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Production of 6-kestose by the filamentous fungus *Gliocladium virens* as affected by sucrose concentration

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ABSTRACT

The filamentous fungus *Gliocladium virens* is able to produce fructooligosaccharides (FOS), fructose-containing sugars, used as functional ingredients to improve nutritional and technological properties of foods. In this work we evaluated FOS production by *G. virens* when grown in a wide range of sucrose concentrations (10–400 g l^{-1}). High sucrose concentrations increased both biomass and FOS production, including 6-kestose, a trisaccharide comprising β (2 → 6) linked fructosyl units, with enhanced stability and prebiotic activity when compared to the typical FOS β (2 → 1) linked. The highest 6-kestose yield (3 g l^{-1}) was achieved in media containing 150 g l^{-1} sucrose after 4–5 days of culture, production being 90% greater than in media containing 10, 30, or 50 g l^{-1} sucrose. After 5 days, FOS production declined markedly, following complete sucrose depletion in the medium. Although most of the β-fructofuranosidases preferentially catalyze sucrose hydrolysis, FOS production in *G. virens* grown in high sucrose concentration, might be attributed to a reverse hydrolysis by these enzymes. In conclusion, high sucrose concentrations increase growth of *G. virens* whilst 6-kestose accumulation in the medium seems to be controlled both by specific properties of β-fructofuranosidases and on the sucrose concentration.

1. Introduction

Quality of life, including attention to the food we consume, is of increasing worldwide interest. In response to the massive demand for healthy and low calorie ingredients, oligosaccharides, especially fructooligosaccharides (FOS), are now widely used to replace sugars in many food products and have received GRAS (generally recognized as safe) status from the Food and Drug Administration (FDA–USA) (Pure Bulk 2010).

FOS are oligomers of fructose mainly represented by 1-kestose (GF_2), nystose (GF_3) and ^{1}F-fructofuranosylnystose (GF_4). These sugars are known as inulin-type FOS (^{1}F-FOS), in which the fructosyl units are attached to sucrose by β (2 → 1) linkages, distinguishing them from other oligomers (Yun 1996; Sangeetha et al. 2005; Ghazi et al. 2007). These oligosaccharides can be obtained by acid or enzymatic hydrolysis of inulin, a linear polymer composed of β (2 → 1) linked fructose residues attached to a terminal sucrose and widely distributed

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in nature as plant storage carbohydrates, mainly in Asteraceae species. The enzymes responsible for inulin hydrolysis are exoinulinases and endoinulinases (EC 3.2.1.7), which promote, respectively, the release of free fructose and FOS, thereby being useful for the production of fructose-enriched syrups and FOS (Carvalho et al. 2007; Singh and Singh 2010).

FOS are usually obtained industrially from sucrose through fructosyltransferases (EC 2.4.1.9) or β-fructofuranosidases (EC 3.2.1.26) with high transfructosylating activity (Sangeetha et al. 2005). These enzymes act on sucrose in a non-proportional reaction, where one molecule of sucrose serves as donor and the other as acceptor of fructose units forming linear or branched chains (Yun 1996).

Frequently the β-fructofuranosidases such as invertases hydrolyze sucrose to glucose and fructose, but depending on its origin may exhibit transfructosylating activity probably due to reverse hydrolysis. Production of FOS by these enzymes occurs under high sucrose concentrations. In contrast, fructosyltransferases have low hydrolytic and high transfructosylating activities even at low sucrose concentrations (Antošová and Polakovi 2001).

The interest in FOS has increased since they were recognized as functional components in foods, acting as prebiotic factors benefiting human nutrition. FOS stimulate the growth of bifidobacteria, reduce plasma levels of cholesterol, phospholipids and triglycerides, relieve constipation and may inhibit the development of tumors and inflammatory bowel diseases (Patel and Goyal 2011). Additionally, FOS are non-cariogenic compounds and excellent calorie-free sweeteners since they are poorly hydrolyzed by digestive enzymes and comprise a safe food for diabetics (Yun 1996; Maiorano et al. 2008; Patel and Goyal 2011). FOS present interesting technological properties since they are easily incorporated into foods, are stable at high and at freezing temperatures, mimic the properties provided by conventional sugars, and may even improve them. In this regard they provide viscosity, humectancy, freezing point depression, thereby increasing the food shelf life (Crittenden and Playne 1996; Yun 1996; Niness 1999; Patel and Goyal 2011).

