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Deciphering the diversity of aerobic culturable thermophiles in hot springs of Manikaran, Himachal Pradesh

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ABSTRACT

For deciphering aerobic culturable diversity of thermophiles from Manikaran hot springs three different types of samples viz water, soil sediment and biomat were collected from upper Manikaran, lower Manikaran and Shangna. The temperature of water was found to be significantly higher at upper Manikaran and lowest at Shangna. But no significant difference was observed in pH of water and soil sediment samples. A total of 108 bacterial isolates were obtained. The percentage of the isolates obtained from on site culturing and standard plate count technique was recorded as 15 and 75 respectively. Only 16 per cent of the total isolates could be obtained through trait specific enrichment technique. Nutrient agar and broth were found to be the most appropriate media for the isolation of thermophiles. Screening of all the isolates for temperature tolerance revealed that majority of them were facultative (58.33%) followed by thermotolerant (21.30%) and obligate thermophiles (20.37%). A significantly higher bacterial load was observed in biomat (5.44 log CFU/ml) as compared to soil sediment (4.84 log CFU/ml) and water (4.13 log CFU/ml) samples. No definite trend was observed in the distribution of actinomycetes population in water, soil sediment and biomat samples. No fungal isolate was obtained from all types of samples. Among all the isolates 77.78 per cent of the isolates were Gram positive, rod shaped, spore forming bacteria while 22.22 per cent were Gram negative, rod shaped, non-spore formers thereby indicating the dominance of these bacteria in Manikaran hot springs.

Keywords: Diversity; thermophiles; Manikaran; hot springs

INTRODUCTION

Temperature is considered as one of the most important environmental variables controlling the activities and evolution of organisms (Abou-Shanab 2007, Takacs-Vesbach et al 2008, Kumar et al 2013). High temperature of water and/or soil exerts pressure on microbial species leading to the selection of specific

flora capable of tolerating and surviving heat under these conditions (Eugene et al 1999, Kumar et al 2013). Thermophiles are the organisms adapted to grow optimally at high temperatures ranging from 55 to 121°C (Sharma et al 2013). The most common and accessible thermal habitats for thermophiles are hot springs, sulfatara and geothermally heated soils (Marteinsson et al 2001, Adiguzel et al 2009, Sen et al 2010).

Among the many extreme environments thermal springs are of considerable interest to researchers worldwide as they harbour a large diversity of microorganisms (Satyanarayana et al 2005). Different physical and chemical conditions, biogeography and geological history influence the different microbial phenotypes in such type of environment (Narayan et al 2008, Lau et al 2009). Therefore it is important to explore microbial diversity from conservation and preservation of valuable germplasm point of view.

Worldwide studies on microbial communities in hot springs have mainly concentrated on habitats at low elevations like Yellowstone National Park (Mitchell 2009), Kamchatka in Russia, Iceland (Reigstad et al 2010), Tunisia (Sayeh et al 2010) etc. But very little is known about the microbial diversity in thermal springs from highly elevated regions. Nature has blessed the state of Himachal Pradesh with a number of hot water springs viz Vashisht , Manikaran, Kasol, Tattapani , Khirganga etc. Manikaran hot springs located at high altitude have not been systematically explored with respect to diversity of inhabitant thermophiles. Therefore the present study was focused on deciphering the diversity of aerobic culturable thermophiles in Manikaran hot springs, Himachal Pradesh by employing a culture-dependent approach.

MATERIAL and METHODS

Collection of samples

Three different types of samples viz water, soil sediment and biomat were collected from upper Manikaran, lower Manikaran and Shangna sites located along the Parvati river valley, district Kullu of Himachal Pradesh.

Sample analysis

Temperature and pH of the samples were recorded at the time of collection with the help of portable temperature probe (Fisher Scientific, Mumbai, India) and pH meter (CyberScan, Singapore) respectively.

Elucidation of microbial diversity

Four different techniques viz on-site culturing, standard plate count, enrichment and membrane filter technique were employed for the elucidation of aerobic microbial diversity. In on-site culturing technique, direct plating of the samples was done immediately after

collecting them on different media viz nutrient agar (NA), potato dextrose agar (PDA) and actinomycetes isolation agar (AIA) as per the method given by Akmar et al (2011).

The aerobic microbial diversity in all types of samples was elucidated by plating serial dilutions of samples (Wollum 1982) on NA, PDA and AIA for the isolation of bacteria, fungi and actinomycetes respectively. The NA and AIA plates were incubated at 55°C for 2 and 7 days respectively while PDA plates were incubated at 28, 37, 45 and 55 ±2°C for 2-7 days.

For enrichment method 10 ml of each type of sample was transferred into 100 ml of potato dextrose and Sabouraud dextrose broth media separately. Incubation was performed at 28, 37, 45 and 55 ± 2°C for 3-7 days. The samples were then serially diluted and plated on PDA and SDA plates. Plates were incubated at 28, 37, 45 and 55 ± 2°C for 3-7 days. The same procedure was repeated five times (Holt and Krieg 1994).

In membrane filter technique 100 ml of each sample was filtered through 0.45 µm polyvinylidene diflouride (PVDF) filter membrane (millipore) to concentrate all the samples in five folds. Five ml of concentrated sample was mixed with PDA and SDA and poured into petriplates (Mulvany 1969). Plates were incubated at 28, 37, 45 and 55 ± 2°C for 3-7 days.

Temperature tolerance profile

All isolates were screened for their temperature tolerance by inoculating them in nutrient broth and then incubating at different temperatures ranging from 37-85°C for 24-72 h. Following incubation, absorbance was recorded at a wavelength of 600 nm using SPECTRONIC® GENESYS ™5 spectrophotometer after regular intervals of time (Kumar et al 2014).

