Int J Clin Exp Med 2019;12(6):7545-7552
www.ijcem.com /ISSN:1940-5901/IJCEM0085379

Original Article

Regulation of SmpB on acidogenic/aciduric ability and expression and activity of aciduric virulence factor in *Streptococcus mutans* from caries-sensitive children

Yu Miao, Zhaojun Wang, Shuangyang Zhang, Lege Mode, Rina Huangsha

Department of Stomatology, The Fourth Affiliated Hospital of Inner Mongolia Medical University, Baotou 014030, China

Received September 11, 2018; Accepted February 8, 2019; Epub June 15, 2019; Published June 30, 2019

Abstract: Objective: The aim of this study was to explore the roles of SmpB on the acidogenic/aciduric ability and expression of aciduric virulence factor in Streptococcus mutans from caries-sensitive children. Methods: Four hundred and seventy-four children, between the ages of 3 and 5, were randomly selected from kindergarten. Non-irritating saliva was collected to identify those with streptococcus mutans. The ability to produce acid of different strains of streptococcus mutans was compared. A mutant strain of streptococcus mutans with ΔSmpB deficiency was constructed. Streptococcus mutans and strains without SmpB were sampled at the logarithmic growth stage and stable stage, respectively. Streptococcus mutans and train SmpB knockout strains were sampled at the logarithmic growth stage and stable stage, respectively. Expression of F-ATPase was determined by real-time PCR. Enzymatic activity of F-ATPase was also determined. Results: Children were divided into the high caries group and caries-free group. When pH was between 6.0 and 7.0, there were no significant differences in acid-producing and acid-resistance between the high caries group and caries-free group at the same PH. When pH was between 5.0 and 5.5, acid-producing and acid-resistant abilities of bacteria, in the two groups, were significantly inhibited by acid. Knockout of SmpB inhibited the growth of streptococcus mutans and significantly reduced acid-producing and acid-resistant abilities of streptococcus mutans. Expression and activity of F-ATPase in ΔSmpB deficiency strain decreased markedly. Conclusion: SmpB plays critical roles in the regulation of acidogenic/aciduric ability and F-ATPase expression activity during the cariogenic process.

Keywords: SmpB, acidogenicity, aciduricity, streptococcus mutans, caries

Introduction

Caries is the most common disease in the oral cavity, with a high incidence and wide prevalence, seriously endangering human oral and physical health. The World Health Organization (WHO) ranks dental caries as the third most important noncommunicable disease, after cardiovascular disease and cancer [1]. In China, although awareness of caries prevention has increased, incidence of caries is still as high as 50%. In some areas, it is more than 90% [2]. Prevalence of caries not only threatens human health, but also brings great loss to the social economy. Thus, prevention and treatment of caries has aroused great attention.

Caries is a chronic infectious disease mainly caused by bacteria and many factors. *Streptococcus mutans* is currently the main cariogenic bacteria recognized throughout the world. It depends on biofilm to live. It is one of the bacteria that forms dental plaque because of its adaptability to various environments in the oral cavity [3, 4]. It can adapt to the changing oral environment, becoming one of the main components of dental plaque. Cariogenic virulence mainly depends on the plaque biofilm adhering and aggregating on the dental face. In the biofilm, bacteria takes full advantage of carbohydrates to produce acid and inhibit the growth of other bacteria. *S. mutans* are also aciduric, which causes enamel demineralization, crystal disintegration, and formation of carious cavities [5, 6].

Acid production and acid tolerance are two major physiological characteristics of *S. mu-*

