ISOLATION AND MOLECULAR CHARACTERIZATION OF THERMOTOLERANT BACTERIA FROM MANIKARAN THERMAL SPRING OF HIMACHAL PRADESH, INDIA

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ABSTRACT

The present study was conducted to isolate and characterize thermotolerant bacteria from Manikaran thermal spring of Himachal Pradesh. Ten thermotolerant bacterial isolates were isolated and the optimal temperature for growth of the isolates was 70°C and the optimal pH was 7.0. 16S rRNA gene sequences of all these thermophilic bacterial isolates showed 98-99% homology with *Geobacillus* sp. The phylogenetic analysis of the 16S rRNA gene sequences also confirmed the affiliation of these thermophilic isolates with the genus *Geobacillus.*

Keywords: Thermal spring, Thermotolerant, 16S rRNA, *Geobacillus,* Phylogenetic analysis.

No: of Tables :1 **No:of Figures:1** **No:of References:10**

Introduction

Advances in molecular biology, bioinformatics and cultivation technologies herald a new age of exploration of microbial world. Since the discovery of *Thermus aquaticus* at Yellowstone National park, newer and different habitats are being exploited to understand microbial diversity over time. A great bacterial diversity has been reported in various solar heated environments and phylogenetic research also showed that thermophiles are abundant in many more extreme environments (Zeikus, 1979; Hugenholtz *et al*., 1998). In India, thermal springs are found scattered throughout the country. Thermophilic strains have also been recovered from various thermal springs in India (Bisht *et al*., 2011; Verma *et al*., 2014). In state of Himachal Pradesh, Manikaran thermal spring has been found to be one of the hottest water spring of India having a temperature as high as 106°C. The present study was undertaken to explore the Manikaran hot water spring of Himachal Pradesh, India.

Materials and Methods

Samples were collected in sterilized screw capped vials from different sites of Manikaran hot water spring and brought to the laboratory and kept at 4°C in refrigerator till further processing. Culture media used for the isolation of thermophilic bacteria were Castenholz TYE medium (Castenholz, 1969). All the incubations were done in covered water bath incubator at 70°C for 3-4 days in Castenholz TYE broth. The temperature range of 40-100°C, pH range of 4.0-10.0 and incubation period of 24-144 hrs was investigated for the best growth of thermophilic bacteria. Thermophilic bacterial isolates have also been studied for various morphological and biochemical characters (Kristjansson *et al.,* 1986; Aneja, 2003). For molecular characterization, DNA was extracted from each thermophilic bacterial isolate using Genomic DNA extraction Mini-Kit (Real Genomics). PCR amplification of the 16S rRNA gene was carried out by using the forward primer (5'-GGTCAGCGGCGGACGGGTGAGTAAC-3') and the reverse primer (5'-GACGGGCGGTGTGTACAGAGGCCCG-3'). PCR products were sequenced using same primers by a commercial sequencing facility (Xcleris lab). The sequences of these ten bacterial isolates after sequencing were blasted using online NCBI BLAST program (Altschul, 1997). Phylogenetic analysis began with aligning of sequences using tools like Clustal W and after alignment, phylogenetic tree was constructed using MEGA 6.0 software.

Results and Discussion

In the present study, ten thermotolerant bacterial isolates belonging to genus *Geobacillus* were investigated in thermal spring of Manikaran, Himachal Pradesh. These isolates were given codes viz., MS1, MS2, MS3, MS4, MS5, MS6, MS7, MS8, MS9 and MS10 and colonies of these thermophilic bacteria were creamish circular on Castenholz TYE medium, cells were gram positive, rod shaped, motile and sporulating. These isolates were catalase and oxidase positive where as rest of biochemical descriptors viz., urease test, MR-VP test and fermentation of sugars

were found to be negative. The optimal conditions observed for the maximum growth of all thermophilic isolates, were found to temperature of 70°C, optimal pH of 7.0 and optimal time 96 hours. The 16S rRNA gene of all isolates was amplified, sequenced, and *insilico* analysis of these ten sequences was carried out. Based on BLAST alignment of 16S rRNA gene sequences of these isolates to GenBank sequences, these all isolates were found to belong to genus *Geobacillus* and showed 98-99% similarity (Table-1).

Table 1. The comparision of the 16S rRNA gene sequences of the obtained *Geobacillus* isolates with the 16S rRNA gene sequences in GenBank and their closest phylogenetic relative identity.