There is an increasing interest in novel molecules with prebiotic and physiological effects. Some fungi are able to synthesize levan-type FOS containing two fructose units linked by β (2 → 6) linkages (^{6}F-FOS: 6-kestose), or neolevan-type FOS containing a fructose unit also linked by this type of linkage to a glucose (^{6}G-FOS: neokestose, neonystose, or neofructofuranosylnystose). Such FOS exhibit increased prebiotic activity when compared to the usual inulin-type FOS (Marx et al. 2000; Kilian et al. 2002). Okada et al. (2010) reported four novel trisaccharides in beverages produced by fermentation of an extract of fruits and vegetables by yeasts (*Zygosaccharomyces* spp. and *Pichia* spp.) and lactic acid bacteria (*Leuconostoc* spp.). The O-β-D-fructopyranosyl-(2 → 6)-D-glucopyranose produced during the fermentation was not cariogenic and was selectively used by the beneficial intestinal bacteria, *Bifidobacterium adolescentis* and *B. longum*, but was not used by unfavorable bacteria.

Gliocladium virens, a saprophytic filamentous fungus (Ascomycota, Hypocreales) isolated from the rhizosphere of *Vernonia herbacea*, a native inulin-accumulating Asteraceae from the Brazilian savanna, has the ability to synthesize FOS in sucrose-based medium. Additionally, invertase activity was detected early during fungal development, indicating that *G. virens* is a promising fungus for the production of FOS and enzymes with activities on fructose-based carbohydrates (Pessoni et al. 2009). In this work we analyzed the influence of sucrose concentration on FOS production by *G. virens* aiming to increase the knowledge on the utilization of filamentous fungi in the production of such sugars.

2. Materials and methods

2.1. Biological material and cultivation

Gliocladium virens J.H. Mill., Giddens & A.A. Foster, originally isolated from the rhizosphere of *V. herbacea* (Cordeiro-Neto et al. 1997), was obtained from the URM culture collection at the Federal University of Pernambuco, Brazil (URM number 3333). To obtain the inoculum, the fungus was cultured in potato dextrose agar (PDA) for 7 d at 28 °C. Plugs (6 mm diameter) of agar-containing the mycelium were transferred to 250 ml Erlenmeyer flasks containing 100 ml Czapek medium constituted by the following components in g l^{-1}: $NaNO_3$ (3), KH_2PO_4 (1), KCl (0.5), $MgSO_4 \cdot 7H_2O$ (0.5), $FeSO_4 \cdot 7H_2O$ (0.01). Sucrose was employed as carbon source at 10, 30, 50, 100, 150, 300, and 400 g l^{-1}. The flasks were kept at 28 °C with constant shaking (140 rpm). After the cultivation period, the mycelial mass was separated by filtration, dried, and the dry matter determined gravimetrically. The culture filtrates were used for sugar, protein, and enzymatic analyses. All experiments were performed in triplicate.

2.2. Quantification of total sugars and extracellular proteins

The concentration of total sugars in the culture filtrates was determined by the phenol–sulfuric method (Dubois et al. 1956). An equimolar mixture of glucose and fructose was used as standard. The protein content was determined according to Bradford (1976), using bovine serum albumin as standard.

2.3. Fructooligosaccharide (FOS) analysis

Samples of culture filtrates, containing 5 mg of total sugars, were deionized by ion exchange chromatography in columns (10 × 1 cm) containing cationic (Dowex® 50Wx8) and anionic (Dowex® 1x8) resins from Sigma–Aldrich. The deionized samples, containing 2 mg ml^{-1} of total sugars, were filtered through nylon membranes (0.45 μm) and analyzed by High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC/PAD) in a Dionex ICS-3000 system with CarboPac PA-1 column (4 × 250 mm), using a gradient of 500 mM sodium acetate in 150 mM sodium hydroxide (Shiomi et al. 1991; Vieira et al. 1995). The identification of the peaks was based on retention times compared to those of standards while their quantification was based on the peak area compared to those of the standards. Additionally, confirmation of the peak identities was carried out by spiking the sample with authentic standards.

The culture filtrates were also analyzed by thin-layer chromatography (TLC). Aliquots containing 200 μg of total sugars were applied on silica gel 60 plates (Merck) and the TLC was carried out using butan-2-ol, propan-1-ol and water (3:12:4, v:v:v) as the mobile phase. Sugars containing fructose were detected using a solution of urea/phosphoric acid as described by Wise et al. (1955).

