Abstract
Dental caries is caused by the disturbance in oral homeostasis, marked by a notable increase in the population of *Streptococcus mutans*. Cells of *S. mutans* adhere to the tooth surface and forms stable biofilms that are resistant to various physical and chemical changes inside the oral cavity. In these areas, dietary sugars are rapidly converted to organic acids. Exposure of tooth surfaces to low-pH for prolonged periods causes demineralization of enamel and the development of carious lesions. The aim of our study was to identify new therapeutic agents and molecular targets that can reduce the virulence of *S. mutans* by decreasing its biofilm forming ability.

We examined the *in vitro* potency of the ethanolic extract of *Morus alba* against the oral pathogens, chiefly *Streptococcus mutans*. The MIC of crude ethanolic extract of *M. alba* against *S. mutans* and *Lactococcus lactis* was 125 mg/l. The growth of *Actinomyces viscosus* was inhibited at concentration as high as 1000 mg/l whereas the growth of *Lactobacillus acidophilus* was not inhibited at all. Effect on the adherence to glass surface and biofilms of *S. mutans* in microtitre plates at sub-MIC concentration of the extract was evaluated. The extract strongly inhibited the biofilm formation of *S. mutans* at the active accumulation and plateau phase.
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The active component from crude extract was purified by silica gel chromatography. The purified fraction of *M. alba* shows a 8-fold reduction (15.6 mg/l) in MIC against *S. mutans*. The structure of compound in purified fraction was analyzed with GC-MS, NMR and IR spectroscopy. The active compound was found to be 1-Deoxynojirimycin (DNJ). Both water-soluble and alkali-soluble glucan formation was estimated in the presence of 5 mg/l of purified DNJ from *M. alba* to determine its effect on the extracellular polysaccharides secreted by *S. mutans*. It was found that in the presence of purified compound the water-soluble glucan was reduced by 23.2% while the reduction in the alkali-soluble glucan was 44.8% compared to the control. Confocal microscopy revealed that biofilm formed in presence of DNJ is thinner than the control and the architecture of biofilm is altered in the presence of DNJ. This reflects a prospective role of DNJ in controlling the overgrowth and biofilm-formation of *S. mutans*.

The effect of plant lectins from edible sources, on the growth and biofilm formation of *Streptococcus mutans* was studied. Lectins were isolated from plant sources by conventional methods of protein purification. The effect on growth of *S. mutans* was evaluated following CLSI guidelines. None of the lectins used in this study inhibited the bacterial growth and multiplication. The adherence and biofilm formation of bacteria to saliva coated polystyrene plates was tested in presence of plant lectins. All the plant lectins tested, inhibited both the adherence
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and biofilm in a concentration dependent manner. Confocal microscopy and Scanning electron microscopy were employed to view the biofilm formation in the presence of plant lectin (glucose/mannose-specific) at sub-MIC concentrations. These evaluations revealed that lectins inhibited the clumping and attachment of *S. mutans*. Lectins tested here, inhibited initial biofilm formation by *S. mutans*. Glucose/Mannose specific lectin altered the adhesion arrangement of the bacteria on the saliva-coated surfaces. The plant lectins used in this study may offer a novel strategy to reduce development of dental caries by inhibiting the initial adhesion and subsequent biofilm formation of *S. mutans*.

To study the role of different cellular proteins on the biofilm formation of *S. mutans*, adherence deficient mutants were generated by EMS-induced mutagenesis. The mutants were characterized on the basis of their initial adherence and biofilm formation on the saliva-coated surface. The time-dependent biofilm studies reflect that of the 30 adhesion defective mutants studied here, 18 mutants showed decreased biofilm formation on saliva-coated surface at 12-, 20- and 24 hours of growth. The *in-vitro* polysaccharide formation by these mutants was found to be reduced as compared to the controls. Such mutants were characterized as sucrose dependent adherence defective mutants. The remaining 12 mutants show decrease in biofilm formation at all phases of growth including the initial phase. The polysaccharides synthesized by these mutants *in-vitro* was
more than sucrose-dependent adherence defective mutants, albeit lower than the parent strain. These 12 mutants showed a decreased initial adherence on the saliva-coated surface and were categorized as sucrose-independent adherence defective mutants.

Three sucrose independent adherence defective mutants BSM3, BSM5 and BSM61 were selected for evaluation of their biofilm by confocal laser scanning and scanning electron microscopy. The microscopic revelations confirmed that BSM3, BSM5 and BSM61 showed altered cellular arrangement and reduction in biofilm formation. The ELISA and Western-blot analysis for cell surface adhesin Agl/II of mutants BSM3, BSM5 and BSM61 showed very low expression in these mutants.

The 2D protein analysis in the pH range 4-7 of total cellular proteins from parent strain, BSM3, BSM5 and BSM61 was done to study the expression of various proteins in this range that aided to alter the biofilm arrangement in the mutants. Of 401 spots identified, 57 protein spots showed differential expression of the parent and mutant strains. 13 proteins were expressed more in the parent strain than in the mutant strains while 44 proteins showed higher expression in mutants than the parent strain. The principal redox protein Rex A that modulates transcription in response to cellular NADH/NAD changes, showed 5.5 folds lower
expression in mutants. The heat shock protein Dna K showed 6.65 folds overexpression in mutants. The other proteins overexpressed in mutants are mainly associated with basic metabolism of S. mutans, which can be attributed to its planktonic mode of growth compared to biofilm formation in parent strain. However, a more targeted approach is required for the validation of role and interaction of these proteins in biofilm deficient mutants.