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Invertase production by *Aspergillus niger* in submerged and solid-state fermentation

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Abstract

Three *Aspergillus niger* strains were grown in submerged and solid state fermentation systems with sucrose at 100 g l^{-1}. Average measurements of all strains, liquid vs solid were: final biomass (g l^{-1}), 11 ± 0.3 vs 34 ± 5; maximal enzyme titres (U l^{-1}) 1180 ± 138 vs 3663 ± 732; enzyme productivity (U l^{-1} h^{-1}) 20 ± 2 vs 87 ± 33 and enzyme yields (U/gX) 128 ± 24 vs 138 ± 72. Hence, better productivity in solid-state was due to a better mould growth.

Introduction

Cultivation of micro-organisms by solid state fermentation (SSF) is an alternative to submerged fermentation (SmF) for the production of enzymes by moulds (Pandey *et al.* 1999). The main advantages of SSF over SmF are minor catabolic repression (Ramesh & Lonsane 1991, Solís-Pereira *et al.* 1993) higher enzyme productivity (Lekha & Lonsane 1994, Acuña-Argüelles *et al.* 1995) and enzyme titres (Pandey *et al.* 1999). To answer why this should be so, polyurethane foam (PUF) as a non-biodegradable support (Zhu *et al.* 1994) has been used to study the synthesis and secretion patterns of enzymes, together with accurate measurement of biomass. Furthermore, invertase (β-D-fructo furanoside fructo hydrolase EC 3.2.1.26) seems to be an appropriate choice as a model system, since it has been studied in detail in *Aspergillus niger* (Boddy *et al.* 1993).

The present communication compares SSF and SmF systems in relation to the production of biomass and secretion of invertase using three different strains of *Aspergillus niger*. Evidence is presented supporting higher titres of invertase by *A. niger* grown in SSF system are due to higher levels of biomass production, as compared to the lower invertase and biomass levels observed in SmF system, when high levels of substrate (100 g l^{-1}) are supplied to both types of culture media. This could be useful for future applications regarding commercial production of enzymes.

Materials and methods

Micro-organisms

Three *Aspergillus niger* strains C28B25 (Boccas *et al.* 1994, Antier *et al.* 1993), N-402 (Debets *et al.* 1993) and Aa20 (IRD-UAM collection) were grown on basic mineral medium (BMM) containing (g^{-1} l^{-1}): $NaNO_3$, 15.0; KH_2PO_4, 1.76; KCl, 0.76; $MgSO_4$, 0.76; $FeCl_2$, 0.001; $CuSO_4$, 0.001; $MnCl_2$, 0.001; $ZnCl_2$, 0.001, supplemented with 100 g sucrose per litre. Inoculation was performed with 2×10^7 spores per g of carbon source. Seed cultures were propagated on PDA culture medium for 72 h before each fermentation described below.

