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Production of Extracellular Protease by *Streptomyces albidoflavus*

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Abstract: Production of protease by *Streptomyces albidoflavus* isolated from laterite soil was studied under submerged fermentation. The strain started protease production after 24 h of incubation and maximum level of enzyme production was found with 72 h old culture. Attempts were made to optimize the cultural conditions for getting high yields of enzyme. The optimum levels of pH and temperature for enzyme production were 7.0 and 35°C respectively. Among carbon sources, maltose (1%) supported maximum production of protease followed by trehalose, glycerol, starch and glucose. High yield of protease was recorded in the medium supplemented with peptone (0.75%) followed by beef extract, casein, yeast extract, tryptone and NaNO₃.

Key words: *Streptomyces albidoflavus*, protease production, optimization of culture conditions

INTRODUCTION

Proteases are the most important class of industrial enzymes and comprise about 25% of commercial enzymes in the world. Two thirds of the industrially produced proteases are from microbial source (Gerhartz, 1990). *Streptomyces* species are heterotrophic feeders which can utilize both simple and complex molecules as nutrients. In addition to antibiotics, *Streptomyces* species liberate several extracellular enzymes (Gupta *et al.*, 1995). They produce a variety of extracellular proteases that have been related to aerial mycelium formation and sporulation (Kim and Lee, 1995) as well as with the utilization of nutrient sources (Bressolier *et al.*, 1999). As the composition of culture medium strongly influenced enzyme production (Giarrhizzo *et al.*, 2007), an attempt has been made to optimize the cultural conditions for getting high yields of protease from *Streptomyces albidoflavus*.

MATERIALS AND METHODS

An actinomycete strain was isolated from the laterite soil of Acharya Nagarjuna University campus and the culture was identified as *Streptomyces albidoflavus* by 16S r RNA analysis and gene sequences are submitted to NCBI genbank with accession number EF 142856. Pure culture of the strain was maintained on yeast extract-malt extract-dextrose medium. The culture suspension was prepared and inoculated in glucose-peptone-yeast extract (GPY) broth (g L⁻¹): glucose, 10; peptone, 5; yeast extract, 5; K₂HPO₄, 1 and MgSO₄.7H₂O (pH 7.2). The culture broth was collected at 12 h interval for 108 h and examined for protease activity as well as for biomass.

Biomass obtained by filtration of culture broth was determined in terms of dry weight (mg mL⁻¹). Protease activity of culture filtrate was determined by a colorimetric method (Yang and Wang, 1999). The reaction mixture containing 1 mL of 1% casein in phosphate buffer (pH 7.0) and 1 mL of enzyme solution was incubated at 37°C for 20 min and the reaction was stopped with 3 mL of 10% trichloroacetic acid. The absorbance of liberated tyrosine in filtrate was measured at 660 nm.

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One IU of protease activity was defined as the amount of enzyme that produced an absorbance at 660 nm equivalent to 1 μ mole of tyrosine in one min. under the assay conditions.

The impact of pH and temperature on protease production by the strain was determined. The influence of carbon sources such as glucose, starch, glycerol, maltose and trehalose were studied on biomass as well as protease production. The respective carbon sources (1% wt./vol.) were amended in GPY broth as a sole carbon source. The nitrogen sources like peptone, casein, yeast extract, tryptone, beef extract, NaNO_3 , KNO_3 , NH_4Cl and $(\text{NH})_2\text{SO}_4$ were examined on cell growth as well as protease production. The respective nitrogen sources were added (1% wt./vol.) in GPY broth as a sole source of nitrogen. The optimum concentration of best carbon and nitrogen sources was also studied on protease production.

RESULTS AND DISCUSSION

The growth pattern and protease production by *S. albidoflavus* was studied in GPY broth (Fig. 1). Protease production started after 24 h and reached maximum level after 72 h of cultivation. There was a gradual increase in biomass as well as protease production during exponential phase. These results confirmed the observation of Petinate *et al.* (1999) and Yang and Wang (1999) who reported the increased protease production by *Streptomyces cyanens* and *S. rimosus* during log phase, indicating that high levels of protease production are observed with increased biomass.

