

**Production of fructooligosaccharides by mycelium-bound
transfructosylation activity present in *Cladosporium cladosporioides*
and *Penicillium sizovae***

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1 **ABSTRACT**

2 Different filamentous fungi isolated from molasses and jams (kiwi and fig) were
3 screened for fructooligosaccharides (FOS) producing activity. Two strains, identified
4 as *Penicillium sizovae* (CK1) and *Cladosporium cladosporioides* (CF₂15), were selected on
5 the basis of the FOS yield and kestose/nystose ratio. In both strains the activity was
6 mostly mycelium-bound. Starting from 600 g/L of sucrose, maximum FOS yield was
7 184 and 339 g/L for *P. sizovae* and *C. cladosporioides*, respectively. Interestingly, the
8 highest FOS concentration with *C. cladosporioides* was reached at 93% sucrose
9 conversion, which indicated a notable transglycosylation to hydrolysis ratio. The
10 main FOS in the reaction mixtures were identified by HPAEC-PAD chromatography.
11 *C. cladosporioides* synthesized mainly 1-kestose (158 g/L), nystose (97 g/L), ¹F-
12 fructosylnystose (19 g/L), 6-kestose (12 g/L), neokestose (10 g/L) and a disaccharide
13 (34 g/L) that after its purification and NMR analysis was identified as blastose [Fru-
14 β(2→6)-Glc]. *P. sizovae* was very selective for the formation of ¹F-FOS (in particular 1-
15 kestose) with minor contribution of neoFOS and negligible of levan-type FOS.

16

17 *Key words:* Fructooligosaccharides; transfructosylation; prebiotics; bioactive
18 oligosaccharides; blastose;

19

1 1. INTRODUCTION

2 Inulin-type fructooligosaccharides (¹F-FOS) are fructose oligomers with a
3 terminal glucose unit in which 2-4 fructofuranosyl moieties are linked by $\beta(2\rightarrow1)$
4 bonds [1;2]. ¹F-FOS are used as food ingredients due to their properties, such as
5 prebiotic action favouring the development of bifidobacteria and lactobacillus, low
6 caloric intake (2 kcal/g), low glycemic index, improved gut absorption of Ca^{2+} and
7 Mg^{2+} , lowering of blood lipid levels, prevention of urogenital infections and reduced
8 risk of colon cancer [3;4].

9 ¹F-FOS are commonly obtained by controlled hydrolysis of inulin or other
10 fructans (typically using inulinases, EC 3.2.1.7) [5] or by enzymatic
11 transfructosylation of sucrose catalyzed by β -fructofuranosidases (EC 3.2.1.6) or
12 fructosyltransferases (EC 2.4.1.9) [6]. Short-chain ¹F-FOS (1-kestose, nystose, ¹F-
13 fructosylnystose, and so on) are currently produced at multi-ton scale from
14 concentrated sucrose solutions using fungal transfructosylating enzymes from
15 *Aspergillus niger*, *Aspergillus oryzae* or *Aureobasidium pullulans* [7;8].

16 Other FOS containing $\beta(2\rightarrow6)$ linkages between two fructose units (⁶F-FOS,
17 also called levan-type FOS, such as 6-kestose or 6-nystose) [9-12] or between a
18 fructose and a glucose (⁶G-FOS, also called neoFOS, such as neokestose or
19 neonystose) have also been described and are commonly produced by enzymes from
20 yeasts [13-15]. Levan-type FOS and neoFOS are reported to exhibit improved
21 prebiotic properties and chemical stability compared to inulin-type FOS [16-18],

1 although more studies are required to elucidate the bioactivity of the different FOS
2 series.

3 Industrial scale production of FOS is performed by either soluble enzymes in
4 batch reactions [19] or by entrapped cells in alginate gel beads using continuous
5 fixed-bed reactors [20;21]. Several immobilized enzymes for FOS synthesis at
6 laboratory scale have also been reported [6;22-24]. The use of biomass (e.g. mycelia)
7 or immobilized biocatalysts minimizes the loss of activity during operation and
8 allows establishing a continuous process.

9 The identification of novel microbial strains with high transfructosylation
10 activity and/or producing a distinctive FOS pattern is currently being investigated
11 [25]. In this work, we have screened different microorganisms able to grow on
12 sucrose-rich substrates such as molasses or jams with the aim of identifying new
13 transfructosylating enzymes able to produce FOS with different composition
14 compared with already known biocatalysts.

