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ORIGINAL RESEARCH PAPER

Fructooligosaccharide production by *Penicillium expansum*

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Abstract Fructooligosaccharides (FOS) production by *Penicillium expansum* was evaluated. In a first stage, the best conditions for *P. expansum* growth and sporulation were established with potato/dextrose/agar being the most suitable medium at between 22 and 25°C, giving good growth and good sporulation. The inocula from this medium were used for FOS production using shake-flask cultures, and yielded 0.58 g FOS/g sucrose (3.25 g FOS/l.h), demonstrating the potential of this strain for sucrose conversion to FOS.

Keywords β-fructofuranosidases · Fructooligosaccharides · *Penicillium expansum*

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Introduction

Fructooligosaccharides (FOS) are non-conventional sugars that cannot be hydrolyzed by gastrointestinal enzymes. They have a low caloric value and can promote beneficial effects to the host through the selective stimulation of indigenous bacteria like bifidobacteria and lactobacilli (Mussatto and Mancilha 2007; Teitelbaum and Walker 2002). They are formed by $\beta(2 \rightarrow 1)$-linked fructose (F) units with a last one, $\alpha(2 \rightarrow 1)$, attached to a terminal glucose (G) moiety. These oligomers are mainly composed of 1-kestose (GF_2), 1-nystose (GF_3), and 1-β-fructofuranosyl nystose (GF_4), which are found in many fruits and vegetables, although in too low a concentration to exert any beneficial effect (Yun 1996). Commercially, FOS can be produced by from sucrose using microbial β-fructosyltransferases (FTases) or β-fructofuranosidases (FFases) with high transfructosylating activity (Chen and Liu 1996). Such enzymes can be obtained from plants, bacteria or fungi, with the latter giving the greatest yields (Yun 1996; Antosová et al. 2008). Several strains, especially those from the *Aspergillus* genus, are good FOS producers (Sangeetha et al. 2005). However, there is no report on FOS production by *Penicillium expansum*. The present work, therefore, has evaluated FOS production by this fungus. Initially, the best conditions for producing an inoculum of fungal spores were established and this was then followed by an examination of FOS production in shake-flask culture.

Materials and methods

Microorganism and culture conditions

Penicillium expansum MUM 02.14 was obtained from Micoteca of the Centre of Biological Engineering, University of Minho, Portugal. Mycelia growth and sporulation at 22 and 27°C, in potato/dextrose/agar (PDA) and Czapek-Dox agar (CZ) media, were evaluated, in Petri dish culture. Plates were inoculated with 40 µl spore suspension containing 7.5×10^7 spores/ml. Six plates (3 PDA and 3 CZ) were incubated at 22°C and six similar ones at 27°C.

Fermentation medium and conditions for FOS production

FOS production was carried out in 500 ml Erlenmeyer flasks containing 100 ml culture medium (% w/v): sucrose (20), yeast extract (2.75), $NaNO_3$ (0.2), K_2HPO_4 (0.5), $MgSO_4 \times 7H_2O$ (0.05), and KCl (0.05), sterilized at 112°C for 15 min. The flasks were inoculated with 1 ml spore suspension containing around 1.8×10^7 spores/ml, and incubated at 25°C with shaking at 160 rpm for 48 h. Experiments were carried out in duplicate; and samples were aseptically collected for determining FOS (1-kestose, 1-nystose, and 1-β-fructofuranosyl nystose) and residual sugars (sucrose, fructose and glucose) concentrations, along with extracellular β-fructofuranosidase activity. Biomass concentration was determined at the end.

Analytical methodology

Microorganism growth and sporulation

Penicillium expansum growth in Petri plates was assessed daily for 7 days. Sporulation was determined by counting the spores in a Neubauer chamber after suspending them in 5 ml 0.1% (w/v) Tween 80. Biomass in the fermentation medium (g/l) was determined after drying at 105°C.