tans. In dental plaque, sugar in the food is metabolized via glycolytic pathways by bacteria, which generates organic acids within a short time. Generated lactic acid can lead to a decrease of pH in oral environment and enamel demineralization, resulting in caries. To adapt to the low pH in oral environment, bacteria maintains a neutral intracellular environment by acid adaptability or resistance [7, 8]. Bacteria adheres to the surface of teeth through adhesion mechanisms (such as sucrose-dependent transfer factor, general transcription factors (GTFs), and sucrose non-dependent factor, PAC) to form an acquired biofilm. Bacteria in the biofilm generates acids via carbohydrate metabolism by aciduric virulence factors (such as lactate dehydrogenase (LDH)), reducing the pH of the dental plaque. Accumulated protons in the environment enter the cells and acidize cell plasma by concentration gradients in the proton channel of F-ATPase on the cell membrane. Furthermore, acidity influences the activity of acid-sensitive enzymes in the cell plasma, greatly reducing the ability of cells to generate ATP. At the same time, acidic pH will lead to the destruction of cellular DNA, proteins, and other molecular structures. This requires that cells have the mechanisms to pump large amounts of protons out of the cytoplasm, which is the acid tolerance ability [8, 9]. Many acid-producing bacteria that are not resistant to acids are killed under low pH. They cannot participate in the occurrence and development of caries. However, *S. mutans*, lactobacillus, and *Streptococcus sobrinus* can conduct glycolysis and continuously produce acids under low pH. Their presence leads to enamel demineralization and remineralization imbalances, finally causing caries [10]. Therefore, bacteria with strong aciduricity can survive in the biofilm of dental plaque under extremely low pH and continuously produce acids, leading to caries. Acidogenicity and aciduricity are two necessary characteristics of cariogenic bacteria. Acid resistance is the precondition of continuous acid production, the most important virulence mechanism of micro-organisms and the most important condition for caries.

Membrane-bound proton-translocating ATPase can maintain a neutral environment in the cells by transporting protons out of cells to protect intracellular acid-sensitive enzymes and proteins. It can guard against acid lethality due to cariogenic bacteria. Under low pH, cariogenic bacteria can survive and continuously generate acids that result in a decrease in the pH until enamel demineralization occurs. Therefore, F-ATPase is the most important acid-resisting virulence factor [11-13]. SmpB protein is ubiquitous in prokaryotic bacteria. It has been found with the study of trans-translation, which is necessary in the trans-translation system of bacteria [14]. SmpB participates in the regulation of many physiological processes in cells. It regulates a wide variety of proteins and has different biological functions in the body [15, 16]. These indicated regulation roles of SmpB are crucial for the survival and environmental adaptability of bacteria, especially expression of the virulence system of pathogenic bacteria.

The current study explored the roles of SmpB on acidogenic/aciduric ability and expression of acid-resisting virulence factors in Streptococcus mutans from caries-sensitive children.

Materials and methods

Sample collection and identification

Four hundred and seventy-four children, between the ages of 3 and 5, were randomly selected from 9 kindergartens in Donghe, Kundulun, and Qingshan District of Inner Mongolia, Baotou City. This study used three-stage stratified cluster sampling. After breakfast, the children did not brush their teeth or gargle for 2 hours. Nonirritant saliva was then collected. The saliva was sealed in an icebox and delivered to the laboratory within 1 hour. Samples were diluted with phosphate-buffered saline (PBS), seeded on mitis-salivarius-bacitracin (MSB) agar plates, and cultured for 48 h at 37°C under anaerobic conditions. Typical colonies were picked and Gram-positive streptococcus, confirmed by microscope, were seeded in each plate at 5 quadrants (upper left, bottom left, upper right, bottom left, and center). One colony from each quadrant was picked to subculture in 10 mL of brain heart infusion fluid medium at 37°C under anaerobic conditions for 24 hours. Bacterial microbiochemical reaction tubes were used to test the ability of bacteria to ferment sorbitol, mannitol, raffinose, and D-melibiose, as well as the ability to hydrolyze aesculin and arginine. Bacteria identified as *S. mutans* were stored at -20°C. Fifty pediatric *S.*

mutans clinical isolates from the previous epidemiological investigations were selected. There were 25 strains in the high caries group and 25 in the caries-free group (dmft ≥ 5 in patients with high caries and dmft = 0 in patients without caries).

Comparison of acidogenic ability

BHI culture media containing 5% sucrose with different pH values (5.0-7.0) was prepared using pH 0.5 as an interval. Clinically isolated *S. mutans* at 1.0 McFarland were seeded in a 15-mL centrifugation tube at ratio of 1:10 (v/v) (bacterial suspension: BHI culture medium) and incubated at 37°C under anaerobic conditions (80% N_2, 10% H_2, and 10% CO_2) for 48 hours. Afterward, the medium was centrifuged at 5,000 rpm for 15 minutes. Final pH values of the culture supernatant were determined and pH change values ΔpH were calculated.