The standards used in the HPAEC/PAD and TLC analyses were fructose, glucose, sucrose, 1-kestose, and nystose, purchased from Sigma–Aldrich (USA), and 6-kestose and neokestose, generously provided by Dr. Norio Shiomi (Rakuno Gakuen University, Ebetsu, Hokkaido, Japan).

2.4. *Enzyme activity*

Hydrolyzing and transfructosylating activities were carried out using sucrose as substrate at final concentrations of 10 g l^{-1} and 200 g l^{-1}, respectively, in 100 mM sodium acetate buffer, pH 5. After incubation for 30, 60 min and 24 h at 40 °C the reaction was stopped by boiling for 10 min. The assay conditions were previously established and were based on Pessoni et al. (2009). The reaction mixtures were appropriately diluted and analyzed by HPAEC/PAD system as described above. For the standard assay conditions, one unit (U) of hydrolyzing activity was defined as the amount of enzyme that releases 1 μmol of glucose from sucrose per minute, whereas one unit (U) of transfructosylating activity was defined as the amount of enzyme that produces 1 μmol of FOS per minute. Both hydrolyzing and transfructosylating activities were expressed as U per mg of total protein.

3. Results and discussion

According to Maiorano et al. (2008), the most studied variables to achieve the best production of FOS are the type and concentration of the carbon source. Based on previous time course experiments, *G. virens* was grown for 3 and 6 d in media containing sucrose at concentrations ranging from 10 to 150 g l^{-1}. The fungal biomass increased linearly with increasing sucrose concentration and presented maximum values at 150 g l^{-1} after 6 d of cultivation (Fig. 1). At this concentration, the fungal growth was 63% higher than in cultures at 10 and 30 g l^{-1} sucrose. When compared to cultures at 50 and 100 g l^{-1} sucrose, the growth was 43 and 18% higher, respectively. As expected, the lowest sucrose concentration resulted in reduced development of *G. virens* as previously reported (Pessoni et al. 2009). Therefore, the fungus was able to counteract the extracellular osmotic pressure, even at high sucrose concentrations and to produce increased biomass under such conditions.

HPAEC/PAD analysis revealed that *G. virens* was able to produce at least two different oligosaccharides with retention times of 5.9 and 8.2 min, the latter clearly identified as 6-kestose, a levan-type FOS (Fig. 2). Pessoni et al. (2009) reported that this microorganism was able to produce 1-kestose, nystose and ^{1}F-fructofuranosylnystose, but not 6-kestose, in medium containing 30 g l^{-1} sucrose, even after 18 d of growth. Under our experimental conditions those sugars were not detected but a relatively high proportion of 6-kestose was

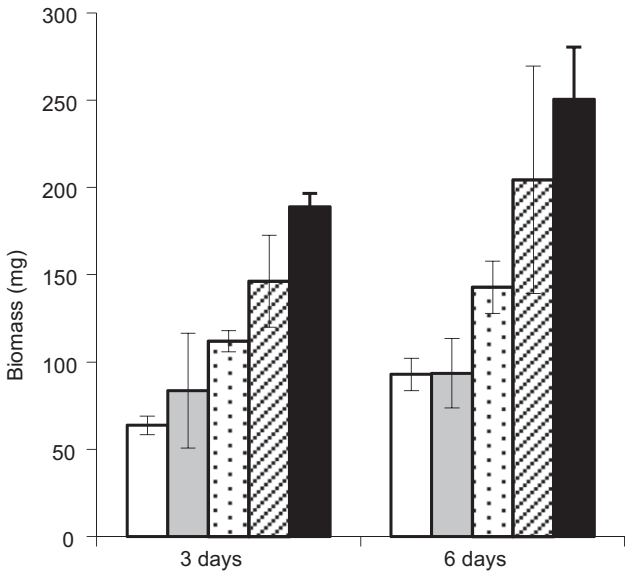

Fig. 1 – Biomass production by *Gliocladium virens* after 3 and 6 days of culture in medium containing 10 (□), 30 (▒), 50 (▒), 100 (▨) and 150 g l^{-1} sucrose (■). Values are means of three replicates (±SD).

detected. These differences in FOS composition could be related to the physiological performance of the fungal strain due to changes during storage, leading to an enhanced growth and higher ability to synthesize FOS at the beginning of the cultivation period. As reviewed by Ryan and Smith (2004), chromosomal abnormalities and polymorphisms in fungi after long-term preservation have been reported by several authors. Such differences in post-preservation stability were associated with changes in morphology and physiology that could explain the differences in *G. virens* behavior detected in the present work.

