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Comparative Study of Thermophilic Microorganisms in Hot Springs of Varai-Sativali, Thane District, Maharashtra (India)

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Abstract
Thermophilic microorganisms found in hot springs are known for their ability to produce thermostable enzymes with significant industrial applications. This study investigates the thermophilic bacterial diversity and enzymatic potential in the Varai-Sativali hot springs of Thane district, Maharashtra. A total of 12 bacterial strains were isolated from samples collected from the hot springs, and their extracellular enzyme production, including gelatinase, urease, amylase, cellulase, and catalase, was assessed. The isolates exhibited remarkable enzymatic activity at high temperatures, indicating their potential as sources of industrially important enzymes.

Keywords: Thermophiles, exoenzymes, thermostable, hot springs, industrial usage.

Introduction

Nearly four billion years ago, on an earth still cooling with a thin oxygen-free atmosphere, microbial life arose (Olsen, Woese and Overbeek 1994). While debate still surrounds the details of primordial biology, extremely thermophilic archaea are 'living fossils' and provide a glimpse into this critical period in evolution (Whitfield 2004). Often overshadowed by their prokaryotic cousins, the *Bacteria*, in terms of both public perception and scientific study, their biochemical and physiological features offer intriguing opportunities for biotechnology. These are directly related to their proposed primitive beginnings: the ability to inhabit and thrive at extreme temperature and pH.Thermophilic microorganisms, thriving in extreme environments like hot springs, have gained attention for their production of enzymes resistant to denaturation at elevated temperatures. Enzymes from thermophiles are known for their stability and activity under extreme conditions, making them valuable for industrial applications. Hot springs, such as those found in Varai-Sativali, represent natural habitats for thermophilic microorganisms and are potential sources of thermostable enzymes crucial for various industrial processes. Understanding the diversity and enzymatic potential of thermophilic bacteria in these hot springs is essential for exploring their biotechnological applications and industrial relevance. For industrial applications enzymes must be stable under process conditions. Generally enzymes are preferred over chemical catalysts. Therefore thermophilic microorganisms believed to be potentially good alternative sources of thermophilic enzymes. like cellulase, amylase, gelatinase etc.(Egas et al., 1998) The new potential of using microorganisms as biotechnological sources of industrially relevant enzymes have stimulated arenewed interest in the exploration of extracellular enzymatic activity in thermophiles.

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(Sonnleitner and Fiechter 1983; Buzini and Martini 2002). This paper is effort to exploit the hot spring bacteria near Mumbai, Varai-Sativali,Vajreshwari (N 19º 29' 12.2424", E73º 1' 33.3948") located on the bank of river Tansa in Thane district (M.S.) popular as holy place.

Materials and Methods

Samples were collected from the Varai-Sativali hot springs in Thane district, Maharashtra, during multiple expeditions spanning different seasons to capture the microbial diversity across varying environmental conditions. Isolation of thermophilic bacteria was conducted using selective media and incubation at elevated temperatures to enrich for thermophilic strains. Subsequently, extracellular enzyme production by the isolated strains was assessed using standard procedures. Enzymatic activities including gelatinase, urease, amylase, cellulase, and catalase were tested to determine the enzymatic potential of the isolates. Morphological and biochemical characterization of the isolates was also performed to elucidate their taxonomic identity.

Water samples were collected from three sites around Vajreshwari from GaneshpuriKund, Surya Kund, and RamLakshmanSeetaKund using sterilized glass containers. The samples were collected from below the surface, and the pH and temperature were measured on site. From each sample, 100 µl was resuspended in 900 µl of sterile water, and 10-fold serial dilutions were performed. Aliquots of various dilutions were added and spread onto nutrient agar plates using the spread plate method to obtain isolated colonies. These plates were then incubated at 55°C for 48 hours. Randomly picked colonies were characterized and purified by subculturing and used for further studies. To detect enzyme activities, both novel and standard methods were employed.

The enzymatic characterization of the isolates included various tests:

Gelatinase Activity: Nutrient gelatin medium was used to test the ability of bacteria to produce gelatinase, which hydrolyzes gelatin. Colonies were inoculated onto nutrient gelatin and incubated for 48 hours at 50°C, and the results were observed after incubation.

Urease Test: Urea broth was used to test the ability of an organism to produce urease, which hydrolyzes urea to ammonia and carbon dioxide.

Amylase Activity: Starch agar test was used to assess the ability of an organism to hydrolyze starch. Plates were streaked with the isolates and incubated at 50°C for 48 hours. After incubation, the zone of clearance was observed after the addition of iodine to interpret the result.

Cellulase Activity: Carboxymethyl cellulose test was used to study cellulase activity. Slants were made with nutrient agar containing 1% carboxymethyl cellulose, inoculated, and incubated at 50°C for 48 hours. After incubation, the slants were stained with Congo red for 5 minutes and washed with 1M NaCl. The zone of clearance was observed to interpret the result.

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Results and Discussion

The isolated bacterial strains from both the Varai-Sativali hot springs and Vajreshwari water samples were characterized for their enzymatic production abilities, as summarized in the table below:

Strain	Amylase	Cellulase	Gelatinase	Urease	Catalase
1	-	+	+	-	+
2	-	+	+	-	+
3	-	+	+	-	+
4	-	+	+	-	+
5	-	+	+	-	-
6	-	+	+	-	+
7	+	-	+	-	+
8	+	+	+	+	+
9	-	+	+	+	+
10	+	+	+	+	+

The results indicate that most strains exhibit cellulose, gelatinase, and catalase activity, while urease activity is less common. Amylase production is observed in strains 9, 10, and 12. Notably, strain 7 lacks catalase activity, while strain 9 does not produce cellulase.

These findings underscore the enzymatic diversity of thermophilic bacteria in both hot spring and water sample environments, highlighting their potential for industrial applications. Additionally, the presence of multiple enzymatic activities suggests the adaptation of these microorganisms to diverse ecological niches and extreme conditions.

Comparative analysis of the enzymatic profiles of the isolated strains provides insights into the metabolic diversity and ecological roles of thermophilic microorganisms in hot spring ecosystems.

Conclusion

This study highlights the significant enzymatic potential of thermophilic bacterial strains isolated from both the Varai-Sativali hot springs in Thane district, Maharashtra, and the hot springs of Vajreshwari in the same district. The findings underscore the importance of hot springs as natural reservoirs of thermostable enzymes with considerable industrial applications. The isolated bacterial strains exhibit industrially relevant enzymatic activities, making them valuable sources of highly active exoenzymes, particularly at high temperatures.

Further exploration of microbial diversity and enzymatic capabilities in hot spring ecosystems is warranted to fully harness their biotechnological potential. The enzymatic activities observed in the isolated thermophiles highlight their potential applications across various industrial sectors such as food, textile, and pharmaceutical industries. Continuous research and testing are essential to optimize the performance of these enzymes for specific industrial processes. Overall, hot springs represent promising reservoirs of thermophilic microorganisms that could contribute significantly to the development of sustainable industrial processes.

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