International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 6, Issue 1, pp: (166-170), Month: April - September 2018, Available at: www.researchpublish.com

Implications for Dental Health: Stress Response of Oral Pathogens to Food Residue

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Abstract: **The study aimed to determine the stress response of oral pathogens to simple and complex sugars, common salts and metal ions residue in the oral cavity.**

Method: Stock solution of three sugars: sucrose, glucose and fructose having concentration of 10x was prepared and sterilized by autoclaving. Salt stock sodium chloride, lead nitrate and copper sulfate of concentration 1mg/ml were prepared in autoclaved distilled water. The salt stock was serially diluted in sterilized distilled water to obtain a concentration of 0.2, 0.4, 0.6, 0.8 and 1 mg/ml. A stock of four metals: calcium, zinc, iron and lead having concentration 0.2mg/ml was prepared. The metals were further diluted in sterile distilled water so as to obtain concentration of 10, 20, 40, 80 ppm. The stock solution prepared from sugars, salts and metals was then used individually as basal media to determine the oral pathogens response.

Results: The data of sugar stress response showed sucrose had the highest bacterial growth attachment and fructose was the lowest. The salinity results showed sodium chloride had the highest bacterial attachment whereas lead nitrate and copper sulfate had moderately attachments.

Metal stress investigation showed that calcium metal had high attachment of biofilm and lead metal had less attachment.

Conclusion: It was evident that the concentration of sugars, salts and metals affected the growth of oral pathogens. The presence of biofilm attachment played a major role to establish the functional and structural integrity of microorganisms. The statistical analysis showed the P-value was 0.09161, F-calculated was 25.4382 and F-critical was 3.2873. Since F critical was smaller than F calculated, the null hypothesis (H_0) was rejected and accepted alternative hypothesis (H_A). Therefore there was a significant difference on how the sugars, salts and metals affected the bacterial growth population in the oral cavity.

Keywords: **Oral pathogens, Stock solution, Biofilm formation, Stress response.**

1. INTRODUCTION

Oral bacterial species mostly lack an environmental niche and are found almost exclusively within the mouth (Rachid et al., 2000). For these bacteria, planktonic growth would cause them to be quickly washed away by saliva, swallowed and destroyed within the acidic juices of the stomach. These bacteria likely spend the majority of their natural existence growing as a biofilm.

Dental plaque development is a natural phenomenon and is known to contribute to the host defence by preventing colonization by exogenous species. The bacteria constituting the oral biofilm vary at distinct sites as a result of the inherent biological and physical properties at these sites. However, the balance of the bacterial population shows a shift in disease condition like dental caries (Marsh, 2003).

The initial attachment of bacteria begins with pellicle formation. The pellicle is a thin coating of salivary proteins that attach to the tooth surface within minutes after a professional cleaning. The pellicle acts like double-sided adhesive tape, adhering to the tooth surface on one side and on the other side, providing a sticky surf ace that facilitates bacterial attachment to the tooth surface. Following pellicle formation, bacteria begin to attach to the outer surface of the pellicle.

Bacteria connect to the pellicle and each other with hundreds of hairlike structures called fimbriae. Once they stick, the bacteria begin producing substances that stimulate other free floating bacteria to join the community. The consistent low pH in dental caries favours the selection of acid producing bacteria. Therefore, non fermentable artificial sugars; metabolic inhibitors or stimulation of saliva flow can bring about the suppression of sugar catabolism and acid and restore the balance favouring normal microflora (Marsh, 2006).

Bacterial accumulation on oral surfaces is a major factor in the development of most of the common dental diseases such as dental caries and periodontal disease (Williams and Cummins, 2003). Worldwide, 60–90% of school children and nearly 100% of adults have dental cavities, often leading to pain and discomfort (World Health Organization, 2012). Severe periodontal (gum) disease, which may result in tooth loss, is found in 15–20% of middle-aged (35-44 years) adults (World Health Organization, 2012).

Dental caries is formed when the balance inside mouth is disturbed. If the proportion of acidogenic and aciduric (acid-tolerating) bacteria increases it leads to demineralization of enamel by rapid metabolism of dietary sugars to acid, resulting into a low pH. Thus, favourable micro environment is created for these organisms enhancing their growth and multiplication whereas most species associated with enamel health are sensitive to acidic environmental conditions (Loesche, 1986; Becker, 2002).

