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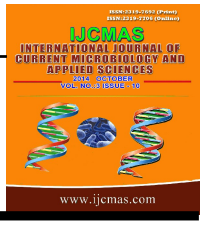

Original Research Article

Control of growth *Streptococcus mutans* isolated from saliva and dental caries

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Keywords

Streptococcus mutans, isolation, characterization, control of growth, xylitol sugar, susceptibility to antibiotics, chlorhexidine and garlic extract.

A B S T R A C T

One of the important virulence properties of *Streptococcus mutans* is their ability to form biofilms known as dental plaque on tooth surfaces and is a primary an etiological agent in dental caries. The aim of the study was to isolation, characterization and control of growth *S. mutan* isolates from saliva and dental caries. A total 70 dental plaque and saliva samples were collected from patient receiving treatment at the outpatient clinic, Faculty of Dental Medicine, Al-Azhar University, Cairo, Egypt, 2012. Out of 100 isolates recovered and identified, *Streptococcus* sp. was represented 56% from total isolates (*S. mutans* 40% and *Streptococcus sobrinus*16%), *Lactobacillus* sp. 15%, *Staphylococcus* sp. 12 %, *Fusobacterium* sp.10% and *Corynebacterium* sp. 7%. Ten *Streptococcus mutans* isolates subjected to different concentration from xylitol sugar 0.001%, 0.01% and 1.0 % has been used as a substitute sugar to inhibit dental caries. Measured growth and calculated inhibition growth percentage the resulted obtained revealed that xylitol sugar inhibited growth of *S. mutans* this inhibition depend on xylitol concentration. The *S. mutans* isolates were grown on sucrose, glucose and xylitol as only carbon source , pH was found drop from 7.2 to 4.3 with sucrose and 3.7 with glucose, while no change on pH when used xylitol as only carbon source after 60h. from incubation period. The susceptibility of *S. mutans* isolates to ten antibiotics revealed that highly sensitive to vancomycin, penicillin, and erythromycin but highly resistant to ciprofloxacin, bacitracin and methicillin. Quantitative studies of chlorhexidine susceptibility exhibit strong activity on *Streptococcus mutans*, isolates range from1-2µg/ml. The inhibitory effect of garlic extract on all *S. mutans* isolates revealed that all isolates sensitive to garlic extract.

Introduction

Streptococcus mutans is a Gram-positive, non-motile, non-spore forming, catalase-negative, facultative anaerobic cocci bacterium commonly found in the human oral cavity, is a significant contributor to tooth decay (David *et al*., 2011). The mutans streptococci comprise a group of seven species, of which *Streptococcus mutans* and *Streptococcus sobrinus* are the predominant species isolated from human saliva and

dental plaque (Loesche, 1986). It has also been reported that *S. mutans* adhere to damaged cardiac tissues which is marked as a significant event in the pathogenesis of chronic infective endocarditis (Miller-Torbert *et al.,* 2008) with a death rate of up to 50% in spite of antibiotic treatments (Nakano *et al.,* 2007). Experiments with gnotobiotic hamsters revealed these to be the main initiator microorganisms in dental caries disease (Fitzgerald and Keyes, 1960). Dental caries is a common infectious disease world-wide. The aetiology of the disease is multifactorial, life habits and mutans streptococcus infection being the most important factors (Johnson, 1991; Bratthall, 1997). In the disease process, the calcified tissues of the tooth are demineralized and the organic substance is broken down. *Streptococcus mutans* metabolize carbohydrates, such as glucose and sucrose, to produce acid and enhance biofilm formation with the early colonizing bacteria to induce dental caries. *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus* are all capable of demineralizing enamel by producing an acidic environment (Loesche, 1986) and (Featherstone, 2008). Therefore, control of the bacterial biofilm on teeth is essential for the maintenance of oral health. Xylitol, a five-carbon natural sugar alcohol, has been used as a substitute for sugar to inhibit dental caries (Twetman and Stecksen-Blicks, 2003; Autio, 2002; Sung *et al*., 2012). Various mechanisms for prevention of dental caries by xylitol have been suggested (Makinen, 1985) and (Makinen and Isokangas, 1988). Not only is xylitol unfermented by most dental plaque microorganisms but it also interferes with in vitro growth of the microorganisms, including mutans streptococci (Knuuttila and Makinen, 1975) and (Vadeboncoeur *et al*., 1983). The antibiotics such as penicillin amoxicillin, ampicillin, erythromycin tetracycline and chloramphenicol are used treatment dental caries In the recent years, a shift from narrows spectrum antibiotic prescriptions which included penicillin to broad-spectrum aminopenicillins which include amoxicillin by dental professionals has been reported and the increase of bacterial isolates resistant to the former antibiotics is blamed for such a shift in prescription practices (AI-Haroni and Skaug, 2007). The antimicrobial effects of dental luting glass ionomer cements on *Streptococcus mutans* (Sina *et al*., 2014). Worldwide, hundreds of plants are used in traditional medicine as treatment for bacterial infections. Conventional drugs usually provide effective antibiotic therapy for bacterial infections, however there is an increasing problem of antibiotics resistance and continuing need for new solution.

