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Biofilm Formation and Species Specific Adherence Characteristics of Microbes Commoly Found in Dental Plaque

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Abstract: ***Dental caries is most likely the result of a polymicrobial infection caused by one or more of the over 500 bacterial species that have been identified from the human oral cavity. The objective of this study was to investigate the characteristics of biofilms formed by different bacteria species commonly found in dental plaque. Primary biofilm screening was done using tube staining assay, cover slip assay and exo polysaccharide (EPS) assay. This study concluded the biofilm forming potential of all isolated strains by cover silp assay, tube assay and by EPS assay. The characteristic feature of biofilm forming bacteria is production of EPS.***

Keywords: Biofilm, Dental caries, Exo polysaccharide assay, cover slip assay, tube assay.

I. INTRODUCTION

Biofilms are usually heterogeneous; in that they contain more than one type of bacterial species, but they can be homogeneous in cases such as infections and medical implants. In nature, bacterial cells are most frequently found in close association with surfaces and interfaces, in the form of multicellular aggregates embedded in an extracellular matrix generally referred to as biofilms (Mirriam, et al., 2012). Biofilm formation is regulated by different factors like bacterial mobility, cell membrane proteins, extracellular polysaccharides and signalling molecules play significant roles in biofilm formation (Ahn et al., 2008). Basic structural units of a biofilm are microcolonies, separate communities of bacterial cells embedded into EPS matrix (Svjetlana and Vrane, 2007). Dental caries and periodontal diseases are the major infectious diseases on a global scale. Oral biofilms mostly consists of multiple bacterial strains include mainly the *Streptococcus* species i.e. *Streptococcus mutans*, which has major role in the formation of dental caries (Sonkusale and Tale, 2015). Dental caries is the single most common biofilm dependent oral infectious disease. The objective of this study was to investigate the characteristics of iofilms formed by different bacterial species commonly found in dental plaque. The results of this study will improve our understanding on the characteristics of biofilms depending on bacteria, such as the amount of biofilms formed and the peak time of biofilm formation. These information will help us decide the treatment plan by using antibiotics.

II. MATERIALS AND METHODS

A. *Collection of Bacteria*

In the present investigation, the pure ATCC microbial culture of gram positive bacteria *Streptococcus mutans* ATCC 25175, *Lactobacillus acidophilus* ATCC 4356, *Streptococcus mitis* ATCC 49456, *Enterococcus faecalis* ATCC 51299 and gram negative bacteria *Pseudomonas aeruginosa* ATCC 27853 were processed from the microbial culture collection of the National Center for Cell Science, Pune, Maharashtra, India.

B. *Biofilm Characterization*

The processed bacterial colonies were re-streaked on Mueller Hinton agar after incubation onto nutrient agar plates to obtain pure cultures. The viability of the isolated cultures was checked in Mueller Hinton agar broth and those found to be viable were screened for biofilm formation. Primary biofilm screening was done using tube staining assay, Cover slip assay and EPS assay (Christensen et al., 1982).

