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Quorum quenching for the management of dental plaque microbes

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ABSTRACT

In the present study, variation among different bacterial strains was observed in the plaques of tobacco chewers (TC) as compared to those from normal persons (non tobacco chewers) (NTC). Bacterial strain J1 (dominant in the plaques of NTC) did not initiate the process of adhesion (biofilm formation) until it appeared to have reached a required population density necessary for the production of adhesive substances by the bacterium, which indicating the involvement of quorum-sensing mechanism. The spent media of bacterial strain J15 (dominant in the plaques of TC) also substantially reduced the adhesion of the respective bacteria to the acrylic particles by bacterial strain J1. This indicates the possibilities of existence of some quorum-quenching molecules in the spent medium of strain J1 as well as strain J15 bacteria. These molecules might be exploited for efficient management of dental plaque-biofilms. It may help to avoid the use of extremely high doses of antibiotics which may be harmful in the long run.

1. Introduction

Many bacteria possess the property of formation of "multicellular surface bound communities called "biofilms". With the formation of biofilms, bacteria get changed in their behaviors such as their ability to resist challenges from predators, antibiotics and host immune system. Thus, an antimicrobial agent for treatment of an infection by microbes becomes usually ineffective when it encounters the microbes in the form of bifilm. The biofilm formation by microbes is regulated by quorum sensing.

Quorum sensing is the ability of bacteria to control gene expression by means of detection of a minimal threshold stimulatory concentration of chemical signal molecules called "auto inducers", which are secreted by self and/or other bacteria. (Ni et al., 2009). This enables them to control behaviors like virulence factors expression, antibiotic production, conjugation, sporulution and biofilm formation. Quorum sensing is a 'population density dependent process' in which each bacterium produces a signaling molecule (inducer), and each bacterium also has a receptor for the signaling molecule. Binding of auto inducer molecule to the receptor activates the transcription of certain genes, including those responsible for the synthesis of the inducer itself.

Oral bacteria have the ability to form biofilms known as "dental plaque" on tooth surface. Biofilm formation is essential for the progression of infection of certain bacteria like *Streptococcus*

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mutans. The early colonizers of the enamel surfaces are predominantly Streptococci, which form mixed-species micro colonies during early plaque development. (Kolenbrander et al., 1993). Quorum sensing and biofilm formation are intimately related to each other and their interaction is important to the pathogenesis of many bacterial infections (Singh et. al., 2000).

Since quorum sensing regulated genes are expressed (or repressed) depending upon bacterial cell density, it might be possible to manipulate the system to one's advantage. If quorum sensing genes are made to express (or repress) at low cell density in response to specific chemical signal molecule (added externally), bacteria may fail to form biofilm/virulence factors. This is called "Quorum quenching." The quorum quenchers may help to attenuate virulence, reduce biofilm formation and increase bacterial sensitivity to drug therapy.

Cigarette smoking and use of smokeless tobacco is quite common all over the world, and in India also, people of different social status smoke and also consume smokeless tobacco (Saini et al., 2009; Huang et al., 2012). Keeping this in view, a study was carried out to find out the impact of tobacco chewing habit on oral micoflora with special references to their capacity to bind with oral implant materials and to quench Quorum sensing system.

2. Materials and Methods

2.1. Chemicals

All the chemicals and reagents were of analytical grade and were procured from Hi Media, Mumbai India.

2.2. Isolation of Bacteria from dental plaques

Dental plaques from 20 chronic TC persons and 20 NTC persons were removed with the help of sterile tooth picks, serially diluted and cultured on Nutrient agar medium at 37ºC for 40-72 hours.

2.3. Isolation and characterization of bacteria

Different types of bacterial cultures growing on the Petri plates were identified, based on their morphological and biochemical tests and were designated as, J1, J2…..J24. From all 24 bacterial strains, the Gram positive strain J1 was found most frequently and highly dominant in dental plaques of NTC persons while J15, a Gram positive, frequent and highly dominant among the dental plaques of TC persons.

