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GROWTH OF AMYLASE PRODUCING THERMOPHILIC *BACILLUS SUBTILIS* AR-27 AT DIFFERENT pH, TEMPERATURE AND CULTURE MEDIA

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ABSTRACT

Growth of amylase producing *Bacillus subtilis* AR-27, a Gram positive strain from soil was found maximum at 60°C after 24 h keeping the medium pH 7.0 in Luria broth containing 1% starch as a substrate.

Key words: Production, Conditions, *Bacillus subtilis*, Thermophilic, α-amylase,

INTRODUCTION

Amylases are considered among most important industrial enzymes and are of great significance in contemporary biotechnology (Mobini-Dehkordi and Javan, 2012). Term amylases refer to different enzymes such as α-amylases, β-amylases and amyloglucosidases which are capable to hydrolyze starch into different saccharides on the basis of linkages (Rameshkumar and Sivasudha, 2011). These enzymes are extensively used in various industries like paper, food, textile, detergent, brewing and distilling (Pandey *et al*., 2000; Rao *et al*., 2007).

There are various sources from where amylase can be obtained such as plants, animals and microorganisms however enzymes of microbial origin are considered important as they generally possess characteristics that meet industrial demands (Vihinen and Mantsala, 1989, Fogarty and Kelly, 1990, Negi and Banerjee, 2006) and specifically *Bacillus* species are the most effective producers of α-amylases (Saha *et al*., 2014). Despite the fact that several microorganisms can produce amylases, obtaining a strain with suitable characteristics remains a challenging task. One of the important attribute of nearly all of the enzymes used in industrial applications is thermostability and that's the reason thermophilic microorganisms considered as potential source for the production of amylases (Srivastava and Baruah, 1986, Sonnleitner and Fiechter, 1983). This study is concerned about the analysis of soil samples obtained from various locations for isolating amylolytic enzyme producing strains, and to characterize the strain productivity and the enzyme produced, particularly their behavior toward temperature and pH.

MATERIAL AND METHODS

Isolation of strain

Soil samples were collected from vegetative fields near Karachi in sterilized containers. Amylase producing strains were isolated and selected according to the method of Min *et al*. (1999).

Selection of the thermophilic amylase producers

Thermophilic strain was selected by streaking all amylase producing cultures on Luria starch agar plates and incubating them on elevated temperatures i.e. from 40°C to 70°C with a difference of 10°C among each temperature. Growth of organisms was observed after 24 h.

Optimization of pH for growth of bacteria

Selected thermophilic amylase producing strain was incubated in Luria broth, supplemented with starch. The values of pH tested were 5, 6, 7, 8, and 9. pH was adjusted using 1N NaOH and 1 N HCl. Flasks were then incubated at temperature 60˚C for 24 hours and the absorbance was taken at 600 nm.

Selection of medium and optimization of substrate concentration

Medium selection was done by inoculating selected culture in Luria broth and Nutrient broth with varying starch concentrations and incubating them for 24 h at temperature 60˚C.

RESULTS AND DISCUSSION

The optimum temperature for the growth of *Bacillus subtilis* AR-27 was found to be 60ºC (Fig.1). It was reported that α-amylase production from *Bacillus* species on commercial scale has optimum temperature in the range of 37- 60ºC (Syu and Chen, 1997). The strain grew better on Luria broth and optimum pH appeared to be 7.

