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Full Length Research Paper

Optimization of growth and extracellular glucoamylase production by *Candida famata* isolate

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Candida famata **was isolated from traditional Moroccan sourdough. It exhibited high glucoamylase and biomass production. Starch induces high glucoamylase production *C. famata* with maximum glucoamylase activity at 5 g/L. Glucose stimulates good production in biomass but strongly inhibits glucoamylase production. Among the sources of nitrogen tested, yeast extract and the $(NH_4)_2HPO_4$ gave maximum glucoamylase and biomass after 72 h of incubation in liquid medium at 30°C, pH 5 and 105 rpm.**

Key words: Glucoamylase, yeast, optimization, biotechnology, *Candida famata*.

INTRODUCTION

Up to the early 1970's it was considered that plant and animal materials were the best sources of enzymes. Nowadays, however, microbial enzymes are becoming increasingly important for their technical and economical advantages (Kelly and Fogarty, 1976). Amylases have important applications in diverse industries such as baking, brewing, detergent, medicine, textile, paper and pharmaceutical (Kennedy, 1987). Extracellular amylase (Tani et al., 1986; Mahmoud, 1993) have been found in various species of fungi (Pandy et al., 2000; Jaffar et al., 1993; Norouzian and Jaffar, 1993; Frandsen et al., 1999; Reilly, 1999), bacteria (Srivastava and Baruah, 1986; Tanaka et al., 1987) and yeasts (Bui et al., 1996; Yamashita et al., 1987; Yamashita et al., 1985; Dohmen et al., 1990; Hostinova et al., 1991).

Different culture conditions greatly affect the production of amylase. So, it becomes necessary to investigate different factors involved in maximum production of amylase. Yeast amylases production have been reported at different pH and temperatures of incubation (Spenser-Martins and Van Uden, 1979; Oteng-Gyang et al., 1981).

In the present study, screening was carried out with *Candida famata*, which was isolated from traditional Moroccan sourdough. Studies were also conducted to determine the optimum culture conditions and factors involved in maximum production of glucoamylase.

MATERIALS AND METHODS

Microorganism

C. famata was isolated from traditional Moroccan sourdough using the following medium (g/L): soluble starch 5, KH_2PO_4 3; $(NH4)_2SO_4$ 1; $MgSO_4$ 0.5; and yeast extract 4. pH was adjusted to 5 with 0.1 M HCl. Medium was solidified by the addition of 1.5% agar, and autoclaved at 121°C for 15 min. Liquid medium was incubated at 30°C in a flask on rotary shaker set at 105 rpm for 72 h.

Isolation and screening of the isolate

1 g of traditional Moroccan sourdough were suspended in 10 ml of sterile physiological water and plated on medium using pour plate method and then incubated at 30°C for 72 h. Starch degradation was detected by the disappearance of the blue color of the medium

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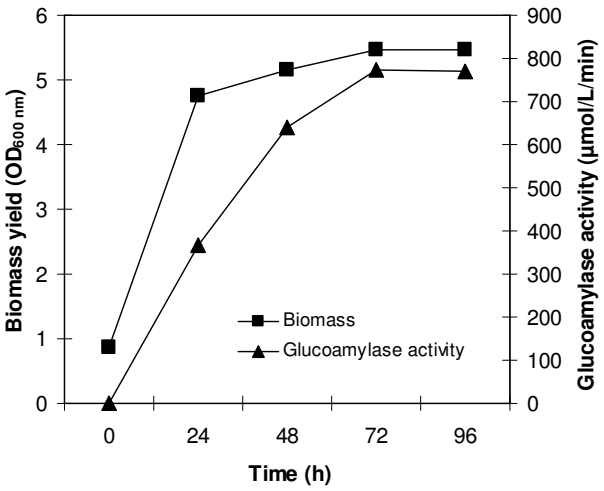

Figure 1. Effect of incubation time on biomass production and glucoamylase activity by *Candida famata*.

around microbial colonies after addition of iodine solution. Colonies with the largest halo-forming zone were isolated for further investigation. Evaluation of the clear zones was estimated as diameter (mm) of the clear zone. Yeast colonies producing large clear zones were picked up and purified three times by streaking on Potato Dextrose Agar (PDA). Pure cultures were maintained on PDA, stored in a refrigerator and sub-cultured at 3 months interval.