Comparison of aciduric ability

BHI culture media with different pH values (5.0-7.0) was prepared with phosphorylate using pH 0.5 as an interval. Clinically isolated *S. mutans* were seeded in a 15-mL centrifugation tube at ratio of 1:10 (v/v) (bacterial suspension: BHI culture medium) and incubated at 37°C under anaerobic conditions (80% N_2, 10% H_2, and 10% CO_2) for 48 hours. Afterward, the medium was centrifuged at 5,000 rpm for 5 minutes. The supernatant was discarded. Bacteria were collected and diluted with 4 mL sterile saline. After being vortexed for 25 seconds, the absorbance of diluted bacteria solution was measured using an ultraviolet spectrophotometer (UV2550) at 540 nm. Sterile saline was used as a negative control.

Establishment of S. mutans ΔSmpB mutant strain

A strain of *S. mutans* with strong acidogenicity and aciduricity from the high caries group was selected to knockout Smpb genes. After a suicide plasmid with two homologous fragments was introduced into the target strain, homologous recombination occurred twice in the genome of the target strain under the effects of recombinase. After the first homologous recombination, the entire suicide plasmid was recombined with the genome of the target strain. After the second homologous recombination, both the back mutation (in the same fragment of the first homologous recombination) and positive mutation (in another homologous fragment) occurred. The *S. mutans* and donor bacteria of *Escherichia coli* WM3064 with recombinant deletion vector were cultured by shaking overnight. They were inoculated into a fresh culture until OD_{600} = 0.4-0.5. *S. mutans* was mixed with WM3064 at ratios of 1:1, 1:2, and 1:4. The volume of the mating system was 400-1000 μL. After mixing, 100-200 μL of the solution was dropped onto a LB/Dap plate and cultured at 30°C for 24 hours until a single colony appeared. The recombinant homologous fragment was confirmed by PCR amplification.

Detection of growth curve

Wild-type *S. mutans* and a single colony of ΔSmpB *S. mutans* were seeded in BHI culture medium at 1% ratio and grown at 37°C with agitation. The culture medium was sampled at 3, 6, 12, 18, 24, and 36 hours, respectively. The culture medium without seeding was used to calibrate the zero point. Absorbance of the bacterial solution at 600 nm was measured 3 times.

Determination on mRNA expression and enzymatic activity of F-ATPase

Bacteria in the stable growth phase were collected. Total RNA was extracted using RNAiso Plμs solution. Moreover, cDNA was synthesized by reverse transcription and real-time PCR was performed on the target area of F-ATPase. Primer sequences were F'-CGGATGCGTGTTGCTCTTACTG and R'-GGCTGATAACCAACGGCTGATG. Three-step PCR amplification was used as follows: pre-denaturation at 95°C for 3 minutes, denaturation at 95°C for 30 seconds, annealing for 30 seconds, and extension at 72°C for 30 seconds, for a total of 40 cycles. Fluorescence signals during extension were measured and solubility curve analysis was performed after the cycle: 95°C for 15 seconds, 62°C for 23 seconds, and 95°C for 15 seconds.

The bacterial suspension (75 μL) at the stable growth phase was collected and the volume was brought to 3.0 mL with 50 mmol/L Tris-maleate and 10 mmol/L $MgSO_4$. After mixing well, the solution was heated to 37°C. The reaction was initiated after adding 30 μL of 0.5

Table 1. Acidogenic ability of S. mutans from different caries susceptibility children cultured in different pH (pH 7.0~5.0)

	Caries-free group (n = 25)	High caries group (n = 25)	*P*-value
pH = 7.0	3.176 ± 0.265	3.196 ± 0.229	0.776
pH = 6.5	2.681 ± 0.182	2.743 ± 0.205	0.263
pH = 6.0	2.097 ± 0.148	2.157 ± 0.126	0.129
pH = 5.5	1.084 ± 0.156	1.618 ± 0.13**	< 0.0001
pH = 5.0	0.765 ± 0.127	1.014 ± 0.134**	< 0.0001

***P < 0.01 VS* caries-free group, indicating differences showed statistical significance.

Table 2. Aciduric ability of S. mutans from different caries susceptibility children cultured in different pH (pH 7.0~5.0)