Although natural product is not necessarily safer than synthetic antibiotics, some patients prefer to use herbal medicines. Thus heath care professionals should aware of the available evidence for herbal antibiotics. Garlic (*Allium Sativa*) is one of the most extensively researched medicinal plants and its typical odor and antibacterial activity depends on allicin produced enzymatic activity of allinase (a cysteine sulfoxide lyase) on alliin after crushing or cutting garlic clove (Ross *et al*., 2001; Ellmore and Feldberg, 1994; Farzaneh *et al*., 2013). They are extensive literature on antibacterial effect of fresh garlic, garlic extract has been reported to inhibition growth of various Gram- positive and Gram- negative bacteria including *Micrococcus, Enterobacter. Escherichia, Klebsiella, Lactobacilli, Pseudomonas, Salmonella, Shigella, Proteus* and *Helicobacter pylori* (Ross *et al*., 2001), (Tsao and Yin, 2001) and (Sivam *et al*., 1997). The paper aims control of growth Streptococcus mutans isolated from saliva and dental caries.

Materials and Methods

Samples collection

The samples used on this study were collected from the tooth of a patient diagnosed with dental caries by a physician. The patient was receiving treatment at the outpatient clinic, Faculty of Dental Medicine, Al-Azhar University, Cairo, Egypt, 2012. The infected area of the tooth was swabbed with sterile cotton wool and saliva transferred to a sterile screw capped tube that contained 5.0 ml of Reduced Transport Fluid (RTF) with aseptic precautions, vortex mixed for 1minute, to disperse the bacteria. A loopful of dispersed samples was inoculated on various media.

Isolation and characterization of *S. mutans*

The samples were collected immediately streaked on Brain Heart Infusion (BHI), Gold's Medium and azide blood agar, an inhibitory media incubated at 37°C for 24, 48–72 h. Characteristic colonies were picked from the plates and purified by repeated sub-culturing. *S. mutans* was identified using cultural, morphological and bio-chemical characteristics as described (Cheesbrough, 2000; Slots and Taubman, 1992; Buchannan and Gibbon, 1974).

Xylitol inhibition test and Effect of carbon sources on pH change

The cells were cultured in 5ml Brain Heart Infusion (BHI) overnight at 37 °C from stocks kept frozen to produce log-phase cells. The cells were transferred to fresh BHI on the morning. The growth medium contained xylitol, the concentration ranging from 0.001, 0.01 and 1.0% xylitol was added to the sterile medium using filter sterilization. The control medium contained no added xylitol. The cells were cultured in shaking water both at 37°C for 8h. Growth was followed by measuring the absorbance at a wavelength of 660 nm and calculated inhibition growth percentage. The estimation effect of carbon sources on pH, prepare BHI medium and BHI without carbon source and add 1% from sucrose or glucose or xylitol and adjacent pH at 7.2, sterilized, inoculation with *S. mutans* isolates and incubation in shaking water both at 37°C measurement pH after 10, 20, 30, 40, 50, 60 and 70 hours from incubation period.

Antibiotics sensitivity screening of *Streptococcus mutans* isolates

The antimicrobial sensitivity profile the forty *S. mutans* isolates to ten antibacterial drugs was determined according to the method Bauer- Kirby (Bauer *et al*., 1966), (Forbes *et al*., 1998), (Lee *et al*., 2004) and (Aqueveque *et al*., 2006) using disks of antibiotics placed on surface of Brain Heart Infusion (BHI) medium seeded with the test organism. Inhibition zone were measured after 72h of incubation at 37 °C in the presence of 5% CO2 interpretation of resistance was based on the National Committee for Clinical Laboratory Standards NCCLS criteria.

