Eco. Env. & Cons. 28 (1) : 2022; pp. (198-202)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i01.025

Physicochemical Analysis of West Coast Thermal Spring of Ganeshpuri and Isolation of Multi Catalytic *Bacillus licheniformis* Strain

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(Received 6 May, 2021; Accepted 5 June, 2021)

ABSTRACT

In India many hot springs are associated with religious importance, people visit and use them. Ganeshpuri hot spring is one of them and present study is the first attempt to analyse spring for physicochemical parameters thoroughly. Water temperature of 57 °C indicates its acro thermal nature having acidic pH value 5.5. TS values were within permissible limits of potability but TDS and conductivity values were high. Dissolved oxygen measured had value of 4.5 mg/l, within recommended range of potability. Temperature, pH, DO was recorded for spring water, kund water had similar values. Iron, chloride, calcium magnesium hardness, bicarbonate content was above the standard value permissible, while total alkalinity, sulphate, nitrate, was within the standard recommended limits of potable water. Spring harbors microbes of biotechnological application, a thermo-pH tolerant multi catalytic potential strain of *Bacillus licheniformis* was isolated and can be explored for diverse biotechnical and environmental applications.

Key words : *Physicochemical analysis, Hot spring, Ganeshpuri, India.*

Introduction

Thermal or hot springs are natural geological phenomena that occur all over the world. Thermal springs are sites where warm or hot ground water issues from the earth on a regular basis for at least a predictable period and is significantly above the ambient ground temperature. The water issuing from a thermal spring is heated by geothermal energy like magma heating surrounding rocks and aquifers due to vicinity of volcanic region (Bisht SS *et al.,* 2011). Depending on the temperature of spring, (Vouk, 1923) classified them as euthermal (warm) 30-50 °C, acrothermal (hot) 50-70 °C, and hyperthermal (steaming) 70 °C or higher.

Geothermal characters of hot springs are not similar to each other and suggest that all hot springs may be independent occurrences. Even springs that look same differ in characteristics such as temperature, flow rate and physiochemical properties of water (Sarolkar, 2005a).

From ancient times they are used for bathing (Atkinson and Davidson, 2002) and other recreational activities. Hot water is believed to have medicinal value curing many ailments like skin diseases. Various temples are found in the vicinity of hot springs in India having religious importance and people visit them for holy bath. In modern time also spas and the growing importance attached to the 'natural' health industry makes thermal springs as centres of balneology (Olivier *et al.,* 2008). In western countries thermal springs are also being used for in-

dustrial processing, agriculture, aquaculture, bottled water and the extraction of rare elements (Abu Zeid, 1998).

Physicochemical and biological studies of hot springs reveal quality of water and its suitability for irrigation, agriculture, drinking and industrial purposes (Mangle and Vijay, 2012). It also contributes to environmental and ecological database of the region as well useful for general health of public who uses these spring water (Yibas *et al.*, 2011). Biotechnological importance lies with the fact that many reports have shown that they harbour microbes (Brock, 1967) capable of producing biomolecules which finds vast number of applications including environmental clean-up and wellbeing. Many of them produce enzymes capable of biodegradation and waste disposal (Pathak and Rathod, 2015).

Numerous hot springs are present in the area of 300 km along the West coast of India. These hot springs are grouped under West Coast geothermal province. Ganeshpuri hot spring is one of them and we set out to perform abiotic analysis of its water for physicochemical parameters.

Isolation of microbes of biotechnological potential especially multi catalytic bacteria useful as whole cell or enzymes in consortia was carried out to explore hot spring for application and ecological significance.

Materials and Methods

Geographical location of spring

The hot spring of Ganeshpuri lies near Vajreshwari village in Thane district of Maharashtra within the radius of five kilometers around Vajreshwari temple with 19.5° North latitude and 73° East longitude. This temple itself is present on a hillock formed by volcanic eruption and is 75 km away from Mumbai, was named after Goddess Vajreshwari. There are around 21 hot water springs in a 5-kilometer radius of the temple. Ganeshpuri hot springs discharge through a concrete enclosure into tanks constructed for bathing purpose. The water is mostly used for hot water bath. Ganeshpuri, besides being a temple is also a place of tourist attraction.

The study involved visit to hot spring location, collection of water sample and transportation to laboratory for analysis.

Collection of hot spring water sample

Water was collected from the site of Ganeshpuri hot spring in new, unused plastic bottles which were rinsed with spring water three-four times before water sample and scraped sediment collection. Water temperature was recorded immediately on the site manually by glass mercury thermometer, pH with pH strips (Hi media) 1 to 14 scale with 0.5 and 1.0 pH increment. Capped bottles were transported to lab and stored at 4 °C in refrigerator until further processing. Separate bottles were used for microbiological and physiochemical studies.