Measurement of enzyme activity

The fermented broth was taken after 72 h and centrifuged at 7000 rpm for 10 min, and then substrate-free supernatant was used for estimation of enzyme activity.

Amylase activity was determined by measuring the reducing sugar formed by the enzymatic hydrolysis of starch using the method of Somogyi and Nelson (Nelson et al., 1944). 0.25 ml soluble starch (1%), 0.15 ml phosphate buffer (0.1 M) and 0.1 ml enzyme solution were mixed and incubated at 40°C in water bath for 30 min. The reaction was stopped with 2 ml of Somogyi reactive, and 1.5 ml of distilled water, followed by boiling for 15 min to develop blue color. The absorbance was measured at 540 nm with a spectrophotometer. The blue color was measured against the control in which no enzyme was added. A calibration curve of absorbance and concentration of glucose was established with known amount of glucose.

One unit (µmol/L/min) of glucoamylase was defined as the amount of µmol of reducing sugar per liter of enzymes per min, measured as glucose under the conditions of assay.

Biomass yield

It was determined by measuring the absorbance of the suspension at 600 nm.

Effect of incubation time, temperature and medium pH

To ascertain the effect of culture conditions the present study was carried out at different incubation periods (24, 48, 72 and 96 h), temperatures (20, 25, 33 and 40°C), and medium pH (3.0, 5.0, 6.0, 7.0 and 8.0). Their effects on biomass yield and glucoamylase production were recorded.

Effect of carbon and nitrogen sources

The production of extracellular amylases under different carbon and nitrogen availability were studied in liquid medium. Seven carbon sources (starch, sucrose, lactose, maltose, galactose, fructose and glucose), and nitrogen sources: organic (3 g/L) yeast extract, peptone, tryptone, meat extract and inorganic: CH_4N_2O; $NaNO_3$; $(NH_4)2HPO_4$; $SO_4(NH_4)_2$ were added at the same concentration of nitrogen (0.318 g/L N equivalent of 1 g of $SO_4(NH_4)2$) and initial medium pH 5; Effects of carbon and nitrogen sources on production of glucoamylase and biomass yield was recorded. Fermentation experiments were carried out for 72 h.

To insure optimum percentage of carbon and nitrogen sources, the study was carried out at different starches (2.5, 5, 7.5, and 10 g/L) and yeast extract concentration (0, 1, 2, 3, 4, and 5 g/L).

Batch fermentation in a laboratory bioreactor

C. famata was grown in a (2 liter Setric Set 002 M Bioreactor) containing 1 L of the production medium. The bioreactor was operated at 30°C, and 105 rpm. The initial pH of the medium was adjusted to 5.5 with 0.1 M of KH_2PO_4 before sterilization; inoculum was prepared in Erlenmeyer flasks in a volume corresponding to 10% of fermentation broth medium and incubated at 30°C at 150 rpm. Samples were withdrawn at 1 h intervals, and centrifuged at 6000 rpm for 10 min after the recording biomass; cell-free supernatant was used for the assay of glucoamylase.

RESULTS AND DISCUSSION

Of the sixteen yeasts isolated, four were considered to be the best amylase producing strains. One of them identified as *C. famata* using API gallery ID 32 C (Biomérieux REF 32 200) was selected for the present work.

Effect of incubation time

C. famata showed different glucoamylase activities at different incubation period; it was found that the isolate produced maximum activity after 72 h of incubation (Figure 1) but highest biomass yield was recorded after 48 h of incubation time. The final pH of the supernatant was found to range from 6 to 7.5. Production of amylase after 3 days of incubation by *Aspergillus* sp. was also reported by Rahman et al. (1993) and Cherry et al. (2004).

Effect of temperature on biomasse and glucoamylase activity

The influence of temperature on glucoamylase activity of the crude enzyme showed that enzyme activity increased progressively with increase in temperature from 25°C reaching a maximum at 30°C (Figure 2). Above 35°C, there was a reduction in the glucoamylase activity. Similar results were also reported by Rene and Hubert (1985) with *Filobasidium capsuligenum*.

