

Production and Partial Characterization of Penicillin Amidase Produced by *Bacillus megaterium* from Temple Puja Wastes

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Abstract Temple puja wastes are released in water bodies or land creating severe environmental pollution and health hazards. The temple wastes extract was used to isolate the *Bacillus megaterium* for the production of enzyme penicillin amidase as it recorded the largest zone of activity. The result obtained showed that on nutrient agar medium the grown colonies were confirmed by gram staining as *Bacillus megaterium* in temple wastes. P.amidase activity was determined using DNSA method. Highest yield of enzyme activity by *B.megaterium* was obtained after 48 hrs. of incubation. The optimum temperature and pH for the enzyme activity was obtained at 60°C (6.0± 0.55) and pH 7.0 (2.04 ± 0.49). The results shown that *B.megaterium* is a good producer of extracellular P.amidase at high temperature which could be an indication of P.amidase produced as thermostable. The study revealed the potential of agricultural wastes' capability to produce amylase by *B.megaterium*.

Keywords *Bacillus megaterium*, Penicillin Amidase, Temple Waste, Temperature

1. Introduction

During good old days, when human population was limited and there were few temples in cities, this practice of disposal of small quantity of the temple waste in rivers or lakes created in significant pollution [1]. Today, however, increased human population and number of temples in cities with trend of collective worships rather than domestic activity, has caused generation and accumulation of massive quantities of temple waste everyday [8].

Penicillin amidase also known penicillin amidase or benzylpenicillin-amidohydrolyse is an enzyme used commercial production of semisynthetic penicillin [3]. *Bacillus* species produce a large variety of extracellular enzymes, such as amylases, which have significant industrial importance members of the genus. *Bacillus* is heterogeneous and it is very versatile in the adaptability to the environment [4]. Therefore, the objectives of the study were to isolate and identify bacteria from temple waste, perform partial characterization of the enzyme production and its properties with regard to the effect of temperature and pH.

2. Materials and Methods

Isolation of Bacterial Stains from Temple Waste

The temple waste materials like flowers, leaves, fruits and items like milk, honey, cord, ghee, jaggery were collected from temples which are then disposed after puja into natural water bodies. The collected samples were brought to isolation of *Bacillus megaterium* bacteria by plating technique [5] using starch casein agar medium. After inoculation and incubation, the colonies were observed under microscope and there it was employed to morphological and biochemical characteristics.

Screening and Extraction of Enzymes

The isolated colonies were screened using screening medium (carboxyl methyl cellulose) is a substrate at 37°C. The extracellular enzyme solution was obtained by centrifugation at 5000 rpm. The supernatant obtained was collected and used in enzymes assay. The enzymes activity was assayed as described [6] by DNSA method.

Effect of Temperature and pH on Enzymes Activity

The optimal temperature for activity was attained by assaying activity of enzymes at different temperature range of 30, 40, 50, 60, and 100°C for 48 hours. The optimum pH was determined by running the assay between 4.0 to 8.0 samples which were taken at regular intervals and analyzed by DNSA method [7].

Effect of Incubation Period

It was carried out by the Dinitro salicylic method at different incubation periods such as 72, 96, 120 and 144 h, after which assay was determined.

3. Results and Discussion

The results were high encouraging where luxuriant growth of *Bacillus megaterium* bacteria from temple wastes. The isolates that had creamy colonies and were all catalase positive, gram positive, rod shape, hydrolyze starch (blue blackish color) and utilized glucose. The bacterial isolate was identified and characterized as *Bacillus megaterium*.

It is recommended by many workers [9] to supplement nutrient media with minerals, phosphorus, energy source and all those essential factors like proteins. It was shown that it contains proteins, amino acids and chemical constituents like milk.

Table 1: Effect of Temp., Incubation Period and pH on Enzyme Activity

Effect of pH	Enzyme Activity	Effect of Incubation Period (hr.)	Enzyme Activity (mg /ml)	Effect of Temp. (°C)	Enzyme Activity
4	0.012 ± 0.02	72	1.04	40	6.0 ± 0.55
5	1.00 ± 0.33	96	0.99	50	7.6 ± 0.59
6	1.50 ± 0.19	120	1.82	60	9.6 ± 1.32
7	2.04 ± 0.49	144	1.32	70	7.0 ± 0.52
8	1.99 ± 0.38			100	8.3 ± 0.09

The enzyme degrading of isolate *Bacillus megaterium* species was screened by culturing medium and supplemented with CMC on this. *B.megaterium* produces a clear zone (5.0 mm) around the inoculated colony indicates a positive result for penicillin amidase producing strain.

The optimum pH for penicillin amidase activity was determined for maximum activity at pH of 7 and minimum activity at pH of 4. The maximum optimized temperature was recorded at range of 60° C (6.0± 0.55) (Figures 1 and 2). This result is in agreement with [8] who stated in their report the most bacterial enzymes function between a pH range of 6 and 8.

The effect of incubation period of the yield of penicillin amidase enzyme (Figure 3) had enzyme activity of 1.04 mg/ml at 72 h; this was followed by an increase in enzyme activity at 120 h (1.82 mg/ml), which was recorded maximum enzyme activity. It is mentioned that the temple wastes consist of a variety of components of plant and animal origin, which can fulfill the requirements of natural media. Different nutrient elements required for microbial growth that are provided by common microbiological media components are compared with temple waste components, and it is highly justifiable that the extract of temple waste organic constituents can be used and that can be industrially exploited for the synthesis of enzyme.

4. Conclusion

India is recognized as has having major issues with water pollution, predominately due to untreated sewerage. Water supply and sanitation continue to be inadequate, despite long-standing efforts by the various levels of government and communities at improving coverage. Ecological issues are an integral and important part of environmental issues challenging India. Water pollution by untreated pond wastes, rivers and garbage- all affect the food and environment quality necessary for ecosystems. It is concluded that the temple wastes consist of variety of components of plant and animal origin, which can fulfill the requirements of natural media to isolate the bacterial isolates. The isolates can be industrially exploited for the synthesis of penicillin amidase enzyme. There is a need to create awareness about temple wastes disposal in the mind of common man and also about the communication by expert persons to deal this problem by keeping all the human ethics and feeling unchanged to solve this problem through ecofriendly ways.

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