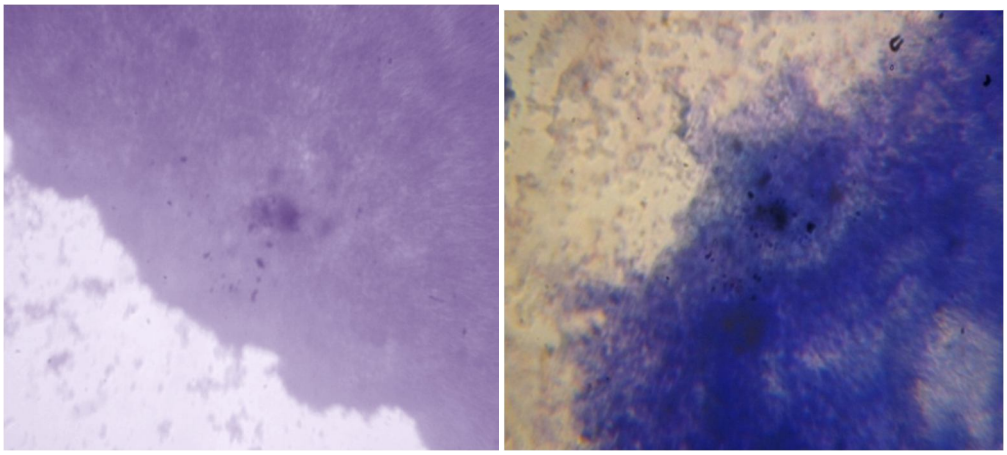
III. RESULTS AND DISCUSSION

A. *Cover Slip Assay*

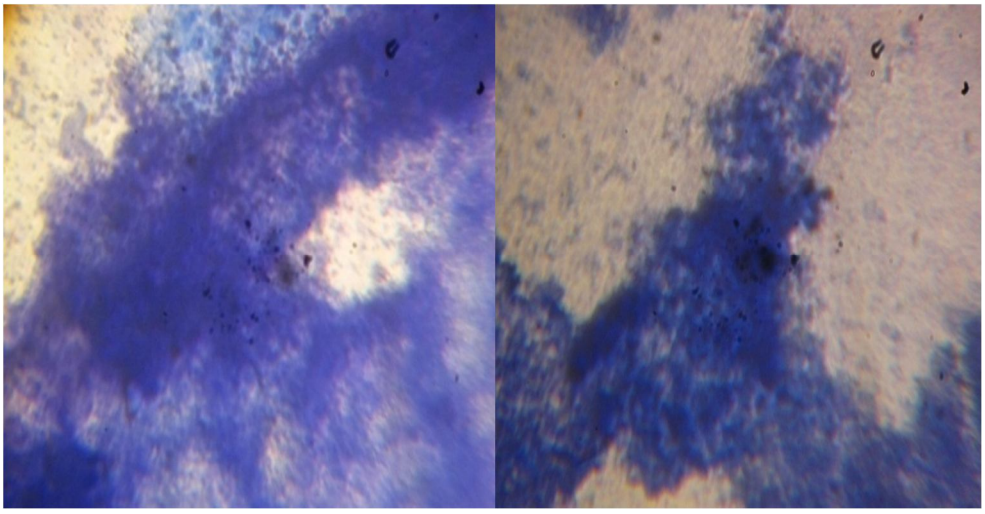
The present data of cover slip assay includes visual observation based on staining as lower levels of attachment with light staining on the coverslip and higher levels of attachment with a visibly defined line of staining at the air-liquid interface observed under the microscope. The results of the coverslip assay are presented in (Figure 1) In the present result, a dense biofilm production on glass cover slip was observed in *S. mutans*, *S. mitis* and *P. aeruginosa* whereas production of moderate biofilms was observed in *L. acidophilus*. The possible explanation for above result is that *P. aeruginosa*, *S. mutans* and *S. mitis* are more prone to form biofilm at the interface between the medium and air.

Minimum biofilm production was observed in *E. faecalis* (Figure 1). The results of coverslip assay are in accordance with the reports of Hossain and Uddin, (2014). Coverslip assay is commonly used assay as it is convenient and economical.

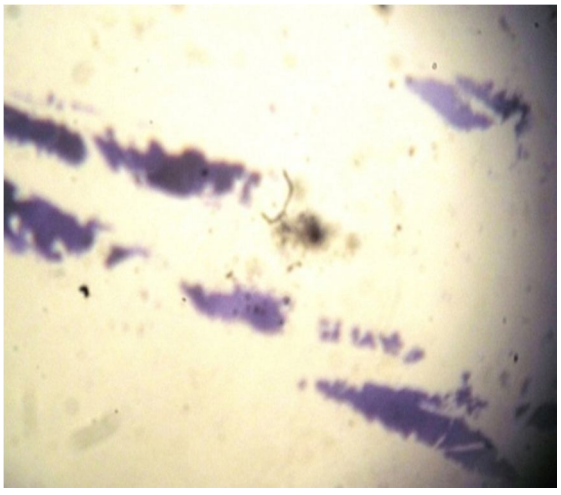
Figure 1: Characterization of biofilm formation by common oral bacteria using cover slip assay



(A) P. aeruginosa *(B) S. mutans*



(C) S. mitis (D) L. acidophilus



(E) E. faecalis

B. Tube Assay

In the present assay, six different sugars (dextrose, sucrose, maltose, lactose, starch and mannitol) as sole carbon source were incorporated in culture media to screen biofilm formation capability by the all test organisms. In the tube assay, visible film form inside the wall of tubes was interpreted as a positive result. The result of tube assay is presented in (figure 2).