15

1 **2. MATERIALS AND METHODS**

2 **2.1. Materials**

3 Sucrose, glucose, fructose and *p*-anisaldehyde were from Sigma-Aldrich.
4 Nystose and 1-kestose were from Fluka. 1^F-fructosylnystose was from Megazyme. 6-
5 Kestose, neokestose and neonystose were synthesized as previously described
6 [9;13;14]. Yeast extract was from Difco and barley malt flour from Diagermal. All
7 other reagents and solvents were of the highest available purity and used as
8 purchased.

9 **2.2. Isolation and screening of microorganisms with transfructosylating activity**

10 The fungal cultures employed in the study were isolated from molasses or from
11 commercial and home-made kiwi and fig jams. The contents of each sample were
12 uniformly mixed, and a sample (1.0 mL) was aseptically withdrawn, mixed with 9.0
13 ml of sterile water, and then diluted for isolation purposes. The inoculum was
14 transferred to Potato Dextrose Agar (PDA) plates containing chloramphenicol (0.05
15 g/L) to inhibit bacterial growth. Plates were repeatedly incubated at 28°C until
16 obtaining homogenous morphological colonies. Identification of the best performing
17 strains was carried out at CBS (Centraal Bureau voor Schimmelcultures, Baarn,
18 Holland) using standard molecular techniques. The microorganisms were routinely
19 maintained on MYA slants (Barley malt flour 100 g/L, yeast extract 5 g/L, agar 15
20 g/L, pH 5.5) at 4°C.

21 The screening for FOS production was performed by inoculating the strains
22 into 1 L flasks containing 100 mL of liquid MY medium (Barley malt flour 100 g/L,

1 yeast extract 5 g/L, pH 5.5) containing 200 g/L of sucrose. The growth was carried
2 out on a reciprocal shaker (150 rpm) at 28°C. Sugar composition was analyzed by
3 HPLC during the growth after filtration of the mycelium.

4 Studies were carried out with lyophilized mycelium obtained after growth on
5 MY medium with and without 200 g/L sucrose) for different times under the
6 conditions described above. Mycelium with the highest activity was found when
7 cultures were performed using MY medium without 200 g/L sucrose after 96 h on a
8 reciprocal shaker (150 rpm) at 28°C. After centrifugation, cells were washed with
9 sodium acetate buffer (20 mM, pH 6.0), lyophilized and used for biotransformations.

10 **2.3. Biotransformations with *Cladosporium cladosporioides* and *Penicillium*** 11 ***sizovae***

12 The activity of mycelium-bound and extracellular enzymes was
13 independently assayed [26]. Biotransformations aimed at FOS production were
14 carried out with growing, re-suspended and lyophilized mycelium and with
15 mycelium-free supernatant of *Cladosporium cladosporioides* and *Penicillium sizovae*. In
16 experiments with whole growing cultures, the substrate was added together with the
17 medium and biotransformation followed during the growth. Experiments with
18 mycelium-free supernatant were accomplished using the liquid fraction obtained
19 after centrifugation of the whole culture and ultrafiltration using a stirred
20 ultrafiltration cell (Model 8050 Amicon, Millipore, capacity 50 ml) with a 10.000 Da
21 cut-off membrane. The extracellular fraction containing 0.41 g/L of total protein
22 (Bradford assay) was used for biotransformation, started by incubating the mixture
23 at 50°C in an orbital shaker at 90 rpm after addition of 200 g/L of sucrose. Freshly

1 suspended (40 g_{dry weight}/L) and lyophilized mycelium (40 g/L) were added to a 200-
2 600 g/L sucrose solution in 20 mM sodium acetate (pH 6.0) in a total reaction
3 volume of 2 mL. The mixture was incubated at 50°C in an orbital shaker at 90 rpm.
4 At different times, aliquots (50 µL) were withdrawn, diluted with 200 µL of water,
5 incubated for 10 min at 90°C to inactivate the enzymes, and analysed by HPLC to
6 determine the total FOS yield and by HPAEC-PAD to identify the synthesized FOS.

