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ISOLATION AND CHARACTERIZATION OF THERMOSTABLE AMYLASE PRODUCING BACTERIA FROM HOT SPRING OF UNKESHWAR, NANDED (MS) INDIA

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Abstract: *Amylase is an industrially important enzyme and applied in many industrial processes such as saccharification of starchy material, pharmaceutical, detergent, food and textile industry. Thermophilic bacteria can survive at high temperature, in which hot spring is one of its habitats. India has many hot springs with potential as a habitat for thermophilic bacteria. The purpose of this study is to isolate a thermophilic bacterium that has ability to produce thermostable amylase enzyme from Unkeshwar hot spring located at Nanded district in Maharashtra, India. Thermophilic bacteria were isolated from unkeshwar hot water spring. These isolates have efficient amylase producing ability. These isolates were Gram positive rod shaped motile bacterium. The isolate was identified as Bacillus Spp. on the basis of microscopic and biochemical characteristics. It showed optimum growth on nutrients agar medium at pH 7 and temperature 60˚C.*

Key Words: Hot spring water, amylase, thermophilic bacteria.

1.0 Introduction

Microorganisms are the most important sources of enzyme production. Amylase is an enzyme that breaks starch down into sugar. Amylase is present in human saliva, where it begins the chemical process of digestion. Foods that contain much starch but little sugar, such as rice and potato, taste slightly sweet as they are chewed because

amylase turns some of their starch into sugar in the mouth. Plants and some bacteria also produce amylase. As diastase, amylase was the first enzyme to be discovered and isolated (by Anselme Payen in 1833).

Amylases are a group of important enzymes which are mainly employed in the starch processing industries for the hydrolysis of polysaccharides like starch into simple sugars (Akpan et al., 1999; Mitchell and Lonsane, 1990; Damien et al., 2010). Conversion of starch into sugar, syrups and dextrin forms the major part of starch industries (Marshall, 1975). The hydrolysates are used as carbon sources in fermentation as well as source of sweetness in a range of manufactured food products and beverages. Hydrolysis of starch products containing glucose and maltose in brought about by controlled degradation (Norman, 1982). In hydrolyses starch and used commercially for the production of

Sugar syrups from starch which consist of glucose, maltose and higher oligosaccharides (Hagihara et al., 2001). Amylases accounts for about 30% of the world's enzyme production (Vander et al., 2002; Rita et al., 2009).

Amylases are important hydrolase enzymes which have been widely used since many decades. These enzymes randomly cleave internal glycosidic linkages in starch molecules to hydrolyze them and yield dextrins and oligosaccharides. Among amylases α-Amylase is in maximum demand due to its wide range of applications in the industrial front. Amylase is an industrially important enzyme and applied in many industrial processes such as saccharification of starchy material, pharmaceutical, detergent, food and textile industry. Present investigation is therefore aimed at exploration of unkeshwar hot water spring for isolation of thermostable amylase producer.

2.0 Materials and Methods

2.1 Sample collection

Water sample was collected by grab sample collection method from Unkeshwar hot water spring, located at Nanded district in Maharashtra. Samples were collected in

clean, presterilized plastic bottles without any air bubble and transported in laboratory within 24 hours and maintained in refrigerator at 4□. The temperature and pH of the samples were measured while sample collection with standard digital thermometer and pH meter respectively. (Pathak *et al.*, 2016)

2.2 Isolation of isolates

Water sample was serially diluted and spreaded on nutrient agar plates. The plates were incubated for 24 hours at 60˚C. After incubation morphologically different colonies were appeared on the agar medium were selected for further screening. (Sonalkar *et al.*, 2015, Khairnar *et al.*, 2012)

2.3 Screening of isolates for amylase activity

Amylase producing organisms were screened by qualitative plate assay method. Isolates were grown on starch agar plates having pH 7.0 and incubated at 60°C for 2 days. After incubation plates were observed for zone of clearance after the addition of iodine solution. Three bacterial strains showing zone of clearance were isolated.

Table No.1 Morphological characteristics

Colony Character	Observed characters
Size	3 mm
Shape	Circular
Colony Color (Surface)	White
Margin	Entire
Surface	Smooth
Elevation	Raised
Opacity	Opaque
Consistency	Sticky
Gram's Nature	+ve , Rod shaped
Motility	Motile

2.4 Biochemical Characterization of the Isolates

The isolate showing remarkable zone of clearance was selected for further analysis. Morphological and biochemical characteristics of the isolate was studied for the identification of the isolate (Khairnar *et al.*, 2012, Pathak *et al.*, 2016)

Table No.2 Biochemical Characteristics

Test	Result	Sugar profile	Result
Starch Hydrolysis	+ve	Glucose	+ve
Indole production	-ve	Maltose	+ve
Methyl red	+ve	Sucrose	+ve
VP	-ve	Xylose	-ve
Citrate utilization	-ve	Ribose	-ve
Nitrate reduction	-ve	Fructose	+ve
Catalase	+ve	Lactose	+ve

Fig. 1 Amylase Test

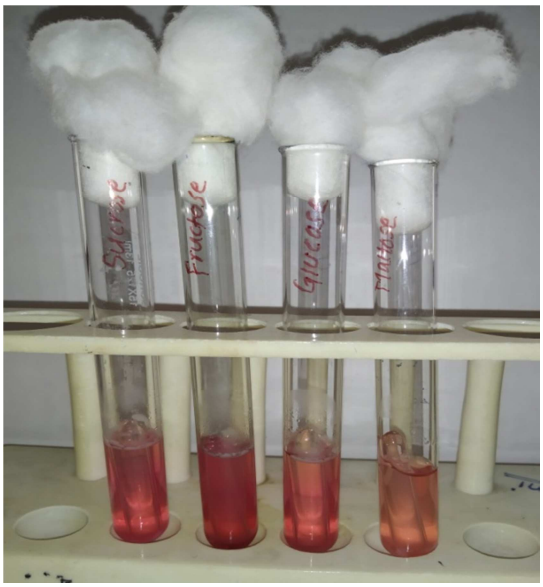

Fig.2 Sugar Test

3.0 RESULTS AND DISCUSSION:

The Unkeshwar hot spring water was odorless. The temperature and pH at the time of sample collection was recorded as 62□ and 7.4 respectively. Water sample was serially diluted and spreaded on nutrient agar plates. The plates were incubated for 24

hours at 60˚C. After incubation morphologically different colonies selected. Amylase producing organisms were screened by qualitative plate assay method. Isolates were grown on starch agar plates having pH 7.0 and incubated at 60°C for 2 days. After incubation plates were observed for zone of clearance after the addition of iodine solution. Three bacterial strains showing zone of clearance were isolated. and was identified by microscopic feature, cultural characteristics, biochemical tests and sugar profile as shown in table 1 and table 2 respectively.

The selected isolate was identified as *Bacillus* species by comparing with Bergey's Manual of systemic bacteriology volume. An efficient amylase producer was isolated from Unkeshwar hot water spring and identified as *Bacillus* species. As amylase production was carried out at high temperature, it can be used in different industries.

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