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PRODUCTION OF a-AMYLASE BY *Aspergillus niger* AND ITS PARTIAL PURIFICATION

M. Javed Asad, M. Asghar, M. A. Sheikh & J.I. Sultan[1]
Departments of Chemistry and [1]Animal Nutrition
University of Agriculture, Faisalabad

The fermentation conditions such as nitrogen source, corn steep liquor, yeast extract and cane molasses were optimized for a-amylase production. *Aspergillus niger* produced maximum alpha-amylase (21.20 IU/ml/min) in the optimum growth medium of waste bread (1.5%), containing 0.2% (NH4hS04, 1.0% corn steep liquor, 0.10% yeast extract and 1.0% cane molasses (added after 12 hr). (NH4hS04 proved to be the best additional nitrogen source amongst urea, NH4H2P04 and NaNOJ. Alpha-amylase produced under optimum conditions was partially purified and maximally isolated by 50% (NH4hS04 precipitation. Specific activity of the enzyme increased by 7.19 folds after (NH4hS04 precipitation.
Key words: alpha-amylase, *Aspergillus niger*;, partial purification, waste bread

INTRODUCTION

Utilization of agro-industrial wastes and by-products for production of enzymes by microbial process is a newly emerging trend in developing countries. Alpha-amylase (I,4-alpha-glucan glucanohydrolase, Ec 3.2.1.\) is a glycoprotein having a single polypeptide chain of about 475 residues. It has two free SH groups and four disulphide bridges and contains tightly bound Ca^{+2} (Granger et al., 1975).

Alpha-amylase acts on reducing ends and rapidly hydrolyzes the amylose portion of starch to maltose. It also acts on large linear polymers of by-products of starch at internal bonds. Amylases have got important industrial applications in extraction of fruit juices, production of wort, sugar syrups, gaseous and liquid fuels (ethanol, butanol etc.), edible acids (acetic acid, citric acid, lactic acid etc.), animal feed proteins and quality chemicals from starch and starchy waste materials. Alpha-amylase is also used in textile industry for desizing of cotton cloth. By maintaining a careful balance of the ratio of glucoamylase to alpha-amylase, these enzymes are used in malting, backing, brewing and confectionery. Complete starch hydrolysis can be achieved if a-amylase, p-amylase (glucoamylase) and debranching enzymes (a 1-6, glucosidase) are present in a proper proportion in the reaction medium (Asghar et aI., 2000).

Generally, fungi secrete a-amylase and other scarifying enzymes depending on the composition of growth medium and fermentation conditions (Poonam and Singh, 1995). A wide variety of microorganisms produce extra-cellular amylases, having different specificities, properties and action patterns. These amylases are used extensively in various industries and suitability of an amylase to a particular process depends on its specific characteristics (Fogarty et aI., 1990). The project was, therefore, planned for optimization of culture conditions for production and partial purification of a-amylase from *Aspergillus niger* in waste bread medium.

MATERIALS AND METHODS

Substrate: Waste bread obtained from the students mess, Ayub Hall, University of Agriculture, Faisalabad was dried and ground to powder form (40mm mesh). It was used as a substrate in fermentation medium for a-amylase production.

Fermentative Organism and Inoculum: Certified culture of *Aspergillus niger* procured from the Department of Microbiology, University of Agriculture, Faisalabad was raised on potato starch-agar slants sporulation medium and incubated at pH4 and 37°C for 72 hr. The spores of *Aspergillus niger* were transferred aseptically to a 500 ml conical flask containing 100ml of pre-sterilized inoculum medium (Asghar et aI., 2000) in laminar air flow. The flask was then kept on shaker (120 rpm) at 37°C for 72 hr.

Submerged Fermentation: The pH of the growth medium of waste bread (1.5%) containing pre-optimized micronutrients was adjusted to 4 and the flasks were plugged with cotton (Asghar et aI., 2000). The medium was autoclaved for 15 min at 1.1 kg/cm/ pressure (121°C). Spore inoculum (5ml) was then added aseptically into each flask and the flasks were incubated at 37°C on orbital shaker (120 rpm) for optimum time period (48 hr).

