ISSN 2320-9984 01 >
9 772320 998015

Volume 1, Issue 1, April 2013 ISSN: 2320-9984 (Online)

International Journal of Modern Engineering & Management Research

Website: www.ijmemr.org

Study the Activity of Thermophilic yeasts on Wheat Flour Media: Production of Extra Cellular Raw Starch Digestive Amylase Enzymes.

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Abstract*—Three thermophilic years were studied for the extra cellular amylase production on Wheat Flour (WF media). The three test yeasts mainly produced α –amylase, β–amylase and α–glucosidase, whereas glucoamylase productivity was found to be negligible. Among various media, WF media supported maximum amylases and biomass production. Analysis of the correlationship among various factors showed significant correlations of the dry weight yield of the test yeast with their starch utilization rate, extra cellular protein content and α–amylase productivity.*

***Keywords*:** *Yeast, Wheat flour, amylase enzymes.*

1. 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 (data presented elsewhere). Three thermophilic yeast *Pichia angusta, Candida hallenica, Candida ishiwadae*. 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.

2. 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).

- WF: media Wheat flour – 1 gm, yeast extract – 0.5 gm
- SYE medium: Soluble starch 1.5, yeast extract - 4, K_2HPO_4 - 3, $MgSO_4$. $7H_2O$ - 0.5, pHi 7.0[5]

- 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
- 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.

3. AMYLASES ASSAY

Alpha amylase activity was measured by the method of Spencer-Martins and VanUden (1979)[7]. A α -amylase unit was defined as the quantity of the enzyme mediating 0.1 Δ E at 550 nm.

β-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 α 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].

4. 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 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].

Days	3rd	5th	7th	9th	12th
a Amylase	27.3	29.2	31.5	35.5	38.8
a Glucosidase	0.18	0.23	0.23	0.26	0.2
b Amylase	0.3	0.33	0.38	0.41	0.4
Starch	40	0	0	0	0
pH	7	7.3	6.5	6.2	5.5
Protein	1	1.3	1.5	1.8	2
Dry wt	30	65	110	190	120

Table 1 : Amylase production and growth stage of *Pichia angusta*

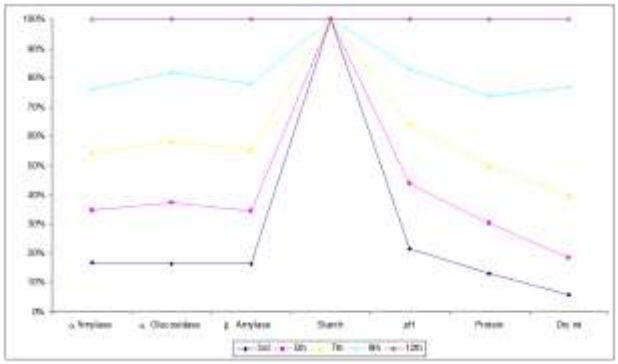

Figure 1 : Production of amylases by Pichia angustain wheat flour medium

The α-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 (Figure. 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 *Pichia angusta* was found to be maximum before the autolytic phase, whereas in *Candida hallenica* and *Candida ishiwadae* the correlations between amylase production and growth stage were not observed. Maximum α-amylase yield of *Candida ishiwadae* was recorded on the 12th day of incubation. *Pichia angusta* product the highest 38.8 EU of a-amylase (Fi. l), followed by Candida hallenica (29.89 EU, Figure. 2), *Candida ishiwadae* (18.12 EU, Figure. 3) β-amylase synthesis was recorded as maximum in Candida hallenica (0.84 SU, 9th day), followed by *Pichia angusta* (0.412 SU 9th day) and *Pichia ceferrii*. The α-glucosidase synthesis was recorded as maximum in *Pichia angusta* (0.266 IU/ml), whereas it was considerably lower in *Candida hallenica and Candida ishiwadae* (Figure. 2 & 3). Test yeast which were selected for the study after preliminary screening based on the clear zone method, produced mainly extra cellular α-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 α-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 (Figure. 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.

Days	3rd	5th	7th	9th	12th
a Amylase	9.8	11.3	13.5	16.1	18.12
a Glucosi-dase	0.15	0.15	0.18	0.21	0.18
b Amylase	0.16	0.2	0.21	0.25	0.23
Starch	42	0	0	0	0
pH	7	7.5	6.5	6.3	5.3
Protein	1.2	1.9	2.6	2.9	3.5
Dry wt	25	80	140	180	170

Table 2: Amylase production and growth stage of *Candida ishiwadae*

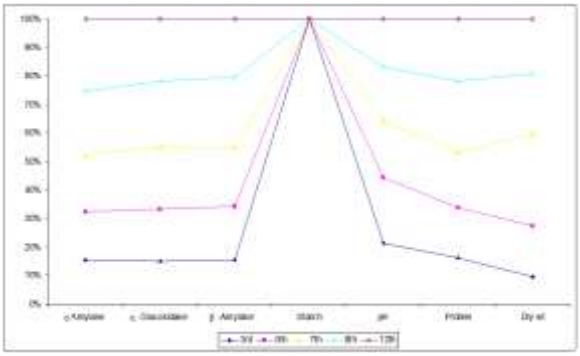

Figure 2 - Production of amylases by *Candida hallenica*in wheat flour medium

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'*.

Days	3rd	5th	7th	9th	12th
a Amylase	18.1	19.9	23.2	26.3	29.89
a Glucosi-dase	0.13	0.16	0.19	0.2	0.19
b Amylase	0.53	0.64	0.71	0.84	0.7
Starch	45	0	0	0	0
pH	7	7.5	6.5	6.2	5.5
Protein	1.5	1.6	1.8	2.3	2
Dry wt	60	110	200	130	120

Table 3: Amylase production and growth stage of Candida hallenica

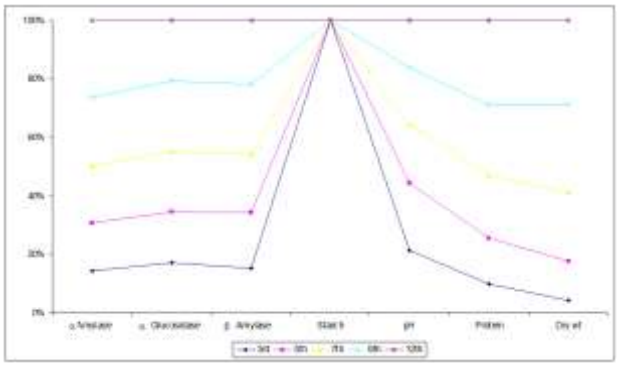

Figure.3 Production of amylases by Candida ishiwadaewheat flour medium

Preliminary data amylase and biomass production by the test in the four studied media, were analyzed for the correlation ship at the probability level 0.001. In each of the test yeast the following three significant correlation ships were recorded, positive correlation between biomass productivity and α-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.

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