	Caries-free group (n = 25)	High caries group (n = 25)	*P*-value
pH = 7.0	1.699 ± 0.259	1.704 ± 0.265	0.94649
pH = 6.5	1.413 ± 0.197	1.451 ± 0.218	0.520943
pH = 6.0	1.195 ± 0.161	1.316 ± 0.189	0.018577
pH = 5.5	0.815 ± 0.176	1.081 ± 0.19**	< 0.0001
pH = 5.0	0.381 ± 0.161	0.719 ± 0.126**	< 0.0001

***P < 0.01 VS* caries-free group, indicating differences showed statistical significance.

mmol/L ATP. In the preliminary experimental stage, this study sampled and tested at 10 minutes, 20 minutes, 30 minutes, and 3 hours. It was found that only the values before 30 minutes were slightly changed, but with a similar trend. Samples (50 μL) at 10 minutes and 3 hours of culturing were taken and the inorganic phosphorus content (Pi) released by ATP hydrolysis was measured. ATP activity is expressed as μmol (Pi)/g/min. Molybdenum acid colorimetry was used to measure the Pi at 660 nm.

Statistical analysis

All results are expressed as mean ± standard deviation (SD). Statistical analysis was conducted with Student's *t*-test for comparisons between the two groups. ANOVA was employed for multiple comparisons. In both cases, differences with *P < 0.05* are considered statistically significant.

Results

Comparison of acidogenic/aciduric ability in S. mutans with different pH sensitivity

Under different pH values, the acidogenic ability of bacteria decreases with a decrease of pH in the growth environment. When the pH was 6.0-7.0, there were no significant differences in the acidogenicity ability at the same pH between the high caries group and caries-free group (P > 0.05). Moreover, there were no significant differences in acidogenic ability between the two groups under different conditions (P > 0.05). When the pH was 5.5 and 5.0, acidogenicity abilities in the high caries group and caries-free group were significantly inhibited by acid, compared with the conditions at pH 6.0-7.0. Additionally, there were significant differences in acidogenic abilities between the two groups (**Table 1**).

At pH 5.5-7.0, the growth of bacteria was not influenced by pH. There were no significant differences in growth between the high caries group and caries-free group at the same pH. Moreover, there were no significant differences under different conditions (P > 0.05). When the pH was 5.0, the growth of *S. mutans* was significantly influenced by environmental pH. Aciduric ability in the high caries group and caries-free group was significantly inhibited (P < 0.05, **Table 2**).

Influence of SmpB on growth activity of S. mutans

F0/R0 primer for the adjacent genome in the SmpB upstream and downstream homologous fragment was used to verify the mutant positive colony. Amplified bands of wild-type bacteria and experimental bacteria (**Figure 1A**) indicated successful knockout.

Results of the growth curve showed that, from 4 hours, the growth rate of bacteria with ΔSmpB knockout was lower than that of wild-type bacteria. Differences in OD_{600} nm were even more significant after overnight culturing. Absorbance at 600 nm was the highest at 12 hours. Afterward, the bacteria entered in to a decline, showing reduced proliferation and death. Absorbance at 600 nm gradually decreased. When most of the bacteria in the culture medium died, the proliferation function of the bacteria began to increase, at which time the OD600 nm increased. Therefore, the value at 24 hours decreased to the lowest point, then slightly increased. The value of bacteria with ΔSmpB knockout was lower than that of wild type. Results showed that knockout of SmpB influ-

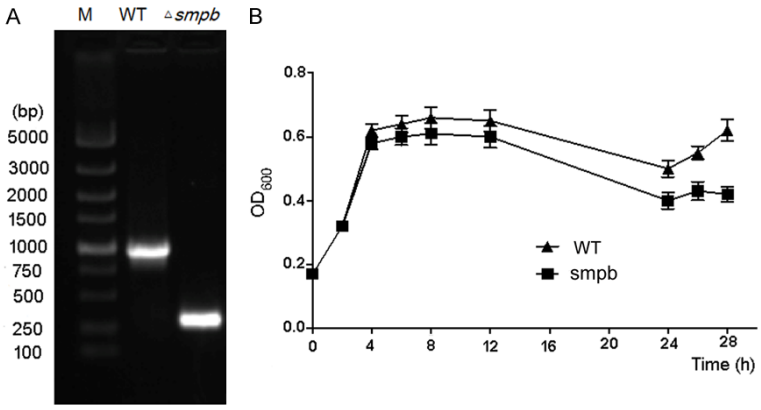

Figure 1. Establishment of S. mutans ΔSmpB mutant strain and influence of SmpB on growth activity. A. Amplified bands of wild-type bacteria and experimental bacteria indicated ΔSmpB was successfully knockouted. B. Growth curve indicated SmpB knockout inhibit the growth of streptococcus mutans. Cells were grown at 37 °C in BHI culture medium and the growth rate of each strain was monitored by measuring OD 600. Error bars represented SD of three independent cultures.