Morphological and biochemical characterization of isolates

All isolates were identified on the basis of morphological, physiological and biochemical characteristics according to the standard methods described by Ludwig et al (2009).

RESULTS

A total of 108 bacteria (11 actinomycetes and 97 bacteria) were obtained from all types of samples collected from selected sites. The temperature of water was found to be significantly higher at upper Manikaran and lowest at Shangna. However no significant difference was observed in pH of water samples collected from these three sites. The temperature of soil sediment at upper Manikaran (84.5°C) was found to be statistically at par with the temperature at lower Manikaran but significantly higher than that of Shangna (72.7°C). No significant differences in soil sediment pH were noticed at the selected sites.

The highest temperature of 89.5ºC was recorded in the water samples collected from upper Manikaran. Earlier workers also reported a temperature of 89°C in the samples collected from the hot springs of Manikaran (Chandrasekharam et al 2005, Cinti et al 2009). However Dwivedi et al (2012) and Kumar et al (2014) reported the temperature range of 89 to 95°C of Manikaran hot spring water which was slightly higher as compared to the present study. This discrepancy in results could be attributed to season of sample collection as well as accuracy of the instrument.

The pH of different samples was recorded to be slightly acidic ranging from 6.1-6.4. This may be attributed to the fact that the thermal discharges at Manikaran were of Na- HCO_3 - Cl which might be responsible for acidic pH (Chandrasekharam et al 2005). Cinti et al (2009) reported the pH of Manikaran hot spring water ranging from 6.8-7.1 while it ranged from 4.0-7.0 according to Verma et al (2014).

The percentage of the isolates obtained from on-site culturing and standard plate count technique was recorded as 15 and 75 respectively. However only seventeen isolates (16% of the total) were obtained through enrichment technique. Thus it can be inferred from these results that on-site culturing method has yielded less number of bacterial isolates probably due to the fact that these plates were incubated at ambient temperature. The higher rate of isolation was observed with standard plate count technique because all the plates were incubated at the optimum temperature in the laboratory as required by the thermophiles (Sharma et al 2012).

In order to obtain a diverse range of nutritional types of bacteria with various metabolic requirements from Manikaran hot springs, different types of media were employed. However nutrient agar and broth were found the most appropriate for isolating thermophiles. These results are in accordance with the observations of Akmar et al (2011) who reported nutrient agar and broth as the most appropriate media for the isolation of thermophiles.

Screening of all the isolates for temperature tolerance revealed that a total of 20.37, 58.33 and 21.30 per cent of the isolates were found as obligate, facultative and thermotolerant thermophiles respectively (Fig 1). Owing to their modest thermophily, facultative thermophiles may be secondarily adapted to hot environments which may be one of the reasons for getting higher percentage of facultative thermophiles.

The bacterial load was found to be significantly higher (5.44 log CFU/ml) in biomat as compared to sediment (4.84 log CFU/ml) and water (4.13 log CFU/ml). However no significant difference was

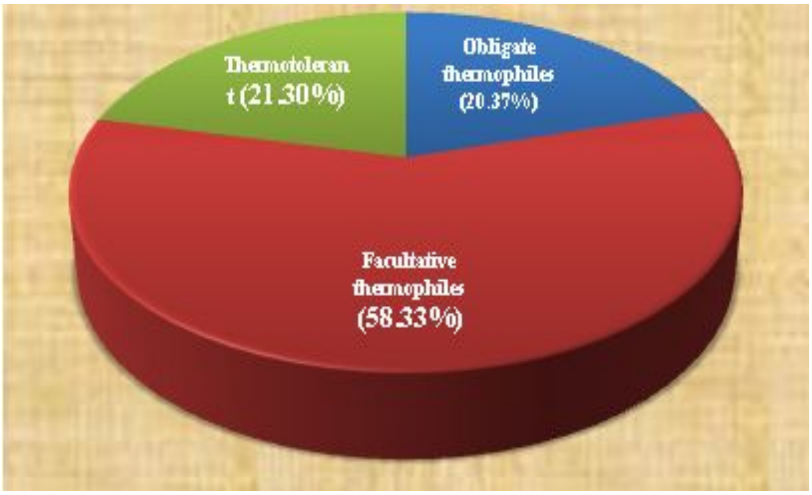

Fig 1. Temperature tolerance profile

observed in bacterial load obtained at different locations. The interaction between types of sample and location was found statistically significant. Highest bacterial load was observed in biomat samples which may be attributed to the fact that microorganisms living in microbial mats can survive under extreme conditions such as high temperatures, high light intensity, salinity and desiccation as reported by other workers also (Zohary 1985, Esteve et al 1992).

No definite trend was observed in the distribution of actinomycetes population in water, soil sediment and biomat samples. In some samples actinomycetes were observed on nutrient agar whereas in others only on actinomycetes isolation agar. Their presence was however observed at all locations. No fungal isolate was obtained from all types of samples. Among all the isolates 77.78 per cent of the isolates were Gram positive, rod shaped, spore forming bacteria while 22.22 per cent were Gram negative, rod shaped, non-spore formers thereby indicating the dominance of these organisms in Manikaran hot springs as observed in other harsh environments also viz deep sea hydrothermal vents, solfataras, geothermally heated soils, man-made thermal systems (Sneath 1986, Khalil 2011). Various other workers have also reported the dominance of Gram positive bacteria in the hot springs like Savusavu hot springs in Fiji (Narayan et al 2008) and Karachi hot springs (Asad et al 2011).

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