The highest production of 6-kestose at 10, 30 and 50 g l^{-1} sucrose occurred after 3 d of cultivation. However, after 6 d the levels of FOS declined at the same sucrose concentrations (Table 1), possibly due to the consumption of 6-kestose as an alternative carbon source since sucrose availability decreased in the medium. At the lowest sucrose concentrations, FOS production was low (approximately 3–300 mg l^{-1}), but the highest production (3 g l^{-1}) occurred after 6 d of cultivation in 150 g l^{-1} sucrose, *ca.* 10–100-fold higher than in media containing lower sucrose concentrations.

According to Maiorano et al. (2008), sucrose is preferably hydrolyzed and used for mycelial growth, but, at high concentrations, the sucrose excess is generally converted into FOS. Chen and Liu (1996) reported increased mycelial development of *Aspergillus japonicus* in the presence of 100 g l^{-1} sucrose. However, higher production of β-fructofuranosidases with transfructosylating activity was achieved only with 250 g l^{-1} sucrose, suggesting that at sucrose concentrations below 100 g l^{-1} most of the sugar is used during mycelial growth, while higher sucrose concentrations resulted in the production of enzymes involved in FOS synthesis.

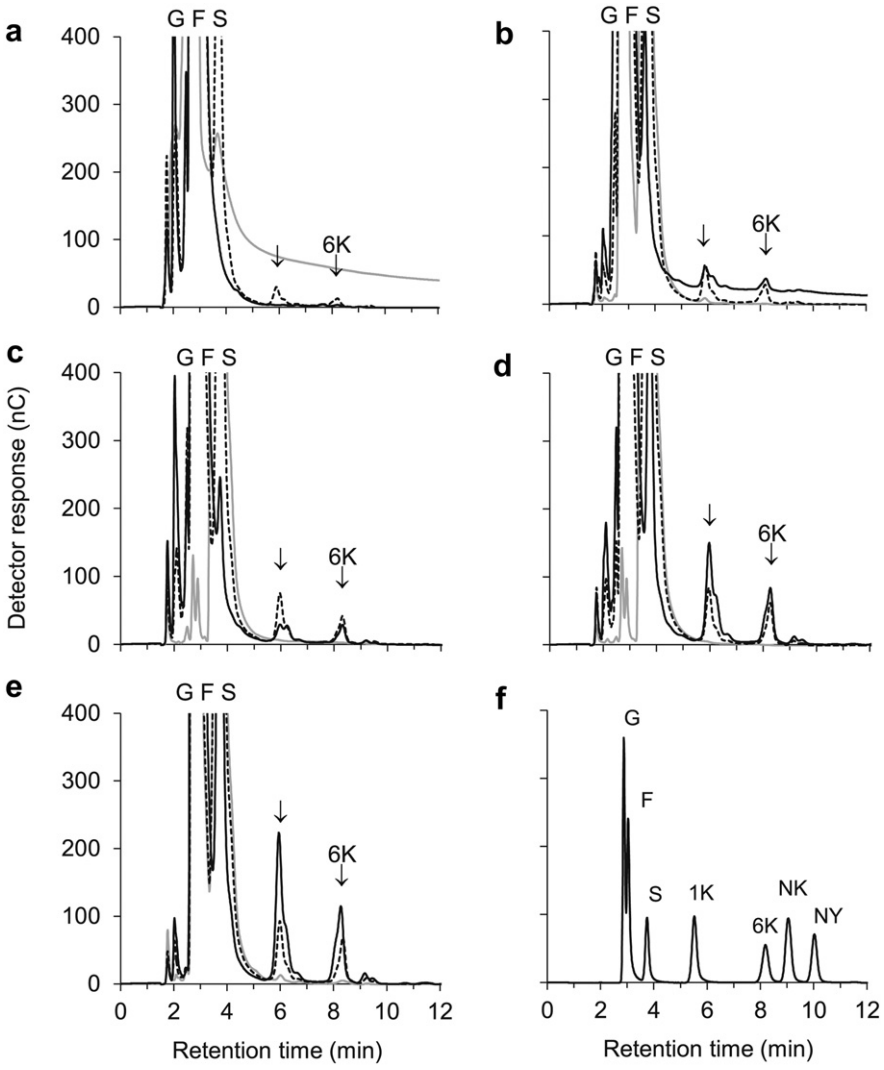

Fig. 2 – HPAEC/PAD analysis of the culture filtrates of *Gliocladium virens* obtained after 0 (—), 3 (- - -) and 6 (—) days of culture in medium containing 10 (a), 30 (b), 50 (c) 100 (d) and 150 g l^{-1} sucrose (e). The peaks were identified by comparison with the retention times of the standards: glucose (G), fructose (F), sucrose (S), 1-kestose (1K), 6-kestose (6K), neokestose (NK) and nystose (NY) (f). The fructooligosaccharides (FOS) produced are indicated by arrows.