All test organisms in the present investigation showed different thickness of biofilm with different sugars. In the present experiment, *P. aeruginosa, S. mitis,* and *S. mutans* showed highest adherence *while L. acidophilus* showed moderate adherence *and E. faecalis* showed less adherence with all sugars to the wall and bottom of the tubes (Figure 2). The present result can be related with conclusion of Hossain and Uddin, (2014), Kaustubh and Vidya, (2015) and Jadhav and Vidya, (2015).

The observations clearly showed that capability of biofilm formation is related to the presence or absence of sugar in the culture medium. Earlier studies suggested that sugars are the major nutritional substrate that results in the development of dental caries. In the investigation, all the tested organisms showed least adherence with maltose and starch and highest adherence with dextrose and sucrose whereas with mannitol and lactose moderate adherence was observed.

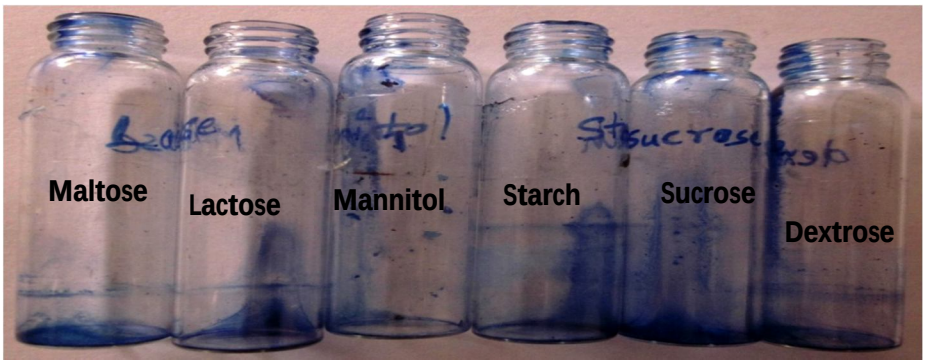
According to the literature, usually tube assay is not suggested for biofilm screening. In this assay biofilm adherence ability in the test tube is noted by observers, and is very difficult to differentiate.

Figure 2: Characterization of biofilm formation by common oral bacteria

(A) Biofilm production in tube assay by *S. mitis*

(B) Biofilm production in tube assay by *S. mutans*

(C) Biofilm production in tube assay by *L. acidophilus*



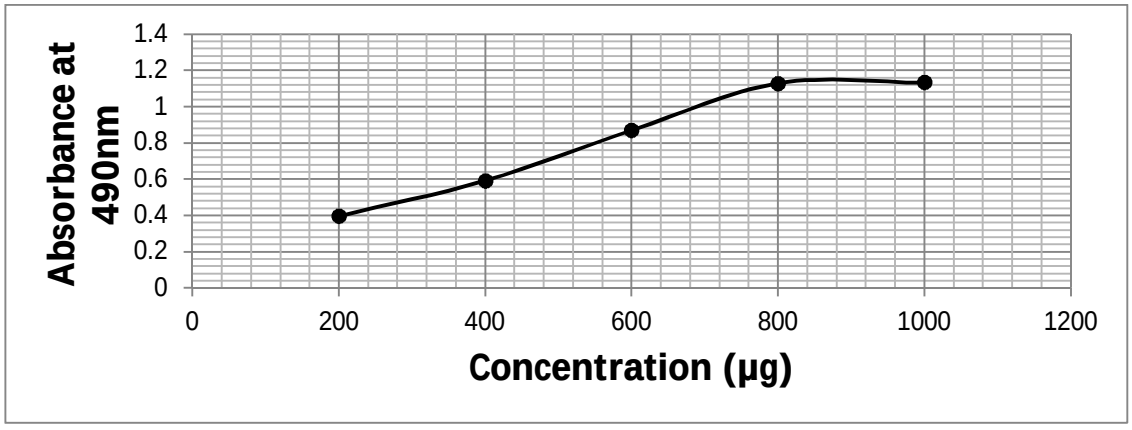
(D) Biofilm production in tube assay by *E. faecalis*

