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Characterization of Two Lytic Bacteriophages of *Streptococcus sobrinus* Isolated from Caspian Sea

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ABSTRACT

The aim of this research was to detect *Streptococcus sobrinus* lytic bacteriophages from Caspian Sea. Dental plaque samples were collected and cultured in Brain Heart Infusion Broth. The Caspian Sea water was filtered through a 0.45 μm membrane filter, added to *Streptococcus sobrinus* and cultured in Brain Heart Infusion Agar. Plaque forming assay suggested the presence of specific bacteriophages in sample. The first isolated bacteriophage for *Streptococcus sobrinus* was hexagonal in shape with approximately 70-98 nm in diameter that was most probably related to family Cystoviridae of bacteriophages. Another lytic bacteriophage was droplet-shape with a core and a coat measuring 75-81.25 nm in diameter and 93.75-100 nm in length that was most probably related to Guttaviridae of bacteriophages. Detection of new lytic phages for a gram positive bacterium and their applications for phage therapy of dental plaque could be considered as the significance and impact of the present study.

Key words: Bacteriophages, *Streptococcus sobrinus*, dental plaque, Caspian Sea

INTRODUCTION

One of the most extensive infectious diseases in humans is dental caries. Regarding worldwide spread of dental caries, its therapy is the most expensive treatments universally (Dinis *et al*., 2004; Hanada, 2000). The mutans Streptococci, related oral pathogenic bacteria such as *Streptococcus mutans, Streptococcus sobrinus* and *Streptococcus mitis* are responsible for initiation of dental plaque formation (Jacques, 1998; Milnes *et al*., 1993; Van der Ploeg, 2008). These strains are prevailed in teeth surfaces. Their concentrations and consequently their virulence are highly increased upon consumption of certain carbohydrates specially sucrose (Tanzer *et al*., 2001). Following production of capsular polymers, fructan and glucan and attachment to teeth, coaggregation of more than 500 sp. of 30 different bacterial genera in teeth surfaces results in acid generation from sugary substances. This triggers enamel decalcification and caries commence (Nyvad and Kilian, 1990; Schaechter, 2004). There are few reports studying on therapeutic vaccines against oral Streptococci such as *Streptococcus sobrinus* (Dinis *et al*., 2004) and *Streptococcus gordonii* (Fischetti *et al*., 1996; Lee, 2003) in order to prevent dental caries. Recently several studies have focused on tooth decay prevention through phage therapy of oral Streptococci (Bachrach *et al*., 2003; Beheshti Maal *et al*., 2010; Hitch *et al*., 2004). Although isolation of three lytic tailed bacteriophage, M102, e10 and f1, from human saliva that could attack various strains

of *Streptococcus mutans* has been reported (Delisle and Rostkowski, 1993) but all of these phages have been arisen as mutant progeny of prophages that had been carried in lysogenic host strains of *Streptococcus mutans* using mitomycin induction (Armau *et al*., 1988). The isolation of bacteriophages against standard strains of oral Streptococci such as *Streptococcus anginosus*, *Streptococcus constellatus*, *Streptococcus cristatus*, *Streptococcus gordonii*, *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus parasanguis*, *Streptococcus pneumoniae*, *Streptococcus salivarius*, *Streptococcus sanguis* and *Streptococcus sobrinus* from human oral cavity has been attempted but no bacteriophage specific for these recognized oral pathogens were found (Hitch *et al*., 2004). Also the detection of bacteriophages for *Streptococcus sobrinus, Streptococcus mutans and Streptococcus salivarius* from human saliva has been unsuccessful (Bachrach *et al*., 2003). The complete genome sequence of M102, a lytic bacteriophage of *Streptococcus mutans*, has been resolved regardless the potential effects of these phages for dental caries phage therapy (Van der Ploeg, 2007). While *Streptococcus salivarius, Streptococcus sanguis, Streptococcus oralis* and *Streptococcus gordonii* are first spp. that are colonized in tooth surfaces, however *Streptococcus sobrinus* along with *Streptococcus mutans* are responsible for the most dental disorders such as tooth decay, periodontitis and gingivitis (Jacques, 1998; Milnes *et al*., 1993; Smith *et al*., 1993; Tanzer *et al*., 2001; Van der Ploeg, 2008). The main goals of this research were isolation and identification of *Streptococcus sobrinus* from dental plaques of healthy individuals as well as patients with mild gingivitis, isolation and identification of its lytic bacteriophages as potential agents for phage therapy of dental plaque.

