

Research Article

# Study of Bacterial Dominance and its Occurrence Frequency in Dental Plaque Sample

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## ABSTRACT

The intention of the present study was to investigate the bacterial dominance of dental plaque. For this study, 20 plaque samples were collected from adult humans of the around Marthandam area, Kanyakumari District, Tamil Nadu. The collected plaque samples were inoculated separately into the Basal salt medium and Basal salt medium agar plates. The morphologically different bacterial colonies were selected, identified by studying cultural, morphological and biochemical characteristics according to Bergey's Manual of Systematic Bacteriology. As a result, 7 bacterial genera such as *Bacillus* sp, *Lactobacillus* sp, *Staphylococcus* sp-I, *Staphylococcus* sp-II, *Micrococcus* sp, *Streptococcus* sp, *Proteus* sp and *Pseudomonas* sp. were identified. Among these, *Streptococcus* sp has been found highest incidence (21.57 %) followed by *Staphylococcus* sp-I (17.65%).

**Keywords:** Dental plaque, Bacterial dominance, Tooth decay and *Streptococcus* sp

## INTRODUCTION

Tooth decay (Dental caries) have plagued human since the dawn of civilization and still constitutes one of the most common human infectious disease in different parts of the world<sup>1</sup>. It is an adherent deposit of bacteria and their products, which forms as a white greenish or even yellow film on all tooth surfaces. Dental plaque accumulates naturally at stagnant or retentive sites formed after one to two days with no oral hygiene<sup>2,3</sup>. Dental caries cause destruction of enamel, dentin or cementum of teeth due to bacterial activities<sup>4</sup>.

Tooth decay is caused by certain types of acid producing bacteria which cause damage in the presence of fermentable carbohydrates such as sucrose, fructose and glucose<sup>5</sup>. Oral *Streptococci*, (*Streptococcus mutans* and *Streptococcus sobrinus*) which are the major members of oral flora, frequently cause bacteremia and infective endocarditis<sup>6</sup>. These microorganisms in dental plaque are embedded in a polymer matrix of host and bacterial origin. It is said to contain a diverse microbial community that may remain stable over time in a healthy state and it can harbor bacterial species that may predominate in diseased states<sup>7</sup>. The intention of this study was to assess the bacterial dominance of dental plaque samples.

## MATERIALS AND METHODS

### Collection of sample

Twenty plaque samples were collected from adult humans of around Marthandam area, Kanyakumari District, Tamil Nadu. Dental plaques from all patients were picked up through forceps and transferred into 2 ml of normal saline

(sterilized). All the collected samples were stored in a cool place then transported to the laboratory.

### Isolation of bacteria

The plaque samples in tubes were inoculated separately into 25 ml of Basal salt medium containing (g/L) 1.0 g yeast extract, 0.3 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.14 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2 g CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.1 g NaCl, 0.05 g KH<sub>2</sub>PO<sub>4</sub>, 0.05 g K<sub>2</sub>HPO<sub>4</sub>, 0.6 mg H<sub>3</sub>BO<sub>3</sub>, 0.17 mg CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.09 mg CuCl<sub>2</sub>·2H<sub>2</sub>O, 0.1 mg MnCl<sub>2</sub>·4H<sub>2</sub>O, 0.22 mg ZnCl<sub>2</sub>, 10 g glucose<sup>8</sup>. The inoculated flasks were incubated at 35<sup>o</sup>C for 48 hours. After incubation, freshly grown culture of 1 ml from each dental plaque was serially diluted up to 10<sup>-5</sup> with distilled water. 100µl of diluted samples were spread over the Basal salt medium agar plates. The plates were incubated at 37<sup>o</sup>C for 72 hours. After incubation, the morphologically different bacterial colonies were picked up and streaked into fresh agar plates.

### Characterization and identification of bacteria

The isolated bacterial colonies were identified by studying cultural (growth on different media such as MacConkey agar, Mannitol salt agar, Cetrimide agar, Eosine methylene blue), morphological (Gram's, spore and capsule staining) and biochemical characteristics (such as coagulase, catalase and oxidase test) according to Bergey's Manual of Systematic Bacteriology<sup>9</sup>.