Data on the effect of pH and temperature on protease production by the strain are presented in Fig. 2. The optimum pH for high protease yield was found to be 7.0, whereas the strain exhibited maximum growth at pH 6.5. The strain showed maximum enzyme production as well as biomass when cultured at 35°C. On either side of this optimum temperature, the productivity was declined. El-Raheem *et al.* (1994) reported that maximum production of protease by *Streptomyces corchorusii* ST 36 was obtained with pH 6.0 at 30°C. Ellaiah and Srinivasulu (1996) described that the high protease yield by *S. fradiae* was found with medium adjusted to pH 7.0 at 28°C.

The impact of different carbon sources on biomass and protease production by the strain is presented in Fig. 3. Among all the carbon sources, maltose proved to be the best for growth as well as protease production followed by trehalose, glucose, glycerol and starch. Nitrogen sources like peptone and beef extract were found to be suitable for maximum production of protease followed by casein, yeast extract, tryptone and NaNO_3 . Medium supplemented with organic nitrogen sources supported high protease production when compared to inorganic nitrogen sources (Fig. 4). Yeast extract and

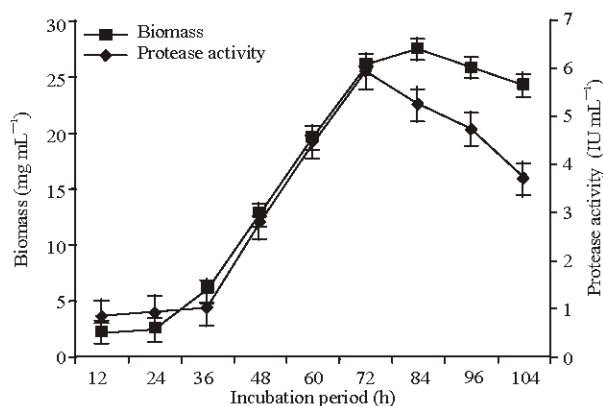


Fig. 1: Effect of incubation period on biomass and protease production by *Streptomyces albidoflavus* (values are means of three replicates \pm SD)

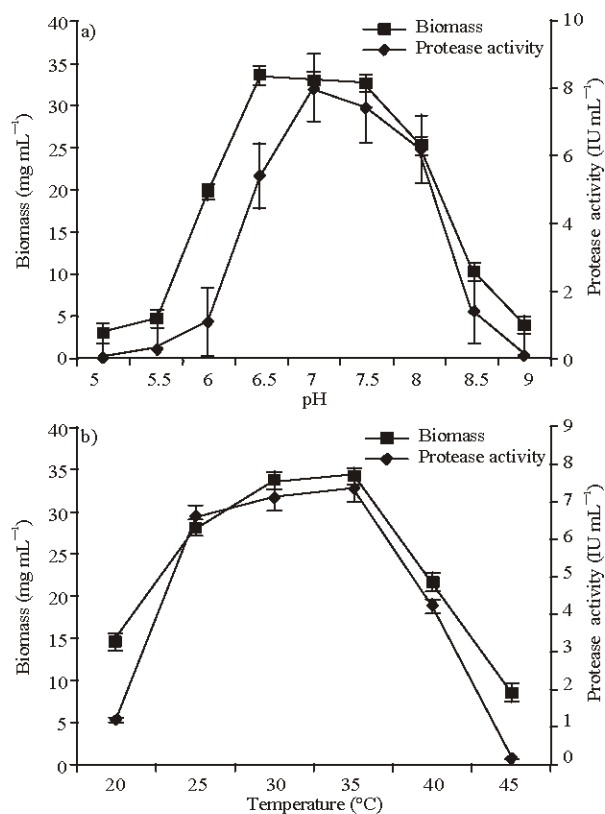


Fig. 2: Impact of a) pH and b) temperature on biomass and protease production by *Streptomyces albidoflavus* (values are means of three replicates±SD)