Katapodis et al. (2004) investigated the ability of the thermophilic fungus *Sporotrichum thermophile* to produce FOS when grown for 7 d at different sucrose concentrations (50–450 g l^{-1}). The increase in sucrose levels stimulated progressively the production of 6-kestose, reaching the highest value (approximately 1.6 g l^{-1}) at 250 g l^{-1} sucrose and declining after that. At 150 g l^{-1}, the fungus produced only ca. 0.5 g l^{-1} of 6-kestose, one sixth than the amount produced by *G. virens* grown in the same sucrose concentration.

Table 1 – Production of 6-kestose (g l^{-1}) by *Gliocladium virens* after 3 and 6 days of culture in medium containing different concentrations of sucrose. Values are means of three replicates (±SD).

Sucrose (g l^{-1})	6-Kestose (g l^{-1})	
	3 days	6 days
10	0.017 ± 0.000	0.003 ± 0.000
30	0.170 ± 0.014	0.062 ± 0.006
50	0.317 ± 0.010	0.200 ± 0.005
100	1.056 ± 0.007	1.526 ± 0.039
150	1.610 ± 0.032	3.080 ± 0.180

The culture filtrates of *G. virens* grown in 150 g l^{-1} sucrose were also analyzed by TLC (Fig. 3). Two fructose-based oligosaccharides were detected, one identified as 6-kestose (R_f 0.269). The unidentified oligosaccharide (R_f 0.325) also contains fructose since the staining reagent employed is specific for free and combined fructose molecules (Wise et al. 1955). TLC analysis confirmed the HPAEC/PAD profile, as the highest FOS production occurred after 6 d. The sucrose conversion into monosaccharides was also observed at 3 and 6 d of growth (Fig. 3).

In order to determine the time course of FOS production, *G. virens* was cultured for 10 d in medium containing 150 g l^{-1} sucrose. Gradual increase of the mycelium biomass and of the

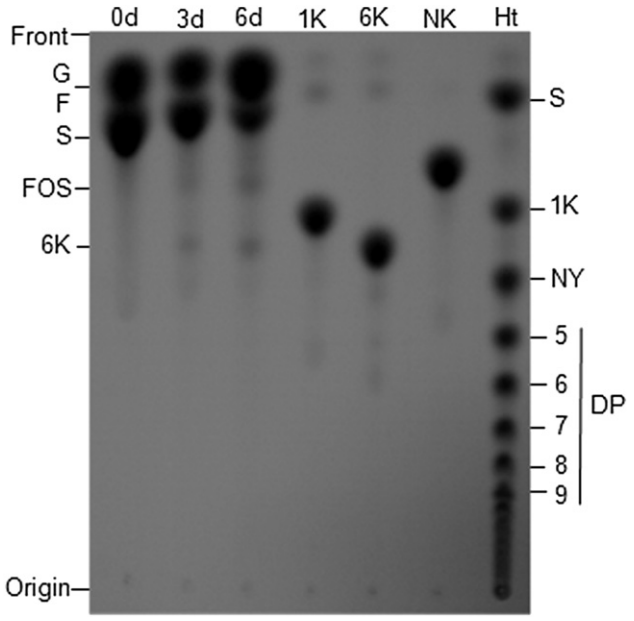

Fig. 3 – TLC analysis of the culture filtrates of *Gliocladium virens* obtained after 0, 3 and 6 days (d) of culture in medium containing 150 g l^{-1} sucrose. G = glucose, F = fructose, S = sucrose, 1K = 1-kestose, 6K = 6-kestose, NK = neokestose, NY = nystose, FOS = fructooligosaccharides. DP number represents the degree of polymerization of the inulin-type oligosaccharides from tubers of *Helianthus tuberosus* (Ht) used as standard.