Table 3. Acidogenic ability of WT and ΔSmpB S. mutans at pH 5.0

	ΔSmpB S. mutans	WT S. mutans	*P*-value
pH = 5.0	0.843 ± 0.082	1.176 ± 0.098**	0.0107

***P* < 0.01 *VS* ΔSmpB S. mutans group, indicating differences showed statistical significance.

Table 4. Aciduric ability of WT and ΔSmpB S. mutans at pH 5.0

	WT S. mutans	ΔSmpB S. mutans	*P*-value
pH = 5.0	0.829 ± 0.073	0.477 ± 0.081**	0.005

***P* < 0.01 *VS* WT S. mutans group, indicating differences showed statistical significance.

enced the growth activity of bacteria, resulting in inhibition of the growth of *S. Mutans* (**Figure 1B**).

Acidogenic/aciduric ability and mRNA expression and activity of F-ATPase reduced by SmpB knockout

In this study, pH 5.0 was used to detect acidogenic/aciduric abilities of the bacteria. It was shown that these abilities were significantly reduced by the knockout of SmpB (**Tables 3**, **4**). *S. mutans* and those with ΔSmpB knockout in the exponential phase and stable phase of growth were sampled. Moreover, mRNA expression levels of F-ATPase were measured via real-time PCR. As illustrated in the figure, during the exponential phase, transcription of F-ATPase in the ΔSmpB mutant strain did not significantly change. However, it was significantly downregulated by approximately 30% during the stable phase (**Figure 2A** and **2B**).

F-ATPase enzymatic activity was consistent with mRNA changes. During the exponential phase, the enzymatic activity of F-ATPase in the ΔSmpB mutant strain was not significantly changed. However, it was significantly downregulated by approximately 21% during the stable phase (**Figure 2C** and **2D**).

Discussion

SmpB widely exists in prokaryotic bacteria. According to research on tmRNA mediating trans-translation, SmpB was found and considered to be a tmRNA-specific affinity molecule. Currently, SmpB has become a subject of intense research in biological chemistry and the functions of SmpB were summarized in **Table 5**. Typical strains include *Bacillus subtilis*, *Yersinia pseudotuberculosis*, *E. coli*, streptomycetes, and *Helicobacter pylori*. SmpB has been proven to survive under stress and starvation. These conditions are especially important for regulation of the virulence system of pathogens. For example, Li et al. [17] found that *E. coli* without SmpB exhibited significantly lower survivability against external stress, such as acid processing, heat, or peroxidation, compared with the wild type. Svetlanov et al. proved that [18], after SmpB was knocked out of *Francisella tularensis*, replication and proliferation in macrophages were damaged and the virulence of invasion in the mouse fever model disappeared. Yang et al. [19] reported that SmpB plays an important role in maintaining the normal growth and development of *Streptomyces syancus*. In the current study, SmpB played a regulatory role in acidogenic/aciduric abilities and aciduric virulence factors in caries-sensitive pediatric-derived *S. mutans*.

In this study, pediatric-derived *S. mutans* were cultured in a medium containing sucrose. The

