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INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol.10, pp.110-114, November, 2010

ISSN: 0975-833X

RESEARCH ARTICLE

PRODUCTION OF AMYLASES BY *STREPTOMYCES TENDAE* TK-VL_333

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ARTICLE INFO

Article History:
Received 5th September, 2010
Received in revised form
7th October, 2010
Accepted 24th October, 2010
Published online 1st November, 2010

Key words:

Actinomycetes, *Streptomyces tendae,* amylases, optimization pattern

ABSTRACT

A study has been undertaken to optimize the amylolytic activity of *Streptomyces tendae* TK-VL_333 isolated from laterite soil samples of Guntur region by using basal medium as inorganic salts-starch medium. Four-day old culture showed maximum enzymatic activity when grown at pH 7.0 and temperature 30°C. The productivity of the strain was enhanced by amending the medium with sorghum flour and peptone at a concentration of 3% (w/v) and 0.5% (w/v) respectively. This is the first report on the production of amylases by *S. tendae*.

INTRODUCTION

Starch is one of the abundant carbon sources in nature and amylases which catalyze the hydrolysis of starch or other carbohydrates to sugar, syrups and dextrin (Sadhukham *et al*., 1992) are the most important group of enzymes. In the present day biotechnological applications, amylases constitute 25% of the total enzyme market (Rao *et al*., 1998). New amylases could be potentially useful in the pharmaceutical and fine-chemical industries if enzymes with suitable properties could be identified (Nigam and Singh, 1995). With the advent of new frontiers in biotechnology, the spectrum of amylase applications has expanded into many other fields such as clinical, medicinal and analytical chemistry (Pandey *et al*., 2000). Though amylases originate from different sources (plants, animals and microorganisms), the microbial enzymes are widely employed in industry due to their reproductivity (Burhan *et al*., 2003). Very little information is available on the production of amlylolytic enzymes such as α-amylases, pullulanase and glucosyltransferase by *Streptomyces* spp. (Hoque *et al*., 2006) when compared to that of bacteria and fungi (Adeyanju *et al*., 2007). As cultural conditions greatly influence the enzymatic activity, optimization of cultural factors for obtaining maximum yields of amylase is a necessary task. Keeping all these in view, an amylolytic actinomycete strain isolated from laterite soils of Guntur region was identified as *Streptomyces tendae* TK-VL_333 by 16S rRNA analysis. The present study deals with the optimization of cultural conditions for amylase production by *S. tendae*.

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MATERIALS AND METHODS

Microorganism: *Strepotmyces tendae* TK-VL_333 was isolated from laterite soils of Guntur region by using soil dilution plate technique on asparagine-glucose agar medium (Williams and Cross, 1971). The 16S rRNA gene sequence of the strain was deposited in NCBI Gene bank with an accession number FJ877150.

***Production medium and cultural conditions*:** Inorganic salts-starch broth (ISP-4) composed of 1% soluble starch, 0.01% (w/v) K_2HPO_4, 0.01% (w/v) $MgSO_4$. $7H_2O$, 0.01% (w/v) NaCl, 0.02% $(NH_4)_2SO_4$, 0.02% (w/v) $CaCO_3$, 0.001% (w/v) $MnCl_2$, 0.001% $ZnSO_4$. $7H_2O$ (pH 7.2) was employed for studying the amylolytic profile of the strain. The culture suspension of the strain, prepared from one week old culture was inoculated into ISP-4 broth and the fermentation was carried at 35^{o}C for 7 days. Production of biomass as well as amylolytic activity was analyzed. Biomass was recorded as cell dry weight (mg/ml) whereas the culture filtrates collected at regular intervals of 24 h served as crude enzyme extract.

***Amylase assay*:** The enzyme assay was based upon the procedure described by Bernfield (1955). The reaction mixture containing 1 ml of 1% (w/v) starch solution and 1 ml of enzyme extract were incubated at room temperature for 15 min. The reaction was terminated by the addition of 2 ml Dinitrosalicylic (DNS) reagent and the tubes kept at zero time incubation served as control. The solution was heated in a boiling water bath for 5 min followed by the addition of 1 ml potassium sodium tartrate solution. After cooling, the volume of the solution was made up to 10 ml and optical density was read at 560 nm. One amylase unit equals to that amount of enzyme needed to release 1 mg of reducing sugar (maltose as standard) for 15 min at 37^{0}C.

Optimization of cultural conditions for amylase production

***Initial pH*:** The effect of initial pH on cell growth of the strain and enzymatic activity was determined by adjusting the pH of production medium ranging from 4-10. The optimal pH achieved at this step was used for further study.

***Temperature*:** Enzymatic yield of the strain was determined by conducting the fermentation at different temperatures varying from 20-40^{o}C. Temperature at which the strain showed maximum amylolytic activity was fixed for further studies.