Sample Harvesting: After the expiry of optimum incubation period, both the control and experimental samples (in each experiment) were filtered and the filtrates were centrifuged at 1000 rpm for 15 min at _10°C temperature. The supernatants were collected and subjected to enzyme assay.

Enzyme Assay and Partial Purification: Alpha-amylase activity was determined by the spectrophotometric method described by Bernfeld (1955). One unit of alpha-amylase activity was defined as the number of l.l moles of maltose liberated by Iml of enzyme solution per minute. The enzyme present in crude extract was purified by (NH4)2S04 precipitations and dialyzed by the method of De-Moraes et al. (1999).

RESULTS AND DISCUSSION

Nitrogen Source : For the production of maximum a-amylase, varying concentrations (0.1, 0.2, 0.3 and 0.4%) of four different nitrogen sources i.e. $(NH_4)_2S0_4$, urea, $NH_4H_2P0_4$ and $NaN0_3$ were used in duplicate under pre-optimized culture conditions. Culture filtrates harvested from the medium containing (NH4)2S04 showed maximum a-amylase yield followed by urea, $NH_4H_2P0_4$ and $NaN0_3$. It was observed that the medium supplemented with 0.2% (NH4hS04 as nitrogen source gave maximum (18.13 IUlmllmin) a-amylase activity (Fig. I) in the presence of pre-optimized concentrations of micronutrients (MgS04.7H20, 0.04; KCI, 0.1 and KH_2P0_4, 0.2%). *Aspergillus niger* utilized (NH4hS04 as the best additional nitrogen source efficiently and showed better a-amylase production as compared to other nitrogen containing compounds. A study conducted by Niziolek (1998) for production of a-amylase using different nitrogen sources, also showed (NH4)2S04 as the best nitrogen source, amongst urea, (NH4hS04, NH_4CI, $KN0_3$, $NaN0_3$ and Ca(NO,l)2'

Corn Steep Liquor: Corn steep liquor at four different levels (0.5, 1.0, 1.5 and 2.0%) was used as an additional carbon and energy source in culture medium of waste bread. It was observed that with the addition of corn steep liquor to the growth medium of waste bread, the production of a-amylase increased steadily and reached its maximum of 18.85 IUlml/min with 1.0% level. Further addition of corn steep liquor (2,0%) caused a decrease in enzyme activity (Fig. 2). Omidiji et al. (1997) also observed an increase in a-amylase production by *Aspergillus niger* B I with the addition of glucose as energy source and suppression of the enzyme by its higher concentrations.

Table I. Activity of a-amylase produced by *Aspergillus niger* with varying concentra-tions of yeast extract under optimum conditions*.

Yeast extract (%)	a-amylase (IV mllmin)
	Mean
0.00	19.14
0.05	19,46
0.10	19.89
0.15	19,41
0.20	19.02

*=(NH4)2S04, 0.2% and yeast extract, 0.10%.

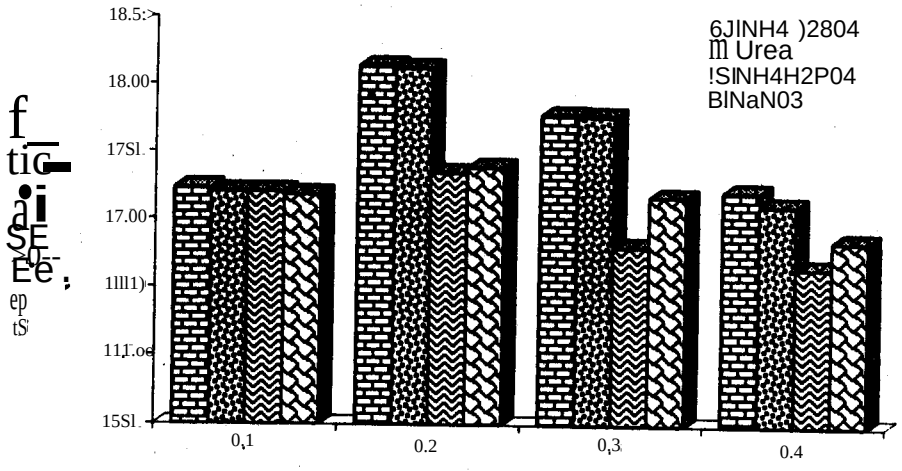
Yeast Extract: Results indicated that addition of yeast extract 0.1% to the waste bread fermentation medium enhanced the production of a-amylase by *Aspergillus niger*, leading to the maximum activity of 19.89 IUlmllmin. Further addition of yeast extract (0.2 and 1.5%) caused a gradual decrease in enzyme production (Table I). Adams (1997) added 0.1% yeast extract to the fermentation medium for optimum production of a-amylase by a mutant of *Alternaria alternata*. Hillier et al. (1996) reported 0.05% yeast extract as optimum for a-amylase production by *Verticillium tricarpus*.