2.4. Comparative ability of most dominant bacterial strains J1 and J15 to adhere on artificial acrylic denture repair polymer particles

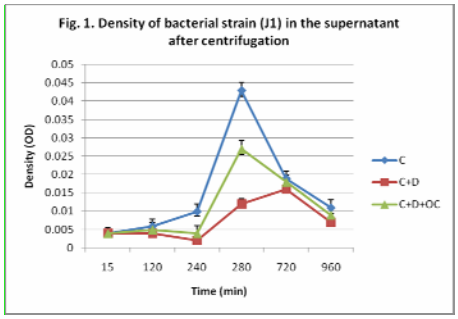
A sterile mortal and pestle were coated by vaseline. Pyrex rapid repair self polymerizing Resin powder and Liquid (as used for denture repair) were thoroughly mixed and kneaded. Glass plates of 15cm X 13 cm dimensions were thoroughly cleaned and coated with Vaseline. On one glass plate, the kneaded material was placed and pressed with another glass plate so that uniform flat layer of polymer was obtained. The glass plate with polymer was allowed to dry so that the material polymerized to form a dry layer. The pieces of this were crushed in a grinder to find fine particles. The powder was passed through a sieve (15 meshes /cm) to obtain fine particles of almost uniform size.

3. Results

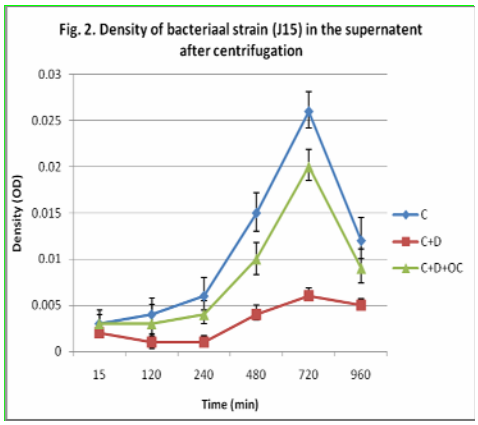
The present study assessed the difference between the bacterial strains colonizing the dental plaques of NTC and TC persons. In total, 7901 CFU strains were isolated from dental plaques of NTC, while 1295 CFU strains were isolated from TC persons. Among the isolated bacterial strains, J1 (*Staphylococcus* sp.) was found to be highly dominant in the plaques of NTC persons, and similarly the bacterial stain J15 (*Streptococcus* sp.) in dental plaques of TC persons. Incidentally and interestingly all the bacterial strains isolated were Gram positive. A total of 24 dominant bacterial strains were isolated, out of which seven were micrococci, 11 streptococci and six were staphylococci.

Fascinatingly, none of the isolated bacteria was bacilli or spiral bacteria. The growth pattern of staphylococci and streptococci along with their adherence to the denture material, a small study was carried out by using spent medium of same bacterial culture on their adherence to the acrylic particles. Both the bacterial strains, J1 and J15 followed normal growth patterns characterized by lag phase, a log phase and death phase (Figures 1 and 2).

The growth of bacterial strain J1 was quite faster compared to strain J15, strain J1 entered death phase much earlier than bacterial strain J15. Both the bacterial strains J1 and J15 exhibited sufficient capacity and ability to adhere to the denture material. The strain J1 exhibited the process of adhesion during the period from 15 min to 120 min and this degree of adhesion continued till 8 hour and later on this activity along with population density decreased. While, strain J15 exhibited much higher adherence power compared to strain J1 and were able to adhere to denture particles at very low population density and continued to increase and retained this capacity till 12 hour, thereafter decreased (Figures 3 and 4).



C = Control; medium inoculated with bacteri
C + D = Medium inoculated with bacteria + Acrylic powder
C + D + OC = Medium inoculated with bacteria + Acrylic powder + Spent media



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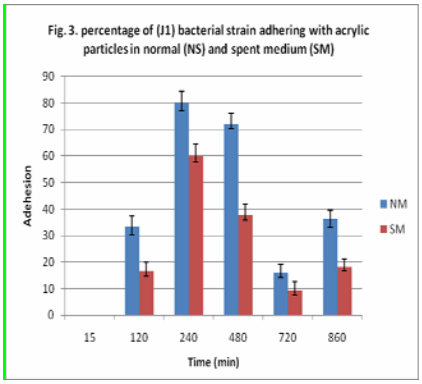

