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RESEARCH ARTICLE

ENHANCED PRODUCTION OF THERMOSTABLE AMYLASE BY THERMOPHILIC *GEOBACILLUS THERMOLEORANS* STRAIN REKADWADSIS ISOLATED FROM UNKESHWAR HOT SPRING SEDIMENT

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ABSTRACT

The thermohilic *Geobacillus thermoleorans* Strain REKADWADSIS isolated from Unkeshwar thermal spring showed optimum temperature 65 ± 0.2 °C at its pH optima 7.5 ± 0.2. *Geobacillus* amylase production was detected on starch nutrient agar plates at 65 °C. *Geobacillus* was produced 8, 623 U/mL amylase under SmF at 68 ± 0.2 °C. The supplementation of additional lactose (1%) and tryptone (1%) with 10% inoculum was enhanced the production of thermostable amylase (14, 223 U/mL). *Geobacillus* thermostable amylase has apparent approximately MW 42 kDa.

Thermophilic amylase producing bacteria isolated from sediment sample collected from thermal gradient of Unkeshwar hot spring located at Unkeshwar, Nanded (19° 85' N, 78° 25' E) using starch nutrient agar at 65 ± 0.2 °C and at pH 7.0 ± 0.2. Thermophile developed colony within 48 h at 65 ± ± 0.2 °C temperature and at pH 7.0 ± 0.2. The colony was light yellow coloured, 4.0 mm in size, circular, smooth, convex and opaque. Long rods were 6.0x1.0 µm in size, Gram positive, arranged either singly or as diplobacilli with terminally placed endospore. Isolated species utilized arabinose, fructose, galactose, maltose, mannitol, lactose, trehlose and sucrose as carbon source. The strain showed positive amylase, oxidase and catalase tests. Thermophilic amylase producer was tentatively identified as *Geobacillus thermoleovorens* using Bergeys manual of systematic bacteriology (Bergeys *et al.,* 1984). Morphologically and biochemically indentified species further confirmed by using 16S rRNA gene sequence analysis. Ribosomal gene bank database showed homology values between 97 to 99%. Confirmed 16S rRNA gene sequence labeled *Geobacillus thermoleorans* Strain REKADWADSIS has been deposited in NCBI repository with the accession number: KP053645. The morphological and biochemical characteristics of *Geobacillus thermoleorans* Strain REKADWADSIS are presented in Table 1.

Table 1. Morphological and biochemical characteristics of *Geobacillus thermoleorans* Strain REKADWADSIS

Accession number	KP053645
Shape	Long rod
Size (Length x Breadth)	6.0x1.0 µm
Gram staining	+
Arrangement	Single, Diplobacilli
Endospore	Terminal
Colour of colony	Light yellow
Size of colony	4 mm
Form of colony	Circular
Margin of colony	Smooth
Elevation of colony	Convex
Density of colony	Opaque
Optimum temperature	65±0.2 °C
Optimum pH	7.5±0.2
Arabinose	+
Fructose	+
Galactose	+
Maltose	+
Mannitol	+
Lactose	+
Trehlose	+
Sucrose	+
Amylase	+
Oxidase	+
Catalase	+

The production of *Geobacillus* amylase was analyzed under submerged fermentation (SmF). The thermostable amylase produced by *Geobacillus thermoleovorens* strain REKADWADSIS showed maximum activity at 68 ± 0.2 °C temperature and at its optimum pH 7.5 ± 0.2 (Bernfeld, 1995).

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Geobacillus has produced 8, 623 U/mL amylase under SmF. Similar type of results were reported by different research group worldwide (Uguru *et al.,* 1997; Pathak and Rekadwad, 2013).

The supplementation of additional carbon source such as lactose (1%) and nitrogen source such as tryptone (1%) along with 10% inoculum size was enhanced the production of thermostable amylase up to 14, 223 U/mL under SmF. The denaturing SDS-PAGE performed as per method described by (Laemmli, 1970) showed thermostable amylase has apparent approximately molecular weight 42 kDa. Najafi *et al.* (2005) purified and characterized extracellular amylase produced by *Bacillus subtilis* AX20.

From the results, it is concluded that isolated *Geobacillus* is produced high quantity of thermostable amylase. The isolated species may be choice for enzyme production on commercial scale under solid state fermentation.

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