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CHARACTERIZATION OF THERMOPHILIC AMYLASE FROM AN OBLIGATE THERMOPHILE, *THERMOACTINOMYCES VULGARIS*

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Abstract: Amylase finds a wide range of applications in starch industries, i.e., baking, brewing, distillery. The wild-type (1227) and mutant strains (1261 and 1286) of *Thermoactinomyces vulgaris* were screened for the production of amylase using 1% soluble starch. The maximum production of amylase was observed after 12 h of incubation at 50°C in wild-type strain 1227 of *T. vulgaris*. The amylase was found to be thermophilic, exhibiting its optimal activity at 75°C and at pH 6.0 in this obligate thermophile; and it preferred soluble starch as its substrate. Among the metal ions tested, Mn^{2+} was most stimulatory, while Hg^{2+} was most inhibitory to the activity of amylase. Thus, *T. vulgaris* amylase is a thermophilic metalloenzyme, requiring Mn^{2+} for its high-temperature catalysis, which can be exploited for amylase-based industries of diverse interests.

Keywords: Amylase, metalloenzyme, *Thermoactinomyces vulgaris*, thermophilic amylase

INTRODUCTION

Amylases are enzymes which hydrolyze starch molecules to give diverse products, including dextrins and progressively smaller polymers composed of glucose units (Windish and Mhatre, 1965). Amylases constitute a class of industrial enzymes, which alone form approximately 25% of the enzyme market (Burhan et al., 2003; Rao et al., 1998; Sidhu et al., 1997). This plays a vital role in many industrial processes such as sugar, textile, paper, brewing, baking and distilling industries. It is also used in food and pharmaceutical industries as a digestive aid (Sivaramakrishnan et al., 2006).

The enzymes from thermophilic microbes have been proved to be more useful in biotechnological applications. Thermophiles represent an obvious source of thermostable enzymes, being reasonable to assume that such character will confer their proteins a high degree of thermal stability. The *Thermoactinomyces* growing at high temperature is thus a good source of industrially important enzymes.

MATERIAL AND METHOD

Strains Used and Culture Conditions

A wild-type strain (Stock no. 1227) and two mutant strains (Stock nos. 1261, and 1286) of *T. vulgaris*, which were kindly supplied by Professor D.A. Hopwood, John Innes Centre, Norwich, U.K., were used for screening the production of extracellular amylase. The auxotrophic strains 1261 (nicotinamide⁻thiamine⁻) was streptomycin resistant. The other auxotrophic strains 1286 (thiamine⁻) was streptomycin sensitive. The media and culture conditions, as described by Hopwood and Wright (1972) were used with certain modifications (Singh, 1980; Sinha and Singh 1980).

Screening of *T. vulgaris* Strains for the Production of Extracellular Hydrolytic Enzymes

Amylase activity was determined, using soluble starch (1%) as a substrate in Hopwood's medium (Hopwood and Wright, 1972). Sterile modified Hopwood's agar medium was poured on a sterile Petri-plate and allowed to solidify at room temperature. It was then inoculated with the wild-type (1227) and auxotrophic mutant strains of *T. vulgaris*, using 0.2 mm cork-borer and incubated for 24-48 h at 50°C. The plates were then stained with Lugol's Iodine (2 g KI and 1 g I_2 crystal dissolved in 300 ml distilled water, filtered and stored in brown bottle). The formation of a halo zone surrounding the colony under blue black background was considered positive for amylase activity.

Culture Condition for Amylase Production

The Hopwood medium, supplemented with 3% starch as sole carbon source was used as a production medium. The 30 ml of sterilized liquid medium was inoculated with 1ml of spore suspension containing 10^7 spores/ml (having O.D. of 0.65 at 600 nm). The contents were mixed thoroughly and incubated at 50°C. Mycelium was filtered out with Whatmann no. 1 filter paper, and the filtrate was used for assaying the amylolytic activity of *T. vulgaris*. In order to investigate the optimum conditions for maximum production of amylase, samples were taken at regular time intervals (i.e., after 4, 8, 12, 16, 20 and 24 h) and assayed at its growth temperature (50°C) for amylase production.

Assay for Amylase Activity

The amylase activity was assayed by the method of Bernfeld (1955) by estimating the reducing sugar (maltose) produced during starch hydrolysis using 3,5 dinitrosalicylic acid (DNS) as a coupling reagent. The reaction mixture consisted of 0.5 ml of 1% (w/v) soluble starch in 20 mM sodium acetate buffer (pH