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SHORT COMMUNICATION

Cellulolytic Activity of Thermophilic Bacilli Isolated from Tattapani Hot Spring Sediment in North West Himalayas

Indu Priya[1] · M. K. Dhar[1] · B. K. Bajaj[1] · Sanjana Koul[1] · Jyoti Vakhlu[1]

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Abstract Eight thermophilic bacterial strains were isolated from Tattapani Hot spring and screened for various hydrolytic enzymes including cellulases. The isolated bacterial strains were identified as *Geobacillus thermodenitrificans* IP_WH1(KP842609), *Bacillus licheniformis* IP_WH2(KP842610), *B. aerius* IP_WH3(KP842611), *B. licheniformis* IP_WH4(KP842612), *B. licheniformis* IP_60Y(KP842613), *G. thermodenitrificans* IP_60A1(KP842614), *Geobacillus* sp. IP_60A2(KP842615) and *Geobacillus* sp. IP_80TP(KP842616) after 16S ribotying. Out of the eight isolates *Geobacillus* sp. IP_80TP grew best at 80 °C whereas rest of the isolates showed optimal growth at 60 °C. *G. thermodenitrificans* IP_WH1 produced a thermotolerant cellulase with maximum activity at 60 °C.

Keywords Tattapani hot spring · Thermotolerant cellulase · *Geobacillus thermodenitrificans* · North West Himalayas and thermophilic bacterial strains

Introduction

Thermophilic bacteria have been isolated from hot springs and hydrothermal vents where the temperature ranges between 40 and 122 °C [1]. Cellulases are important industrial enzymes and their applications include manufacture of paper, textile, pulp, food and biofuel [2]. As most of the industrial enzymatic reactions are carried out at high temperature, therefore cellulases isolated from thermophilic bacterial strains are important for industrial applications [3].

North West Himalayas have abundant unexplored environmental niches (both extreme hot and cold) that can be a source of novel organisms and biomolecules. With this background, thermophilic bacteria were isolated from previously unexplored Tattapani hot spring sediment. Tattapani hot spring (Lat. 33.2500°N, Long. 74.2500°E) is situated in Rajouri district of Jammu and Kashmir (India). The temperature of this hot spring ranges between 50 and 100 °C. The sediment was collected by using the protocol developed by Cummins [4]. At the time of the sample collection the temperature of the sediment was 60 °C and pH was 7. The samples were immediately shifted on dry ice and transported to the laboratory for further use. The bacterial strains (incubated for 48 h) were isolated by dilution plate method on the nutrient agar at 60 and 80 °C [5]. The bacterial load calculated at 60 °C and pH 7 was 62×10^5 cfu/g of sediment that is much higher than Moroccan hot springs 6×10^2 cfu/g [6] but relatively higher than Uttranchal hot springs 2×10^5 cfu/g [7]. All the bacterial isolates were identified by amplifying 16S rRNA gene [8] and their sequences were submitted to GenBank (Accession Numbers: KP842609, KP842610, KP842611, KP842612, (KP842614, KP842615 and KP842616). Among the isolates *Geobacillus thermodenitrificans* IP_WH1, *Bacillus licheniformis* IP_WH2, *B. aerius* IP_WH3, *B. licheniformis* IP_WH4, *B. licheniformis* IP60Y, *G. thermodenitrificans* IP_60A1 were obtained at 60 °C and two strains *Geobacillus* sp. IP_60A2 and *Geobacillus* sp. IP_80TP at 80 °C. The phylogenetic

M. K. Dhar, B. K. Bajaj and Sanjana Koul have contributed equally to this work.

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✉ Jyoti Vakhlu
jyotimetagenomic@gmail.com; jyotivakhlu@gmail.com