Garlic extract preparation

Garlic extract was prepared according to method described by (Bakri and Douglas, 2005). Briefly the peeled fresh garlic 50.0 g was chopped and homogenized in 50 ml sterile distilled water, centrifuged, filtered through Wattman No. 1 filter paper and keep in frozen until used

Garlic extract sensitivity screening of *Streptococcus mutans* isolates

The antimicrobial activity of garlic extract on

S. mutans isolates determined by the conventional paper disk diffusion method by using paper disk (266812 W. Germany 12.7 mm in diameters) was soaked in 0.5 ml garlic extract, placed on Brain Heart Infusion (BHI) medium inoculated with *Streptococcus mutans* isolates and incubated at 37°C in presence of 5% CO_2 for 72h. The inhibition zone around the disk was measured in mm and recorded.

Chlorhexidine sensitivity screening of *Streptococcus mutans* isolates

The antimicrobial activity of chlorhexidine on *S. mutans* isolates determined by the conventional paper disk diffusion method by using paper disk (266812 W. Germany 12.7 mm in diameters) was soaked in solution contains 2 µg/ml chlorhexidine, placed on Brain Heart Infusion (BHI) medium inoculated with *Streptococcus mutans* isolates and incubated at 37 °C in presence of 5% CO_2 for 72h. The inhibition zone around the disk was measured in mm and recorded

Results and Discussion

A total 70 dental plaque and saliva samples were collected from patient diagnosed with dental caries by a physician (Table 1). Faculty of Dental Medicine, Al-Azhar University, Cairo, Egypt, 2012. Out of 100 isolates recovered and identified prevalence of *Streptococcus* sp. was 56% from total isolates (*S. mutans* 40% and *Streptococcus sobrinus* 16%), *Lactobacillus* sp. 15%, *Staphylococcus* 12 %, *Fusobacterium* sp. 10% and *Corynebacterium* sp. 7%. The various tests and results of the characterization scheme on table 2 shown that *S. mutans* is a Gram-positive, non-motile, non-spore forming, catalase-negative, facultative anaerobic, cocci, ferment of wide range of carbohydrates sucrose, mannitol, melibiose, raffinose, cellobiose sorbitol, lactose, salicin trehalose and inulin

The xylitol used as substitute sugar for growth ten isolates *Streptococcus mutans* the result raveled that inhibition *of Streptococcus mutans*. The percentage of inhibition growth depends on xylitol concentration the data recorded on table 3. The effect of carbon sources on pH formation were found that sucrose and glucose drop of pH after incubation period 60 hours from 7.2 to 4.3 and 3.7 while alcohalic sugar xylitol no change on pH value after incubation period 60 hours (Fig. 1). The susceptibility of *S. mutans* isolate to ten antibiotics revealed that highly sensitive to vancomycin with percentage 95%, penicillin 80% and erythromycin 72.5% but highly resistant to ciprofloxacin with percentage 80%, bacitracin 75% and methicillin 55% (Table 4).

The inhibitory effect of garlic extract on 40 isolates from *S. mutans* revealed that inhibition zone around the disks varied from 20mm to 36mm this indicated that all isolates of *S. mutans* sensitive to garlic extract (Table 5).

Streptococcus mutans is one of the most important oral bacterial which plays a major role in dental caries, bacteremia and consequently bacterial endocarditis among predisposed patient (Natagta *et al.,* 2006) and (Tanzer *et al.,* 2001). Prevention of dental caries can be achieved by proper and regular tooth brushing and rinsing with mouth rinses containing antibacterial agent such as chlorhexidine, sodium hypochlorite are widely used as mouthwashes and irrigating agents, respectively but this antibacterial agent is widely used have side effect such as cytotoxic to human periodontal ligament cells, inhibition protein synthesis and affect mitochondrial activity of these cells (Chany *et al.,* 2001). The

