Potassium in dental plaque, an old story with a new perspective

Dental plaque or biofilm is home to caries causing bacteria such as *Streptococcus mutans*. Interestingly, the fluid in the biofilm is like an aqueous reservoir of material shared between different species. Although the fluid content is derived from the host and inhabitant bacteria, the fluid in mature biofilm is distinct from other oral micro-environments. By 1970s scientist knew that potassium is the most abundant cation in dental plaque fluid. Since the micro-environment of cariogenic species is so distinct, a question that arises is if potassium levels in the plaque fluid affected the pathogenic attributes of *S. mutans*? Moreover, the presence of multiple genes encoding potassium transporters in the *S. mutans* genome had not been characterized in relation with its ability to form a sticky biofilm and produce acid that demineralizes enamel. We have demonstrated that potassium is critical for *S. mutans* cariogenic attributes. Furthermore, we have identified its key player in transport of potassium, which is annotated as the Trk2 system.

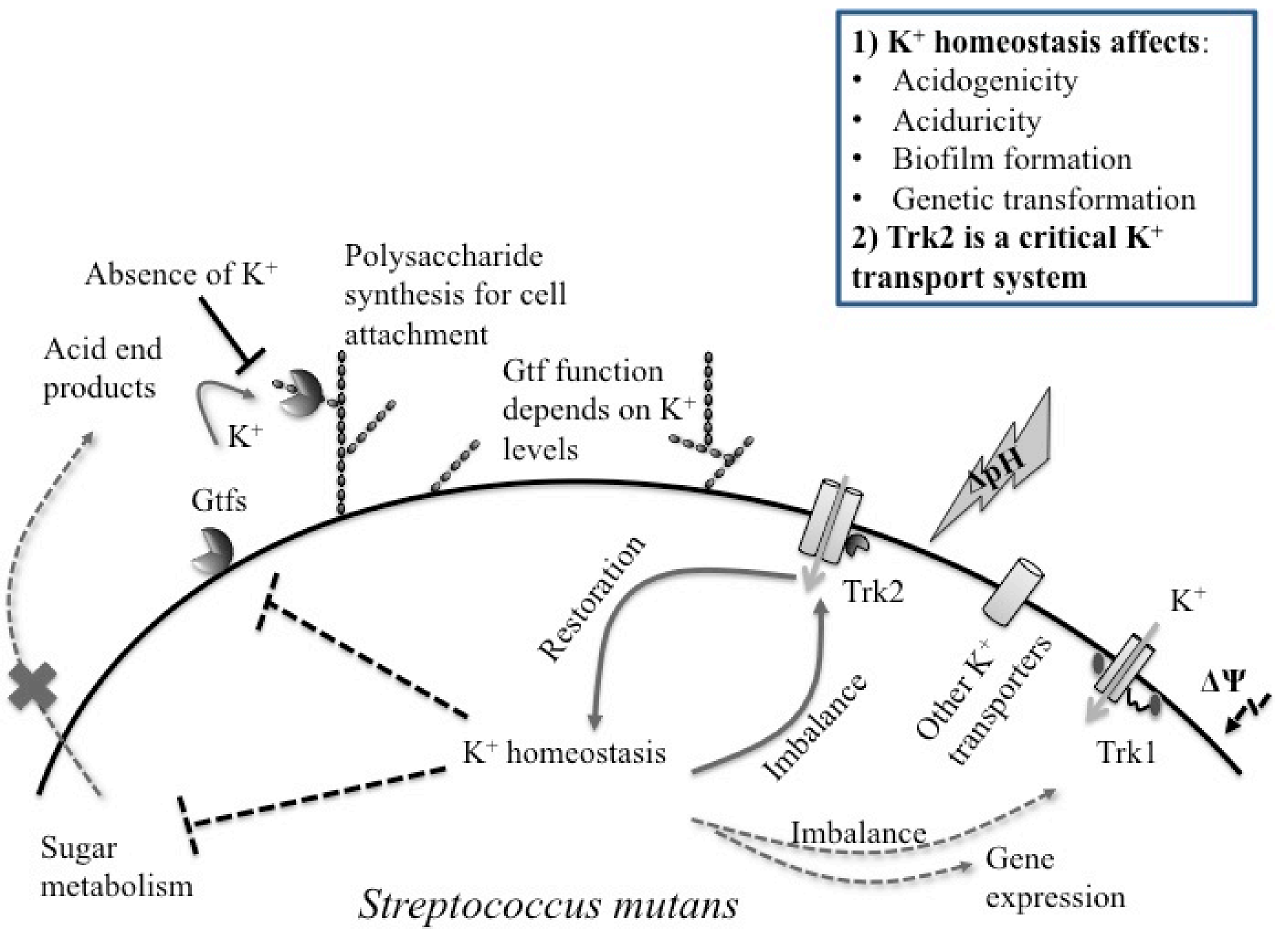

Fig. 1. Trk2 mediated potassium homeostasis is critical for various physiological functions in *S. mutans*. Imbalance in potassium (K^+) homeostasis affects Gtfs, which further affects the

exopolysaccharide production.

In this study, we constructed *S. mutans* strains, which were deficient of individual putative potassium transporters. Using wild-type and the mutant strains we first quantified the amount of potassium required for growth and stress tolerance. The specific concentration was 25 mM for growth of *S. mutans* and this concentration was in the range of potassium levels found in human saliva. Concentrations of 5 mM or 50 mM resulted in significant slowing of growth, thus 5 mM was used to see the effect of low potassium and 50 mM was used to see the effect of high potassium levels. With the normal required potassium determined, we next wanted to test the specific roles of each of the four putative potassium transporters. The strains were grown under limiting potassium conditions, ranging from 0 mM to 50 mM. No potassium in the minimal growth medium abolished