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Research Article

Isolation and Characterization of antibiotic producers from Vajreshwari Hot Spring, Mumbai

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Abstract

In the era of assessment of thermophiles and their characteristics, we have selected Vajreshwari hot spring, Mumbai. Vajreshwari town (19°29′12″N 73°1′33″E) is located in Thane district, Maharashtra, India. Water sample from this hot spring was screened for isolation of antibiotic producers by crowded plate technique. Two thermophiles namely, VHSI-1 and VHSI-2 were isolated and characterized thoroughly. On the basis of morphological characters, microscopic features and biochemical profile, VHSI-1 and VHSI-2 were identified as *Bacillus aeolius* and *Bacillus thermoamylovorans* respectively. These isolates can be explored for detection of possibly new promising antibiotic as well as production of various industrially important thermostable enzymes.

INTRODUCTION

Vajreshwari town (19°29′12″N 73°1′33″E) is situated on bank of the river Tansa, in Bhiwandi city of Thane district, Maharashtra, India. There are approximately 21 hot springs within 5 km radius of Vajreshwari shrine. It is reported that, temperature of these hot springs ranges from 43 to 80ºC. Many religious people and tourists visit to this place (Patil and Unnikrishnan, 2015). Antibiotics producers mostly belong to various genera of filamentous actinomycetes and Gram positive bacilli (Gebreyohannes *et al.*, 2013, Denyer *et al.*, 2004). According to literature thermal springs are habitats of many actinomycetes and bacilli. *Streptomyces* sp., *Micromonospora* sp., *Microbispora* sp. and *Planosporangium* sp. are antibiotic producing actinomycetes previously reported from Thai hot spring sediment by Thawai *et al.*, (2005). Chaudhary and Prabhu, (2016) have previously reported thermophilic actinomycetes from Vajreshwari hot springs. Very few species of *Bacillus* from hot springs are known to produce antibiotic. In this context, we attempted isolation and characterization of antibiotic producers from Vajreshwari hot spring, Maharashtra. Moreover, the isolates reported by us have also shown the ability to produce a wide array of enzymes to be applied in biotechnological industries and biomedical fields.

MATERIALS AND METHODS

Isolation, characterization and preliminary identification:

Water samples from one of the Vajeshwari hot springs were collected in Jan. 2018. Temperature and pH of water sample was recorded *in situ* by using digital thermometer and pH meter respectively. Crowed plate technique was used for isolation of antibiotic producer (Aneja, 2006; Kamoldeen, 2013). In this method, water samples from Vajeshwari hot spring ranging in volume 0.1 to 2.0 mL were inoculated on nutrient agar plates aseptically.

All the plates were incubated in a bacteriological incubator (Kumar make, Mumbai) at 65ºC for 18 h for further studies. Morphological, microscopic, biochemical and physiological characters of selected isolate were determined as per the standard methods which are already described in the following cited references. Preliminary identification of selected isolates was carried out by using Bergey's Manual of systematic bacteriology (Vos *et al*., 2009; Rathod and Pathak, 2014; Rathod and Pathak, 2014a; Rathod and Pathak, 2016; Rathod and Pathak, 2016a; Pathak and Rathod, 2015; Pathak and Rathod, 2015a; Pathak and Rathod, 2013; Pathak and Rathod, 2014; Pathak *et al*; 2014, Pathak *et al*., 2015; Pathak *et al*., 2015a; Pathak and Rathod, 2016; Pathak *et al*., 2016; Pathak *et al*., 2016a; Pathak *et al*., 2016b; Rathod and Pathak, 2016b; Rathod and Pathak, 2016c; Rathod and Pathak, 2017; Pathak and Rathod, 2016a; Pathak and Rathod, 2017).

RESULTS AND DISCUSSION

Isolation of antibiotic producer:

Temperature and pH of vajreshwari hot spring was recorded 65ºC and 7.89 respectively. After incubation, two microbial colonies were observed that showed zone of growth inhibition (Table 1). These colonies were designated as VHSI-1 and VHSI-2 and further subcultured on nutrient agar slants.

Table 1: Size of zone of growth inhibition

Volume of water sample inoculated (mL)	Total colony count	Designation of Antibiotic producers	size of zone of growth inhibition (mm) in crowed plate technique
0.1	37	-	-
0.5	189	VHSI-1	11
1.0	292	VHSI-2 and VHSI-3	10 and 09
1.5 and 2.0	Mat growth	-	-

Characterization and preliminary identification:

Colonies of VHSI-1 were circular and cream-colored. VHSI-1 was Gram stain variable and motile rod. Length and width of a cell was 2.0 and 0.5 µm respectively. VHSI-1 formed terminally located endospore on sporulation medium. VHSI-1 was catalase-negative and oxidase positive. Temperature range for growth of VHSI-1 was found 35 to 65°C, with an optimum at 55 °C. pH range for growth of VHSI-1 was found 7 to 9, with an optimum at pH 8. NaCl concentration range for growth of VHSI-1 was found 0.5 to 5%, with an optimum at 2% NaCl. Acid without gas is produced by VHSI-1 from a wide range of carbohydrates viz. glucose, mannitol, mannose, salicin, starch and xylose. Casein, gelatin and starch substrates were hydrolyzed by VHSI-1. On the basis of morphological characters, microscopic features and biochemical profile, VHSI-1was identified as *Bacillus aeolius.* This organism was previously reported from a shallow marine hydrothermal vent, Vulcano Island and Eolian Islands, Italy (Vos et al. 2009).

Colonies of VHSI-2 were white, lenticular, and 2 mm in diameter. VHSI-2 was Gram stain positive and weakly motile rods. Length and width of a cell of VHSI-2 was 3.0 and 0.5 µm respectively. Endospore formation was not observed. VHSI-2 showed optimum growth at 60ºC and pH 7. VHSI-2 was catalase-positive and oxidase-negative. Starch was efficiently hydrolyzed by VHSI-2 (Figure 1). Acid without gas was produced by VHSI-2 in presence of glucose and starch. On the basis of morphological characters, microscopic features and biochemical profile, VHSI-2 was identified as *Bacillus thermoamylovorans.* This organism was previously reported from palm wine (Vos et al. 2009). Identification of VHSI-3 isolate is in progress.

Figure 1: Starch hydrolysis by *Bacillus thermoamylovorans* VHSI-2 isolate from Vajreshwari hot spring, Maharashtra.

Conclusions

In conclusion, two bacterial isolates from Vajreshwari hot spring designated as VHSI-1 and VHSI-2 were found antibiotic producers as screened by crowded plate technique. On the basis of morphological characters, microscopic features and biochemical profile, VHSI-1 and VHSI-2 were identified as *Bacillus aeolius* and *Bacillus thermoamylovorans* respectively. These isolates can be explored for detection of possibly new promising antibiotic. Moreover production of various thermozymes can also be achieved from these isolates.

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