antibiotics for prevention of dental caries is not recommended, since there is risk of development bacterial resistant. The data obtained from study revealed that xylitol sugar was inhibited growth of *Streptococcus mutans* and not used as an energy source by the cariogenic bacteria and acid production is reduced. Furthermore, xylitol may stimulate the existing defense mechanisms against the pathogenesis of dental caries, such as an increase in salivary flow and maintenance of high pH in the oral fluid and the Plaque (Scheinin and Makinen, 1976). Acid produce play crucial roles in the pathogenesis of dental. Acidogenesis (acid production) and aciduracity (acid tolerance) are key cariogenic virulence factors of *S. mutans* (Kuramitsu, 1993). Bearing these properties, *S. mutans* has an upper hand over less-acid-tolerant species and hence impose physiological stress on them. Thus, even in stress conditions, it emerges out to be most prevalent inhabitant of cariogenic plaque. Thus, stress tolerance plays a crucial role in its pathogenesis. Therefore, xylitol is widely considered as an anticariogenic agent and used to partially substitute sucrose in human diet to prevent dental caries (Scheinin and Makinen, 1976).The susceptibility of *S. mutans* isolates to ten antibiotics exhibited highly sensitive to vancomycin with percentage 95%, penicillin 80% and erythromycin 72.5% but highly resistant to ciprofloxacin with percentage 80%, bacitracin 75% and methicillin 55%. The resistant of *Streptococcus mutans* to penicillin with percentage 22.8 % and erythromycin 23.9 was recorded by (Fani *et al*., 2007). Hasan *et al.*, (2014) used quercitrin in combination with deoxynojirimycin is synergistic across the range of cariogenic mechanisms of *S. mutans* compared to their individual effect combination to suppress the cariogenic pathways of *S. mutans*. The in vitro susceptibility of *S. mutans* isolates to chlorhexidine revealed that all isolates sensitive to chlorhexidine 2µg/ml (Table 6). Susceptibility of *S. mutans* to chlorhexidine were studied by Grönroos *et al*., (1995) and found that *S. mutans* is more susceptible than *S. sobrinus, S. cricetus* and *S. rattus*. Of the 379 clinical isolates studied, 50% were inhibited at 1 µg of chlorhexidine per ml, 90% were inhibited at 2 µg/ml, and all were inhibited at 4 µg/ml. The in vitro antibacterial, antifungal and antiviral activities of garlic extract have been widely recognized (Ross *et al*., 2001; Tsao and Yin, 2001; Sivam *et al*., 1997; Jain, 1998; Weber *et al*., 1992). Moreover in vivo studies on experimental animals also documented the inhibitory activity of garlic extract on various infectious agents such as methicillin resistant *Staphylococcus aureus* (Tsao *et al*., 2003) *Cytomegalovirus* (Fang *et al*., 1999) and *Shigella* sp. (Chowdhury *et al*., 1991). In vitro data obtained in this study revealed that garlic extract is good inhibited growth of *Streptococcus mutans*. It is thought that tooth paste or mouth wash containing garlic extract might be useful for prevention of dental caries.

Table.1 Ratio of bacteria isolated from dental caries

Bacterial species	**Prevalence (%)**
Streptococcus mutans	40
Streptococcus sobrinus	16
Lactobacillus sp.	15
Fusobacterium sp.	10
Corynebacterium sp.	7
Staphylococcus sp.	12

Table.2 Characterization of *S. mutans* isolates

Test	Result	Test	Result
Gram stain	Gram- positive	Growth on 6.5 NaCl	-ve
Shape	Cocci	Acid from:-	
Motility	Non motile	Sucrose	+ve
Oxygen requirements	Facultative anaerobic	Mannitol	+ve
Catalase	-ve	Melibiose	+ve
Hemolysis	α- β	Raffinose	+ve
Esculin hydrolysis	+ve	Cellobiose	+ve
Arginine hydrolysis	-ve	Lactose	+ve
Urea hydrolysis	-ve	Sorbitol	+ve
Hippurate hydrolysis	-ve	Salicin	+ve
Starch hydrolysis	+ve	Trehalose	+ve
Voges Proskauer test	+ve	Inulin	+ve
Glucan	+ve	Xylitol	-ve

ve = negative, +ve = positive

Table.3 Inhibition growth Percentage of ten isolates *S. mutans* in presence different concentration from xylitol

Bacterial isolates	0.01% xylitol	0.1% xylitol	1% xylitol
G12	2.4	27.5	40.3
G20	1.7	22.7	34.2
G22	2.8	30.2	45.0
G28	4.1	43.0	62.0
G32	1.4	20.5	32.0
G35	1.9	23.0	38.4
G40	3.5	36.4	52.7
G45	2.7	31.0	44.8
G50	1.3	19.5	30.5
G55	3.6	35.0	49.3