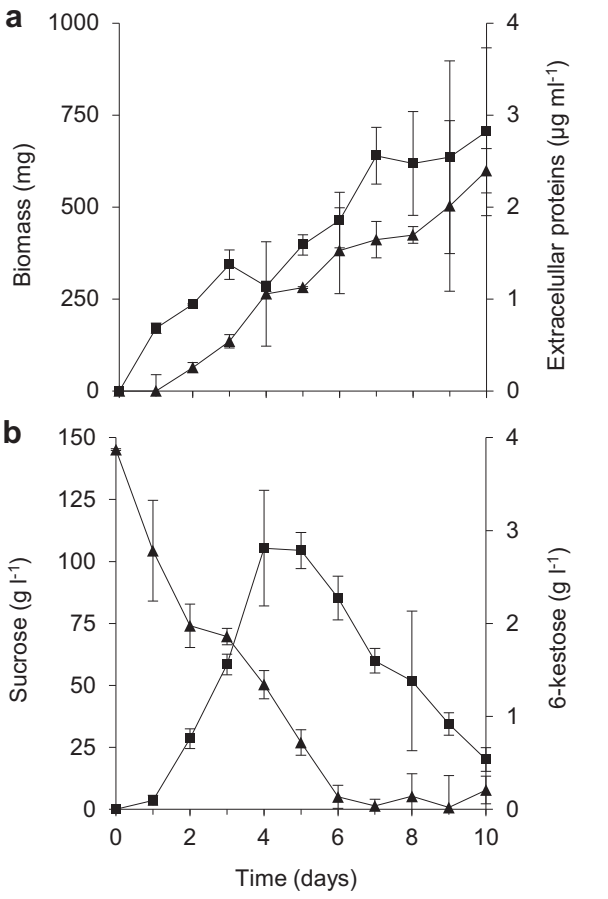

Fig. 4 – Time courses of biomass production (■) and extracellular protein content (▲) (a) and sucrose depletion (▲) and 6-kestose production (■) (b) by *Gliocladium virens* during 10 days of culture in medium containing 150 g l^{-1} sucrose. Values are means of three replicates (±SD).

extracellular proteins levels in the culture medium was observed. After 10 d, the fungus produced about 700 mg of biomass and secreted to the medium approximately 240 µg of proteins per flask, corresponding to 2.4 µg ml^{-1} (Fig. 4a).

The analyses of the culture filtrates by HPAEC/PAD showed a high rate of sucrose hydrolysis and the consequent release of glucose and fructose. The production of 6-kestose was detected early (in the first day of culture), however, the highest production (2.8 g l^{-1}) was achieved only after 4–5 d, decreasing after that (Fig. 4b). The reduction of FOS after 5 d coincided with the sucrose depletion in the medium. The amount of FOS decreased after the accumulation of glucose in the medium (data not shown), which in turn might have inhibited the transfructosylating activity and increased the hydrolytic activity. According to Yoshikawa et al. (2006), in *Aureobasidium pullulans*, glucose is a strong repressor of the FOS production since it was found to cause the repression of four of the five forms of β-fructofuranosidases. *A. pullulans* was able to produce 1-kestose after 1 d of growth in 50 g l^{-1} sucrose, accumulating glucose in the culture medium. FOS was then consumed together with glucose, producing free fructose later on.

To determine if sucrose concentrations higher than 150 g l^{-1} improves FOS production, *G. virens* was cultured for 5 d in 150, 300 and 400 g l^{-1} sucrose. The growth in sucrose concentrations higher than 150 g l^{-1} resulted in increased fungal biomass, however, under our experimental conditions, the content of extracellular protein remained unchanged (Fig. 5), possibly as a consequence of nitrogen limitation. FOS production was also unaffected by sucrose concentrations higher than 150 g l^{-1} (data not shown). Previous work showed that changes in the sources and concentrations of nitrogen increased extracellular proteins without affecting FOS production by *G. virens* (Pessoni et al. 2009). In fact, limitation of FOS production by transfructosylating activities when exceeding 400 g l^{-1} sucrose has already been reported for *Rhodotorula* sp. (Alvarado-Huallanco and Filho 2011) and *Xanthophyllomyces dendrorhous* (Linde et al. 2012). The decline of FOS production at very high levels of sucrose might be explained by the decline of enzymatic reactions rates due to non-ideal thermodynamic behavior, inhibition by sucrose, mass transfer limitations or low water activity at high sugar concentration (Antošová et al. 2008; Vega-Paulino and Zúniga-Hansen 2012).