2. MATERIALS AND METHODS

Stress Response Study:

(a) Sugars

Stock solution of three sugars: sucrose, glucose and fructose having concentration of 10x was prepared and sterilized by autoclaving. The sugars were used individually as the only carbon source in basal media to determine the pathogens response. 100µl of the same concentration of inoculum was inoculated in 96 well microtitre plates and was incubated at 37ºC for 72 hours. After incubation, the optical density of the adherent biofilm was taken using a micro ELISA auto reader at 570nm. The tubes were stained in similar manner and results obtained with different sugars were compared.

(b) Salinity

Salt stock sodium chloride, lead nitrate and copper sulfate of concentration 1mg/ml were prepared in autoclaved distilled water. The salt stock was serially diluted in sterilized distilled water to obtain a concentration of 0.2, 0.4, 0.6, 0.8 and 1 mg/ml. 200µl of same inoculum concentration was inoculated into 96 well microtitre plates and incubated at 37ºC for 72 hours. The optical density was read after incubation to determine the response of the pathogens.

(c) Metals

A stock of four metals: calcium, zinc, iron and lead having concentration 0.2mg/ml was prepared in autoclaved distilled water and filtered through 0.22µm membrane filters and stored at 4ºC. The metal stock was further diluted in sterile distilled water so as to obtain concentration of 10, 20, 40 and 80 ppm. The media was inoculated with overnight broth culture as incubated for 72 hours followed by staining and analysis of stress response similar to (a) and (b).

(d) Statistical Analysis

The data obtained was analyzed using SPSS software and the significance difference of bacterial growth attachment was calculated using one way Anova.

3. RESULTS

Stress Response of Oral Opportunistic Pathogens:

(a). Simple and complex sugars

The pathogens stress condition that was screened using three different sugars (glucose, fructose and sucrose) obtained the following results. Fructose was found to be a poor source of carbon as most of the strains showed very less biofilm attachment. Sucrose showed best attachment whereas glucose showed moderate attachment.

(b). Salinity

The pathogens were also screened using three different salts (Sodium chloride, Lead nitrate, Copper sulfate) as the sole basal media. Sodium chloride was found to have a high attachment whereas lead nitrate and copper sulfate had less attachment of biofilm.

(c). Metal ions

Three different metal ions (Calcium, Zinc, Iron and Lead) were used as the sole basal media to screen the isolates and the result obtained was as follows: Calcium was found to have a high attachment; Zinc, Iron had moderate attachment whereas lead had less attachment of biofilm.

(d). Statistical data

The statistical analysis using Anova two-factor without replication, showed that the P-value was 0.09161, F-calculated 25.4382 and F-critical 3.2873.

4. DISCUSSION AND CONCLUSION

This study provides additional knowledge on stress response of six opportunistic oral pathogens: *Streptococcus mutans, Staphylococcus aureus, Methicillin Resistant Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterococcus spp.* Based on the findings, the pathogens responded differently to stress environment. The bacterial strains showed an interesting pattern of utilization of different energy sources influencing the pattern of bacterial growth attachment. The highest attachment was observed in presence of sucrose and the lowest was of fructose (Figure 1). Sucrose is considered the most cariogenic dietary carbohydrate, because it is fermentable, and also serves as a substrate for the synthesis of extracellular (EPS) and intracellular (IPS) polysaccharides in dental plaque (Newbrun, 1967; Bowen, 2002). There was very poor biofilm formation in case of fructose which is in agreement with earlier studies (Cury et al., 2000).

The findings for salt stress response showed sodium chloride had the highest bacterial attachment whereas lead nitrate and copper sulfate had moderately attachments. The results indicated that sodium ions and chloride ions had less effect on bacterial growth rate; hence more biofilm attachment (Figure 2). The metal stress investigation showed that calcium metal had high attachment and lead metal had less attachment (Figure 3). Bacterial cells produce RpoS a stress response regulator and also accumulate in response to environmental stresses such as low pH and osmotic stress. The maintenance of intracellular homeostasis is the basis of the enhanced physiological status and acid tolerance of biofilm cells (McNeill and Hamilton, 2004).