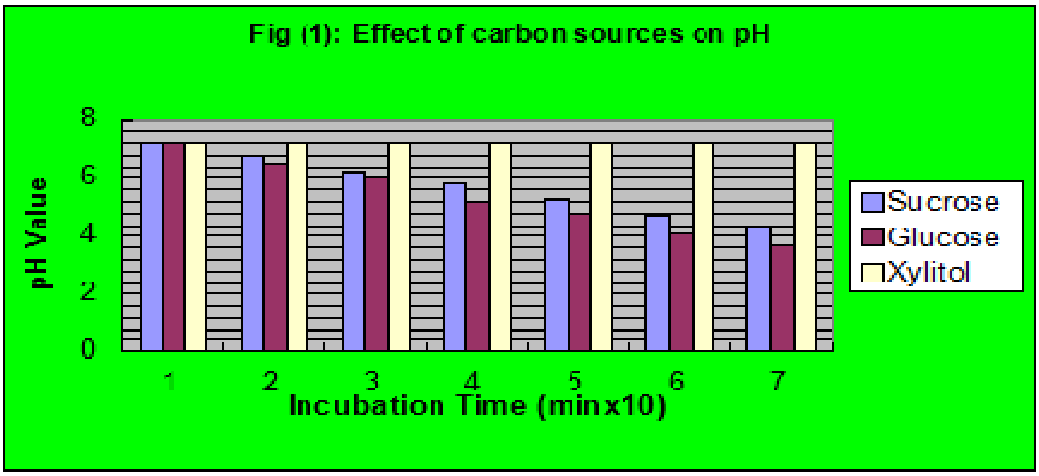

Table.4 Antibiotics sensitivity rate of 40 *Streptococcus mutans* isolated from dental caries by Bauer- Kirby disc diffusion methods

Antibiotics disc	Disk-potency µg/ml	Number of resistant isolates (%)	Number of sensitive isolates (%)
Penicillin	10	8(20)	32(80)
Ampicillin	10	15 (30)	22 (70)
Ciprofloxacin	30	32 (80)	8 (20)
Streptomycin	10	19(47.5)	21 (52.5)
Erythromycin	15	11(27.5)	29 (72.5)
Tetracycline	30	14 (35)	26 (65)
Bacitracin	10	30 (75)	10 (25)
Methicillin	10	22 (55)	18 (45)
Chloramphenicol	30	17 (42.5)	23 (57.5)
Vancomycin	30	2 (5)	37 (95)

Table.5 Inhibitory effect of garlic extract on the 40 *Streptococcus mutans* isolates isolated from dental caries by disc diffusion methods

Range of inhibition zone mm	Number of isolates (%)
20 -24	7 (17.5)
24 – 28	15 (37.5)
28- 32	10 (25)
32- 36	8 (20)

Table.6 Inhibitory effect of chlorhexidine 2µg on the 40 *Streptococcus mutans* isolates isolated from dental caries by disc diffusion methods

Range of inhibition zone mm	Number of isolates (%)
16 -20	5 (12.5)
20 – 24	12 (30.0)
24- 28	13 (32.5)
28- 32	10 (25.0)

References

AI-Haroni, M., Skaug, N. 2007. Incidence of antibiotic prescribing in dental practice in Norway and its contribution to national consumption. *J. Antimicrob. Chemother.,* 59: 1161–1166,

Aqueveque, P., Becerra, J., Palfner, G., Silva, M., Alarcon, J., Anke, T., Sterner, O. 2006. Antimicrobial activity of metabolites from mycelial cultures of Chilean basidiomycetes. *J. Chil. Chi. Soc.,* 51: 1057–1060.

Autio, J.T. 2002. Effect of xylitol chewing gum on salivary *Streptococcus mutans* in preschool children. *ASDC J. Dent. Child.,* 69(1): 81–86.

Bakri, I.M., Dougles, C.W. 2005. Inhibition effect of garlic on oral bacteria, *Arch. Oral. Biol.,* 50: 645–651.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol*., 45: 493–496.

Bratthall, D. 1997. Discovery! A *Streptococcus mutans* safari! J. Dent. Res., 76: 1332–1336.