biofilms of all test *S. mutans* strains. Trk2 deficient strain required concentrations greater than 25 mM to form a biofilm. One way that *S. mutans* sticks to the tooth and makes biofilms is by generating sticky glucan polymers from dietary sucrose. A closer look at the colonies on sucrose rich agar plates revealed that the surrounding glucan puddle, usually seen for the wild-type strain, was almost negligible for Trk2 deficient strain. To translate this information into numbers, we quantified the glucan produced by wild type and Trk2 deficient strains under three potassium levels: 5 mM that resulted in significantly reduced glucan production in both the strains, 25 mM resulted in normal glucan levels for wild-type but lowered levels produced by the Trk2 deficient strain, and 50 mM resulted in matrix recovery for Trk2 deficient strain. This experiment confirmed that potassium is required for production of biofilm polysaccharides by *S. mutans*. These polysaccharides are produced by specific enzymes called glycosyltransferases (Gtfs), and *S. mutans* has three types of Gtfs that are responsible for producing water soluble and stickier water insoluble glucans. We examined the expression of S-glucosyltrasferase in supernatant of both wild-type and Trk2 deficient strains under three test potassium concentrations. S-Gtf is responsible for the production of soluble glucans, which also act as primer for production of insoluble glucans. In the absence of Trk2 or under low potassium levels a reduced expression of S-Gtf was observed, which convinced us that potassium homeostasis is required for biofilm formation by sucrose-mediated attachment of *S. mutans*.

Maintaining potassium homeostasis is also a requirement for *S. mutans* to tolerate saline and acid osmotic stress. Absence of Trk2 resulted in low potassium uptake by *S. mutans*, which impaired its tolerance to environmental stresses. This study revealed that Trk2 is a key regulator, which links potassium homeostasis with the ability of *S. mutans* to adhere, form a biofilm and tolerate acid and oxidative stressors. Taking forward, Trk2 may serve as an attractive target to curb *S. mutans*-mediated cariogenicity.

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