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Selective fructose production by utilization of glucose liberated during the growth of *Cladosporium cladosporioides* on inulin or sucrose

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The growth of *Cladosporium cladosporioides* in sucrose, inulin and extract of Jerusalem artichoke as sole carbon source have been investigated. The invertase (EC 3.2.1.26) and inulase (EC 3.2.1.7) activities present in the cells produced a glucose-fructose mixture from which glucose was preferentially removed. Results showed that 62-83% of the initial fructose can be recovered from the fermentation in sucrose or an extract of Jerusalem artichoke. The fructose recovered (5g/litre per batch fermentation cycle of 16 h) corresponded to nearly 80% of the total fructose theoretically available. This yield was almost constant in 11 batch cycles using the same cells and fresh sterilized extract of Jerusalem artichoke (0·8% total carbohydrates, w/v) to replace the spent medium and start a new batch cycle. The same high fructose recovery (70%) was achieved with higher total carbohydrate concentration (5%, w/v) in the extract of Jerusalem artichoke, in five batch cycles. However, the time for hydrolysis of all fructans and glucose consumption was much longer (48 h/batch cycle of fermentation at 28°C).

INTRODUCTION

Fructose syrups (95% fructose) are prepared from glucose-fructose mixtures, via chromatographic enrichment procedures or enforcement of displacement of a glucose isomerase product equilibrium. The acid or enzymatic hydrolysis of plant fructose polymers such as inulin offers another possibility for the production of such syrups. The selective utilization of glucose in the glucose-fructose mixture has been reported as an alternative way for fructose enrichment from invert sugar or high-fructose corn syrups (HFCS). *Mucor* sp. M105, *Fusarium* sp. F5 (Ueng *et al.*, 1982), *Zymomonas mobilis* Fru-mutants (Bringer-Meyer *et al.*, 1985; Suntinanalert *et al.*, 1986), and *Saccharomyces cerevisiae* ATCC 36859 Fru-mutant (Duvnjak & Koren, 1987) displayed selective utilization of glucose from a glucose-fructose mixture with alcohol production and fructose accumulation. *Pullularia pullulans* was the only microorganism reported that utilizes glucose selectively with fructose accumulation directly from fructan containing extracts (Fan, 1988). The authors now report an alternative for the production of fructose syrup by the selective utilization of glucose by *Cladosporium cladosporioides* grown in sucrose or an extract of Jerusalem artichoke. Also shown are the results of repeated batch fermentation for the production of fructose from an extract of Jerusalem artichoke, using the same cells. This strain of *Cladosporium cladosporioides* also displayed an intracellular invertase (β-D-fructofuranosidase, (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) and inulase (2,1-β-D-fructan fructanohydrolase, EC 3.2.1.7) activities. The properties of thermostable non-specific fructofuranosidase produced by *Cladosporium cladosporioides* cells for the hydrolysis of a Jerusalem *Artichoke* extract have also been outlined (Ferreira *et al.*, 1991).

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MATERIALS AND METHODS

Cladosporium cladosporioides URM (DEMUFPE) 2830 was isolated from *Dioscorea* sp. (Yam) (Lacerda Filho, 1987) and has been deposited in the Collection of Microorganisms of The Mycology Department of the Federal University of Pernambuco (DEMUFPE). The culture was maintained on potato dextrose agar (PDA) slants. The following media at pH 5·5–6·0 were used for the investigation of the utilization of several carbon sources: sucrose (5·5%, w/v) + yeast extract (1%, w/v); inulin (5%, w/v) + yeast extract (1%, w/v); extract of Jerusalem artichoke (12%, w/v of total carbohydrates). This extract was prepared by soaking for 15–20 min the powder of Jerusalem artichoke tubers (kindly supplied by Dr J. O. B. Carioca, University Federal of Ceara, Brazil) in hot (80°C) distilled water (16% g of powder: 100 ml of distilled water) followed by homogenization in a blender, filtration with squeezing in a gauze cloth and centrifugation at 8000 *g* for 15 min. Aliquots were withdrawn for total carbohydrates and free reducing sugar assays.