***Carbon and nitrogen sources*:** The effect of carbon sources on cell growth and amylolytic production was studied by supplementing the production medium with different carbon sources such as dextrose, glycerol, lactose, mannitol, rice flour, sorbitol, sorghum flour, starch, sucrose and xylose each at a level of 1% (w/v). Influence of various levels of best carbon source (1-5% w/v) on enzymatic production was examined. Likewise, the impact of nitrogen sources on enzymatic yield was investigated by adding different nitrogen sources like ammonium oxalate, ammonium sulphate, beef extract, glutamine, glycine, malt extract, peptone, potassium nitrate, tryptone, tyrosine, urea and yeast extract each at a concentration of 0.2% (w/v) to the production medium containing an optimum amount of the superior carbon source. Besides, the actual concentration of nitrogen source (0.2-1.5% w/v) supporting optimal yields of amylase was recorded.

Statistical analysis: Statistical data was recorded on cell growth of the strain and enzymatic activity by using One-way Analysis of Variance (ANOVA).

RESULTS AND DISCUSSION

***Production profile of amylases*:** Growth pattern as well as the amylolytic activity of the strain TK-VL_333 was determined in ISP-4 broth. The production of amylases by the strain started from 24 h of incubation and gradually peaked at 96 h. Thereafter, the productivity of the strain was declined. The graph value statistically significant at 5 levels (Fig. 1). The optimal incubation period for obtaining high yields of amylases was found to be 96 h for *Streptomyces albidoflavus* (Narayana and Vijayalakshmi, 2008) whereas in *S. aureofaciens* 77, the amylolytic activity was maximum after 7 days of incubation (Shatta *et al*., 1990).

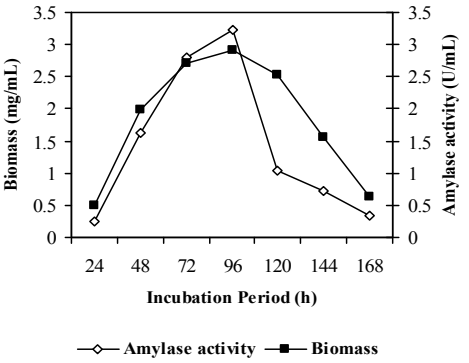

Fig. 1. Growth pattern of *Streptomyces tendae* TK-VL_333 and production of amylases on inorganic salts-starch broth

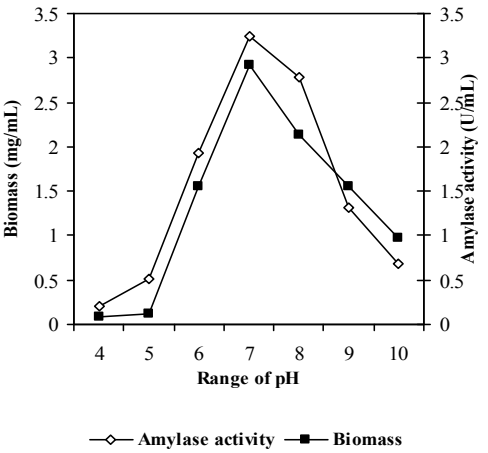

Fig. 2. Effect of pH on cell growth of *Streptomyces tendae* TK-VL_333 and amylase production

***Effect of initial pH and temperature on amylase production*:** The optimum pH for cell growth and amylase production were found to be 7.0 (Fig. 2) which is in consistent with the result of Shatta *et al*. (1990) in *S. aureofaciens* 77. The strain cultured at different temperatures showed maximum yields of

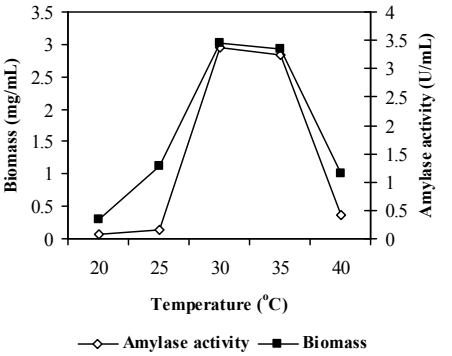

Fig. 3. Effect of temperature on cell growth of *Streptomyces tendae* TK-VL_333 and amylase production

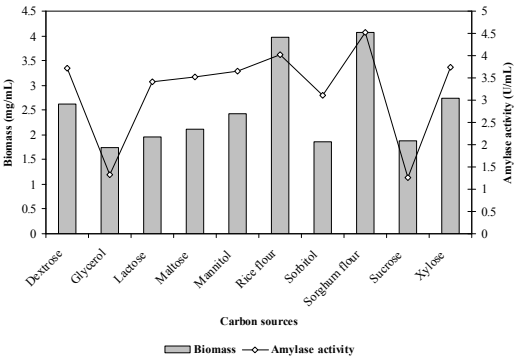

Fig. 4. Impact of carbon sources on cell growth of *Streptomyces tendae* TK-VL_333 and amylase production

optimal temperature for amylase production was reported as 30°C and 28°C respectively (Narayana and Vijayalakshmi, 2008; Shatta *et al*., 1990). In the present study, four-day old culture showed maximum enzymatic activity when grown at pH 7.0 and temperature 30°C. The graph values statistically significant at 5 levels.