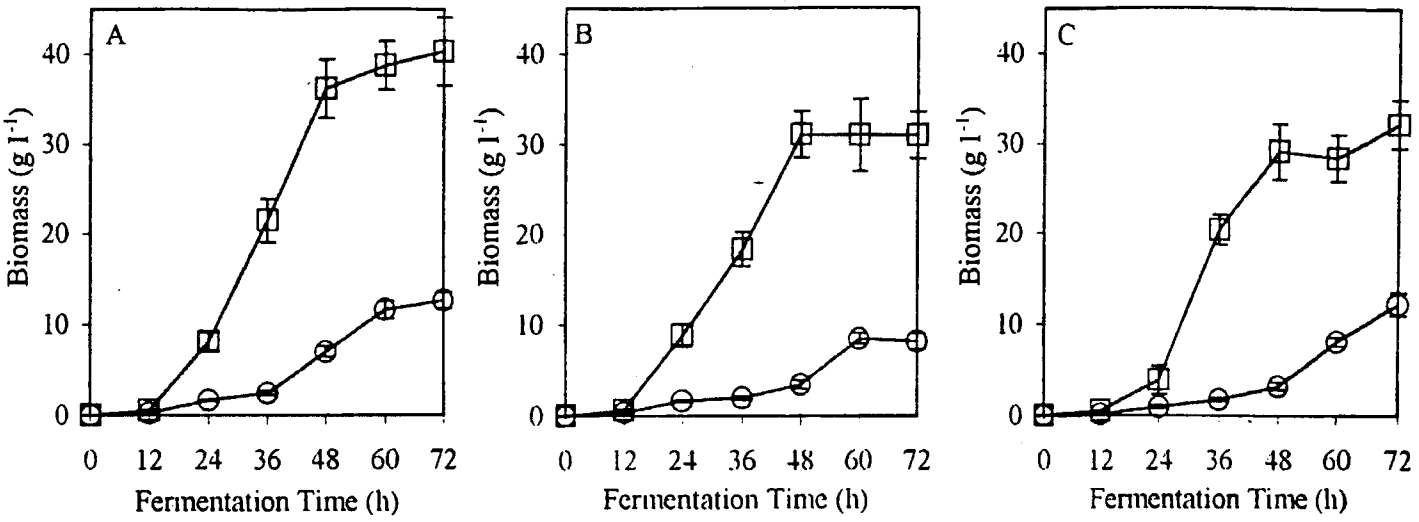

Fig. 1. Biomass production by *Aspergillus niger* strains. Aa20 (A), N402 (B) and C28B25 (C) in SmF (O) and SSF (□). Plots were done using the best fit of logistic equation, $X = X_{max}/(1 + Ce^{-\mu t})$, $C = X_{max} - X_0/X_{max}$, where X_{max}, X_0, and μ were parameters identified by Solver programme (Microsoft Excel).

Fermentation systems

Submerged fermentations were done in 125 ml flasks filled with 25 ml of BMM, at 30 °C in an orbital shaker at 200 rpm.

For solid state fermentation, polyurethane foam (PUF) with an average density of 17 mg ml^{-1} was cut into 0.5 cm cubes. The cubes were washed three times with boiling water and dried overnight in an oven at 75 °C. PUF cubes (1 g dry) were placed in 250 ml Erlenmeyer flasks and autoclaved at 121 °C for 15 min. BMM was autoclaved separately at 121 °C for 10 min and inoculated with *Aspergillus niger* spores. Inoculated medium were added (25 ml) to each flask and thoroughly stirred manually before incubation at 30 °C. *Aspergillus niger* did not grow on PUF cubes when the medium was lacking either sucrose or sodium nitrate, showing that PUF cannot be used as a major nutrient for the growth of this organism.

Enzyme extracts

Extracellular enzyme fractions from SmF were harvested at desired incubation times and filtered through a Whatman paper No. 1. Biomass retentate was washed with 100 ml of distilled water and used for dry weight biomass determination. For SSF experiments, extracts were obtained by gentle pressure of the PUF cubes, within a 60 ml syringe (going from 60 ml to 10 ml volume). The filtrates were maintained at 4 °C and assayed, for invertase activity within 24 h after harvest. Solid residue was washed out several times with distilled water and used for dry weight determination, subtracting the weight of the polyurethane from the final weight. Control experiments with aspartate amino transferase as intracellular marker (data not shown) indicated that biomass lysis was lower than 1% after harvest.

Enzyme assays

Invertase activity was measured by estimating the release of reducing sugars due to invertase activity over sucrose by technique after Miller (1959). One enzyme unit was defined as the amount of enzyme needed to produce one μmol reducing sugar per min.

Results and discussion

Figure 1 shows the growth profiles of three strains of *Aspergillus niger* in SSF and SmF culture. Comparison of growth curves (Figures 1A–C and Table 1) indicates that each given strain grew faster and with higher biomass level in SSF than in SmF system. Microscopic examination of the PUF cubes, showed mycelia tangled in the polyurethane network having large spaces filled with air (data not shown). Thus, it seems reasonable to assume that SSF culture is not as limited by oxygen transfer as in the case of SmF culture (Marsh *et al.* 1998).

Figure 2 (A–C) shows the evolution over time of invertase titres for each strain. Apparent enzyme yields $Y_{E/X} = E_{max}/gX$ (U/gX), calculated at the maximal invertase production time, were not statistically different among SmF and SSF experiments. Strain N402

Table 1. Production ([a]) and kinetic ([b]) parameters of three strains of *A. niger* cultured by SmF and SSF techniques.

Strain	X_{max} (g l^{-1})[a]		E_{max} (U l^{-1})[a]		μ ($l\ h^{-1}$)[b]		Prod (U l^{-1} h^{-1})[b]		$Y_{E/X}$ (U/gX)[b]	
	SmF	SSF	SmF	SSF	SmF	SSF	SmF	SSF	SmF	SSF
Aa20	12.7 ± 0.9	40.3 ± 3.7	1262 ± 95	3411 ± 301	0.08	0.15	21	71	108	94
N402	8.1 ± 0.6	31 ± 2.6	1020 ± 59	3089 ± 265	0.06	0.14	17	64	120	100
C28B25	12.2 ± 1.3	32.2 ± 2.7	1258 ± 148	4488 ± 25	0.08	0.15	21	125	154	221
Average	11 ± 0.25	34 ± 5.1	1180 ± 138	3663 ± 732	0.07 ± 0.01	0.15 ± 0.01	20 ± 2.3	87 ± 33	128 ± 24	138 ± 72
Significance					**		*		NS	

[a]Average values ± standard deviations of triplicates.
[b]Kinetic parameters were calculated as indicated in the text.
Results were compared as groups between SmF and SSF by single way ANOVA analysis where: **, means $p < 0.01$; *, means $p < 0.05$; NS, means $p > 0.05$.

