Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2011, 2 (2) :314-318
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0976-1233
CODEN (USA): ABRNBW

Indian River water action on *Streptococcus*; a microbiological prospective

Pradeep kumar singh[1], Akila Pranathi Parripati[2], Suman Bareth[1] and R. Balaji Raja[1*]

[1]*Department of Biotechnology, National Institute of Technology (NIT), Raipur, Chhattisgarh, India*
[2]*Department of Biotechnology, SRM University, Kattankulathur, Tamilnadu, India*

ABSTRACT

Ganga water is believed to be the purest water since time immemorial according to Indian mythology. It has wide medicinal uses in local therapy. Ganga water is believed to be self puricatory in nature which is the reason behind the uniqueness of it. The study was aimed at demonstrating the self puricatory action of Ganga water against a clinical pathogen such as Streptococcus with Millipore water as the standard. Millipore water is considered to be the most pure water available for research purpose around the world. Ganga water is active against various clinical pathogens due to its unique organic and inorganic constituents. The studies about these constituents are under way at various institutes in India and other countries. 8 year old Ganga water sample produced 14 mm zone of inhibition against Streptococcus and 10 year old ganga sample produced 16 mm zone of inhibition. The antimicrobial activity which is naturally present for Ganga water is not observed in any other perennial river anywhere in the world. Development of new antimicrobial agents from Ganga water is a future prospect, considering the result it has yielded in this study undertaken.

Key words: Ganges, self puricatory, Millipore water, antimicrobial, therapy.

INTRODUCTION

The water of the River Ganga is frequently used for drinking, cooking, and bathing purposes due to ancient knowledge that Ganges water does not putrefy, even after long periods of storage. Water has been used from time immemorial for remedial purposes. Most religious beliefs involve some ceremonial use of ‘‘holy’’ water, and in India the water of the River Ganga is treated with such reverence. Under the continuous Saraswati-Indus civilization going back to 7500 BC, the River Ganga is mentioned in Rigveda [10]. Hippocrates, going back to 500 BC, wrote about the healing of disease with water. Bathing held a prominent place in the law that was prepared by Moses under divine instruction for the government of the Hebrew nation. The role of the bath in the treatment of leprosy also would lead one to believe that water was used for

curative effects [11]. Outbreaks of acute diarrheal disease have been identified as causes of fatal disease dating back as far as the Sanskrit literature and during Hippocratic times [15].

Ernest Hankin, a British bacteriologist, reported in 1896 on the presence of marked antibacterial activity against *Vibrio cholerae*, which he observed in the water of the River Ganga river India, and he suggested that it might help to decrease the incidence of cholera in people using water from the Ganges. Though invisible, it was possible to show that this principle was particulate and D'Herelle called it ''bacteriophage'' [6]. Thus in a way the world owes the discovery of bacteriophages to the Ganges water. Overuse in human medicine and for agricultural purposes has become a recognized medical problem, and scientists have become increasingly concerned about the occurrence of antibacterial resistance in the environment. To curtail the development and spread of antimicrobial resistance will require both the preservation of current antimicrobials through their appropriate use and the discovery and development of new agents. Technologies for accessing and screening new sources of badly needed and novel antibiotics have improved dramatically during the past decade [10, 12, 14, 22]. This study was conducted to validate our ancient knowledge about the antimicrobial effect of Ganga water and to evaluate the potential of Ganga water in our endeavor to explore the possibility of using it as a novel source of antimicrobial compounds.

Streptococcus is a genus of spherical Gram-positive bacteria belonging to the phylum Firmicutes [23] and the lactic acid bacteria group. Cellular division occurs along a single axis in these bacteria, and thus they grow in chains or pairs, hence the name — from Greek στρεπτος streptos, meaning easily bent or twisted, like a chain (twisted chain). Contrast this with *Staphylococci*, which divide along multiple axes and generate grape-like clusters of cells. *Streptococci* are oxidase- and catalase-negative, and many are facultative anaerobes. In 1984, many organisms formerly considered *Streptococcus* were separated out into the genera *Enterococcus* and *Lactococcus* [24]

MATERIALS AND METHODS

Sample collection

Water samples for the present study were collected form the upper stretch of River Ganga (hilly region) at Haridwar, having the geographical coordinates of longitude 76º 32' E and latitude 29º 48' N. A 10-year-old sample was collected from a devotee. The samples thus collected were stored at in clean glass bottles fitted with screw caps indoors in a cool dark place. A fresh Ganga water sample was filtered with a sterilized filtration unit, through a 0.22-lmpore-size membrane filter (diameter, 47 mm; Millipore Inc., Billerica, MA, USA). The purpose of this filtration was to allow viruses to pass through the filter while removing organic and inorganic chemicals, natural organic matter, protozoans, algae, zooplankton, and free-living aquatic bacteria. Boiled water was obtained by boiling a fresh Ganga water sample for 20 min to kill micro-organisms [13].