MATERIALS AND METHODS

Bacterial strains, culture media and chemicals: The standard bacterial strain *Streptococcus sobrinus* ATCC27607 provided from Iranian Research Organization for Science and Technology (IROST), Karaj, Iran was used. The culture media used were Brain Heart Infusion Broth (BHI), Brain Heart Infusion Agar (BHA). Mitis-Salivarius Agar (sucrose, 50 g L^{-1}; agar, 15 g L^{-1}; enzymatic digest of protein, 10 g L^{-1}; proteose peptone, 10 g L^{-1}; K_2HPO_4, 4 g L^{-1}; dextrose, 1 g L^{-1}; trypan blue, 0.08 g L^{-1}; crystal violet, 0.8 g L^{-1}; Na_2TeO_3 solution, 1 mL; distilled water, 1000 mL) (Atlas, 2004) and Blood Agar Base medium, all from Himedia, India, NaCl, glycine, $CaCl_2$, anaerobic class A gas pack, all from Merck, 0.45 μm membrane filter (Millipore, white gridded), api 20 Strep kit (bioMerieux, France) and H_2O_2 from Shimifan, Iran.

Dental plaque samples: The dental plaque samples were collected from healthy volunteers (10 students, aged 22-26, 8 females, 2 males at the Faculty of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran) and patients with mild gingivitis and periodontitis (aged 29-54, 6 females and 4 males). The dental plaque samples were obtained using sterile explorers from the upper right first molars in all individuals that had not applied antibacterial rinse and routine brushing 12-14 h before sampling. The samples were taken in accordance with ethical guidelines and regulations prepared by Department of Operative Dentistry, Faculty of Dentistry, Isfahan University of Medical Sciences, which have been authorized by Iranian Ministry of Health and Medical Education.

Enrichment, isolation and primary identification of dental plaque Streptococci: The dental plaque samples were cultured in BHI and then incubated at 37°C in 5% CO_2 for 24 h. After

bacterial enrichment, the turbid broth media were cultured to Mitis-Salivarius Agar (MSA) using streak plate method and incubated at 37°C in 5% CO_2 for 48 h. The colonies were examined for catalase reaction using hydrogen peroxide.

Macroscopic, microscopic and biochemical characterizations: The individual colonies were examined for their macroscopic traits such as color, size, morphology, light reflection and hemolysis on 10% sheep blood agar. The microscopic morphology and arrangement of purified bacteria on selective media such as MSA were examined using gram staining method. For biotyping, the bioMerieux SA api 20 Strep kit, an identification system for Streptococcaceae was used. Before using api 20 Strep kit, a 48 h well isolated colony was picked from blood agar medium and suspended in 300 μL sterile distilled water, homogenized well and then swabbed aseptically the entire surface of blood agar culture media. Next procedures were followed using api 20 Strep instruction.

DNA extraction of isolated *Streptococcus*: For extraction of DNA from isolated oral *Streptococcus*, a modified protocol of previous published method was followed (Hoshino *et al*., 2004). So the organisms were grown in 4 mL BHI at 37°C in 5% CO_2 for 24 h to optical density of 1.00 at 550 nm. Half and one milliliters of BHI was transferred to a sterile Eppendorf tube and centrifuged at 11000 g for 5 min. The supernatant was discarded and 500 μL of ultrapure distilled water was added to pellet. The suspension was boiled for 12 min using a microwave oven at 500 W. The suspension was being pipetted while boiling for homogenizing the extracted DNA and then centrifuged at 11000 g for 5 min to precipitate the cell debris. The supernatant containing the DNA was transferred to another sterile Eppendorf and stored at -20°C for further processing.