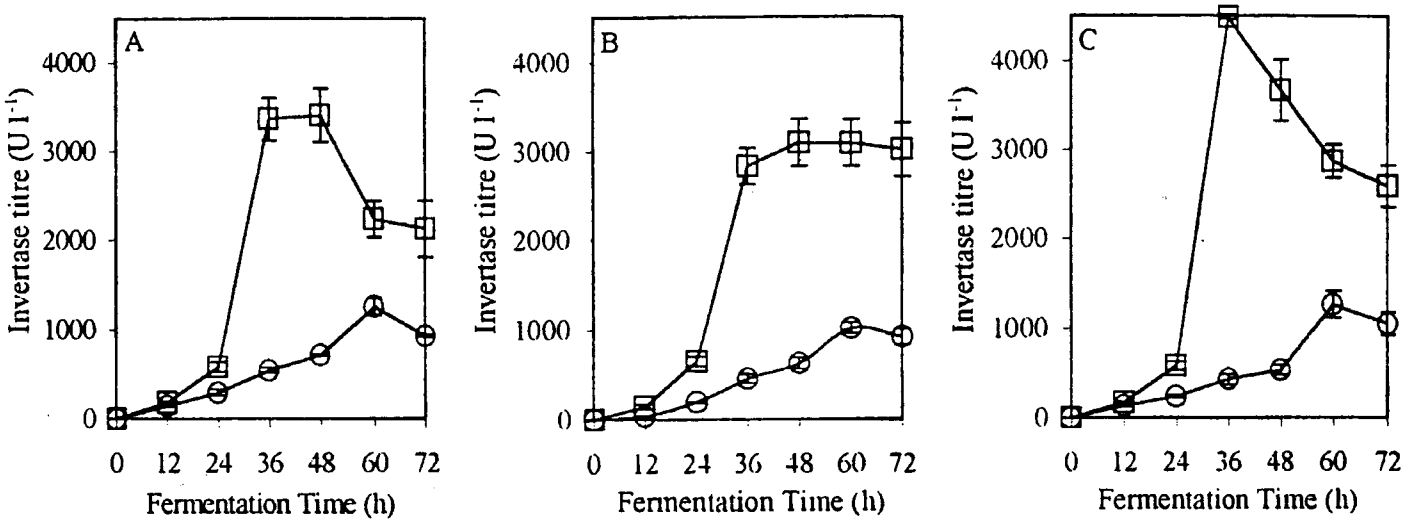

Fig. 2. Invertase titres produced by *Aspergillus niger* strains. Aa20 (A), N402 (B) and C28B25 (C) in SmF (O) and SSF (□).

showed a higher $Y_{E/X}$ value in SmF than in SSF and strains Aa20 and C28B25, the other way around. This is consistent with the proposal that certain strains may be more adapted to one particular culture system (Shankaranand *et al.* 1992, Antier *et al.* 1993).

Enzyme productivity (P = U l^{-1} h^{-1}) was estimated in terms of spent liquor at the peak value of enzyme titres in both culture systems. All strains had higher productivity in SSF (Table 1). Such differences were found statistically significant (Table 1). Higher enzyme productivity of SSF over SmF cultures has been previously reported (Solís-Pereira *et al.* 1993, Lekha & Lonsane 1994, Acuña-Argüelles *et al.* 1995). It was attributed to differences in the induction and repression of enzyme synthesis (Solís-Pereira *et al.* 1993) or to differences in the quality of the enzymes (Lekha & Lonsane 1994, Acuña-Argüelles *et al.* 1995). Our results support that the main difference is a better biomass production without glucose repression in SSF system. This suggests further studies of mass transfer phenomena in SSF and SmF for a deeper understanding of such differences.

Present data may be useful for industrial production of enzymes, because of the advantage of using concentrated fermentation broth in SSF system with a lower risk of contamination and a lower recovery cost related to higher enzyme titres as compared to SmF system.

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