7

8 **2.4. HPLC analysis**

9 The screening of transfructosylation activity and the measurement of the FOS
10 production were carried out analyzing the corresponding reaction mixtures using
11 hydrophilic interaction chromatography (HPLC-HILIC) with a Delta 600 quaternary
12 pump (Waters). The chromatographic column used was a 5-µm Luna-NH₂ 100A (4.6
13 × 250 mm) from Phenomenex and the HPLC detector was a refraction index 2410
14 from Waters. The mobile phase was acetonitrile/water 78/22 (v/v) at 1 mL/min.
15 The temperature of the column was set at 30°C.

16

17 **2.5. Analysis of FOS by HPAEC-PAD**

18 Analysis of FOS composition was carried out by high-performance anion-
19 exchange chromatography coupled with pulsed amperometric detection (HPAEC-
20 PAD) on a Dionex ICS3000 system consisting of an SP gradient pump, an
21 electrochemical detector with a gold working electrode and Ag/AgCl as reference
22 electrode, and an autosampler (model AS-HV). All eluents were degassed by

1 flushing with helium. A pellicular anion-exchange 4 × 250 mm Carbo-Pack PA-1
2 column (Dionex) connected to a 4 × 50 mm CarboPac PA-1 guard column was used
3 at 30°C. Eluent preparation was performed with Milli-Q water and NaOH. The
4 initial mobile phase (at 0.5 mL/min) was 100 mM NaOH. A gradient from 0 to 200
5 mM sodium acetate was performed in 50 min at 0.5 mL/min, and 200 mM sodium
6 acetate was maintained for 25 min. The chromatograms were analyzed using
7 Chromeleon software. The identification of the different carbohydrates was done on
8 the basis of standards commercially available or purified in our laboratory.

9 **2.6. Isolation of an unknown carbohydrate**

10 The biocatalytic reaction with the *C. cladosporioides* CF₂15 enzymatic preparation
11 was scaled up to 10 mL. At the point of maximum concentration of the unknown
12 oligosaccharide, the reaction was stopped by inactivation at 100°C (10 min) followed
13 by filtration. The mixture was purified by semi-preparative HPLC using a system
14 equipped with a Waters Delta 600 pump coupled to a 5 µm Kromasil-NH₂ column
15 (10 × 250 mm; Analisis Vinicos). A three-way flow splitter (model Accurate, Dionex)
16 and a refraction index detector (Waters, model 2410) equilibrated at 30°C were used.
17 Acetonitrile/water 70:30 (v/v), degassed with helium, was used as mobile phase at
18 4.7 mL/min for 40 min. The column temperature was kept constant at 30°C. After
19 collecting the different oligosaccharides, the mobile phase was eliminated by rotary
20 evaporation in a R-210 rotavapor (Buchi).

1 **2.7. Mass Spectrometry**

2 The unknown carbohydrate was analyzed by MALDI-TOF mass spectrometry
3 (Bruker, model Ultraflex III TOF-TOF) using 2,5-dihydroxybenzoic acid doped with
4 NaI as matrix, in positive reflector mode.

5 **2.8. Nuclear Magnetic Resonance (NMR)**

6 The structure of the unknown carbohydrate was elucidated using a
7 combination of ^1H , ^{13}C and 2D-NMR (COSY, TOCSY, NOESY, HSQC, HMBC)
8 techniques. The spectra of the sample (ca. 10 mM), dissolved in deuterated water,
9 was recorded on a Bruker AVANCE DRX500 spectrometer equipped with a tuneable
10 broadband $^1\text{H}/\text{X}$ probe with a gradient in the Z axis, at a temperature of 298 K.
11 Chemical shifts were expressed in ppm with respect to the 0 ppm point of DSS, used
12 as internal standard. COSY, NOESY, HSQC, HSQC-TOCSY, DEPT-HSQC and
13 HMBC sequences were provided by Bruker. COSY, TOCSY (80 ms mixing time), and
14 NOESY (500 ms mixing time) experiments were performed with 8, 32, and 64 scans,
15 respectively, with 256 increments in the indirect dimension and with 1024 points in
16 the acquisition dimension. The spectral widths were 5 ppm in both dimensions. The
17 HSQC and related experiments (16 scans) also used 256 increments in the indirect
18 dimension and 1024 points in the acquisition dimension. The HMBC (64 scans) used
19 384 increments in the indirect dimension and 1024 points in the acquisition
20 dimension. The spectral width for the heteronuclear correlations was 120 ppm in the
21 indirect dimension and 5 ppm in the acquisition one.