All media were respectively distributed (100 ml) in 250 ml Erlenmeyer flasks and sterilized at 100°C for 15 min. Growth was started with 10% (v/v) inoculum and was carried out in a shaker at room temperature (28–30°C). At appropriate time intervals of growth, samples were withdrawn and used for assay of total carbohydrates, reducing sugars, glucose, fructose and also for thin-layer chromatography.

Total carbohydrates were determined after acid hydrolysis, and total reducing sugars (RT) by the DNS method which involves the reaction of reducing sugars with dinitrosalicylic acid (Bernfeld, 1955). Glucose was assayed with the glucose oxidase–peroxidase kit (Merck) and fructose was determined by the difference between free reducing sugars and glucose in the sample broth. Residual sucrose or fructans were determined by the difference between total carbohydrates (assayed after acid hydrolysis) and free reducing sugars. Thin-layer chromatography was carried out on silica gel H (Merck) impregnated with 0·4M NaH_2PO_4 (Ghebregzabher *et al.*, 1976). The solvent system, acetone: butan-1-ol: deionized distilled water (80 : 10 : 10), allowed the separation of sucrose and monosaccharides. The revelator (DPA) prepared with diphenylamine: aniline and H_3PO_4 gave distinct coloration with glucose (grey-blue) and fructose (reddish). The biomass was determined by dry weight, at the end of the growth period.

In view of the time consumed for the total enzymatic hydrolysis of fructans in a Jerusalem artichoke extract with high total carbohydrates concentration, the repeated batch fermentation with fructose accumulation using extracts with 0·8% and 5% total carbohydrates (w/v) was investigated. The same cells, obtained after four times culturing, were used in each batch cycle, which was started by the replacement of the spent medium with fresh sterilized extract of Jerusalem artichoke (100 ml). The end of each cycle was achieved when all the fructans were hydrolysed and the glucose released had been consumed.

RESULTS AND DISCUSSION

Cladosporium cladosporioides grown in sucrose (Fig. 1) and Jerusalem artichoke extract (Fig. 2) displayed release of reducing sugars and selective utilization of glucose with fructose accumulation. In the sucrose medium complete hydrolysis occurred within 7 days of growth but the fructose accumulated was still mixed with glucose because of the chemical composition of the carbon source used (Fig. 1). However, with the extract of Jerusalem artichoke containing 12% (w/v) total carbohydrates, and a fructose: glucose ratio of 2:3, fructose syrup practically free from glucose was obtained after six days' growth. Under the conditions of growth used, the complete hydrolysis of the fructans was achieved in eight days (Fig. 2). The biomass yields were 1·7 and 2·57 g (dry weight/100 ml respectively). The accumulation of fructose in the fermentation broth from sucrose or the Jerusalem artichoke extract was in the range of 62–83% initial fructose (Table 1). Thin-layer chromatography of samples withdrawn during growth in sucrose or an extract of Jerusalem artichoke also showed accumulation of fructose and selective utilization of glucose by *C. cladosporioides*.

The growth in inulin (fructose: glucose ratio of 2:1) also displayed hydrolytic activity, but the fructose released was consumed at approximately the same rate

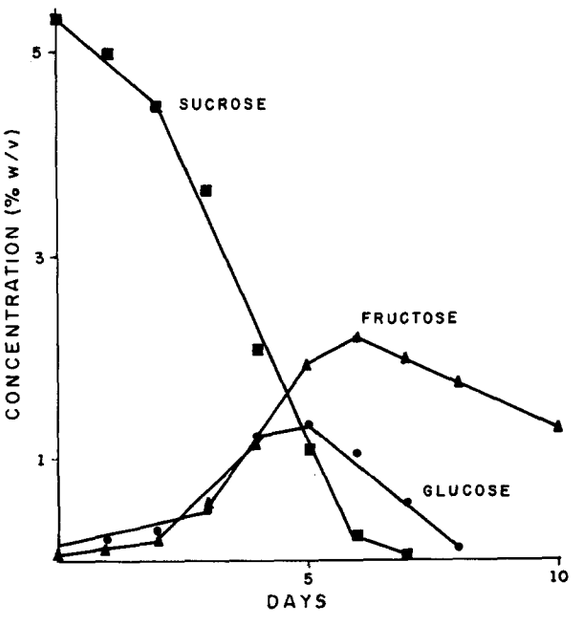

Fig. 1. Hydrolysis of sucrose and fructose accumulation as a function of time growth of *Cladosporium cladosporioides*.