(E) Biofilm production in tube assay by *P. aeruginosa*

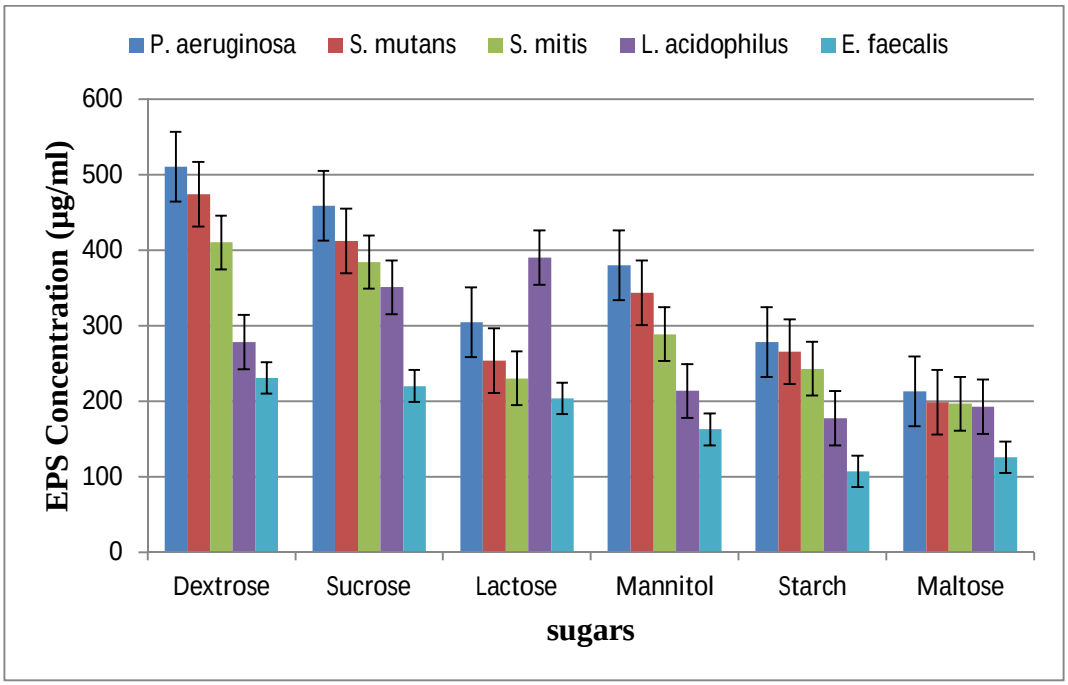
C. Exo Polysaccharide Assay

In this assay, all the test organisms were grown on Tryptic Soy Broth media containing different sugars. The result is summarized in (Graph 2). Glucose was used as a standard to estimate the EPS quantity (Graph 1).

In the present assay, *P. aeruginosa*, *S. mitis* and *S. mutans* showed highest EPS production, indicating the highest biofilm forming potential while *L. acidophilus* produced a moderate quantity of EPS and *E. faecalis* produced the least amount of EPS with all sugars (Graph 2). From the obtained data, it was observed that EPS production by all the tested organisms differs according to supplemented sugars. The present findings can be aligned with the result given by Onbasli and Aslim, (2008), Hossain and Uddin, (2014) and Kaustubh and Vidya, (2015).All the tested organisms showed maximum EPS formation in the presence of glucose and sucrose whereas in presence of mannitol and lactose EPS production was moderate and in presence of maltose and starch lowest EPS formation was observed. Such results are in concurrence with the studies conducted on oral biofilm formation by Hossain and Uddin, (2014). Biofilm formation in the presence of glucose, explains that glucose enhances the adherence of bacteria with the formation of glycocalyx. Many authors in their work justified that with the increasing concentration of glucose biofilm formation also increased. Sucrose is a fermentable sugar that serves as a substrate for the synthesis of extracellular EPS in dental caries Leme et al., (2006). Biofilm producing bacteria are more resistant towards antibiotics, therefore biofilm examination is one of the crucial step used in modern therapies.