22

1 3. RESULTS AND DISCUSSION

2 3.1. Screening of transfructosylation activity

3 Filamentous fungi (45 strains), isolated from sucrose-rich environments (jams
4 and molasses), were firstly grown on a MY medium (see Experimental Section)
5 containing 200 g/L sucrose and FOS production was followed during the growth.
6 The carbohydrate composition of the supernatant was analyzed by HPLC-HILIC,
7 showing that 14 strains (Table 1) were able to produce FOS with different total
8 concentrations (referred to the total amount of sugars in the sample) and
9 compositions (indicated in the table by the kestose/nystose ratio, K/N). Two strains
10 (CF₂15 and CK1, identified as *Cladosporium cladosporioides* and *Penicillium sizovae*,
11 respectively) gave maximum FOS production in shorter times (48 h) and were
12 selected for their ability to synthesize FOS with different K/N ratios (0.8 and 6.0,
13 respectively), at the point of maximum FOS concentration. Notably, *Cladosporium*
14 *cladosporioides* gave the highest production of total FOS, whereas *Penicillium sizovae*
15 was very selective, furnishing kestose as the main product.

16 3.2. FOS production with lyophilized mycelium of *C. cladosporioides* and *P.* 17 *sizovae*

18 The extracellular and cell-bound transfructosylating activity of *P. sizovae* and *C.*
19 *cladosporioides* to produce FOS was evaluated using cultures grown on liquid MY
20 medium with and without 200 g/L sucrose. Cell-free supernatant (broth fraction)
21 and washed mycelium were independently assayed to locate the transfructosylating
22 activity. The supernatant gave conversions into total FOS lower than 5% for both

1 strains, showing that the activity was mostly mycelium-bound; no significant
2 differences were observed in the activity of mycelium grown in the medium with or
3 without sucrose. Therefore, mycelium of cultures grown in liquid MY medium
4 without sucrose was used for next experiments aimed at optimization and product
5 characterization. Mycelia did not lose any significant activity upon lyophilisation
6 (data not shown). It is well reported that lyophilized mycelia of fungi are easy-to-
7 handle biocatalysts often showing remarkable long-term stability [27,28].
8 Lyophilized mycelia of the two strains were used for FOS production using various
9 initial sucrose concentrations (200-600 g/L), and the highest yields of total FOS
10 produced were obtained with 600 g/L of sucrose (Table 2). The time course of total
11 FOS formation with *P. sizovae* and *C. cladosporioides* using 600 g/L sucrose was
12 followed by HPLC-HILIC and is represented in Figs. 1A and 1B, respectively.

13 *P. sizovae* produced 184 g/L of total FOS (31% w/w of total sugars, after 24 h),
14 which was obtained at 53% sucrose conversion, whereas *C. cladosporioides*
15 synthesized 339 g/L of FOS (56.5% w/w, after 72 h) at 93% sucrose conversion. The
16 fact that maximum FOS concentration with *C. cladosporioides* enzyme was obtained
17 when only 7% of initial sucrose remains in the mixture indicates that the
18 transglycosylation to hydrolysis ratio of this enzyme is notable [29]. The FOS yield
19 obtained with *C. cladosporioides* is close to the maximum values reported (around
20 60%) for the industrial processes with *Aspergillus* or *Aureobasidium* sp. enzymes [29-
21 31].

22

1 3.3. Characterization of synthesized FOS

2 HPAEC-PAD was employed for the characterization of the FOS synthesized in
3 the reactions with *P. sizovae* and *C. cladosporioides* using 600 g/L sucrose. According
4 to the chromatograms presented in Figure 2, we detected at least 13 different
5 carbohydrates in the reactions mediated by *P. sizovae* (Fig. 2A) and *C. cladosporioides*
6 (Fig. 2B) mycelia. Peaks 1, 2, and 3 corresponded to glucose, fructose and sucrose,
7 respectively. As illustrated in the chromatogram 2B, the main products present in the
8 reaction mixture with *C. cladosporioides* were peaks 4 (1-kestose) and 9 (nystose).
9 Peaks 7, 8 and 10 were identified as 6-kestose, neokestose and neonystose,
10 respectively, using standards previously purified in our laboratory as described
11 [9;13]. Peak 12 was the pentasaccharide ¹F-fructosylnystose. The oligosaccharides
12 corresponding to peaks 6, 11 and 13 could not be identified so far. Figure 3 illustrates
13 the structures of the different carbohydrates obtained in these reactions.