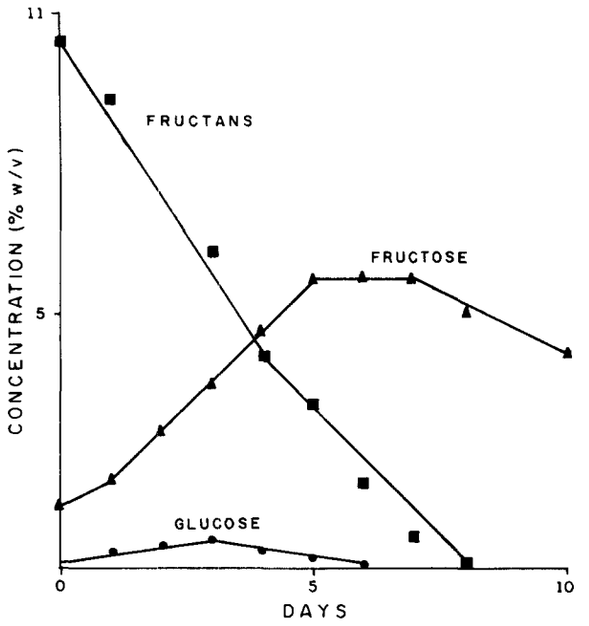

Fig. 2. Hydrolysis of fructans in the extract of Jerusalem artichoke and accumulation of fructose practically free of glucose as a function of time growth of *Cladosporium cladosporioides.*

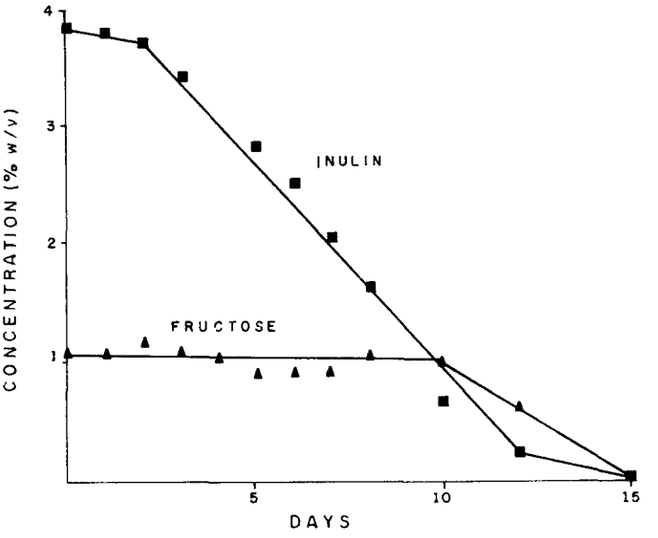

Fig. 3. Hydrolysis of inulin and fructose accumulation as a function of time growth of *Cladosporium cladosporioides.*

since the initial level of fructose was maintained over 10 days' growth. When the complete hydrolysis of inulin was achieved, nearly all the fructose had been consumed (Fig. 3). The biomass yield (1·8 g dry weight/100 ml) was approximately the same as that found with sucrose. Free glucose was not detected during growth in this inulin. Based on the total carbohydrate content, it was found that this inulin possessed a lower glucose content (4·5%) than the extract of Jerusalem artichoke (30%). Because of these results the authors believe that the presence of glucose probably decreases the activity of the fructose transport system by repression or catabolic inhibition. This has been suggested (Ueng *et al.*, 1982) for interpretation of the fructose accumulation during growth of Mucor or Fusarium in glucose-fructose mixtures.