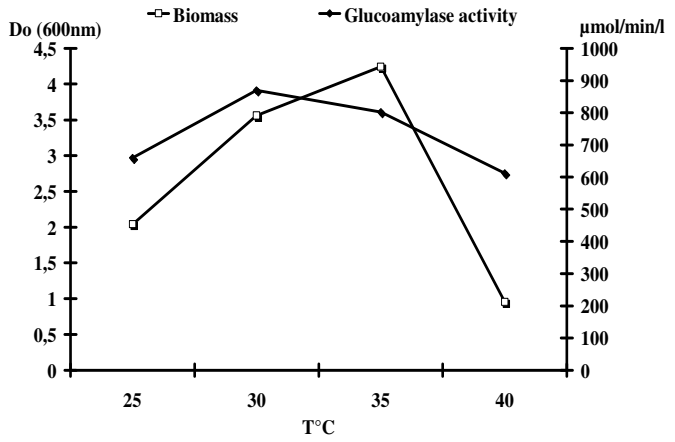

Figure 2. Effect of temperature on biomass production and glucoamylase activity by *Candida famata.*

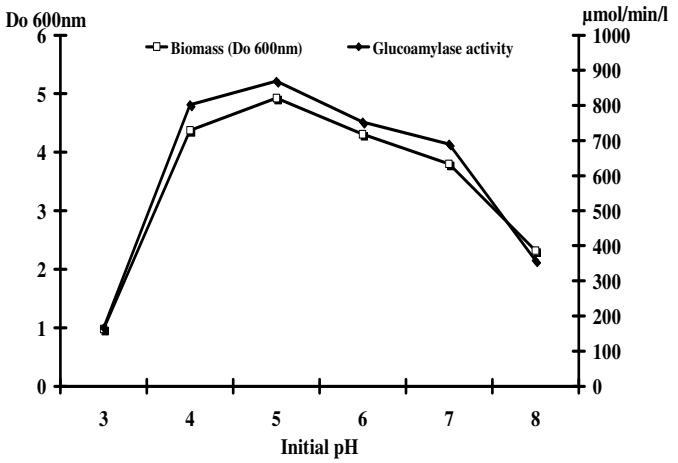

Figure 3. Effect of initial pH of medium on biomass production and glucoamylase activity by *Candida famata.*

Other researchers (Bertrand et al., 2005) also reported that maximum glucoamylase production occurred at the same temperature. Temperature for optimal production of amylolytic enzymes by *Yarrowia lipolytica* was 28°C (Cheon et al., 1997). These results disagree with results found by Chandra et al. (1980). This organism did not produce α-amylase at 30°C although it grew very well at this temperature.

Effect of initial pH of the medium

Medium pH also plays an important role on the production of microbial enzymes. In the present study, maximum production was achieved at medium with initial pH 5 (Figure 3); the pH of the culture filtrates after 72 h were ranged from 6 to 7.5. Similarly, Quang et al. (2000) found that optimum pH of fermentation medium of *Thermomyces lanuginosus* ATCC 34626 was found to be 4.9. In the same way Reiser and Gasperik (1995) reported that optimum activity of *Saccharomycopsis fibuligera* glucoamylase was observed at pH 5.5.

Comparable results were found by Rene and Hubert (1985) with *F. capsuligenum* at pH ranging from 5 to 5.6. Others found that the optimum pH for a better production of amylolytic enzymes by *Lipomyces kononenkoae* is 5.5 (Isabel, 1982). Also, Taylor et al. (1978) reported that *Humicola lanuginosa* produces the two shapes of amylolytic enzymes, with optimum of pH of 4.9 and 6.6, whereas for other groups of bacteria, optimum pH of growth and production of the enzyme by *Bacillus* sp. is 7 (Carlos and Souza, 2001).

Effect of starch concentration

Amylase became more active in relation to the increase in starch concentration from 2.5 g/L to 5 g/L. Beyond 5 g/L, there was a decline in amylase activity (Figure 4).

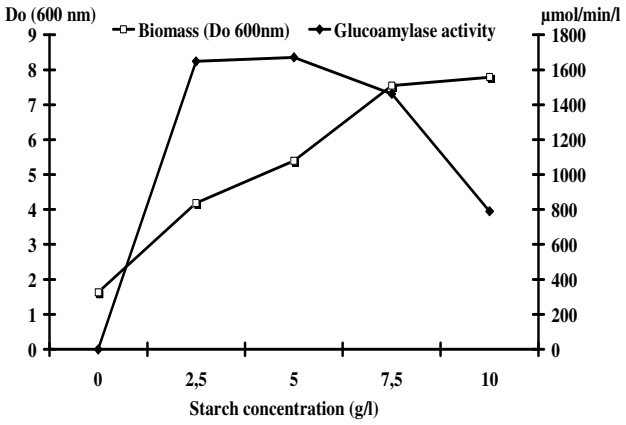

Figure 4. Effect of starch concentration on biomass production and glucoamylase activity by *Candida famata.*

Similar starch concentration (5 g/L) was used for the production of amylase by *F. capsuligenum* (Rene and Hubert, 1985), *Clostridium thermosulfurogenes* (Hyun and Zeikus, 1985) and *Clostridium* sp. (Madi et al., 1987).