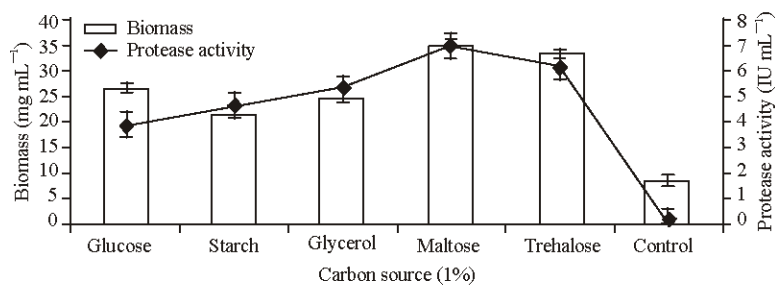


Fig. 3: Effect of carbon sources on cell growth and protease production by *Streptomyces albidoflavus* (values are means of three replicates±SD)

tryptone supported good growth as well as enzyme production. The highest yield of protease production was obtained with maltose at 1% (wt./vol) as a sole carbon source. Peptone at 0.75% (wt./vol) as a sole nitrogen source was found to be suitable for maximum production of protease (Fig. 5).

The carbon and nitrogen sources play an important role in protease production by *Streptomyces* species. Ellaiah and Srinivasulu (1996) suggested that medium amended with Jowar starch (3%) and tryptone (0.5%) with pH 7.0 cultured at 28°C supported maximum protease

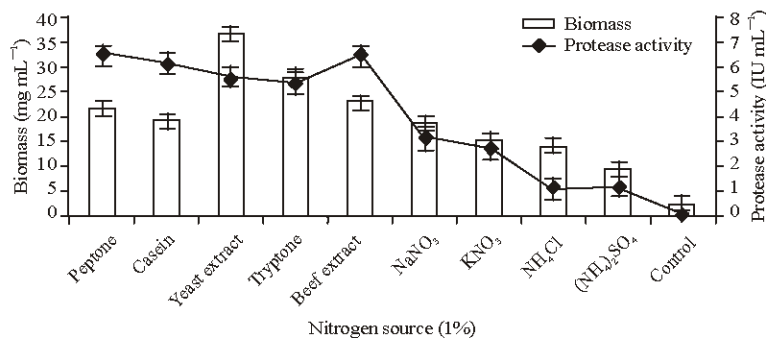


Fig. 4: Effect of nitrogen sources on cell growth and protease production by *Streptomyces albidoflavus* (values are means of three replicates \pm SD)

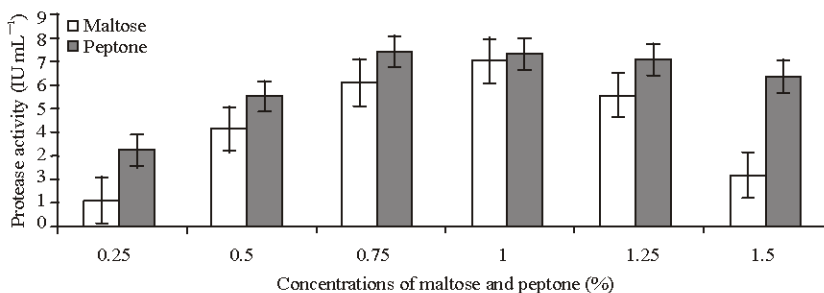


Fig. 5: Impact of different concentrations of maltose and peptone on protease production (values are means of three replicates \pm SD)

yield. De Azeredo *et al.* (2003) stated that in protease production C/N ratio seemed to play a significant role. Nascimento *et al.* (2005) described that the high yields of protease by *S. malaysiensis* was obtained when using wheat bran (2.5%) and yeast extract (0.1%) in culture medium incubated for 5 days at 30°C. Patel *et al.* (2006) suggested that glucose (1% wt./vol.) and peptone (0.5% wt./vol.) are the suitable carbon and nitrogen sources respectively for getting high yields of protease by an alkalophilic actinomycete. In the present study, the strain *S. albidoflavus* was found to elaborate maximum protease after 72 h of incubation at pH 7.0 and temperature 30°C. Maltose (1%) and peptone (0.75%) were proved to be best carbon and nitrogen sources for high levels of protease production by the strain.

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