For certain kind of analysis e.g. TS, TDS, TSS, sulphate, etc. water bottles were stored at room temperature as prescribed in the standard methods (APHA, 2005). Water sample was collected during April month for the study purpose.

Physiochemical analysis of water sample

TS, TDS and TSS of water samples was measured using evaporation method. EC values of the water sample under investigation was measured using Digital Conductivity meter. Dissolved oxygen was measured by Winkler's method at the time of sample collection in situ. The carbon dioxide determined by titrating with NaOH using phenolphthalein as an indicator. The total hardness was determined by titration with EDTA using Erichrome black T as an indicator. The calcium hardness and calcium of the water sample were determined by complexometric titration with EDTA using Murexide as an indicator. Total alkalinity and acidity of the water samples was determined by titrating with H_2SO_4. The iron, Sulfate, total phosphorus and nitrate were estimated using UV-Visible spectrophotometer. Determination of chloride was done by titration using silver nitrate method and carbonate, bicarbonate, CO_2 by acid titration method. Colony forming units were determined by spreading water on nutrient agar media followed by incubation at 50 °C (Jadhav and Pathak, 2015 and Pathak and Rekadwad, 2011).

Isolation of thermophilic bacterial species

The 100 µl water sample was inoculated and spread onto nutrient agar medium containing (g/l) peptone 5, beef extract 3, NaCl 5, and agar 27. The plates were incubated for 24 hours at 55 °C and morphologically different colonies on the medium were isolated. Isolated colonies were cultivated on nutrient agar slants and glycerol stocks were made for maintaining culture (Pathak and Rathod, 2015).

Screening for enzymes

Bacterial isolates were screened for different enzymes production. Basal media used was nutrient agar containing (g/l) peptone 5.0, sodium chloride 5.0, Yeast extract 1.5, Beef extract 1.5, agar 27. Substrates used were starch for amylase, dextran for dextranase, pectin for pectinase (Jadhav and Pathak, 2019), locust bean gum for mannanase (Rattanasuk *et al.*, 2009) caboxy methyl cellulose for cellulase (Kasana, 2008) and tributyrin for lipase (Imamura and Kitauras, 2000). These were added to nutrient agar media separately for testing each enzyme in concentration 10 g/l. Isolates were spot inoculated on agar plate and incubated at 55 °C for 24-48 hrs. Plates were flooded with grams iodine except for tributyrin agar plate and zone of clearance was observed for each enzyme with respect to colony size of isolate. For each enzyme, hydrolytic activity was determined as enzymatic index (EI) by calculating diameter of hydrolysis zone divided by diameter of colony. Efficient isolate was selected for identification purpose.

Morphological and biochemical characteristics of bacteria

Morphological, microscopic and biochemical pattern of efficient thermo stable multi enzyme producer were recorded by observing colony characteristics, grams staining, sugar utilization pattern (Cappucino and Sherman, 2005) and compared with standard reference strain from Bergey's manual of systematic bacteriology (Sneath *et al.*, 1984) for the tentative identification purpose. To confirm the identity MALDI-TOF MS analysis of cell proteins was done and compared with database software to get similarity value. The strain showing ≥ 1.7 log value with strain in database were confirmed as the member of that genus and strains showing ≥ 2.0 log values were confirmed as the member of that species (Rahi *et al.*, 2016 and Suarez *et al.*, 2013).

Results and Discussion

Abiotic analysis of water sample

Water sample was collected, analytical values for various parameters were calculated as mean of three readings and summarized as in Table 1. The water sample from Ganeshpuri hot spring was colorless, odorless, tasteless and clean, no turbidity in appearance. The temperature recorded using mercury thermometer was 57 °C while atmospheric temperature recorded was 42 °C at the time of sample collection. pH tested was found to be in acidic range of 5.5-6.0 with two types of pH strips, one with 1.0 and second with 0.5 pH increments. Dissolved oxygen (DO) measured by chemical kit immediately after collection had value 4.5. Temperature, pH, DO was recorded for spring water, kund water separately and were found to be of similar value. TDS and conductivity values were above the standard values permissible while TS was within the limits.Iron, chloride, calcium magnesium hardness, bicarbonate content was above the standard value permissible. Total alkalinity, sulphate, nitrate, was within the standard recommended limits of potable water. From the hardness values calculated it can be described as hard water. Similar studies are done for other west coast hot springs as well (Sarolkar, 2005b; Sulabha and Pathade, 2018).