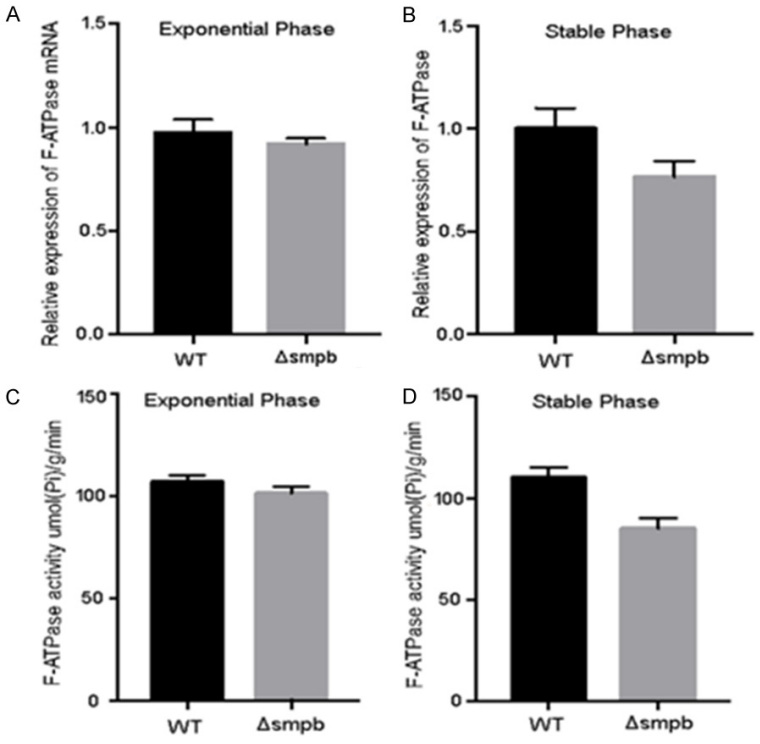

Figure 2. mRNA expression and activity of virulence factor F-ATPase reduced by SmpB knockout. A and B. Comparative qRT-PCR analyses of F-ATPase mRNA in Wild type and knockout strains at the exponential growth stage and stable stage by real-time PCR. C and D. F-ATPase enzymatic activity in Wild type and knockout strains at the exponential growth stage and stable stage. Wild type and knockout strains S. mutans were cultured at the exponential phase and stable phase of growth and harvested for F-ATPase enzymatic activity.

final pH was below 4.5, much lower than the critical value of 5.5, at which enamel demineralization could be tolerant. When the initial pH was 5.5 or 5.0, the bacteria still possessed acidogenic ability. Ability in the high caries group was higher than that the other group, suggesting that the acidogenic virulence factor was strong in children with caries in Inner Mongolia. Acid production amounts and abilities at pH 4.5 and 4.0 were not compared between the two groups. A previous aciduric ability experiment indicated that, for pH < 5.0, bacterial growth with different caries sensitivity was poorer. Additionally, this study compared the growth of bacteria at different initial pH values. Results showed that cells grew very well at pH 5.0-7.0. At the same pH but different conditions, there were no significant differences in growth conditions between the two groups. However, growth was inhibited with decreasing pH. When the pH was 5.0, the bacterial growth of *S. mutans* under the floating state was significantly inhibited. Present results are consistent with previous studies. They also proved that, when pH was close to or above the critical value, cariogenic bacteria grew normally. Analysis of results indicated that, under different conditions, *S. mutans* derived from children aged 3-5 years from the high caries group had strong competitive capacities when pH was low enough to cause enamel demineralization. Additionally, pH could be sufficiently low enough to cause enamel demineralization in the local dental plaque biofilm.

One of the resident bacteria in dental plaque, the aciduric ability of *S. mutans* was achieved by F-APTase. F-APTase is a very important cariogenic enzyme and a multiple subunit complex. It has dual functions of synthesis and hydrolysis of ATP. F-ATPase contains 8 structural genes, atpHGFEDCBC, and the order of coding protein subunits is same as that of other streptococcus: c (atpH: 1459344-1459547), a (atpG: 1458595-1459314), b (atpF: 1458050-1455577), (atpE: 1457544-1458080), α (atpD: 1456023-1457528), γ (atpC: 1455126-1456004), β (atpB: 1453694-1455100), (atpC: 1453264-1453680). β subunit and corresponding atpB genes are genes that code for ATP synthesis and the highly conserved subunit F-ATPase in oral bacteria, in which F-ATPase exhibits very high homology [20]. It has been proven that gene structures of F-ATPase operons in *Streptococcus sanguis*, *S. mutans*, *Streptococcus oralis*, and *Streptococcus pneumonia* are almost identical.

