CHAPTER: VI
SUMMARY

Dental caries is one of the most common prevailing disease in humans and a significant public health problem worldwide. Mutans streptococci are a group of oral bacteria and among this group *S. mutans* and *S. sobrinus* are frequently associated with dental caries.

This study was aimed to understand the genotypes of mutans streptococci prevailing in human dental caries subjects in order to develop new treatment strategies for prevention and treatment of caries. The diversity study at serotype level will throw light on the development of potential vaccine against caries. Diversity study is also important to understand quorum sensing (QS) behavior among the genotypes.

Four levels of strategies were followed in the present investigation to study the frequency or dynamics of mutans streptococci distribution in the dental caries. The lower two levels include morphological and biochemical identification of mutans streptococci. The third level of strategy included multiplex PCR and AP-PCR studies to corroborate the result of first two levels. Fourth level of strategy included 16S rDNA sequencing followed by phylogenetic analysis. The strategies were designed in such a way that the clinical isolates will respond to any one of them and get characterized.

Dental plaque samples were collected from caries subjects and cultured on MSB agar. The bacteria were identified based on colony morphology, biotyping, species specific multiplex PCR, serotyping of *S. mutans* isolates, AP-PCR fingerprinting and finally 16S rDNA sequencing followed by phylogenetic analysis.

In thirty eight isolates, twenty five isolates were identified as *S. mutans* and eight as *S. sobrinus* and five remained unidentified. Among the five isolates, four could neither be identified by biotyping nor by multiplex PCR while one isolate was
identified as *S. sobrinus* by biotyping remained unidentified by multiplex PCR. The results showed that the multiplex PCR for species identification was found to be simple and reliable method for detection and differentiation of *S. mutans* and *S. sobrinus* when compared to morphological and biochemical methods.

The proportion of *S. mutans* (65.78%) was higher than *S. sobrinus* (21.05%) and biotype I (60.52%) was most frequently isolated from the study population. Twenty five clinical strains of *S. mutans* showed that serotype ‘c’(13) was most prevalent in the study population followed by ‘e’(6), ‘f’(1), multiple serotype ‘c’/‘k’(03) and ‘f’/‘k’(02).

Among the eight primers (OPA-2, OPA-3, OPA-5, OPA-12, OPA-13, OPA-17, OPA-18 and 970-11) used in AP–PCR fingerprinting, only the primer 970-11 identified and differentiated the *S. mutans* and *S. sobrinus* clinical isolates. The 970-11 primer generated characteristic and unique AP-PCR fingerprints for *S. mutans* and *S. sobrinus*. By 16S rDNA sequencing it was found that the proportion of mutans streptococci was 89.5% and non-mutans streptococci was 10.5%. The five unidentified isolates were identified by 16S rDNA sequencing and it was found that four isolates were non-MSO and one was mutans streptococci. The phylogenetic tree distinctly highlighted five clusters among the clinical isolates and clearly showed that each species have evolved distinctly.

*In vitro* QS studies using reference strains (MTCC 497) confirmed the biofilm formation and the role of CSP in biofilm as detected by MALDI TOF/TOF signifies the quorum sensing mechanism.

Pomegranate dried peeled methanolic extract powder was used as quorum quenching agents. The MIC of PME against reference strain and clinical isolates was found to be 230 µg/ml. PME does not possess minimum bactericidal effect upto 500 µg/ml concentration. The sub-MIC concentration of PME was found to be 110 µg/ml and the highest level of quorum inhibition was expected at this level. In growth curve study assay, the PME at a concentration of 110 µg/ml did not alter the growth of *S.*
mutans but it significantly interfered in biofilm formation. The biofilm inhibition studies were confirmed by crystal violet assay, light microscopic and confocal laser scanning microscopy analysis.

The HPLC chromatogram of PME showed many components and the presence of ellagic acid in PME, which can be suspected for antibiofilm activity as reported by Bakkiyaraj et al., 2013. The compound (ellagic acid) with possible quorum quenching ability holds the potential for use as anticaries drug as it prevents the formation of biofilm. PME may be a promising quorum quenching candidate which holds the potential for use as anticaries drug.