[1] School of Biotechnology, University of Jammu, Jammu, J&K 180006, India

tree (Supplementary Fig. S1) of the isolated bacterial strains was constructed by using MEGA6 [9] to investigate their relationships with each other. The isolated strains were screened for various hydrolytic enzymes including cellulases at 60 °C by well diffusion method [10] and all were found to be cellulase producers. All the isolated strains were grown at different temperatures viz, 80, 60, 40 and 20 °C for 48 h to check the temperature optima for growth. *Geobacillus* sp. IP_80TP showed optimum growth at 80 °C, while others had optimum growth at 60 °C. The optimal cellulases activity of all the bacilli were assayed quantitatively at 60 °C by following the protocol developed by Miller [11] (Fig. 1). *Geobacillus* sp. IP_80TP though grew best at 80 °C but the cellulase was produced at 60 °C which was less in comparison to all others that grew and produced cellulase at 60 °C. Maximum cellulase was produced by *G. thermodenitrificans* IP_WH1 (0.94 IU/ml) which was higher than other thermophilic cellulases reported in literature such as *Bacillus* sp. with 0.14–0.37 IU/ml [12] and *Bacillus* sp. SMIA-2 with 0.29 IU/ml [13] at 50 °C and pH 7.0. Though *G. thermodenitrificans* has been reported in the literature for the production of thermostable lipase [14], alpha-amylase and alpha-glucosidase [15] but there are no reports of cellulase