Sugars and fructooligosaccharides determination

FOS and residual sugar concentration in the fermentation broth were determined by HPLC (Mussatto et al. 2009). FOS yield was calculated as the proportion of the sum of 1-kestose, 1-nystose and 1-β-fructofuranosyl nystose to the initial sucrose concentration ($Y_{P/St}$ in g/g), and to consumed sucrose concentration ($Y_{P/Sc}$ in g/g). FOS productivity (Q_P in g/l.h) was calculated as the total FOS production (g/l) by fermentation time (h).

Enzyme activity

Samples of the filtered fermentation broth were used as extracellular enzyme source. The β-fructofuranosidase (FFase) activity was determined by measuring the amount of glucose produced from sucrose (Yoshikawa et al. 2006).

Statistical analysis

The results were analyzed by the Tukey test using the software Statgraphics version 4.1. A P value less than 0.05 indicated significant differences among samples.

Results and discussion

Growth and sporulation conditions for *Penicillium expansum*

Penicillium expansum grew well when at 22 and 27°C in both PDA and CZ media; however, the sporulation occurred only on PDA medium (as shown in Supplementary Figs. 1 and 2). At the same temperature, there was no statistically significant differences between growth in the two media (Table 1). Nonetheless, the growth was greater at 22°C. On the other hand, sporulation was statistically different when varying the culture medium and temperature: CZ was less suitable than PDA for sporulation at both temperatures. Such results can be explained by the low N concentration in PDA medium (Supplementary Table 1).

In a final step, fungal growth and sporulation was also evaluated at 25°C which was similar to that at 22°C. Therefore, PDA is suitable for good growth and sporulation of *P. expansum* between 22 and 25°C.

Fructooligosaccharides production

Figure 1a shows the time course of sucrose consumption and FOS production by *P. expansum* grown at the previously established cultivation conditions.

Table 1 Multiple comparison analysis of mycelial growth rate and sporulation results of *Penicillium expansum* cultivated in Potato Dextrose Agar (PDA) and Czapek-Dox Agar (CZ), at 22 and 27°C

Culture media/temperature (°C)	Radial growth rate (mm/h)* Average**	Sporulation (10^7 spores/ml) Average**
CZ/27	0.157[a]	0[a]
PDA/27	0.148[a]	14[b]
CZ/22	0.242[b]	3.4[a]
PDA/22	0.249[b]	29[c]

* Each value was calculated as the overall average of colony diameter measurements (in 3 different directions) taken every day, during 7 days, for each of the three replicates

** Same letters denotes no statistically significant differences at 95% confidence level

There was an initial lag phase up to 12 h before FOS formation began. Between 12 and 24 h, sucrose conversion to FOS commenced and in the following 12 h, the process attained the maximum FOS production (117.7 g/l) with almost total depletion of sucrose. Maximum production of FOS corresponded to a final product containing 1-kestose (GF_2—80%), 1-nystose (GF_3—19%), and 1-β-fructofuranosyl nystose (GF_4—1%) (Fig. 1b). According to Cruz et al. (1998) FOS synthesis always occurs in the sequence $GF \rightarrow GF_2 \rightarrow GF_3 \rightarrow GF_4$, as a consequence of the increasing Km values for such products presented by the transfructosylase. Thus, high concentrations of the preceding oligosaccharide are necessary for the synthesis of the homolog with one more fructose unit. After 36 h, due to sucrose exhaustion from the media, the microorganism started consuming the produced FOS and, therefore, the total FOS concentration decreased.

FOS production by *P. expansum* occurred with good yield ($Y_{P/St} = 0.58$ g/g; $Y_{P/Sc} = 0.68$ g/g) and productivity ($Q_P = 3.25$ g/l.h) when compared to the results obtained for other producer strains like *Aspergillus japonicus* ($Y_{P/St} = 0.61$ g/g, $Y_{P/Sc} = 0.64$ g/g, $Q_P = 5.36$ g/l.h) (Mussatto et al. 2009), being similar to the maximum yield normally found for microorganisms (55–60%, w/w) (Yun 1996; Nishizawa et al. 2001). Nevertheless, the values reported here can probably be improved by establishing the best conditions for performing this fermentative process.