The comparison of the authors' results with those of microorganisms cited in the literature as having preferential consumption of glucose with fructose accumulation shows that only *Pullularia pullulans* (Fan, 1988) and this strain of *C. cladosporioides,* were able to hydrolyse the fructans preferentially using the glucose released. However, *P. pullulans* released inulinase into the culture medium while *C. cladosporioides* retained it in the cells which were stable to reuse in several batch fermentation cycles. In 11 cycles of 16 h each, each one started with a fresh sterilized extract of Jerusalem artichoke (0·8% total carbohydrates, w/v) the fructose accumulated (5 g/litres) was constant (Fig. 4). This amount corresponds to 80% of the fructose theoretically available and was practically free from glucose, since in the residual total carbohydrates more than 90% was fructose. The cell stability and fructose yield offers an alternative inexpensive route for fructose production since it compares well with cells of *Kluyveromyces* marxianus SM 16-10 which in seven batch fermentation cycles of 7 h each, gave a product yield (ethanol) of nearly 94% of the theoretical (Bajpai & Margaritis, 1986). The *C. cladosporioides* cells cultured in the extract of Jerusalem artichoke, sucrose or extract of yam, produced protein with both invertase and inulinase activities which are thermostable.

Table 1. Fructose accumulation during the growth of *Cladosporium cladosporioides* in sucrose and inulin extract of Jerusalem artichoke

	Total carbohydrates (%, w/v)	Fructose available (%, w/v)	Complete hydrolysis (days)	Fructose accumulated (%, w/v);	yield (%)	Glucose residual (%, w/v);	Biomass g cell dry wt/100 ml
Sucrose	5·5	2·4	7	2·03	83	0·608	1·7
Jerusalem artichoke extract	3·2	2·24	3	1·68	75	0·002	1·65
	11·8	8·18	8	5·07	62	—	2·57
Inulin	4·9	4·68	15	0·02	—	—	1·8

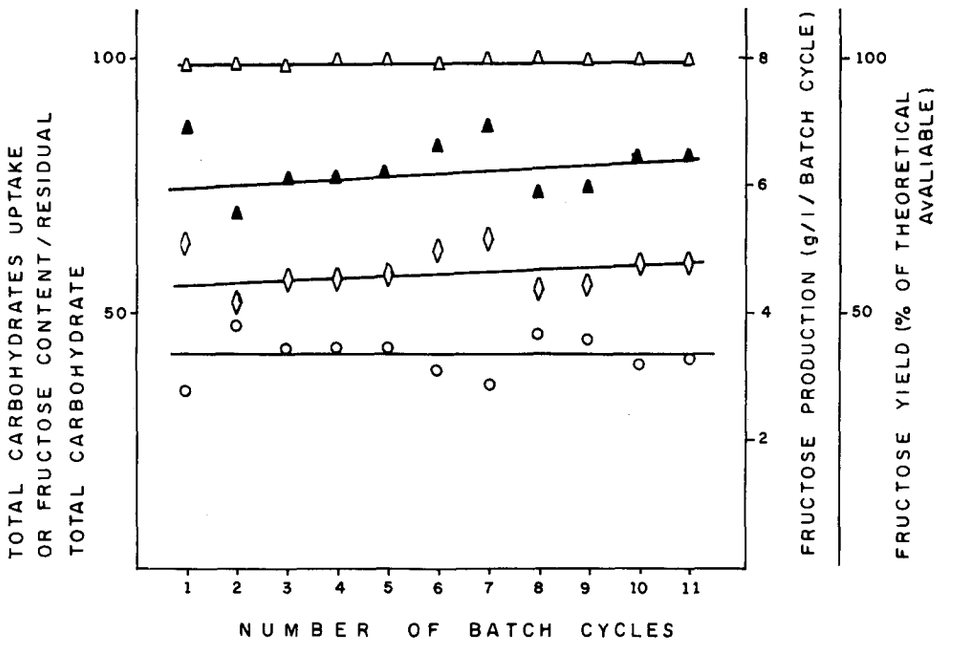

Fig. 4. Repeated use of *Cladosporium cladosporioides* cells in batch fermentation cycles for the production of fructose from extract of Jerusalem artichoke. O-O, total carbohydrate uptake; △-△, fructose content/residual total carbohydrate; ▲-▲, fructose yield (% of theoretical available); ◇-◇, fructose production (g/litre per batch cycle of 16 h).

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