As the highest levels of FOS in the medium were achieved between 4 and 6 d of growth (Fig. 4b), this period was selected for the investigation of the hydrolytic and transfructosylating activities by incubation of the culture filtrates with sucrose at 10 and 200 g l^{-1}. Under low sucrose concentration, the highest hydrolytic activity was observed on day 5 (94.5 U mg of $protein^{-1}$), after 30 min of incubation, decreasing approx. 50%

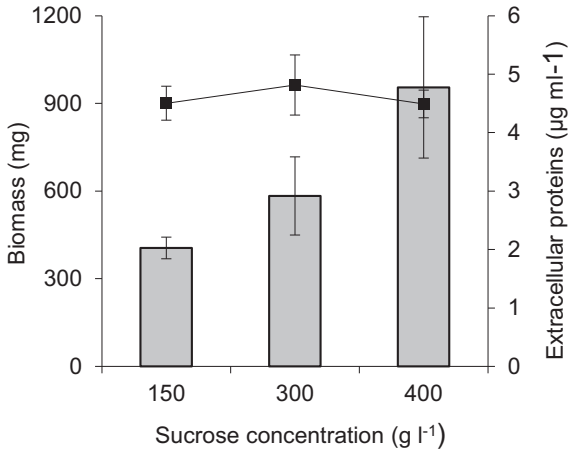

Fig. 5 – Biomass production (bars) and extracellular proteins (line) by *Gliocladium virens* after 5 days of growth in medium containing 150, 300 and 400 g l^{-1} sucrose. Values are means of three replicates (±SD).

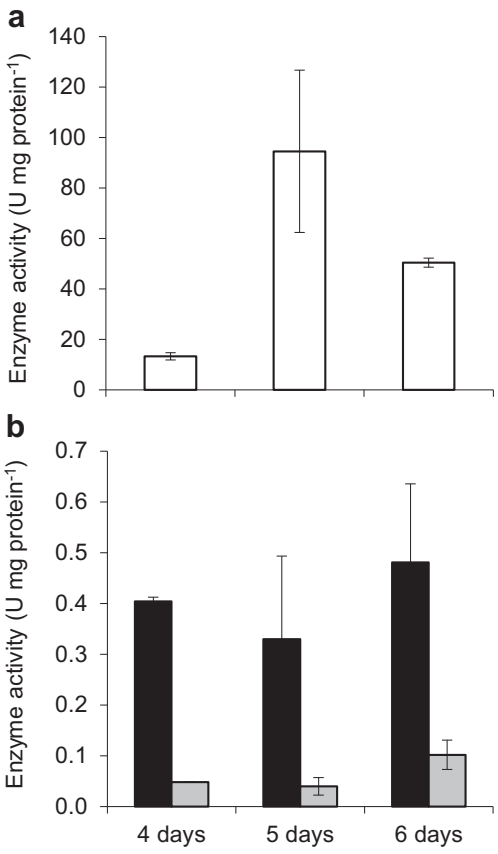

Fig. 6 – Enzyme activity of *Gliocladium virens* culture filtrates obtained after 4, 5 and 6 days of growth in medium containing 150 g l^{-1} sucrose. Hydrolyzing activity (10 g l^{-1} sucrose for 30 min at 40 °C) (a) and transfructosylating activity (200 g l^{-1} sucrose for 24 h at 40 °C) (b). Values are means of three replicates (±SD). Glucose (□), 6-kestose (■) and neokestose (▒).

on day 6 (Fig. 6a). Transfructosylating activity was not detected at 10 g l^{-1} sucrose, however the synthesis of 6-kestose was observed at 200 g l^{-1} sucrose after 24 h of incubation (Fig. 6b), but there were no significant differences in the enzymatic activity during 3 d of culture (average of 0.405 U mg $protein^{-1}$). In addition, neokestose, a neolevan-type FOS, was also produced being about one sixth the amount of 6-kestose (Fig. 6b). Neokestose production was not previously detected in the culture filtrates of *G. virens* growing in medium containing sucrose as carbon source.

The production of FOS by *G. virens* is probably catalyzed by β-fructofuranosidases such as invertases that primarily hydrolyze sucrose to glucose and fructose, but at high sucrose concentrations these enzymes may exhibit reverse hydrolysis, acting on sucrose by cleaving the β (2 → 1) linkage, transferring the fructosyl group to sucrose and releasing glucose. A typical fructosyltransferase can transfer fructosyl units to sucrose, even at low sucrose concentrations (Antošová and Polakovi 2001). However, this was not observed in the present study.