Bacteria

Heterogeneous bacterial counts in the water were elucidated by serial dilution plating directly on nutrient agar (from HI-MEDIA Laboratories Pvt. Ltd., Mumbai, India) plates. After 72 h of incubation at 28º C, the colonies that developed on the plate were counted [17]. The bacterium used was *coccus* (ATCC 51740) procured from LGC promochem pvt. Ltd. Banglore.

Survival of Streptococcus

Inoculation of E. coli in Ganga water and Milli-Q water was carried out using overnight grown culture to assess the impact of water on survival of *Streptococcus*. Overnight grown culture was centrifuged and the pellet was washed three times using 0.85% sterile saline (w/v NaCl), then inoculated in Ganga water and Milli-Q water to a starting concentration of about 9 x 10^6 CFU/mL, and the mixture placed in three replicate (sterile) polypropylene tubes at 30º C under static conditions. *Streptococcus* surviving in the water was quantified at the designated time up to 30 days, diluted, and plated on Hi-Crome ECC agar plates (from HI-MEDIA Laboratories Pvt. Ltd.). All experiments were performed in triplicates.

Determination of antimicrobial activity

The bacteria was grown as 106 bacteria CFU/ml in 1.5% LB agar plates. Wells were produced in the media and 100µl CPP was added along with same volume of streptomycin as the control. The zone of inhibition for control and test were measured in terms of mm with the standard disc diffusion being followed. Antibacterial activity was assayed by the suppression of bacterial growth dependent on application of fractions to the top agar surface.

RESULTS AND DISCUSSION

It is ancient knowledge that Ganges water does not putrefy, even after long periods of storage, thus water from the Ganges has for millennia been regarded as incorruptible [4, 6, 19]. To facilitate a fair assessment of the potential of its self purificatory and incorruptible abilities, Ganga water having a resident bacterial population of 4 x 10^2 CFU/mL was spiked with about 5 x 10^7 CFU/mL *Streptococcus*. The incorruptible nature of the water was studied in fresh, 8-year-old, and 10-year-old Ganga water samples spiked with *Streptococcus*. Figure 1 and 2 shows the zone of inhibition formed of *Streptococcus* in fresh, 8-year-old, and 10- year-old Ganga water during the course of the experiment.

In general, the number of culturable *Streptococcus* declined over time but tended to be greater in fresh water than in 8- and 10-year-old water. Survival of *Streptococcus* over the course of the experiment was 3, 7, and 15 days for fresh, 8-year-old, and 10-year-old Ganga waters, respectively. On the contrary, in Milli-Q water the decrease in the viable count of *Streptococcus* up to 30 days was 5 x 10^4 CFU/mL. Age of the water seems to influence survival of *Streptococcus,* thus its fate was further studied in boiled water and after passage through a 0.2-lm-pore-size membrane filter. To elucidate the involvement of active principals and their sensitivity to high temperatures, the water was boiled. Water samples thus prepared were spiked with *Streptococcus* to evaluate the antibacterial ability of the water. Boiling water at 100º C kills microbes; filtration is becoming increasingly the method of choice for sterilization of biologicals, especially when the product is heat labile, because the filtration process is inherently non-destructive.

In general, 0.2 lm will remove algae, protozoa, and most bacteria, while a 0.01-lm filter is needed to remove viruses [13]. Overall, survival was higher in boiled water (12 x 10^4 CFU/mL for up to 25 days) than in water after passage through a 0.2-lm-pore-size membrane filter (23 x 10^2 CFU/mL for up to 15 days), indicating that heat-labile agents influence the survival of *Streptococcus* in Ganga water. An interesting observation was the ability of the 8- and 10-year-old Ganga water to influence survival of *Streptococcus*.

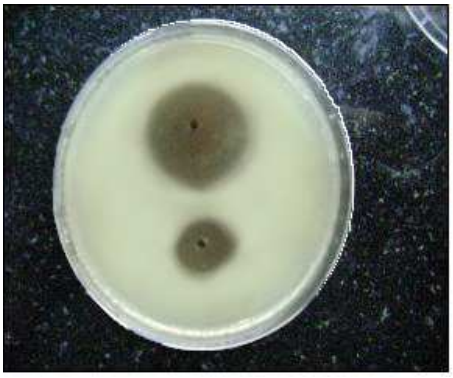

Figure 1 Antimicrobial activity of ganga water (8 year old sample) against *Streptococcus*