On the contrary, other investigators reported that maximum amylase production was produced at 10 g/L, with *Lipomyces kononenkoae*, (Isabel, 982), *Schwanniomyces alluvius* (Jeffrey and Michael, 1982) and 15 g/L with *Chaetomium thermophilum* (Jing et al., 2005). Also, growth of the studied yeast increases according to the concentration of the starch to reach a maximum value at 10 g/L.

Effect of yeast extracts concentration

Figure 5 shows that maximum of glucoamylase activity is obtained with the concentration 2 g/L of yeast extract.

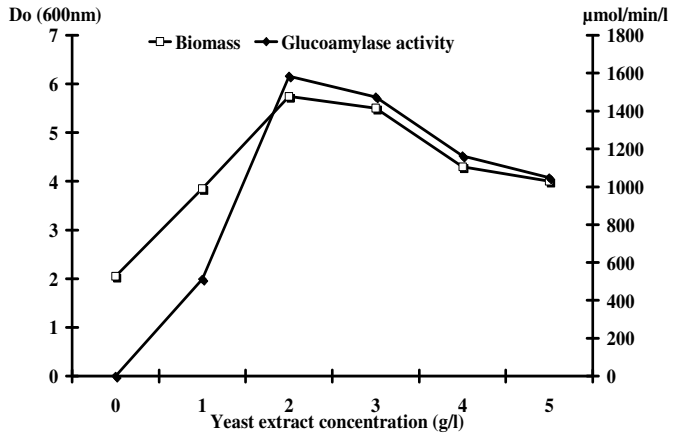

Figure 5. Effect of yeast extract concentration on biomass production and glucoamylase activity by *Candida famata*.

Production of α-amylase by *Aspergillus fumigatus* was obtained at 2 g/L (Cherry et al., 2004), *Pichia subpelliculosa* ABWF-64 (Sanjeev-Kumar and Satyanarayana, 2001), *Lactobacillus amylovorus* at 1 g/L (Oda et al., 2002), and 1.5 g/L with *Penicillium* sp. (Capuccino and Sherman, 2001). Also, Suzuki and Chishiro (1983) reported that *Endomycopsis fibuliger* gives maximum growth and glucoamylase activity at 3 g/L of yeast extract.

These results disagree with the maximum enzyme production obtained with yeast extract by *T. lanuginosus* (Nguyen et al., 2000) and *Aspergillus niger* (Djekrif-Dakhmouche et al., 2006).

Effect of nitrogen sources on glucoamylase production

Yeast extract was replaced in separate medium with 0.3% of different organic and equivalent of 1 g/L of sodium sulphate of inorganic compounds as nitrogen source keeping the rest of the medium composition the same. Among the nitrogen sources, yeast extract was the best organic one followed by meat extract, peptone and tryptone (Table 1). On the other hand, urea was the best inorganic nitrogen source followed by $(NH_4)_2PO_4$.

Yeast extract has been reported to significantly influence enzyme production (Hamilton et al., 1999; Nguyen et al., 2000). Similarly, Han et al. (2005) reported that *Basidiomycete Ganoderma lucidum* gave the highest starch degradation with yeast extract. This is in agreement with the observation of Cherry et al. (2004) who reported that the fungus *A. fumigatus* produces high amylase activity with yeast extract.

The effects of combination of organic and inorganic compounds on amylase production were also studied. We observed that the combination of urea and yeast extract gives highest amylase yields (Table 1), followed by combination of beef extract and urea.