PCR identification of isolated *Streptococcus* using *gtf*T gene: The Primers used for molecular identification of *Streptococcus sobrinus* were the same as a previously published data for PCR identification of oral Streptococci (Hoshino *et al*., 2004). These primers were related to glucosyltransferase enzyme gene, *gtf*T, of *S. sobrinus* and were as follow: forward primer: 5'GATGATTTGGCTCAGGATCAATCCTC3' and reverse primer: 5'ACTGAGCCAGTAGTAGTAGACTTGGCAACT3'. PCR was performed in 20 μL of a reaction mixture containing 1 μL (< 10 ng μL^{-1}) of DNA template, 0.5 μM of each forward and reverse primers, 1.5 mM of $MgCl_2$, 0.5 mM of dNTP mixture, 0.5 U of Smar-Taq DNA polymerase (Cinagen, Iran) and 4.2 mM of 10x PCR buffer. Amplification was done using a gradient thermal cycler (Eppendorf Mastercycler) with the following program, 30 cycles of denaturing step at 98°C for 1 min, primer annealing and extension steps at 70°C for 2 min. The PCR final products were run in electrophoresis in 80 V, 40 mA, for 35-45 min using 1% agarose gel.

Bacteriophage resource sampling: The water sample was collected using a sterile 1000 mL bottle from the depth of 50 cm inframarine surface of the Caspian Sea at Anzali Swamp, Guilan province, north of Iran. The sampling was undertaken for 5 min and the cap of the bottle was fitted below the water surface. Then the Caspian Sea water sample was transferred to our laboratory at 4°C.

Preparation of bacteriophage samples and bacterial treatment: Fifty milliliters of well shaked Caspian Sea water pipetted to sterile falcons and centrifuged at 1000 g for 15 min. The supernatants were filtered through 0.45 μM milipore membrane filter using sterile milipore filtration system and the filtrate was stored at 4°C. Then the identified bacterial isolate from dental

plaque and *Streptococcus sobrinus* ATCC27607 were cultured in 10 mL of BHI separately and incubated at 37°C for 24 h in order to be activated. After growth and obtaining appropriate turbidity, the bacterial inoculum was cultured to 250 mL Erlenmeyer flasks containing 100 mL BHI and incubated in a shaker incubator at 37°C and 120 rpm shaking speed for 16-18 h. The shaking was then stopped at bacterial logarithmic phase and 10 mL of Caspian Sea filtrate were added to flasks aseptically. The shaking at 37°C was continued for another 45-60 min for attachment of probable bacteriophages to their specific bacterial hosts. Then 1 mL of BHI was added to 5 mL preheated 45°C BHA (0.7% agar), as top agar, vortexed and immediately overlayed on BHA plates. The BHAs were incubated at 37°C for 24-48 h until lysis zones, bacteriophage plaques, were appeared.

Bacteriophage isolation and purification: After appearance of bacteriophage plaques, they were cut aseptically and washed with 1.25% glycine in sterile Eppendorf tubes, vortexed well and the aforementioned procedures were repeated for 3 times. The overall BHI from the last trial after complete clearance, 24 h incubation at 37°C with 120 rpm shaking speed, were centrifuged at 1000 g for 15 min and supernatants were passed through 0.45 μM milipore membrane filter. Ten milliliters of the filtrates were used for further purification using a previously described method (Sambrook *et al*., 1989).

Transmission electron microscopy: A drop of purified filtrated bacteriophage suspension transferred on a formvar coated grids (EM standard, 3.2 mm diameter). The additional suspension was removed by drying paper. One of the grids was then stained using uranyl acetate solution (uranyl acetate, 2 g; methanol 35%, 10 mL) and another grid was stained using 2% phosphotungstic acid (pH: 7.2 regulated with KCl, 0.5 molar) and dried following the same method and then observed through Transmission Electron Microscope (Philips, CM 10, Netherlands) at 39 K magnification.

RESULTS

The dental plaque bacteria were increased in BHI at 37°C and 5% CO_2 after 48 h. The cultivation of these enriched bacteria on MSA after 24-48 h incubation at 37°C and 5% CO_2 resulted in emerging of colonies that were pale blue to bluish in color. Further examinations of colonies using stereomicroscope revealed that they were smooth, convex and intermediate with 0.8-0.9 mm in diameter. Microscopic observations showed large gram positive Streptococci. The negative catalase test indicated that the isolates were related to Streptococcaceae family. The macroscopic and microscopic characterization of isolated oral Streptococci indicated that they were members of the genus *Streptococcus* (Table 1).