Cane Molasses: Sterilized cane molasses was added aseptically after 12 hr of incubation to pre-optimized culture medium of waste bread to increase the production of a-amylase by *Aspergillus niger*. It was observed that with the addition of cane molasses, the production of a-amylase increased gradually and reached a maximum of 21.20 IUlmllmin with 1.0% level (Fig. 2), Further addition of cane molasses caused a decrease in enzyme activity (Fig. 2). Molasses is a mixture of soluble sugars and also contains some mineral nutrients and heavy metal ions (Moo- Young, 1985). Supplementation of culture medium with molasses up to 1.0% level supplied additional energy source in the form of soluble sugars and promoted the growth of fungus and thus secretion of a-amylase by it (Zouari and Jaoua, 1999).

Partial Purification of a-Amylase: Alpha-amylase produced by *Aspergillus niger* under optimum conditions was subjected to (NH4hS04 precipitation and the pellets were then dissolved in distilled water (0.1 g/ml) and dialyzed using EDT A. A single step purification of a-amylase by (NH4)2S04 precipitation resulted in almost 7 times increase in specific activity of a-amylase. Initially 86 units of a-amylase were present in crude enzyme extract and finally 29.26 units of a-amylase were recovered after $(NH4)2S0_4$ precipitation. The initial specific activity of a-amylase was 0.605 which increased up to 4.35 (Table 2). The pellets obtained after 50% of $(NH_4hS04$ precipitation were found to contain maximum amount of a-amylase. The purification factor as calculated from the present study is in line with that of De-Moraes et al. (1999) who reported the purification factor to be 6.33 for (NH4)2S04 precipitation and 1.08 for dialysis of a-amylase produced. from *Cellulomonas biazotae* NIAB 442.

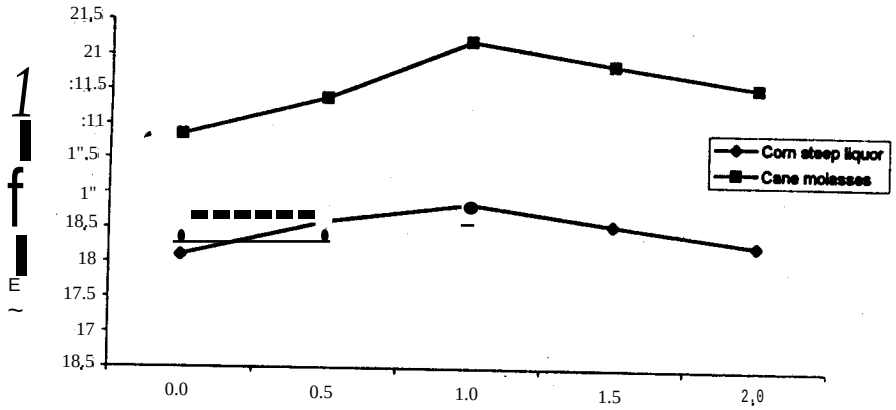
Table 2. Partial2urification of a-amylase from *A~erf.!l , 'lJus ntger*

Treatments	Volume (ml)	Activity (Ulml)	Total units	Protein (~ml)	Specific activity	Total protein (mg/ml)	Purification factor
Crude	200	0.43	86	0.71	0.605	142	1.00
(NH4)2S04 precipitation	14	2.09	29.26	0,48	4.35	6.72	7.19



Nitrogen source (%)

Fig. I Effect of different nitrogen sources on the production of a-amylase by *Aspergillus niger*



Concentration (%)

Fig. 2 Effect of corn steem -liquor and cane molasses on a-amylase production by *Aspergillus niger*

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