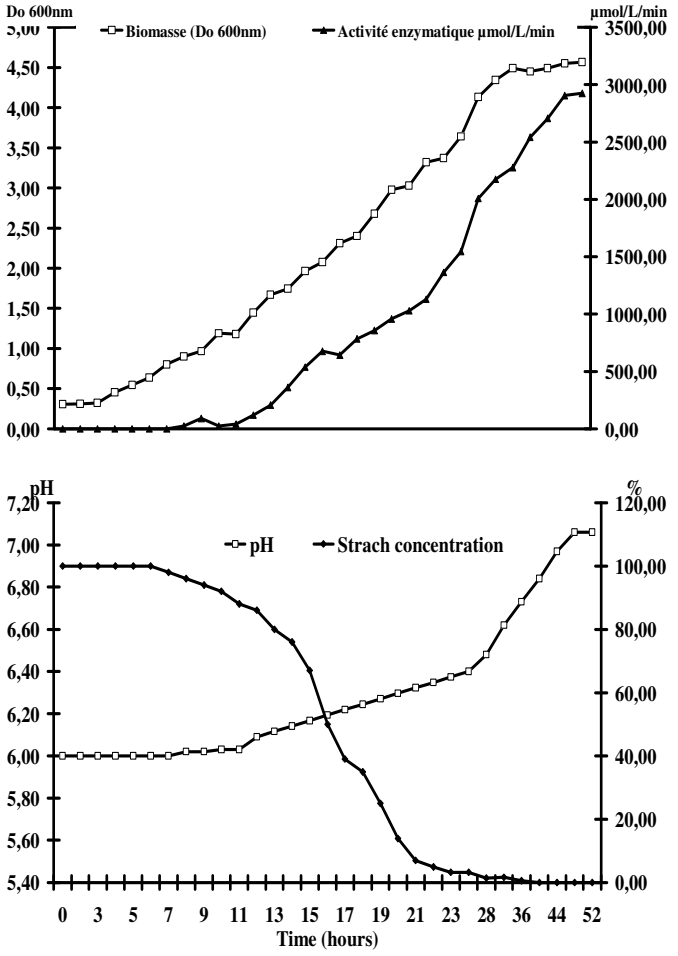

Figure 6. Bioreactor kinetic cultivation of *Candida famata*.

Batch fermentation in laboratory bioreactor

Optimum conditions of growth and of production of enzymatic activity are limited in Erlenmeyer because of the consumption of the substrates and the accumulation of cellular metabolites. To control various parameters of culture, the kinetics of growth and enzymatic production of *C. famata* were carried out by bioreactor under the optimum conditions with the culture previously established.

Results reported in Figure 6 show that enzymatic activity was related to the cell multiplication. Glucoamylase activity increased gradually in the medium after 8 h of culture time and reached a maximum value of 2926.19 µmol/L/min at the end of the exponential phase. The initial pH of the nutrient solution was adjusted to 5. The results showed that pH had an important effect on glucoamylase production. pH increase from 5 at 0 h of culture to 7.8 at 62 h was conversely proportional to starch degradation. In parallel starch is gradually consumed in the medium during the growth. Its hydrolysis is noticeable during logarithmic phase. Starch degradation

Table 1. Effect of nitrogen sources on biomass production and glucoamylase activity by *Candida famata.*

Sources of nitrogen	Biomass (Do 600 nm)	Glucoamylase activity (µmol/L/min)
$(NH_4)_2 HPO_4$	3.485	2587.169
$(NH_4)_2HPO_4$ + YE	5.505	2429.568
Urea + YE	4.115	2420.878
tryptone + urea	3.510	2329.686
Urea	3.345	1938.097
Yeast extract	4.415	1725.673
Beef extract + urea	5.030	1667.740
peptone + urea	3.330	1557.236
Beef extract	5.715	1411.329
$NaNO_3$+ YE	5.480	1311.555
Peptone	4.980	635.661
Tryptone	5.180	362.086
$NaNO_3$	2.005	0.000

YE: Yeat extract.

starts from 7 h to draw near at its maximum after 38 h of the fermentation period.

Conclusion

Yeast extract, soluble starch and urea would be beneficial for glucoamylase production; the enzyme activity was increased by urea. The yeast grown on glucose as well as on all tested simple sugars showed considerable growth production but strongly inhibited glucoamylase activity. Based on these results, the proposed compositions of the fermentation medium (w/v) are soluble starch 5 g/L; yeast extract 3 g/L, urea 0.77 g/L, KH_2PO_4 3 g/L and $MgSO_4$ 0.5 g/L. The pH should be adjusted to 5, and cultivation should be at 30°C under 105 rpm agitation. To reach maximum glucoamylase activity, 72 h fermentation time is needed.

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