Table 1: The main macroscopic and microscopic characteristics of isolated oral *Streptococcus* from dental plaque on mitis-salivarius agar after 48 h incubation at 37°C and 5% CO_2

Characteristics	Macroscopic	Microscopic
Colony traits	Pale blue to bluish, smooth, mucoid with shiny reflection, convex, intermediate colonies with 1-1.5 mm in diameter	
Morphology		Large cocci
Arrangement		Long Streptococci and few Diplococci
Gram reaction		Positive

The results of BioMerieux SA api 20 Strep kit after 4 and 24 h incubation periods at 37°C indicated two 7-digit identification numbers, 6020010 and 6020710, respectively. Using the database (V7.0), analytical profile index and apiweb™ identification software on the internet, it was confirmed that the isolated oral *Streptococcus* was *Streptococcus sobrinus*. The expected size of *gtf*T gene PCR product was ~328 bp (Hoshino *et al*., 2004) and regarding this, the species specific amplified DNA of *Streptococcus sobrinus* was observed in agarose gel after staining with ethidium bromide. The specific bond of *S. sobrinus* glucosyltransferase T gene on agarose gel was located between 300 and 400 bp bonds of ladder. Regarding its molecular weight that was suitably fitted with the expected size, 328 bp, the species of isolated oral *Streptococcus* was reconfirmed as *S. sobrinus* (Fig. 1).

The addition of Caspian Sea filtrate to 16-18 hour *Streptococcus sobrinus* and standard strain *Streptococcus sobrinus* ATCC27607 in BHI growth medium resulted in complete clearance of BHI after 12 h shaking incubation at 37°C. The cultivation of *S. sobrinus*-Caspian Sea filtrate inoculums after 45-60 min shaking, on BHA plates showed the bacteriophage plaques after 24 h incubation at 37°C (Fig. 2). The continuous contamination of new logarithmic growth of *Streptococcus sobrinus* by bacteriophages obtained from individual plaques resulted in more purification of them. The results showed that these specific bacteriophages were lytic phages.

Transmission electron microscopy of purified and concentrated bacteriophage suspension revealed two kinds of specific lytic bacteriophages. The capsid of the first *Streptococcus sobrinus* bacteriophage was hexagonal with approximately 70-98 nm in diameter (Fig. 3). These results suggested that the first specific bacteriophage of *Streptococcus sobrinus* isolated from Anzali Swamp in Caspian Sea is most probably related to family *Cystoviridae* of bacteriophages. The second *Streptococcus sobrinus* bacteriophages were droplet-shape with a core and a coat. Their approximate width and length were measured 140-168×196-266 nm, respectively using negative staining with phosphotungstic acid (Fig. 3). Using uranyl acetate staining of the same

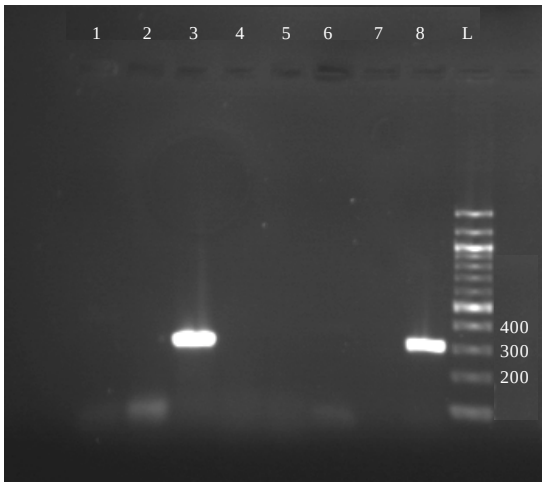

Fig. 1: The molecular identification of *Streptococcus sobrinus* using *gtf*T gene PCR amplification. Columns 1-6 were included the products of *gtf* gene PCR of *S. mutans*, *S. sanguis, S. sobrinus, S. gordonii, S. salivarius* and *S. oralis* respectively. The column 7 is negative control and 8 is positive control (*S. sobrinus* ATCC27607)