14 The compound corresponding to peak 5 was purified by semi-preparative
15 HPLC. Its mass spectrum showed that it was a disaccharide. The 1D and 2D ¹H NMR
16 spectra displayed two anomeric signals, arising from the typical α/β equilibrium and
17 a signal pattern recognizable as fructose and glucose residues. From the combination
18 of the signals from COSY, TOCSY, NOESY, HSQC and HMBC spectra, full
19 assignment of the ¹H and ¹³C resonance signals belonging to the different residues
20 was achieved. The glycosylation position was determined from the existence of a
21 crosspeak between the H6 from glucose and the quaternary carbon C2 from fructose
22 in the HMBC spectrum. The NMR data unequivocally permitted to identify the
23 compound as blastose [Fru- β (2 \rightarrow 6)-Glc] (Figure 4), a sucrose isomer member of the

1 neoFOS series. To our knowledge this is the first report in which the isolation and
2 chemical characterization of blastose is described.

3 **3.4. Kinetics of FOS formation**

4 The FOS formation was analyzed in detail using HPAEC-PAD. Figure 5
5 illustrates the profile of the biotransformation with lyophilized mycelium of *C.*
6 *cladosporioides* starting from 600 g/L of sucrose. At the point of maximum FOS
7 concentration (72 h), the FOS fraction was mainly composed of 1-kestose (158 g/L)
8 and nystose (97 g/L), with formation of lower amounts of the disaccharide blastose
9 (34 g/L), ¹F-fructosylnystose (19 g/L), 6-kestose (12 g/L) and neokestose (10 g/L).
10 Neonystose was only slightly detected at the end of the reaction (96 h). Such a
11 complex mixture of ¹F-FOS, ⁶F-FOS and ⁶G-FOS has only been described with the β -
12 fructofuranosidase from *Rhodotorula dairenensis* [32].

13 The concentration of neokestose never surpassed 10 g/L throughout the
14 reaction; however, blastose concentration was significantly higher (> 30 g/L) after 48
15 h. This result suggests that blastose is not formed by hydrolysis of neokestose, but by
16 the transfer of fructosyl moiety to the released glucose in the medium. In fact, the
17 biosynthetic activity detected in the *C. cladosporioides* mycelium is not very efficient
18 to hydrolyze the $\beta(2\rightarrow6)$ linkages between a fructose and a glucose, as the neoFOS
19 concentration is not diminishing throughout the process, in contrast with ¹F-FOS
20 (Fig. 5). To our knowledge, this is the first report describing the formation of blastose
21 in this kind of transfructosylating reactions.

22 Figure 6 shows the kinetics of FOS formation with lyophilized mycelium of *P.*
23 *sizovae*. At the point of maximum FOS yield (24 h), 156 g/L out of the total FOS

1 concentration (184 g/L) corresponded to 1-kestose. The FOS fraction was completed
2 with nystose (11 g/L), neokestose (6 g/L) and neonystose (11 g/L). The *P. sizovae*
3 enzyme displays a more typical profile with major formation of ¹F-FOS. However, its
4 transglycosylation to hydrolysis ratio is less favourable than that of *C. cladosporioides*.
5 It is interesting to note the negligible presence of blastose with the *P. sizovae* enzyme,
6 which indicates its much lower tendency to use glucose as acceptor to form $\beta(2\rightarrow6)$
7 linkages.

8

9 **4. CONCLUSION**

10 The main enzymes used for industrial production of FOS generally provide a
11 mixture of molecules with the inulin-type structure, ¹F-FOS, whereas those from
12 yeasts usually form levan-type FOS (⁶F-FOS) or neoFOS (⁶G-FOS). In this work, two
13 filamentous fungi (*Cladosporium cladosporioides* and *Penicillium sizovae*) showing
14 mycelium-bound transfructosylating activity were isolated. Maximum FOS yields
15 were 56% and 31% for *C. cladosporioides* and *P. sizovae* respectively. Interestingly, *C.*
16 *cladosporioides* synthesized a mixture of ¹F-FOS, ⁶F-FOS and ⁶G-FOS, including the
17 presence of a non-conventional disaccharide (blastose). Considering that the FOS
18 yield with *C. cladosporioides* is close to that obtained with typical *Aspergillus* or
19 *Aureobasidium* enzymes, the formation of a mixture of FOS with different glycosidic
20 linkages could give rise to certain benefits regarding their bioactivity.