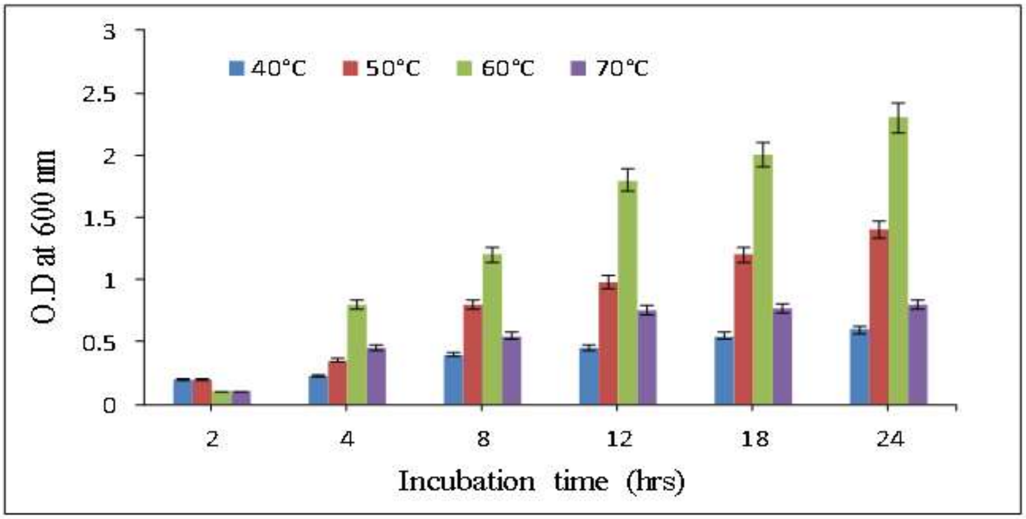

Fig. 1. Effect of different temperatures and time intervals on growth of *Bacillus subtilis* AR-27 (means ± S.E., n = 6).

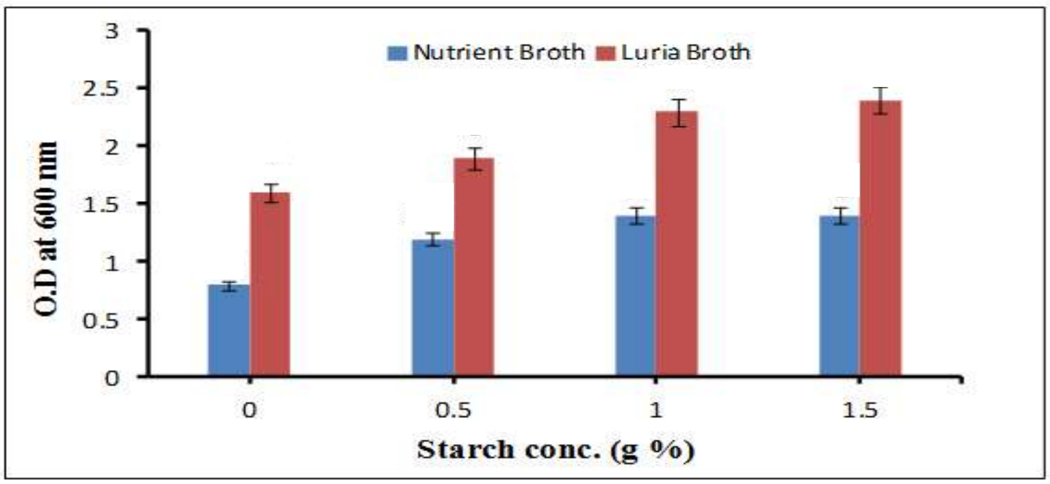

Fig. 2. Effect various concentrations of starch on bacterial growth in two different media.

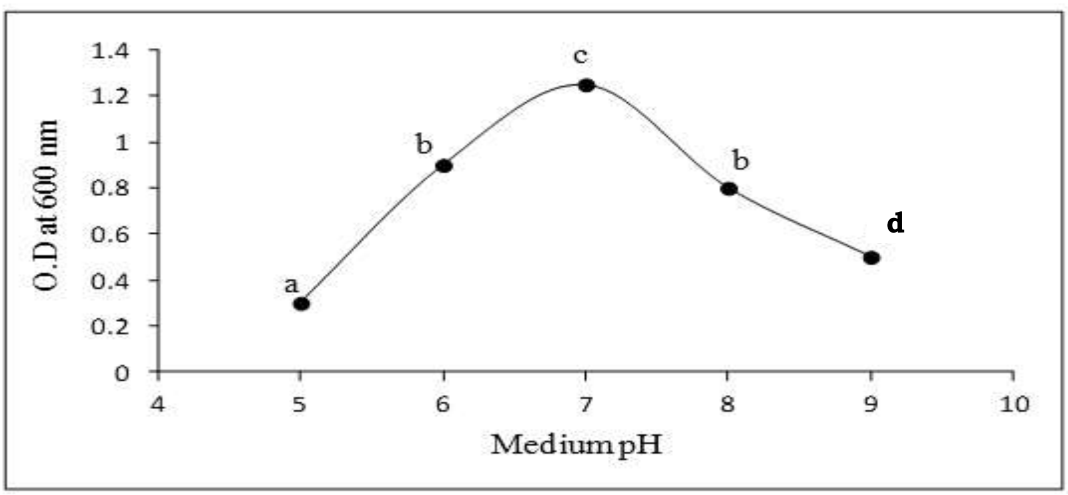

Fig. 3. Effect of different pH on the growth of thermophilic *Bacillus subtilis* AR-27. Symbols (means ± S.E., n = 6) having similar letters are not significantly different from each other (Bonferroni test, $P < 0.05$).

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