Kurakake et al. (2008) reported the presence of two β-fructofuranosidases (F1 and F2) in a strain of *Aspergillus oryzae*, F1 producing 1-kestose, nystose and ^{1}F-fructofuranosylnystose by transfructosylating activity on sucrose, and F2 mainly hydrolyzing sucrose. At sucrose concentrations less than 20 g l^{-1}, the activity of F2 was greater than that of F1, while the activity of F1 increased significantly at higher sucrose levels. In our work we demonstrated that sucrose concentration also affects FOS production, possibly due to the up-regulation of β-fructofuranosidases with transfructosylating activity.

Many fungal species are known to produce enzymes involved in the biosynthesis of FOS but currently β-fructofuranosidases and fructosyltransferases from *Aspergillus*, *Aureobasidium* and *Penicillium* are the main industrial source of mixtures of inulin-type FOS, such as 1-kestose, nystose or ^{1}F-fructofuranosylnystose (Sangeetha et al. 2005; Ghazi et al. 2007). The enzymatic synthesis of levan and neolevan-type FOS such as 6-kestose and neokestose is uncommon in filamentous fungi but relatively well documented in yeasts such as *Saccharomyces cerevisiae*, *Schwanniomyces occidentalis* and *X. dendrorhous*. The β-fructofuranosidase from *X. dendrorhous* (Linde et al. 2012) produces mainly neokestose and those from *S. cerevisiae* (Farine et al. 2001) and *S. occidentalis* (Álvaro-Benito et al. 2010) produce mainly 6-kestose, with neokestose or 1-kestose, respectively, being by-products of the reaction. *S. occidentalis* produces β-fructofuranosidases with transfructosylating activity, which efficiently produces 6-kestose and 1-kestose in the ratio 3:1, generating the highest 6-kestose yield yet reported. After 24 h, the enzyme converted approximately 64% of the available sucrose producing high levels of 6-kestose (76 g l^{-1}) (Álvaro-Benito et al. 2010).

As far as we aware, besides the present work, the production of levan and neolevan-type FOS by filamentous fungi has been reported only in *Thermoascus aurantiacus* (Katapodis and Christakopoulos 2004) and *Sporotrichum thermophile* (Katapodis et al. 2004). The microbial production of 6-kestose and neokestose has not been widely reported probably because they are either not produced or represent only a minor biosynthetic product (Linde et al. 2009).

Mutagenesis studies with *S. cerevisiae* invertase gene (*SUC2*) improved the transfructosylating activity enhancing the production of 6-kestose 6–10-fold by modifying specific amino acids in two conserved motifs. In addition, it was demonstrated that the amino acid Trp 291 in cooperation with Asn 228 constitutes a key component to specially orient sucrose in the acceptor-substrate-binding site promoting the synthesis of 6-kestose instead of FOS with β (2 → 1) linkages (Lafraya et al. 2011). Therefore, the production of 6-kestose and neolevan-type FOS by *G. virens*, instead of inulin-type FOS, as reported in the present work, suggests the specific and precise changes in the enzyme molecule that could be related to its preference for synthesizing 6-kestose based oligosaccharides, as shown for *S. cerevisiae* by Lafraya et al. (2011). The metabolic alterations observed here when compared to our previous work (Pessoni et al. 2009) might be explained by possible changes occurred during fungus storage as already mentioned.

The physiological significance of FOS production from sucrose by microorganisms is uncertain. It has been suggested that these sugars might confer tolerance to environmental stresses such as water and nutrient deprivation (Velázquez-Hernández et al. 2009). It is also possible that FOS production by microorganisms isolated from sucrose-rich environments might protect them against osmotic stress. The osmotic concentration of the medium increases when sucrose is hydrolyzed to glucose and fructose due to the increased number of molecules released. In contrast, the osmolarity of the medium does not change when sucrose is transformed into FOS. In addition, microorganisms able to produce FOS may have advantages against competitor microorganisms able to use only sucrose since FOS is much less susceptible to microbial hydrolysis (Yoshikawa et al. 2006).

The results reported here highlight the ability of *G. virens* to convert sucrose into 6-kestose, depending on the relative substrate concentration of the medium. Investigation of factors other than the carbon source influencing 6-kestose production by *G. virens* and the molecular characterization of its β-fructofuranosidases are important for future applications of this microorganism.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.myc.2012.09.012.

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