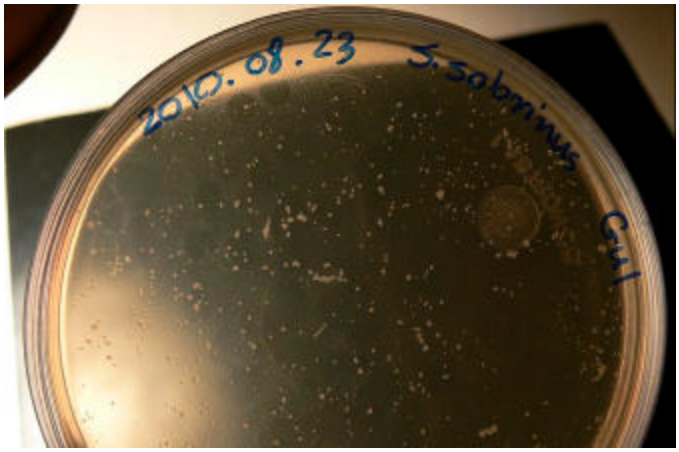

Fig. 2: The plaques of *Streptococcus sobrinus* bacteriophages isolated from Caspian Sea on brain heart infusion agar after 24 h incubation at 37°C

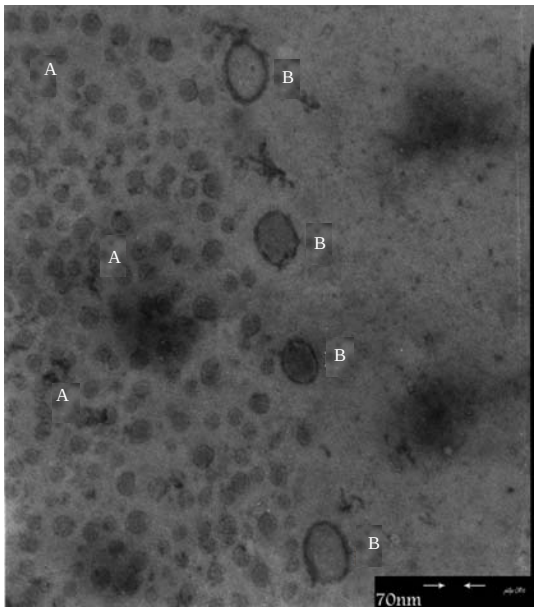

Fig. 3: Transmission electron micrograph of the lytic hexagonal bacteriophages with approximately 70-98 nm diameter (A) Droplet-shape bacteriophages containing a core and a coat with approximate width and length as 140-168×196-266 nm (B) Isolated from Caspian Sea specific for *Streptococcus sobrinus* isolated from dental plaque and *Streptococcus sobrinus* ATCC27607 (Bar = 70 nm)

bacteriophages showed droplet-shape particles with a core and a coat measuring 75-81.25 nm in diameter and 93.75-100 nm in length (Fig. 4). These results suggested that the second specific bacteriophage of *Streptococcus sobrinus* isolated from Caspian Sea is most probably related to family Guttaviridae of bacteriophages.

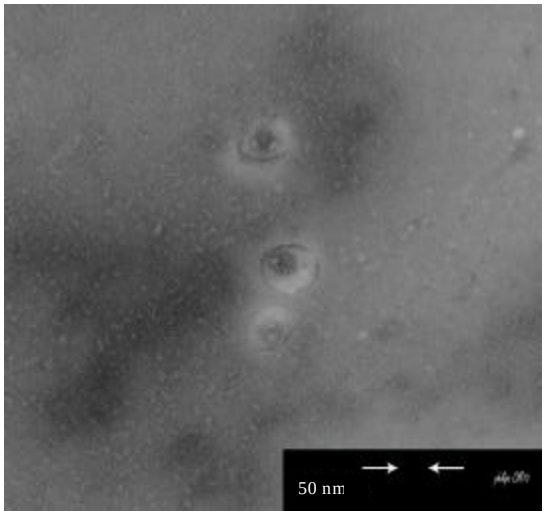

Fig. 4: Transmission electron micrograph of the droplet-shape bacteriophages with a core and a coat measuring 75-81.25 nm in diameter and 93.75-100 nm in length isolated from Caspian Sea specific for *Streptococcus sobrinus* isolated from dental plaque and *Streptococcus sobrinus* ATCC27607 (Bar = 50 nm)