FFase activity, responsible for sucrose conversion to FOS, increased throughout the fermentation, reaching a maximum value of 41 U/ml at the end (Fig. 2). Such a value compares well with other activities for FOS producers such as *A. japonicus*

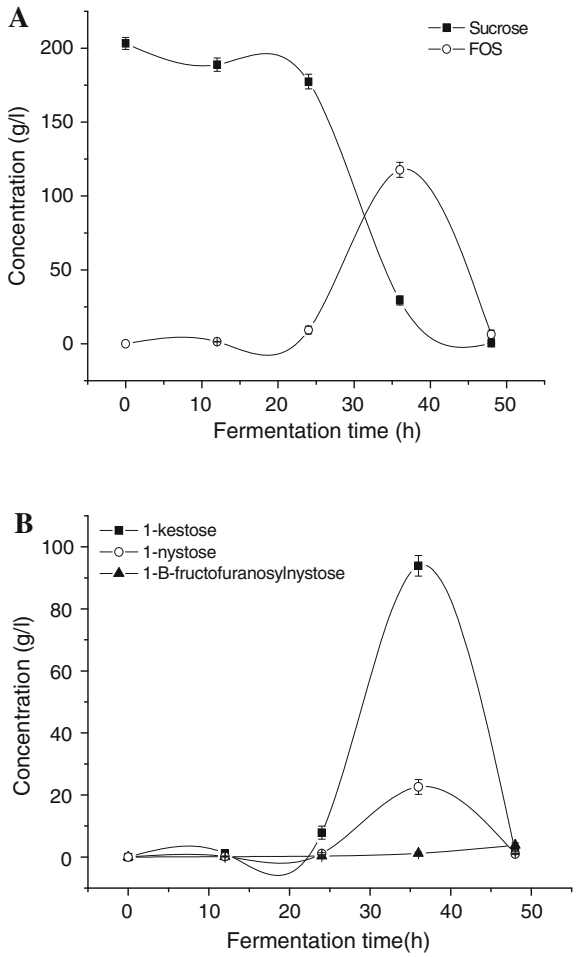

Fig. 1 Kinetic behavior of fructooligosaccharides production by *Penicillium expansum* using shake-flask cultures: (**a**) sucrose consumption and fructooligosaccharides production (as the sum of 1-kestose, 1-nystose, and 1-β-fructofuranosyl nystose); (**b**) 1-kestose, 1-nystose, and 1-β-fructofuranosyl nystose production

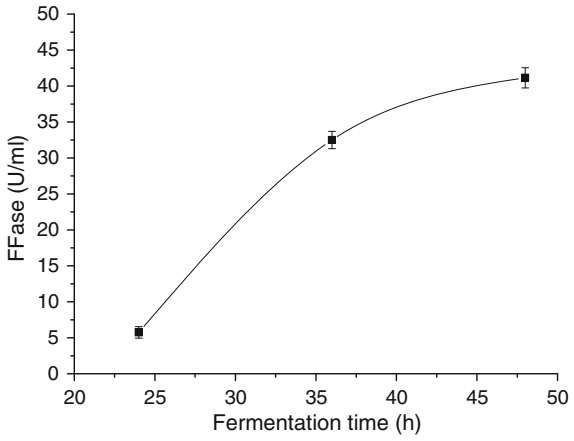

Fig. 2 Activity of β-fructofuranosidase during fructooligosaccharide production from sucrose by *Penicillium expansum* using shake-flask cultures

(55 U/ml) (Wang and Zhou 2006), *A. japonicus* (46 U/ml) (Mussatto et al. 2009), and a mutant *Saccharomyces cerevisiae* (46 U/ml) (ul-Haq et al. 2008).

In conclusion, *P. expansum* has potential for sucrose conversion to FOS. The present results can probably be improved by establishing the best conditions for the fermentation process. Nevertheless, since this is the first study on the FOS production by *P. expansum*, the obtained results open up possibilities to develop an efficient process for producing FOS with yields higher than that usually found in batch processes (55–60%, w/w) (Yun 1996), which is the main challenge for the FOS industries nowadays.

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