The presence of bacterial attachment was due to biofilm formation that varied among bacterial species. *Enterococcus spp.*, had the highest biofilm formation followed by *Streptococcus mutans* and *Pseudomonas aeruginosa* had the lowest biofilm formation (Figure 4). Biofilm formation act as a defensive mechanism of pathogens during various stress conditions (O'Toole et al., 2000). Microorganisms within biofilms can withstand nutrient deprivation, pH changes, oxygen radicals, disinfectants, and antibiotics (Lewis et al, 2001).

Statistical analysis of the data showed the P-value was 0.09161, F-calculated was 25.4382 and F-critical was 3.2873. It was concluded that there was a significant difference on how the sugars, salts and metals affected the bacterial growth population in the oral cavity.

Therefore, the analysis was significant to provide knowledge needed for efficient control and treatment of oral health infections and other systemic diseases.

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APPENDIX - A

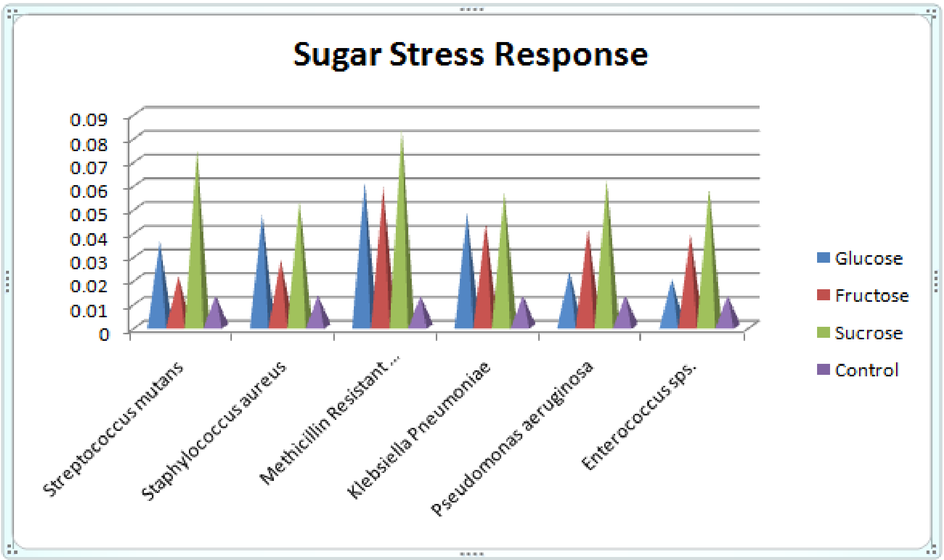

Figure 1

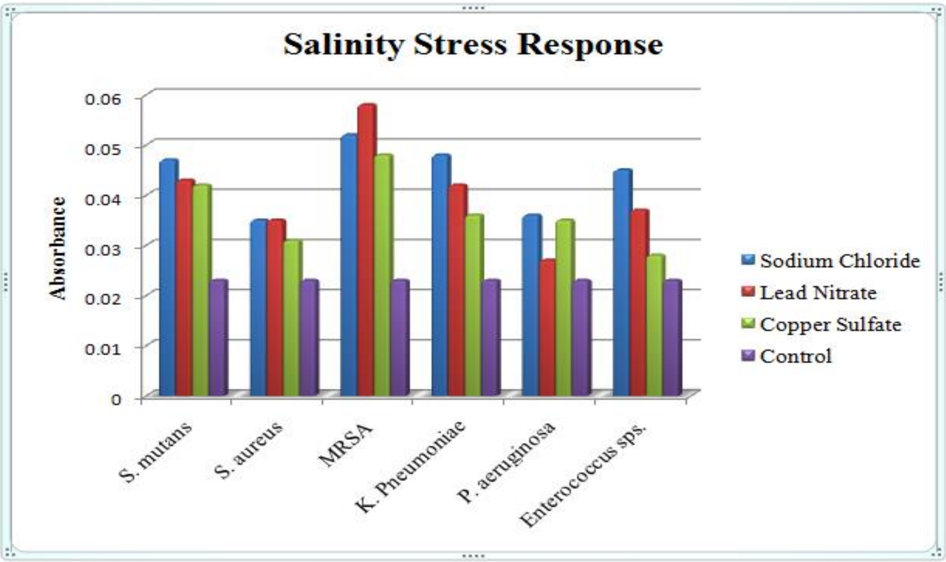

Figure 2

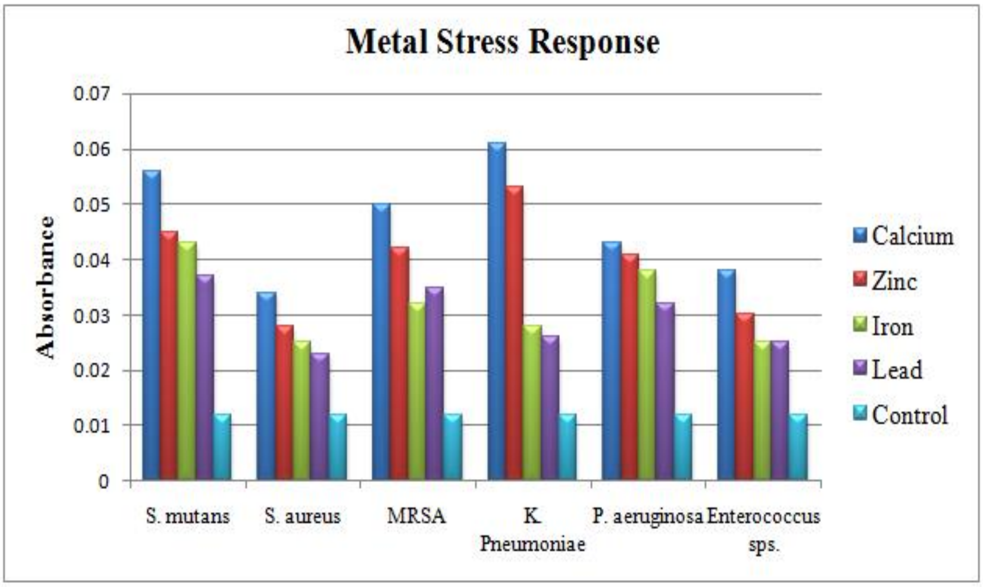

Figure 3

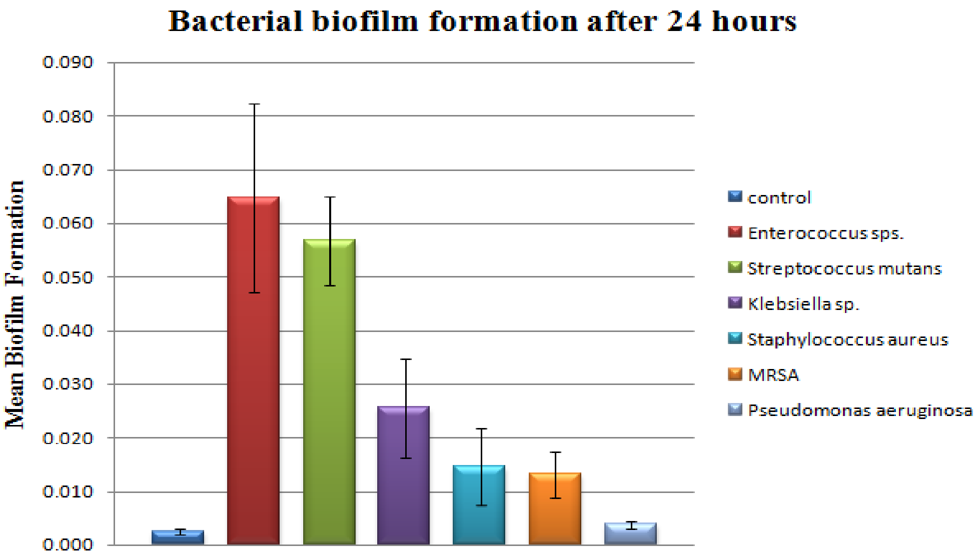

Figure 4