DISCUSSION

The use of bacteriophages for curing the microbial infections or phage therapy has its roots in the 1940s at the middle of the World War II when many of injured solders from Soviet Union and Germany were saved using these antibacterial agents (Bahador *et al*., 2007; Marks and Sharp, 2000). Since the discovery of bacteriophages by Twort and d'Herelle, several bacterial infections from different aspects of medicine such as stomatology, ophthalmology, dermatology, gynecology, laryngology, pediatrics and surgery have been treated using phage therapy (Chanishvili *et al*., 2001). Bacteriophage base therapies have been used against *E. coli* diarrhoeal diseases in calves (Smith *et al*., 1987; Smith and Huggins, 1983), skin burns from *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Soothill, 1992) and water borne pathogens such as *Vibrio cholera* (Marks and Sharp, 2000). The inhibitory effects of bacteriophages on the growth of *Vibrio* sp. as pathogens of shrimp (Pasharawipas *et al*., 2011; Srinivasan *et al*., 2007) and *Salmonella enteritidis*, as a pathogen of poultry, have been investigated (Bielke *et al*., 2007; Donoghue *et al*., 2007; Higgins *et al*., 2007). The lytic bacteriophages of human pathogens such as *Salmonella typhi*, *Salmonella typhimurium* and *Shigella boydii* have been isolated and characterized. These bacteriophages were considered as the first choice of prophylaxis against nosocomial and secondary infections occurred by these pathogens (Abdulla *et al*., 2007; Sagor *et al*., 2005; Sundar *et al*., 2009). There are a few reports indicating the potential role of bacteriophages for controlling oral Streptococci and dental disorders. Armau *et al*. (1988) isolated lytic bacteriophages of *Streptococcus mutans* through screening several human saliva samples. Delisle and Rostkowski (1993) reported the characterization of three lytic bacteriophages of *Streptococcus mutans*, all of them were tailed and related to family Siphoviridae of bacteriophages. Bachrach *et al*. (2003) have investigated the isolation of bacteriophages from human saliva. While their aims were detection of bacteriophages for oral pathogens such as *Streptococcus sobrinus*,

Streptococcus mutans and *Streptococcus salivarius* but they didn't succeed to isolate their bacteriophages from human saliva instead they isolated *Enterococcus faecalis* phages as spherical enveloped structures with 70 nm in diameter without suggesting their viral family. Hitch *et al.* (2004) have tried to isolate bacteriophages against standard oral Streptococci include *Streptococcus sobrinus* NCTC1227, *Streptococcus salivarius* NCTC8618, *Streptococcus mutans* NCTC1049 as well as other nine spp. from human saliva, dental plaque and mature biofilms. They didn't detect any bacteriophage for the mentioned oral Streptococci species. Instead they isolated a lytic bacteriophage for *Proteus mirabilis* as a transient microorganism in oral cavity. Their isolated bacteriophages had icosahedral head with 70×76 nm in diameter and sheath with 97 nm in length. They suggested that the bacteriophages for control of oral infections must be isolated from other resources than oral specimens. According to latest classification of bacteriophages, the genus *Streptococcus* has 290 different bacteriophages among which 1 bacteriophage has been related to Myoviridae family, 276 phages have been Siphoviridae family members, 12 phages were dependent to Podoviridae family that all were related to tailed phages (Ackermann, 2007, 2009). While we recently identified a Cystovirus with ~84 nm in diameter we isolated from Persian Gulf that was specific for *Streptococcus salivarius* (Beheshti Maal *et al.*, 2010) there is not any report in literature review indicating the isolation of lytic bacteriophages for oral Streptococci except for *Streptococcus mutans* (Armau *et al.*, 1988; Delisle and Rostkowski, 1993). In this study we isolated two specific lytic bacteriophages for *Streptococcus sobrinus* from Anzali Swamp in Caspian Sea located at the north of Iran. The first isolated bacteriophages for *Streptococcus sobrinus* were hexagonal in shape with approximately 70-98 nm in diameter that were most probably related to family Cystoviridae of bacteriophages (Fig. 3a). Also we isolated another specific lytic bacteriophage for *Streptococcus sobrinus* from Anzali Swamp in Caspian Sea that was droplet-shape contain a core and coat approximately measuring 140-168×196-266 nm for width and length respectively (Fig. 3b). Uranyl acetate staining of the same bacteriophages showed droplet-shape particles with a core and a coat measuring 75-81.25 nm in diameter and 93.75-100 nm in length (Fig. 4). These results suggested that the second specific bacteriophage of *Streptococcus sobrinus* isolated from Caspian Sea is most probably related to family Guttaviridae of bacteriophages. So far there is no report indicating the isolation of pleomorphic phages (PFP) like Guttaviridae that specifically attack to Streptococci (Ackermann, 2007, 2009). While the Streptococci are very sensitive bacteria to environmental conditions and are absent from sea water resources, the isolation of two specific bacteriophages for an oral *Streptococcus, Streptococcus sobrinus*, from Caspian Sea water is a very considerable finding. In conclusion this is the first report indicating the isolation of a Guttavirus and a Cystovirus from Caspian Sea located at the north of Iran that had lytic effects on *Streptococcus sobrinus* as a gram positive bacterium. Although there are few documents in literature indicating the isolation of *Streptococcus mutans* lytic bacteriophages from salivary samples there has not been any report so far showing the isolation and identification of lytic bacteriophages of other oral Streptococci species. We suggested that the isolation and identification of these lytic bacteriophages of *Streptococcus sobrinus* as well as other oral Streptococci could be considered as biological and powerful agents for phage therapy of dental caries and other dental and periodontal disorders in modern virology, dentistry and medicine.