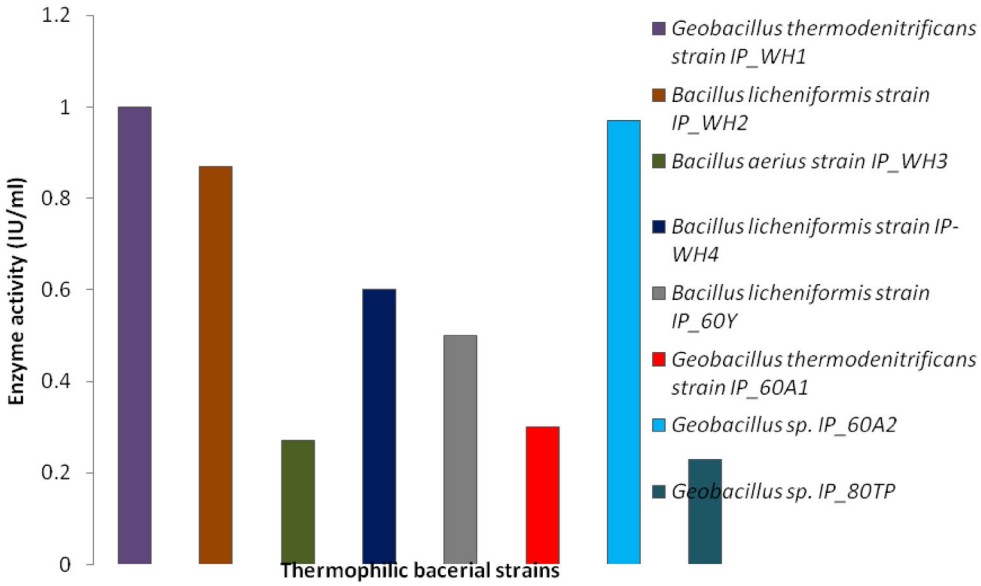

Fig. 1 Comparative cellulase production by eight thermophilic bacteria

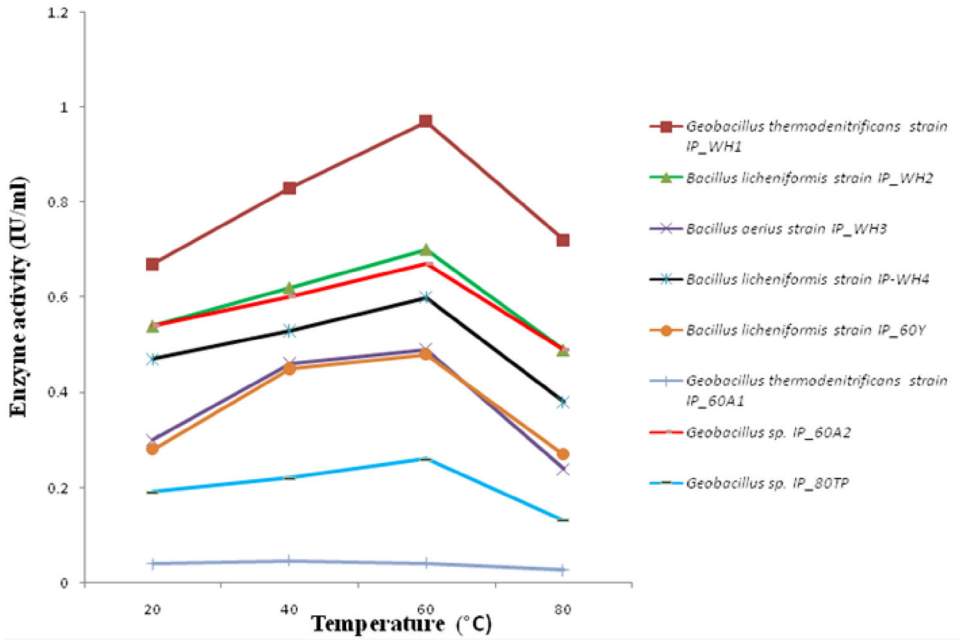

Fig. 2 Temperature profile of the cellulases produced from the isolated bacterial strains

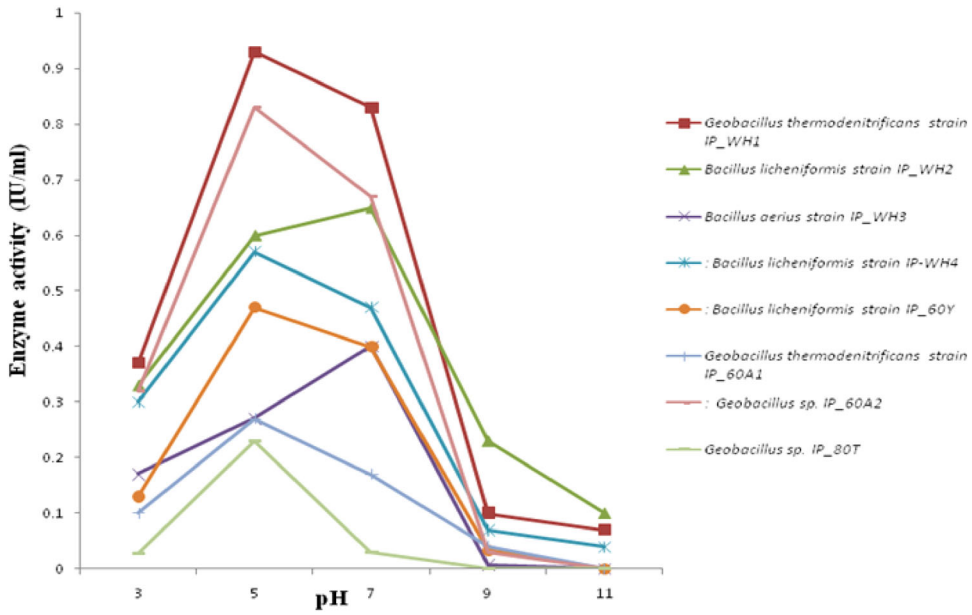

Fig. 3 pH profile of the cellulases produced from the bacterial strains

production. However, there are reports on cellulase production from *Bacillus* sp. and other *Geobacillus* sp. [16–18]. In addition all the thermophillic cellulases produced were analyzed for temperature optima (Fig. 2) and pH optima (Fig. 3). Interestingly, cellulases produced by all the bacteria isolated in present study, had temperature optima at 60 °C, even for *Geobacillus* sp. IP_80TP that has optimal growth at 80 °C. pH optima for cellulases from all the bacterial strains was 5.0 except *B. licheniformis* IP_WH2 and *B. aerius* IP_WH3 which showed pH optima near 7. *G. thermodenitrificans* IP_WH1 has pH optima at 5.0 as is true for many thermostable cellulase produced by various species of Geobacilli [16, 17].

This is the first report of *G. thermodenitrificans* as acid active cellulase producer at 60 °C, approximately 10 °C higher than most reported cellulases. Though the present study indicates that *G. thermodenitrificans* IP_WH1 is the most suitable thermostable acid active cellulase producer but other isolates from Tattapani hot spring can further be screened for the presence of other thermophilic/stable enzymes of industrial importance.

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