Figure 2 Antimicrobial activity of ganga water (10 year old sample) against *Streptococcus*

Eight-year-old water had a better ability to kill *Streptococcus* compared with boiled water and water passed through a 0.2-lm-poresize membrane filter. While antibacterial activity of 10- year-old water was better than that of boiled water and almost comparable to that of water passed through a 0.2- lm filter, indicating that a combination of factors controls the rate of decline and does not let the water putrefy, even after long periods of storage. Further studies should be undertaken to establish which factors are the key regulators influencing the death of *Streptococcus* in Ganga water. To investigate the well-known self-purificatory characteristic of Ganga water, the impact of the addition of *Streptococcus* on the microbial community structure in Milli-Q water and Ganga water after incubation for 0, 3, 5, and 7 days was assessed, using disc diffusion method.

The zone of inhibition in case of 8 year old Ganga water sample was 14 mm (Figure 1) and it was 16 mm in the case of 10 year old Ganga water sample compared with the standard streptomycin (Figure 2). These results established that the antimicrobial potential of the Ganga water sample. The involvement of heat-labile agents influencing survival of *Streptococcus* in Ganga water seems to indicate a role of antimicrobial peptides (AMPs). AMPs are part of the innate immune system, and an important component of immune defense. They are produced by plants, animals, insects, and single-celled organisms, and possess antimicrobial properties. As such, they are an ideal target for future antibiotic production [5, 18, 20]. The encouraging results of these experiments demonstrating the Ganga's antimicrobial capacity, indicating involvement of heatlabile agents, if carefully developed, could eventually provide a much-needed basis for the development of new antimicrobial compounds.

REFERENCES

[1] MD Aitken; MD Sobsey; NA Van Abel. *Water Res*, **2007**, 41, 1059–666.
[2] CJ Beauchamp; AM Simao-Beaunoir; C Beaulieu C. *Water Res*, **2006**, 40, 2452–2462.
[3] H Chart. *J Appl Microbiol*, **2000**, 88:12S–23S [Symposium Supplement]
[4] SG Darian. The Ganges in myth and history. University Press of Hawaii, Honolulu, **2000**.
[5] SM DasGupta; PS Chauhan; CS Nautiyal. "Search for an ever elusive guardian-angel novel antibiotic: myth or reality". IK International, New Delhi, **2007**, 311–335.
[6] F D'Herelle (translated to English by Smith GH). "The bacteriophage: its role in immunity". Williams and Wilkins/Waverly Press, Baltimore, **1922**.
[7] KP Flint. *J Appl Bacteriol* **1987**, 63, 261–270.
[8] JL Garland. *Soil Biol Biochem*, **1996**, 28, 213–221.
[9] JL Garland; AL Mills. *Appl Environ Microbiol*, **1991**, 57, 2351–2359.
[10] T Ha¨usler. "Viruses vs. superbugs: a solution to the antibiotics crisis?" Macmillan Science, New York, **2006**.
[11] J Kloss J. Back to Eden. Back to Eden books, Loma Linda, **1929**.
[12] CG Lerner; PJ Hajduk; R Wagner. *Chem Biol Drug Des*, **2007**, 69, 395–404.
[13] D Liberman; Berman T. *Filtrat Sep*, **2006**, 43, 439–440.
[14] RL Lock; EJ Harry. *Nature Rev Drug Dis*, **1997**, 7, 324–338.
[15] ZH McMahan; HL Dupont. *Aliment Pharmacol Ther*, **2007**, 25, 759–769.
[16] CS Nautiyal; PS Chauhan; YL Nene. *J Ethnopharmacol*, **2007**, 114, 446–451.
[17] CS Nautiyal; R Govindarajan; M Lavania. *J Agr Food Chem*, **2008**, 56, 4474–4481.
[18] LM Rossi; P Rangaswamy; J Zhang. *J Pharm Sci*, **2008**, 97, 1060–1070.
[19] Y Sharma. "The Ganga, India. In: Helmer R, Ivanildo H (eds) Water pollution control—a guide to the use of water quality management principles". WHO/UNEP, Geneva, **1997**.
[20] C Sheridan. *Nat Biotechnol*, **2006**, 24, 1494–1496.
[21] WJ Staddon; LC Duchesne; JT Trevors. *Microb Ecol*, **1997**, 34, 125–130.
[22] M Vicente; J Hodgson; O Massidda. *FEMS Microbiol Rev*, **2006**, 30, 841–852.
[23] KJ Ryan, CG Ray. Sherris Medical Microbiology (4th ed.). McGraw Hill. ISBN 0-8385-8529-9, **2004**.
[24] R Facklam. *Clin. Microbiol. Rev*, **2002**, 15 (4), 613–30.