***Geobacillus* isolates**	**Sequence**		
	No. of Nucleotides	**% identity**	**Closest phylogenetic relative (GenBank accession no.)**
MS1	1142	98%	*Geobacillus thermoleovorans* (FN428646.1)
MS2	1141	99%	*Geobacillus caldoxylosilyticus* (NR_028708.1)
MS3	721	99%	*Geobacillus thermocatenulatus* (FN538989.3)
MS4	693	99%	*Geobacillus vulcani* (NR_025426.1)
MS5	1151	99%	*Geobacillus thermocatenulatus* (FN538989.3)
MS6	1067	98%	*Geobacillus kaustophilus* (JX522539.1)
MS7	1141	99%	*Geobacillus caldoxylosilyticus* (NR_028708.1)
MS8	1141	99%	*Geobacillus stearothermophilus* (FN428694.1)
MS9	1148	98%	*Geobacillus lituanicus* (JX298768.1)
MS10	1136	97%	*Geobacillus toebii* (NR_025143.1)

The phylogenetic analysis of the 16S rRNA gene sequences confirmed the affiliation of these thermophilic isolates with the genus *Geobacillus* (Figure-1).

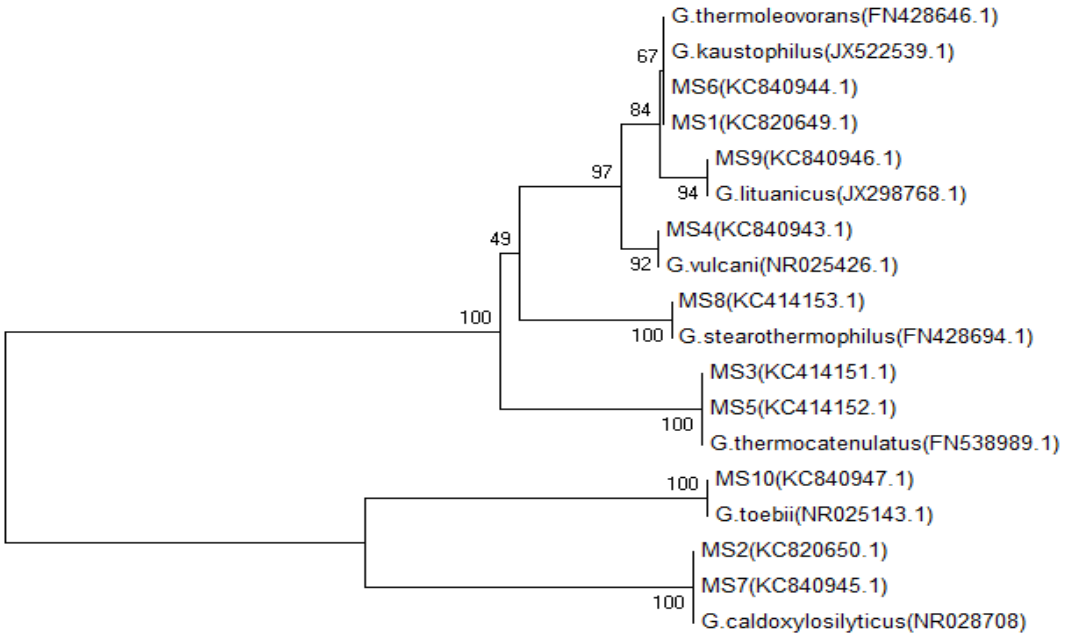

Figure 1: Phylogenetic tree showing the relationships between the 16S rRNA gene sequences of the ten *Geobacillus* isolates and other related *Geobacillus* sequences previously published in the NCBI database.

The identification of these isolates as *Geobacillus* species is in agreement with the findings of previous study (Nazina *et al*., 2004). It was also observed previously that the accurate taxonomic assignment depends upon the region of 16S rRNA gene that it targeted during sequencing (Liu *et al*., 2008). Phylogenetic research showed that thermophiles are abundant in many more extreme environments (Hugenholtz *et al.,* 1998). The 16S rRNA gene, partial sequences of these ten isolates were then submitted to NCBI under these accession numbers., KC820649 (MS1), KC820650 (MS2), KC414151 (MS3), KC840943 (MS4), KC414152 (MS5), KC840944 (MS6), KC840945 (MS7), KC414153 (MS8), KC840946 (MS9) and KC840947 (MS10), and all these isolated were isolated from the soil and sediment samples of natural thermal springs of Manikaran, Himachal Pradesh, India.

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