Graph. 1 Standard graph of glucose for exopolysaccharide estimation



Graph 2: Estimation of exo-polysaccharide production by common oral bacteria in the presence of different sugars (µg/ml)

IV. CONCLUSION

In the current work, it was found that in dental carries the characteristic feature of biofilm forming bacteria is production of EPS. Analysis of the result clearly indicates that all the test organisms are able to form biofilm in the presence of different sugar substrate confirming their roles in different dental problems. *S. mutans*, *S. mitis* and *P. aeruginosa* produced higher biofilm formation justified that they are one of the most potent microorganisms involved in dental caries. It can be concluded that the biofilm formation helps bacteria to form rigid dental plaques and provides resistance to survive against different antibiotics.

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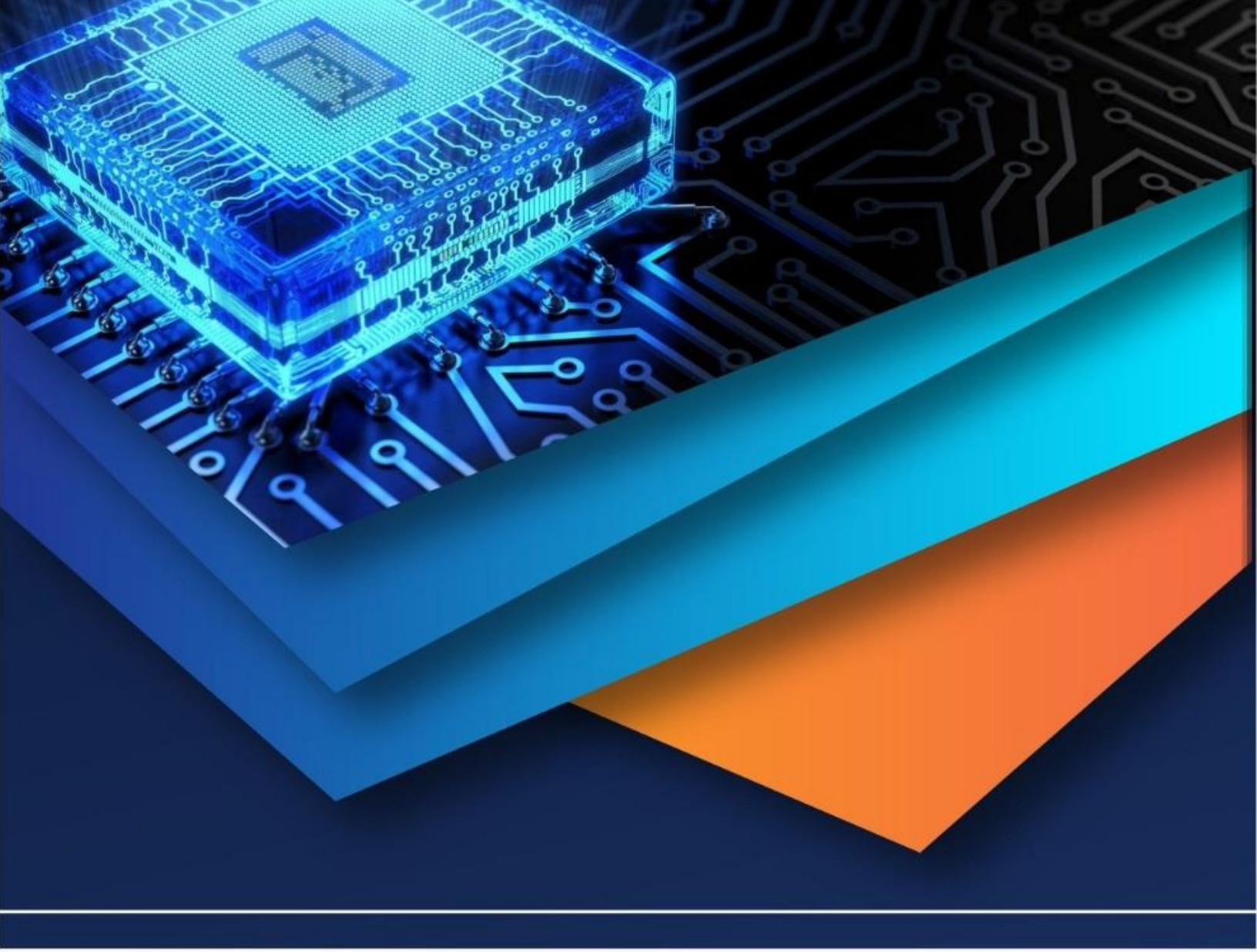

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