Buchannan, R.E., Gibbon, M.E. 1974. Bergey's Manual Of Determinative Bacteriology, 8th ed. Williams and Wilkins Baltimore, USA, Pp. 450–860

Chany, Y.C., Huang, F.M., Tai, K.W., Chou, M.Y. 2001. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells. *Oral Surg. Oral Med. Oral Patho. Oral Radial Endod.,* 92: 446–480.

Cheesbrough, M. 2000. District Laboratory Practice in Tropical Country (PT 2). University Press, Cambridge, Pp. 157–255.

Chowdhury, A.K., Ahsan, M., Islam, S.N., Ahmed, Z.U. 1991. Efficacy of aqueous extract of garlic and allicin in experimental shigellosis in rabbits. *Indian J. Med. Res.,* 93: 33–36.

David, B.U, Linda, O.O., Charles, O.E. 2011. Isolation, Characterization and Antibiotic Susceptibility Studies of Clinical Isolates of *teptococcoccus mutans* Obtained from Patients Visiting Major Dental Clinics in Nsukka, Nigeria . AJPSP 2(1): 1–15.

Ellmore G.S., Feldberg R.S., 1994. Alliin lyase localization in bundle sheaths of garlic cloves (*Allium sativum*) *Am. J. Bot.,* 81: 89–94.

Fani, M.M, Kohanteb, J., Dayaghi, M. 2007. Inhibitory activity of garlic (*Allium sativum*) extract on muiltdrug resistant *Streptococcus mutans*. *J. Indian Soc. Pedod Prevent Dent.,* 25: 164–168.

Fang, F., Li, H., Cui, W., Dong, Y. 1999. Treatment of hepatitis caused by cytomegalovirus with allitridin injection, an experimental study. *J. Tougji Med. Univ.,* 19: 271–274.

Farzaneh, H., Afsoon, A., Fariba, S. 2013. Antibacterial activity of *Pistacia atlantica* extracts on *Streptococcus*

mutans biofilm. *Int. Res. J. Biol. Sci*., 2(2): 1–7.

Featherstone, J.D.B. 2008. Dental caries: a dynamic disease process. Aust. Dent. J., 53: 286–291.

Fitzgerald, R.J., Keyes, P.H. 1960. Demonstration of the etiologic role of *Streptococci* in experimental caries in the hamster. *J. Am. Dent. Assoc.,* 61: 9–19.

Forbes, B.A., Sahm, D.F., Weissfeld, A.S. 1998. Laboratory methods for detection of antibacterial resistance. In: Roche, J., Parker, S.J., McAdam, L., (eds), Baily and Scott's diagnostic microbiology, 10th edn. Mosby Inc., St Louis, Pp. 250–272.

Grönroos, L., Mättö, J., Saarela, M., Luoma, A-R, Luoma, H., Jousimies-Somer, H., Pyhälä, L., Asikainen, S., Alaluusua, S. 1995. Chlorhexidine susceptibilities of *mutans Streptococcal* serotypes and ribotypes. *Antimicrob. Agents Chemother.,* 39: 894–898.

Hasan, S., Singh, K., Danisuddin, M., Verma, P.K., Khan, A.U. 2014. Inhibition of major virulence pathways of *Streptococcus mutans* by quercitrin and deoxynojirimycin: A synergistic approach of infection control. PLoS One, 9(3): e91736. doi: 10.1371/journal.pone.0091736

Jain, R. 1998. Antitubercular activity of garlic oil. *Indian J. Pathol. Microbial.,* 43: 3045.

Johnson, N.W. 1991. Introduction: the nature of the caries process and the need for markers of risk. In: Risk markers for oral diseases. Vol. I. Dental caries: Markers of high and low risk groups and individuals. Johnson, N.W., (ed). Cambridge University Press, Cambridge, Pp. 1–12.

Knuuttila, M.L., Makinen, K. 1975. Effect of xylitol on the growth and metabolism of *Streptococcus mutans*. *Caries Res.,* 9(3): 177–89.

Kuramitsu, H.K. 1993. Virulence factors of mutans streptococci: role of molecular genetics. *Crit. Rev. Oral Biol. Med.,* 4: 159–176.

Lee, S.S., Zhang, W., Li, Y. 2004. The antimicrobial potential of 14 natural herbal dentrifices: Results of an *in vitro* diffusion method study. *J. Am. Dent. Assoc.,* 135: 1133–1141.