***Impact of carbon and nitrogen sources on amylase production*:** Different carbon and nitrogen sources were amended individually in the production medium to determine their

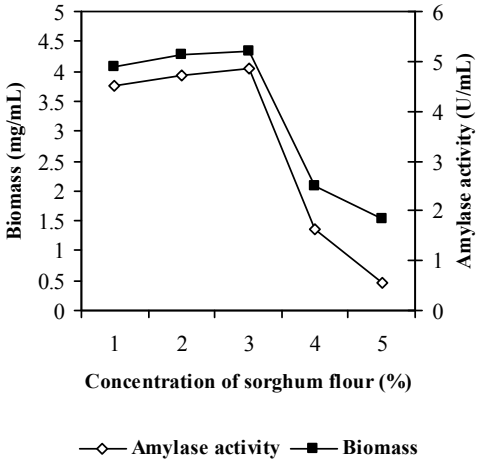

Fig. 5. Effect of different concentrations of sorghum flour on cell growth of *Streptomyces tendae* TK-VL_333 and amylase production

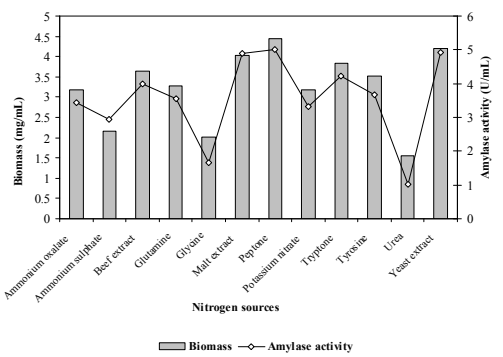

Fig. 6. Impact of nitrogen sources on cell growth of *Streptomyces tendae* TK-VL_333 and amylase production.

effect on amylase production. Sorghum flour was found to be the best carbon source for amylolytic activity of the strain followed by rice flour, xylose, dextrose, mannitol, maltose and lactose. The graph

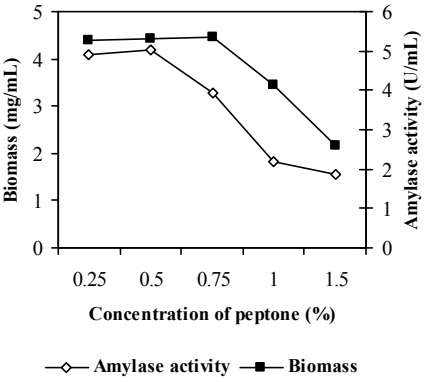

Fig. 7. Effect of different concentrations of peptone on cell growth of *Streptomyces tendae* TK-VL_333 and amylase production.

value statistically significant at 5 levels (Fig. 4). Utilization of carbon sources for the production of amylases by *Streptomyces* spp. was found to vary. Shatta *et al*. (1990) and Narayana and Vijayalakshmi (2008) noted high amounts of amylases with starch in *S. aureofaciens* 77 and *S. albidoflavus* respectively. However, in the present work, maximum yields of amylase were obtained with cheap and easily available carbon sources such as sorghum flour and rice flour which may be useful in the development of cost-effective and high quality biotechnological processes for amylase production. As sorghum flour supported high yields of amylolytic activity, the effect of different concentrations of sorghum flour was further analyzed. Optimal productivity of amylase by the strain was observed when cultured in the medium containing 3% sorghum flour. The graph value statistically significant at 5 levels (Fig. 5). Culture medium amended with 3% (w/v) soluble starch favored high yields of amylase production by *S. aureofaciens* 77 (Shatta *et al*., 1990).

Study on the effect of different nitrogen sources proveQ peptone as the suitable nitrogen source for amylolytic activity of the strain. The graph value statistically significant at 5 levels (Fig. 6). Besides, the production of amylase by the strain was

enhanced by increasing the concentration of peptone in the medium to 0.5% (w/v). The graph value statistically significant at 5 levels (Fig. 7). Similarly, Aiyer (2004) recorded peptone as the best organic nitrogen source for amylase production by *Bacillus lichenformis* SPT 27. 0.5% yeast extract was reported to be the optimal nitrogen concentration for amylolytic activity of *S. albidoflavus* (Narayana and Vijayalakshmi, 2008).

Conclusion

In the present study, *S. tendae* yielded high amounts of amylase in the medium containing sorghum flour and peptone as best carbon and nitrogen sources with pH 7.0 at 30^{o}C for 96 h of incubation. This is the first report of amylase production by *S. tendae* and further studies regarding the isolation and purification of amylases produced by the strain are in progress.

Acknowledgements

We are grateful to the Department of Botany & Microbiology, Acharya Nagarjuna University, Guntur, India for providing laboratory assistance. One of the authors (AK) expresses her gratitude to Indian Council of Medical Research (ICMR), New Delhi, India for awarding SRF.

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