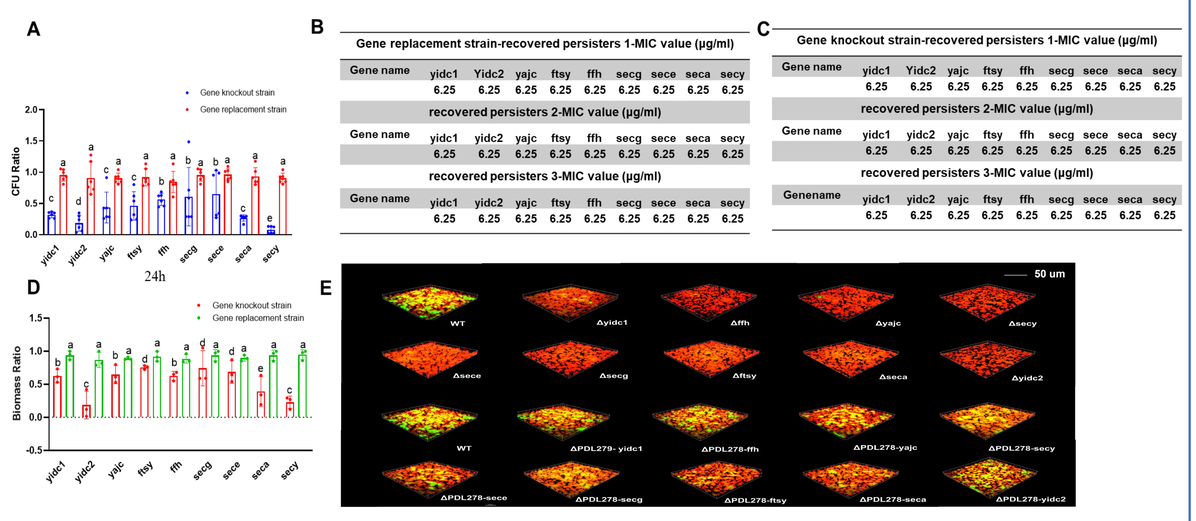


Figure 4 Compared with WT, the percentage of sec secretion system gene knockout strains forming persisters was significantly decreased A, The CFU ratio of sec secretion system gene knockout and replacement strains compared to WT.(n = 4). **B,** Minimal inhibitory concentration of sec secretion system gene knockout strains. **C,** Minimal inhibitory concentration of sec secretion system gene replacement strains. **D,** The ratio of fluorescence intensity of live bacteria compared with wild type (n = 3). **E,** Representative live/dead staining images of DMADDM induced *S. mutans* persister cells and untreated seed control biofilm (live bacteria, stained green; dead bacteria, stained red). Data are presented as mean ± standard deviation. . (The different letters indicate the significant difference between the bars)

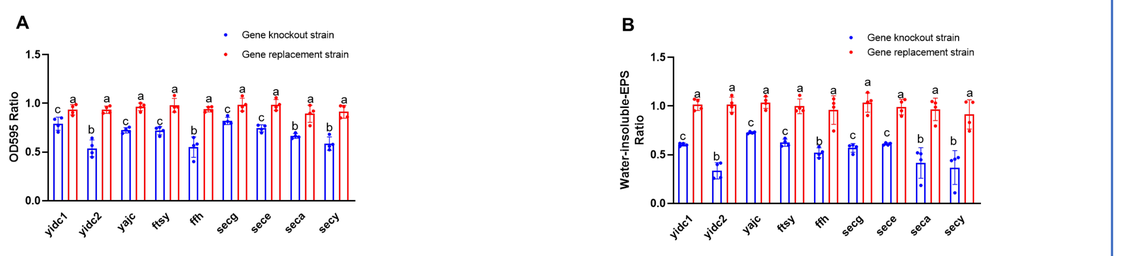


Figure 5 DMADDM-induced sec secretion system gene knockout mutants significantly reduce the extracellular polysaccharides (EPS). A, The ratio of crystal violet staining of biofilms (n = 4). **B,** The ratio of water-insoluble glucans of persisterd of sec secretion system gene knockout and replacement (n = 4). Data are presented as mean ± standard deviation.. (The different letters indicate the significant difference between the bars)

CONCLUSIONS

In conclusion, an extremely high concentration of DMADDM could induce the formation of persister cells in biofilms of *Streptococcus mutans*. Compared with untreated seed control biofilms, the extracellular polysaccharide (EPS) production of persister cells was significantly increased. The sec secretion system gene knockout strains could affect the extracellular polysaccharide (EPS) production in *Streptococcus mutans*, thereby reducing the number of persister cells in biofilms. secA, secY, and yidC2 played essential roles in EPS production and secretion in the Sec secretion system. In short, extracellular polysaccharide (EPS) is essential for the formation of persister cells in biofilms, and the sec secretion system plays an important role in it.