Loesche, W.J., 1986. Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev., 50: 353–380.

Makinen, K.K. 1985. New biochemical aspects of sweeteners. *Int. Dent. J.,* 35(1): 23–35.

Makinen, K.K., Isokangas, P. 1988. Relationship between carbohydrate sweeteners and oral diseases. *Prog. Food Nutr. Sci.,* 12(1): 73–109.

Miller-Torbert, T.A., Sharma, S., Holt, R.G. 2008. Inactivation of a gene for a fibronectin-binding protein of the oral bacterium *Streptococcus mutans* partially impairs its adherence to fibronectin. *Microb. Pathog.,* 45: 53–9. doi: 10.1016/j.micpath.2008.02.001.

Nakano, K., Nomura, R., Nemoto, H., Mukai, T., Yoshioka, H., *et al.* 2007. Detection of novel serotype k *Streptococcus mutans* in infective endocarditis patients. *J. Med. Microbiol.,* 56: 1413–1415. doi: 10.1099/jmm.0.47335-0

Natagta, E., Okayama, H., Ito, H., Inoue, M., Oha, T. 2006. Serotype- specific polysaccharide of *Sterptococcus mutans* contributes to infectivity in endocarditis. *Oral Microbial. Immunol.,* 21: 420– 423.

National Committee for Clinical Laboratory Standard. 1995. Methods for dilution antimicrobial tests for

bacteria that grow aerobically. Standard M7-A3. NCCLS. Villanova.

Ross, Z.M., O'Gara, E.A., Hill, D.J., Sleigholme, H.V., Maslin, D.J. 2001. Antimicrobial properties of garlic oil against human enteric bacteria. Evaluation of methodologies and comparisons with garlic oil sulfides and garlic powder. *Appl. Environ. Microbial.*, 67: 475–480.

Scheinin, A., Makinen, K.K. 1976. Turku sugar studies. An overview. *Acta Odontol Scand*, 34(6): 405–408.

Sina, K., Markus, A., Bettina, S., Annette, A., Elmar, H., Ali, A. 2014. Antimicrobial effects of dental luting glass ionomer cements on *Streptococcus mutans*. *The Sci. World J.*, 2014, Article ID 807086, 7.

Sivam, G.P., Lampe J.W., Ulness, B., Swanzy, S.R., Potter, J.D. 1997. *Helicobacter pylori* in vitro susceptibility to garlic (*Allium sativum*) extract. *Nutr. Cancer.*, 27: 118–121.

Slots, J. Taubman, M.A. 1992. Contemporary Oral Microbiology and Immunology. Mosby Year Book, St. Louis.

Sung, H.L., Bong, K.C., Young, J.K. 2012. The cariogenic characters of xylitol-resistant and xylitol-sensitive *Streptococcus mutans* in biofilm formation with salivary bacteria. *Arch. Oral Biol.*, 57: 697–703.

Tanzer, J.M., Livingston, J., Thompson A.M. 2001. The microbiology of primary dental caries in human. *J. Dent. Educ.*, 65: 1028–1037.

Tsao, S.M., Hsu, C.C., Yin, M.C. 2003. Garlic extract and two diallyl sulfide inhibit methicillin resistant *Staphylococcus aureus* infections in BALB/cA mice. *J. Antimicrobial. Chemother.*, 52: 974–980.

Tsao, S.M., Yin, M.C. 2001. In vitro antimicrobial activity of four diallyl sulfides occurring naturally in garlic and Chinese leek oil. *J. Med. Microbial.*, 50: 646–649.

Twetman, S., Stecksen-Blicks, C. 2003. Effect of xylitol-containing chewing gums on lactic acid production in dental plaque from caries active pre-school children. Oral Health Prev. Dent., 1(3): 195–199.

Vadeboncoeur, C., Trahan, L., Mouton, C., Mayrand, D. 1983. Effect of xylitol on the growth and glycolysis of acidogenic oral bacteria. *J. Dent. Res.*, 62(8): 882–884.

Weber, N.D., Aderson, D.O., North, J.A., Murray, B.K., Lawson, L.D., Hughes, B.G. 1992. In vitro virucidal effects of *Allium sativum* garlic extract and compound. *Planta Med.*, 58: 417–423.