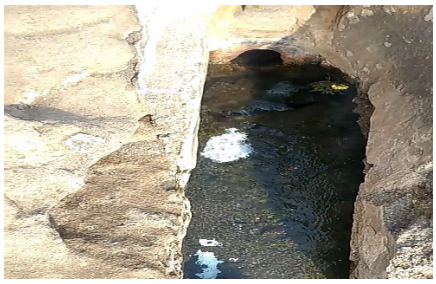

Fig. 1. Hot spring location in river

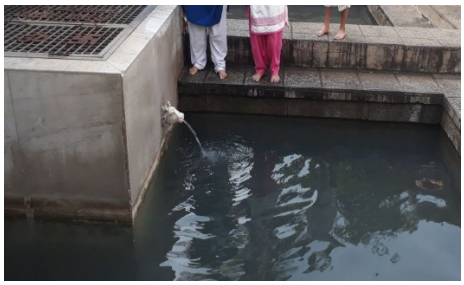

Fig. 2. Hot water kund near temple

Enzyme Screening results

The greater is the clearance zone, the higher is the EI value. The isolates with hydrolysis zone ≥ 1.0 cm is considered signicant. Hydrolysis zone was found to be 1.1 cm for pectinase, for cellulase 2.3 cm, lipase 1.8 cm and mannanase 1.4 cm. Isolate was able to grow up to 60 ºC in nutrient media broth, sodium chloride concentration 3.0% and could withstand pH range of 5 to 9 at 55 ºC incubation temperature

Table 1. Physiochemical analysis of water pre monsoon

Sr.No	Parameter	Values	Permissible by WHO
1	Temperature	57 ºC	-
2	pH	5.5-6.0	6.5-8.5
3	Dissolved Oxygen	4.5	≥ 4.6
4	TS	1352	1500
5	TDS	1219	500
6	TSS	133	-
7	Acidity	584	-
8	Iron	1.3	0.3
9	Total phosphorus	5.28	-
10	Conductivity	2310 µmhos/cm	250
11	Total Hardness	380	150-500
12	Total Alkalinity	28	200
13	Sulphate	292	500
14	Nitrate	0.1	50
15	Chloride	660	250
16	Calcium Hardness	138	75
17	Carbonate	0	-
18	Bicarbonate	28	0.01
19	Magnesium Hardness	142	30
20	Carbon dioxide	11	-

Note: All parameters are expressed in mg/l except pH and temperature, conductivity.

for 24-48 hrs. These results indicate that strain can survive harsh conditions, hence can be explored for certain industrial applications.Preliminary results of pectinase and mannanase activity in liquid media are showing promising results, hence it can be studied for enzyme related as well as whole cell applications.

Identification of the isolate

Morphological characters of the isolate observed are shown in table1.Good quality MALDI-TOF MS spectra was generated for the bacteria and its comparison with the Bruker taxonomy database using Biotyper 3.1 software resulted in identification of bacteria as *Bacillus licheniformis* with best match score of 2.237 with *Bacillus licheniformis DSM 13T.* Strains showing ≥ 2.0 log values are confirmed as the member of that species, hence strain belongs to *Bacillus licheniformis* species.

Table 2. Morphological characters

Sr.No	Characters	Isolate
1	Size	2mm
2	Shape	Circular
3	Color	White
4	Opacity	Opaque
5	Elevation	Raised
6	Surface	Rough
7	Consistency	Butyrous
8	Margin/Edge	Irregular
9	Gram's nature	+ve rods
10	Motility	Motile

Conclusion

The study of hot spring water is an important aspect to find new renewable source of energy and it is beneficial for medical tourism in India. In the present investigation, it was found that few water quality parameters lie within potability range as recommended by WHO but other parameters were not in standard permissible limits of potable water. Hence maintenance of proper sanitary conditions, protection of water source of hot spring is recommended. This water analysis will help to design suitable experiments for determination of community structure of Ganeshpuri hotspring and will help to design suitable experiments for exploration of microbiota of this system. Initial attempts have succeeded in isolation of thermo stable, multi enzyme producer strain of *Bacillus licheniformis*.

It can be utilized not only for production of screened enzymes separately but also for applications where multienzyme action is needed for the process like solid waste composting. Temperature

plays a very critical role in composting. The high temperature phase supports the growth of high microbial activity resulting in maximum biodegradation and killing of pathogenic microbes (Elango *et al.*, 2009; Sutripta Sarkarab *et al.*, 2016). The strain can be used for this purpose and forms the part of future research study.

Acknowledgement

The authors are thankful to the Honorable Vice-Chancellor, Swami Ramanand Teerth Marathwada University, Principal Vivekananda College of arts, commerce and science Aurangabad for providing the infrastructure and necessary facilities.

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