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REFERENCES

Abdulla, H., I. Khafagi, M. Abd El-Kareem and A. Dewedar, 2007. Bacteriophages in engineered wetland for domestic wastewater treatment. Res. J. Microbiol., 2: 889-899.

Ackermann, H.W., 2007. 5500 phages examined in the electron microscope: Brief review. Arch. Virol., 152: 227-243.

Ackermann, H.W., 2009. Phage Classification and Characterization. In: Bacteriophages: Methods and Protocols, Isolation, Characterization and Interactions, Clokie, R.J. and A.M. Kropinski (Eds.). Humana Press, New York, pp: 127-140.

Armau, E., J.L. Bousque, D. Boue and G. Tiraby, 1988. Isolation of lytic bacteriophages for *Streptococcus mutans* and *Streptococcus sobrinus*. J. Dent. Res., 67: 121-124.

Atlas, R.M., 2004. Handbook of Microbiological Media. 3rd Edn., CRC Press, New York, Pages: 1282.

Bachrach, G., M. Leizrovici-Zigmond, A. Zlotkin, R. Naor and D. Steinberg, 2003. Bacteriophage isolation from human saliva. Lett. Appl. Microbiol., 36: 50-53.

Bahador, N., M. Baserisalehi and B.P. Kapadnis, 2007. Application of phages. Res. J. Microbiol., 2: 445-453.

Beheshti Maal, K., M. Bouzari and F.A. Zavareh, 2010. Identification of *Streptococcus salivarius* bacteriophage isolated from Persian Gulf as a potential agent for dental caries phage therapy. Afr. J. Microbiol. Res., 20: 2127-2132.

Bielke, L.R., S.E. Higgins, A.M. Donoghue, T. Kral, D.J. Donoghue, B.M. Hargis and G. Tellez, 2007. Evaluation of alternative host bacteria as vehicles for oral administration of bacteriophages. Int. J. Poult. Sci., 6: 758-761.

Chanishvili, N., T. Chanishvili, M. Tediashvili and P.A. Barrow, 2001. Review: Phages and their application against drug resistant bacteria. J. Chem. Technol. Biotechnol., 76: 689-699.

Delisle, A.L. and C.A. Rostkowski, 1993. Lytic bacteriophages of *Streptococcus mutans*. Curr. Microbiol., 27: 163-167.

Dinis, M., D. Tavares, A.J.M.M. Fonseca, R. Faria, A. Ribeiro, A.M.S. Cabrita and P. Ferreira, 2004. Therapeutic vaccine against *Streptococcus sobrinus*-induced caries. J. Dent. Res., 83: 354-358.

Donoghue, A.M., L.R. Bielke, S.E. Higgins, D.J. Donoghue, B.M. Hargis and G. Tellez, 2007. Use of wide-host-range bacteriophages to reduce *Salmonella* on poultry products. Int. J. Poult. Sci., 6: 754-757.

Fischetti, V.A., D. Medaglini and G. Pozzi, 1996. Gram-positive commensal bacteria for mucosal vaccine delivery. Curr. Opin. Biotechnol., 7: 659-666.

Hanada, N., 2000. Current understanding of the cause of dental caries. Jap. J. Infect. Dis., 53: 1-5.

