PRODUCTION OF EXTRA CELLULAR RAW STARCH DIGESTIVE AMYLASE ENZYMES BY THERMOPHILIC YEASTS ON WHEAT FLOUR MEDIA

*** Dr. Rani Ayachi,**

Introduction-Thermophilic microorganisms are being studied extensively for their thermostable products. The use of thermophilic microorganisms in industrial fermentations has two distinct advantages; saving of expenditure on the cooling system and the minimum chances of contamination[1, 2] Also, high solute concentrations can be used without processing unduly viscous substrates[3] Amylases of the moulds have been preferred in industrial production due to their activity and stability at low pH and their ability to produce high amounts of maltose from liquefied starch[3] In our laboratory, various thermophilic yeasts were screened for the extra cellular production of amylases, by the clear zone based method[4] (data presented elsewhere). Three thermophilic yeast *P. anamala, E. fibuliger, P. ceferrii*. Exhibited a large clear zone and were selected for the study. The present communication describes the Production of various amylases by these three yeasts. The relationship among various factors affecting the kinetics of amylase production by the test yeast was also studied.

Material and Methods Production of amylases - The test yeasts were grown in the following media to select one which supports maximum production of amylases (Medium constituents in g/L).

1. WF: media Wheat flour – 1 gm, yeast extract – 0.5 gm 2. SYE medium: Soluble starch 1.5, yeast extract - 4, K_2HPO_4 - 3, $MgSO_4$. $7H_2O$ - 0.5, pHi 7.0[5] 3. Soluble starch - 10, (NH_4) SO_4 - 2, $MgSO_4$, $6H_2O$ - 0.2, Biotin - 0.001, K_2HPO_4 - 0.001, pHi - 7.05 4. YN base medium with Soluble starch 10, pHi 6.8[6]

Fifty ml of each of the medium was dispensed separately in the 150 ml Erlenmayer flask and sterilized by autoclaving at 15 lb for 15 min. The flasks were inoculated by test yeast prepared from a 7 day old culture of the respective grown on medium. The flasks were incubated at 45 ± 2°C, without shaking and triplicate flasks were withdrawn. on day 3, 5, 7, 9 and 12 of incubation. The medium was separated yeast culture by filtration through preweighed whatman no. 42 paper. The filter papers were dried to a constant weight in an oven at 80 DC and biomass yield was recorded as dry weight in mg. The filtrates were analyzed for amylase activities, starch and extra cellular protein content and change in the pH of the media.

Amylases assay-Alpha amylase activity was measured by the method of Spencer-Martins and VanUden (1979)[7]. A a-amylase unit was defined as the quantity of the enzyme mediating 0.1 Δ E at 550 nm. b-amylase activity was measured by the method of Bernfeld[8] (1955) and expressed as specific units. For the measurement of glucoamylase activity one ml of one percent starch solution (prepared in 0.05 M citrate-phosphate buffer pH 5.2) was incubated with one ml of enzyme extract for 20 min at 50 DC, the released glucose was measured by the GOPOD method of Hugget and Nixon (1955)[9]. Controls were prepared using boiled enzyme extract in the reaction mixtures. A glucoamylase unit was defined as the amount of enzyme which liberates one micro mole of glucose per minute from starch. P-Nitro-phenyl-a-d-glucopyranoside (pNPG) was used as substrate for a-glucosidase activity measurement. 25 mM pNPG solution was prepared in 0.05 M citrate-phosphate buffer pH 4.8. One ml of enzyme sample was incubated with one ml of pNPG solution for 30 min at 50 °C. The liberated glucose was measured by the GOPOD method (Hugget and Nixon 1955)[9]. A a-aglucosidase unit was defined as the amount of enzyme releasing one micro mole of glucose from pNPG per minute.

Analytical methods-Starch and protein concentrations in the culture filtrates were measured by the method of Smith and Roe (1948)[10] and Lowry, *et al*. (1951), respectively[11].