## RESULT AND DISCUSSION

In this present investigation, a total of 51 morphologically different bacterial isolates were obtained from plaque 20 samples. All the bacterial isolates were identified by following standard microbiological techniques such as cultural, morphological and biochemical characteristics

Table 1: Cultural, morphological and biochemical characteristics of *Bacillus species*

S. No.	Characteristics	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>	S <sub>7</sub>	S <sub>8</sub>
1	Gram staining	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
2	Morphology	Rod	Rod	Cocci	Cocci	Cocci	Cocci	Rod	Rod
3	Motility	+ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve
4	Capsule staining	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
5	Spore staining	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
6	Coagulase test	-	-	+ve	-ve	-ve	-ve	-	-
7	Growth on MacConkey agar	NLF	NLF	LF	LF	LF	NLF	NLF	NLF
8	Growth on Mannitol salt agar	-	-	Golden yellow pigment	Golden yellow pigment	-	-	-	-
9	Growth on Cetrimide agar	-	-	-	-	-	-	+ve	+ve
10	Growth on EMB agar	-	-	-	-	-	-	-ve	-
11	Growth on blood agar	$\alpha$ -hemolysis	-	$\beta$ -hemolysis	$\beta$ -hemolysis	$\beta$ -hemolysis	$\alpha$ -hemolysis	-	$\beta$ -hemolysis
12	Catalase test	+ve	-ve	+ve	+ve	+ve	-ve	-ve	+ve
13	Oxidase test	-ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
	Probable identity	<i>Bacillus sp</i>	<i>Lactobacillus sp</i>	<i>Staphylococcus sp-I</i>	<i>Staphylococcus sp-II</i>	<i>Micrococcus sp</i>	<i>Streptococcus sp</i>	<i>Proteus sp</i>	<i>Pseudomonas sp</i>

'LF'= lactose fermentation; 'NLF'= Non lactose fermentation

Table 2: Percentage frequency of isolated bacterial strains

S. No.	Bacterial species	Occurrence of the Bacteria	% occurrence of the bacteria
1	<i>Bacillus sp</i>	6	11.76
2	<i>Lactobacillus sp</i>	4	7.84
3	<i>Staphylococcus sp-I</i>	9	17.65
4	<i>Staphylococcus sp-II</i>	5	9.80
5	<i>Micrococcus sp</i>	7	13.73
6	<i>Streptococcus sp</i>	11	21.57
7	<i>Proteus sp</i>	6	11.76
8	<i>Pseudomonas sp</i>	3	5.88
Total		51	100

(Table 1). In this study, seven bacterial genera were identified viz. *Bacillus sp*, *Lactobacillus sp*, *Staphylococcus sp-I*, *Staphylococcus sp-II*, *Micrococcus sp*, *Streptococcus sp*, *Proteus sp* and *Pseudomonas sp*. The percentage frequencies of isolated bacterial strains from 20 plaque samples were calculated. In this, *Bacillus sp*, *Lactobacillus sp*, *Staphylococcus sp-I*, *Staphylococcus*

*sp-II*, *Micrococcus sp*, *Streptococcus sp*, *Proteus sp* and *Pseudomonas sp* were found as 11.76%, 7.84%, 17.65%, 9.80%, 13.73%, 21.57%, 11.76% and 5.88% respectively (Table 2).

In this present study, *Streptococcus sp* was found as high as higher percentage (21.57 %) of occurrence followed by *Staphylococcus sp-I* (17.65%). This result was supported

by Omolaja *et al.*, in that study, *Streptococcus mutans* was found highest percentage frequency of 53.13%, followed by *Staphylococcus albus* (25%), *Klebsiella pneumonia* (9.34%). *Pseudomonas sp* was found at least percentage (5.88%) of occurrence<sup>5</sup>. This result was also supported by the results of Reyes and Dalmacio<sup>10</sup>. Nwakanma *et al.*<sup>11</sup> identified three bacterial strains *Streptococcus*, *Staphylococcus* and *Lacto bacilli* from the mouth of students. Several members of the *Streptococcaceae* family were identified in the saliva and plaque and they are as considered initial colonizers of the oral cavity<sup>12</sup>. The results of this study confirmed previous reports that some *Streptococcus* species are associated with healthy states<sup>12</sup>. The most commonly associated and strongly implicated member of this family with dental caries is *Streptococcus mutans*<sup>14</sup>.

### CONCLUSION

It was isolated and identified, 8 genus of bacterial strain includes *Bacillus sp*, *Lactobacillus sp*, *Staphylococcus sp-I*, *Staphylococcus sp-II*, *Micrococcus sp*, *Streptococcus sp*, *Proteus sp* and *Pseudomonas sp* were highly prevalent in dental caries and among these, *Streptococcus sp* has been found highest frequency.

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