To study the regulatory roles of SmpB with respect to acidogenic/aciduric abilities and aciduric virulence factors in caries-sensitive pediatric-derived *S. mutans*, the current study established ΔSmpB *S. mutans*. A homologous recombinant gene knockout technique was

Table 5. Summary of SmpB functions

Strain	Biological Functions
Bacillus subtilis	Regulation of protein synthesis system; maintenance of normal cell growth and differentiation; regulation of spore germination.
Yersinia pseudotuberculosis	Regulation of protein synthesis system; maintenance of pathogenicity of pathogens.
Escherichia coli	Regulation of expression of RNase R by binding RNase R-specific proteases HslUV and Lon; assistance with regulation of non-coding RNA tmRNA on RpoS expression; regulation of protein synthesis system; remission of cell pressure during antibiotic stress.
Streptomyces spp.	Regulation of protein synthesis system; maintenance of pathogenicity of pathogen; regulation of cellular circulation and differentiation.
Helicobacter pylori	Regulation of protein synthesis system; maintenance of pathogenicity of pathogen
Helicobacter pylori	Regulation of protein synthesis system; maintenance of pathogenicity of pathogen; remission of pressure and starvation of stress cells.
Salmonella spp.	Influence on protein synthesis system; participation in protein expression in critical metabolic pathways involving DnaK, RNase I, RpsA, and PheS and the two-component system consisting of PhoB and CpxR.
Aeromonas veronii	Regulation of BvgSS promoter.

used whereby a homologous recombination process was mediated by a suicide plasmid. In practical operation, the introduction of recombinant plasmid into *S. mutans* and screening are important steps. For *S. mutans*, it is likely that the introduction of a plasmid by binding and appropriate sucrose concentrations were critical in achieving successful knockout. Results indicate that deletion of SmpB significantly reduced the acidogenic/aciduric ability of *S. mutans*. During the growth stable phase, F-ATPase transcription and enzymatic activity expression in the ΔSmpB mutant were significantly reduced by 30% and 21%, respectively. This reduction suggests that SmpB is very important in maintaining acidogenic/aciduric ability and F-ATPase expression activity in *S. mutans*. The above findings elucidate a new function for SmpB.

During development, prokaryotes exhibit high adaptability to the environment. They can rapidly regulate expression levels of different genes, according to changes in the environment. This suggests that they possess strict gene expression regulatory mechanisms. Numerous investigations have proven that gene expression regulation could be on the transcriptional level (including pre-transcription, transcription, and post-transcription) or on the translational level (including translation and post-translation). The genome and chromosomal structure of prokaryotes is simpler than that of eukaryotes. Transcription and translation can occur at the same time and at the same site. Gene regulation is mainly at the transcriptional level [21]. The current study showed that, for *S. mutans*, regulation of SmpB to control expression of F-ATPase is at the transcriptional level. This is in accordance with the mainstream opinion regarding regulation of prokaryotic genes.

Conclusion

SmpB plays a critical role in maintaining acidogenicity, aciduricity, and F-ATPase expression activity during the cariogenic process. The current study aimed to provide new ideas for exploration of therapeutic targets of bacterial diseases, development of new types of antibiotics, and safeguarding human health.

Disclosure of conflict of interest

None.

Address correspondence to: Yu Miao, Department of Stomatology, The Fourth Affiliated Hospital of Inner Mongolia Medical University, Baotou 014030, China. E-mail: miaoyu1970@yeah.net

References

[1] Aleksunes LM, Goedken MJ, Rockwell CE, Juergen T, Manautou JE, Klaassen CD. Transcriptional regulation of renal cytoprotective genes by Nrf2 and its potential use as a therapeutic target to mitigate cisplatin-induced nephrotoxicity. J Pharmacol Exp Ther 2010; 335: 2-12.