Higgins, S.E., J.P.S. Higgin, L.R. Bielke and B.M. Hargis, 2007. Selection and application of bacteriophages for treating *Salmonella enteritidis* infections in poultry. Int. J. Poult. Sci., 6: 163-168.

Hitch, G., J. Pratten and P.W. Taylor, 2004. Isolation of bacteriophages from the oral cavity. Lett. Appl. Microbiol., 39: 215-219.

Hoshino, T., M. Kawaguchi, N. Shimizu, N. Hoshino, T. Ooshima and T. Fujiwara, 2004. PCR detection and identification of oral Streptococci in saliva samples using *gtf* genes. Diag. Microbiol. Infect. Dis., 48: 195-199.

Jacques, N., 1998. Molecular biological techniques and their use to study streptococci in dental caries. Aust. Dent. J., 43: 87-98.

Lee, S.F., 2003. Oral colonization and immune responses to *Streptococcus gordonii*: potential use as a vector to induce antibodies against respiratory pathogens. Curr. Opin. Infect. Dis., 16: 231-235.

Marks, Y. and R. Sharp, 2000. Bacteriophages and biotechnology: A review. J. Chem. Tech Biotech., 75: 6-17.

Milnes, A.R., G.H. Bowden, D. Gates and R. Tate, 1993. Microbiota on the teeth of preschool children. Microbiol. Ecol. Hlth. Dis., 6: 213-227.

Nyvad, B. and M. Kilian, 1990. Comparison of the initial *Streptococcal microflora* on dental enamel in caries-inactive individuals. Caries Res., 24: 267-272.

Pasharawipas, T., J. Manopvisetcharean, T.W. Flegel 2011. Phage Treatment of *Vibrio harveyi*: A General Concept of Protection against Bacterial Infection Res. J. Microbiol., 6: 560-567.

Sagor, M.M., K.K. Islam, M.R. Ali, S.M. Abdul-Awal, P.P. Adhikary, P.K. Sarker and A.S.M. Rakib, 2005. Bacteriophage: A potential therapeutic agent (A Review). J. Medical Sci., 5: 1-9.

Sambrook, J., E.F. Fritsch and T.A. Maniatis, 1989. Molecular Cloning: A Laboratory Manual. 2nd Edn., Cold Spring Harbor Laboratory Press, New York, USA., ISBN-13: 9780879695774, Pages: 3.

Schaechter, M., 2004. The Desk Encyclopedia of Microbiology. 1st Edn., Elsevier Academic Press, New York.

Smith, D.J., J.M. Anderson, W.F. King, J. van Houte and M.A. Taubman, 1993. Oral streptococcal colonization of infants. Oral Microbiol. Immunol., 8: 1-4.

Smith, H.W. and M.B. Huggins, 1983. Effectiveness of phages in treating experimental *Escherichia coli* diarrhoea in calves, piglets and lambs. Genet. Microbiol., 129: 2659-2675.

Smith, H.W., M.B. Huggins and K.M. Shaw, 1987. The control of experimental *Escherichia coli* diarrhoea in calves by means of bacteriophages. J. Genet. Microbiol., 133: 1111-1126.

Soothill, J.S., 1992. Treatment of experimental infections of mice with bacteriophages. J. Med. Microbiol., 37: 258-262.

Srinivasan, P., P. Ramasamy, G.P. Brennan and R.E.B. Hanna, 2007. Inhibitory effects of bacteriophages on the growth of *Vibrio* sp., pathogens of shrimp in the Indian aquaculture environment. Asian J. Anim. Vet. Adv., 2: 166-183.

Sundar, M.M., G.S. Nagananda, A. Das, S. Bhattacharya and S. Suryan, 2009. Isolation of host-specific bacteriophages from sewage against human pathogens. Asian J. Biotechnol., 1: 163-170.

Tanzer, J.M., J. Livingston and A.M. Thompson, 2001. The microbiology of primary dental caries in humans. J. Dent. Edu., 65: 1028-1037.

Van der Ploeg, J.R., 2007. Genome sequence of *Streptococcus mutans* bacteriophage M102. FEMS Microbiol. Lett., 275: 130-138.

Van der Ploeg, J.R., 2008. Characterization of *Streptococcus gordonii* prophage PH15: Complete genome sequence and functional analysis of phage-encoded integrase and endolysin. Microbiology, 154: 2970-2978.