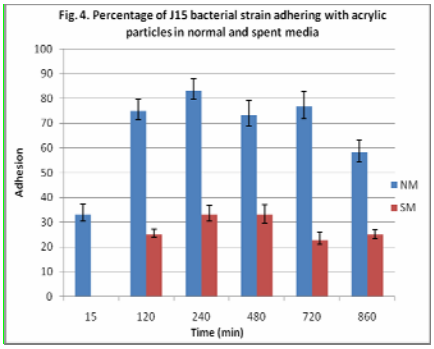

4. Discussion

This is the first study reported regarding the oral micro biota and dental plaque formation among smokeless tobacco users compared with NTC persons. Saini et al., (2009) reported a substantial reduction in the normal oral micro biota of the persons who are chronic tobacco eaters, taking 25-30 Gutkhas (flavored tobacco) per day. In our study also, substantial decrease in the bacterial CFUs were also observed in the plaques of tobacco chewers. Huang et al., (2012) also reported inhibition of a broad spectrum of oral microbes by nicotine. However, in our study greater variety of strains were observed in place of TC compared to NTC persons. Thus, there was steep decrease in bacterial population of TC persons while; the greater variations of bacterial strains were observed among plaques of TC persons compared to NTC persons. All the dominant bacterial strains were Gram positive, which could be due to difference in their cell wall compared to Gram negative bacteria,

which aid in competitively surviving the nicotine rich environment.

Yusuf et al., (2005) had reported that smokeless tobacco could serve as a growth substrate for some species of oral streptococci which are frequently associated with human dental caries. Huang et al., (2012) found that while some streptococci were highly susceptible to nicotine, some others were slightly inhibited or were not affected at all. Interestingly, in the present study, there was a dominance of staphylococci and micrococci in the plaques of NTC, while the plaques of TC were dominated by streptococci. Thus, the results of presnt study, to a large extent, are in agreement with observations of earlier workers.

The adhesion, co adhesion and formation of bacterial bio-film are regulated by quorum sensing. In the present study also, the J1 strain did not initiate the process of adhesion until it appeared to reach a required population density (quarate) necessary for the production of adhesive substances by the bacteria. However, bacterial strain J15 which was dominating among TC persons could able to initiate the process of adhesion at much lower population density, which could be due the adaptability in nicotine rich environment. It is a well known fact that quorum sensing enables bacteria to turn off and on the secretion of extracellular polymeric substances.

Xavier, (2011) predicted that extracellular polysaccharides secretions (EPS) provided and advantage to secreting strains in competition with non secreting strains. EPS binds with bio-film together and protects it again external threats (Nadell et al., 2008). It can also be assumed that since in the early stages of development the dental plaque bio-film provide protection, in case of tobacco chewers the bacteria which produce the adhesive substance at low population densities might have been selected and adopted for the protection of teeth. It would be worthwhile to study the production of EPS by J15 and J1 strains to throw further light on this issue.

A number of other factors like Vander Wal forces, electrostatic forces and hydrophobic interaction are involved in the attachment of bacterial cell surface polymer with the solid surface. In view of the result of the present study, it is desirable to compare these traits in J1 and J15 strains to find out the possible reasons of the greater adhesive power of J15 strain as compared to strain J1. Kodjikian et al., (2003) have reported that different patterns of adhesion exist in isolated bacteria and clusters of bacteria. J15 bacteria are streptococci, while J1 bacteria are staphylococci. Thus, the differences in their adhesive capacities can also be looked at from the angle of Kodjikian et al., (2003).

However, the degree of adhesion of J1 strain was consistently much higher that that of strain J1, despite greater population density of J1. As is clearly evident from the Figures 3 and 4, the presence of spent medium in the test tubes greatly reduced the adhesive power of strains. The spent medium of J1 and J15 strains substantially reduced the adhesion of the respective bacteria to the acrylic particles. It must be recalled that Nelson et al., (1970) observed luminescence in Vibrio fischeri by spent medium. In that case the spent medium induces luminescence at low population density of bacteria. It is now known that the same autoinducer can make quorum sensing gene express at low cell densities (if added externally) thereby, reducing the chances of production of a given substance later at higher population densities (quorum quenching) (Cvitkovitch et al., 2003). The results of the present study indicate the possibilities of existence of such quorum quenching molecules in the spent medium of J1 as well as J15 bacteria. Thus, the results of the present study raised the possibilities of discovery of quorum quenching molecules which might prove useful for the management of dental plaque / bio-film. This may help us avoid the use of extremely high doses of antibiotics, which are harmful in long run.

5. Conclusion

In this work, we have developed a simple technique to study quorum sensing and quenching in dental plaque biofilm formation among tobacco chewers and non chewers. The future confront lies in the aptitude of the dental plaque exploration to build up additional mechanisms for interfering with bacterial quorum sensing which can be used as precautionary and therapeutic tools for combating oral polymicrobial diseases.

Conflict of interest: No conflict of interest amongst the authors.

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