[2] Zhang S, Liu J, Lo EC and Chu CH. Dental caries status of bulang preschool children in southwest China. BMC Oral Health 2014; 14: 16.
[3] Matsumoto-Nakano M. Role of Streptococcus mutans surface proteins for biofilm formation. Jpn Dent Sci Dental Science Rev 2018; 54: 22-29.
[4] Kamiya RU, Napimoga MH, Rosa RT, Höfling JF and Gonçalves RB. Mutacin production in Streptococcus mutans genotypes isolated from caries-affected and caries-free individuals. Oral Microbiol Immun 2005; 20: 20-24.
[5] Ding Y, Wang W, Fan M, Tong Z, Kuang R, Jiang W and Ni L. Antimicrobial and anti-biofilm effect of Bac8c on major bacteria associated with dental caries and Streptococcus mutans biofilms. Peptides 2014; 52: 61-67.
[6] Murata RM, Branco-De-Almeida LS, Franco EM, Yatsuda R, dos Santos MH, de Alencar SM, Koo H and Rosalen PL. Inhibition of Streptococcus mutans biofilm accumulation and development of dental caries in vivo by 7-epiclusianone and fluoride. Biofouling 2010; 26: 865-872.
[7] Shan J, Shuai C, Chengfei Z, Xingfu Z, Xiaojing H and Zhiyu C. Effect of the biofilm age and starvation on acid tolerance of biofilm formed bystreptococcus mutansisolated from caries-active and caries-free adults. Int J Mol Sci 2017; 18: 713.
[8] Guo L, Mclean J S, Lux R, He X and Shi W. The well-coordinated linkage between acidogenicity and aciduricity via insoluble glucans on the surface of Streptococcus mutans. Sci Rep 2015; 5: 18015.
[9] Korithoski B, Lévesque CM and Cvitkovitch DG. Involvement of the Detoxifying Enzyme Lactoylglutathione Lyase in Streptococcus mutans Aciduricity. J Bacteriol 2007; 189: 7586-7592.
[10] Ribeiro CC, Ccahuana-Vásquez RA, Carmo CD, Alves CM, Leitão TJ, Vidotti LR, Cury JA. The effect of iron on Streptococcus mutans biofilm and on enamel demineralization. Braz Oral Res 2012; 26: 300-305.
[11] Gao J and Huang W. [Dynamic changes of aciduric virulence factor membrane-bound proton-translocating ATPase of Streptococcus mutans in the development of dental caries]. West China J Stomatol 2016; 34: 200-204.
[12] van Loveren C, Hoogenkamp MA, Deng DM, ten Cate JM. Effects of different kinds of fluorides on enolase and ATPase activity of a fluoride-sensitive and fluoride-resistant Streptococcus mutans strain. Caries Res 2008; 42: 429-434.
[13] Kuhnert WL, Zheng G, Faustoferri RC and Quivey RG. The F-ATPase Operon promoter of streptococcus mutans is transcriptionally regulated in response to external pH. J Bacteriol 2004; 186: 8524-8528.
[14] Peng L, Yong C, Dan W, Yanqiong T, Hongqian T, Haichao S, Qun S, Yueling Z and Zhu L. Genetic selection of peptide aptamers that interact and inhibit both small protein b and alternative ribosome-rescue factor a of aeromonas veronii C4. Front Microbiol 2016; 7: 1228.
[15] Mraheil MA, Frantz R, Teubner L, Wendt H, Linne U, Wingerath J, Wirth T and Chakraborty T. Requirement of the RNA-binding protein SmpB during intracellular growth of Listeria monocytogenes. Int J Med Microbiol 2017; 307: 166.
[16] Yang J, Liu Y, Liu Z, Meng C and Lin D. Backbone and side-chain resonance assignments for the tmRNA-binding protein, SmpB, from Mycobacterium tuberculosis. Biomol NMR Assigm 2017; 11: 1-5.
[17] Li J, Ji L, Shi W, Xie J and Zhang Y. Trans-translation mediates tolerance to multiple antibiotics and stresses in Escherichia coli. J Antimicrob Chemother 2013; 68: 2477-81.
[18] Svetlanov A, Puri N, Mena P, Koller A and Karzai AW. Francisella tularensis tmRNA system mutants are vulnerable to stress, avirulent in mice, and provide effective immune protections. Mol Microbiol 2012; 85: 122-141.
[19] Yang C and Glover JR. The SmpB-tmRNA tagging system plays important roles in Streptomyces coelicolor growth and development. PLoS One 2009; 4: e4459.
[20] Quivey RG Jr, Kuhnert WL, Hahn K. Adaptation of oral streptococci to low pH. Adv Microb Physiol 2000; 42: 239-274.
[21] Dorman CJ. Genome architecture and global gene regulation in bacteria: making progress towards a unified model? Nat Rev Microbiol 2013; 11: 349-55.