Results and Discussion-Amylases production by the three test yeast was better on the, Wheat flour media as compared to the synthetic media used in the present study. Test yeast produced maximum

*** Takshshila Institute of Engineering and Technology – Jabalpur (M.P.)**

Fig:-01 (a) PRODUCTION OF a-AMYLASE, STARCH & DRY Wt BY *PICHIA ANAMALA* IN WHEAT FLOUR MEDIA

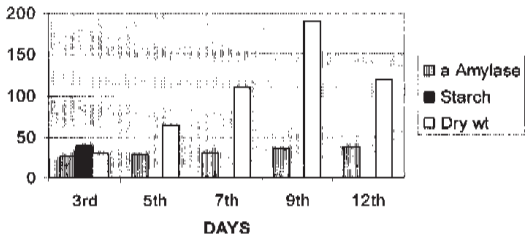


Fig:-02(a) PRODUCTION OF a-AMYLASE, STARCH & DRY Wt BY *ENDOMYCES FIBULIGER* IN WHEAT FLOUR MEDIA

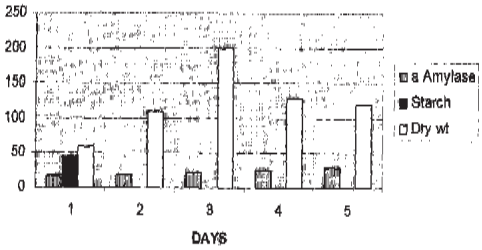


Fig:- 01(b) PRODUCTION OF a-GLUCOSIDASE & b-AMYLASE BY *PICHIA ANAMALA* IN WHEAT FLOUR MEDIA

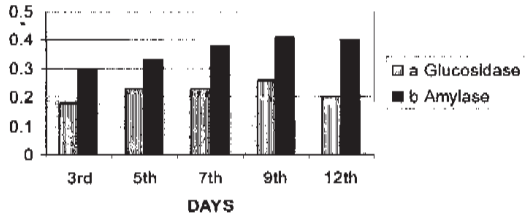


Fig:-02(b) PRODUCTION OF a-GLUCOSIDASE & b-AMYLASE BY *ENDOMYCES FIBULIGER* IN WHEAT FLOOR MEDIA

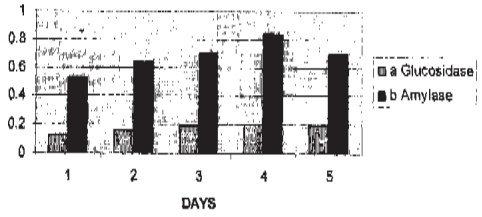


Fig:-01(C) PRODUCTION OF PROTEIN BY *PICHIA ANAMALA* IN WHEAT FLOUR MEDIA

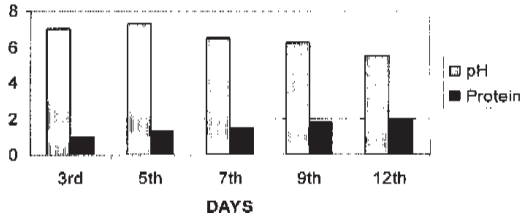
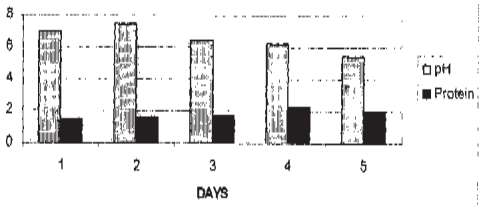
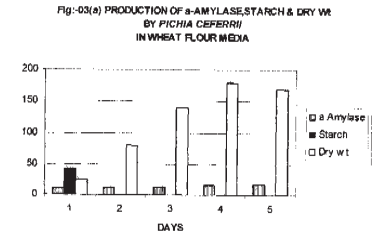
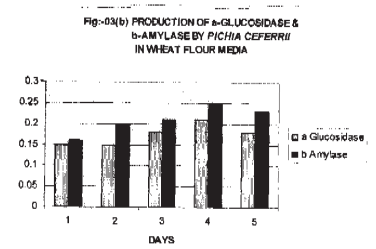