21

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6

1 **Figure legends**

2

3 **Figure 1.** Time course of the reaction of sucrose with *P. sizovae* (A) and *C.*
4 *cladosporioides* (B) mycelia. Reaction conditions: 600 g/L sucrose, 40 g/L lyophilized
5 mycelium, 20 mM sodium acetate buffer (pH 6.0), 50°C.

6 **Figure 2.** HPAEC-PAD analysis of the reaction of sucrose with *P. sizovae* (A) and *C.*
7 *cladosporioides* (B) mycelia at the point of maximum FOS concentration. Peaks: 1:
8 glucose; 2: fructose; 3: sucrose; 4: 1-kestose; 5: blastose; 7: 6-Kestose; 8: neokestose; 9:
9 nystose; 10: neo-nystose; 12: ¹F-fructosylnystose; 6, 11, 13: unknown.

10 **Figure 3.** Structure of the fructooligosaccharides produced by *P. sizovae* and *C.*
11 *cladosporioides* transfructosylating activity.

12 **Figure 4.** 2D-NMR DEPT-HSQC spectra of blastose [Fru-β(2→6)-Glc]. The signals are
13 assigned and labelled. The key points for identifications are also shown.

14 **Figure 5.** Kinetics of FOS formation using 600 g/L sucrose catalyzed by lyophilized
15 *C. cladosporioides*. Reaction conditions: 40 g/L lyophilized mycelium, 20 mM sodium
16 acetate buffer (pH 6.0), 50°C.

17 **Figure 6.** Kinetics of FOS formation using 600 g/L sucrose catalyzed by lyophilized
18 *P. sizovae* (40 g/L). Reaction conditions: 40 g/L lyophilized mycelium, 20 mM
19 sodium acetate buffer (pH 6.0), 50°C.

Table 1. Initial screening of FOS-synthesizing microorganisms in fruit jams and molasses. Experimental conditions: biotransformations with growing cells in MY medium (including 200 g/L sucrose), 28°C, 150 rpm. Carbohydrates: F, fructose; G, glucose; S, sucrose; K, total kestoses; N, total nystoses; FN fructosylnystose; K/N, kestose/nystose ratio.

1

Strain	Time (h) ^a	Composition (%) ^b						K/N ratio
		F (%)	G (%)	S (%)	K (%)	N (%)	FN (%)	
CF ₂ 3V	96	2	33	9	32	24	-	1.3
CF ₂ 4V	96	8	43	9	12	18	9	0.7
CF ₂ 15	48	5	34	6	23	27	5	0.8
CK1	48	12	25	35	24	4	-	6.0
M1A	96	4	30	13	36	17	-	2.1
CF ₁ 1	72	2	18	50	21	9	-	4,4
CF ₁ 2	48	11	14	69	3	-	-	-
CF ₂ 7	96	3	12	67	13	7	-	-
CF ₂ 9V	48	48	47	3	3	-	-	-
CF ₂ 11	72	3	5	87	4	-	-	-
CF ₂ 12	96	8	20	57	10	5	-	2.0
CF ₂ 14	96	6	31	43	18	4	-	4.0
CF ₂ 16	48	2	24	47	12	5	-	2.4
SD4	96	5	26	58	16	5	-	3.2

2

^aTime of maximum FOS production

^bWeight percentage referred to the total amount of sugars in the mixture

1 **Table 2.** FOS production using lyophilised mycelium of *Cladosporium cladosporioides*
2 and *Penicillium sizovae* using different substrate concentrations.

3

Strain	[S] (g/L)	Time (h) ^a	Composition (%) ^b					
			F (%)	G (%)	S (%)	K (%)	N (%)	FN (%)
<i>C. cladosporioides</i>	200	24	8	30	10	23	7	7
<i>C. cladosporioides</i>	400	48	4	30	18	32	11	5
<i>C. cladosporioides</i>	600	72	3	33	12	30	18	4
<i>P. sizovae</i>	200	24	19	30	25	24	2	-
<i>P. sizovae</i>	400	24	12	23	36	25	4	-
<i>P. sizovae</i>	600	24	4	17	47	27	5	-

^aTime of maximum FOS production

^bWeight percentage referred to the total amount of sugars in the mixture