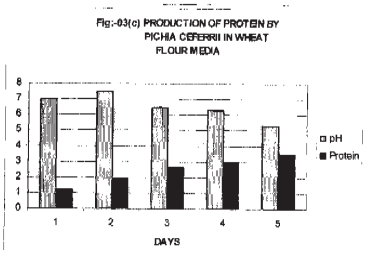
Fig:-02(c) PRODUCTION OF PROTEIN BY *ENDOMYCES FIBULIGER* IN WHEAT FLOOR MEDIA



amylases and biomass in the yeast extract containing WF media (preliminary data about the amylases productivity in the other three media are not included). Yeast extract contains complex organic substances arid is a rich vitamin source with a stimulatory effect on microbial amylases synthesis[12, 13]. The a-amylase production by the test yeast increased with an increase in the dry weigh and was found to be maximum when biomass yield was the highest or after the autolysis (Fig. 1-3). Adams and Deploey (1976) and Adams (1985) a similar relationship is also observed between amylase productivity and the growth stage of the thermophilic fungi[14, 15]. In the present study amylases productivity of *P. anamala* was found to be maximum before the autolytic phase, whereas in E*ndomyces fibuliger* and *Pichia ceferrii* the correlations between amylase production and growth stage were not observed. Maximum a-amylase yield of *Pichia ceferrii* was recorded on the 12th day of incubation. *Pichia anamala* product the highest 38.8 EU of a-amylase (Fig. 1 a, b, c), followed by E*ndomyces fibuliger* 29.89 EU, (FIg. 2 a, b, c), *Pichia ceferrii* 18.12 EU, (FIg. 3 a, b, c) b-amylase synthesis was recorded as maximum in E*ndomyces fibuliger* (0.84 SU, 9th day), followed by Pichia anamala (0.412 SU 9th day) and *Pichia ceferrii*. The a-glucosidase synthesis was recorded as maximum in Pichia anamala (0.266 IU/ml), whereas it was considerably lower in E*ndomyces fibuliger* and *Pichia ceferrii* (Fig. 2 & 3). Test yeast which were selected for the study after







preliminary screening based on the clear zone method, produced mainly extra cellular a-amylase, whereas glucoamylase activity was negligible (hence the data is not included). It suggests that the clear zone based screening in method has an edge for the selection of a-amylase producers.

All the three test yeasts exhibited rapid starch utilization. Starch was completely utilized from the medium on or before five days of incubation (Fig. 1-3). Notably, in the early exponential growth phase, when the starch utilization rate was rapid, lower levels of extra cellular amylases were recorded in the case of all the test yeast. In all the three test yeast the highest amylases yield was recorded only after complete utilization of starch, it indicates that starch hydrolysis products are better amylase inducer than starch *'per se'*. Preliminary data amylase and biomass production by the test in the four studied media, were analyzed for the correlattionship at the probability level 0.001. In each of the test yeast the following three significant correlationships were recorded, positive correlation between biomass productivity and a-amylase production, positive correlation between biomass productivity and extra cellular protein content and negative correlation between biomass production and starch utilization rate. Biomass productivity was common among all the three significant correlations and appears to be a key factor influencing the kinetics of amylase production by the test yeast on wheat flour media.

References-

1. Sorenson, S. G. and Crisan, E. V. (1974): Thermostable lactase from thermophilic fungi. *J. Bacteriol.* 62, (247-249).
2. Cooney, C. L. and Wise, D. L. (1975): Thermophilic anaerobic digestion of solid waste for fuel gas production. *Biotech. Bioeng*. 17, (1119-1135).
3. Sadhukhan, R. K., Manna, S., Roy, S. K. and Chakraborty, S. L. (1990): Thermostable amylolytic enzymes from a cellulolytic fungus Myceliophthora thermophila D 14 (A TCC 48104). *Appl. Microbiol. Biotechnol*. 33: (692-696).
4. Barnett, E. A. and Fergus, C. L. (1971): The relation of extra~ cellular amylase, mycelium, and time, in some thermophilic and mesophilic Humicola species. *Mycopath. Mycol. Appl.* 44 (2), (131-141).
5. Glymph, J. L. and Stutzenberger, F. J. (1977): Production, purification and characterization of a-amylase from Thermomonospora curvata. *Appl. Environ. Microbial*. 34 (4): (391-393).
6. Wickerham, L. J. (1951): In 'Taxonomy of Yeasts' us Department of Agriculture *Tech. Bull*. No. 1029, p. (56).
7. Spencer-Martins, Land VanUden, N. (1979): Extra cellular amylolytic system of yeast Lipomyces kononenkoae. *Eu. J. Appl. Microbiol. Biotechnol*. 6, (241-250).
8. Bernfeld, P. (1955): Amylases a and *b*. In *'Methods in enzymology'* 1: (149-154).
9. Hugget, A. B. C. and Nixon, D. A. (1955): Glucose oxides method for measurement of glucose. *Biochem*. J. 6, (12-19).