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Inulin and Microbial Inulinases from the Brazilian Cerrado: Occurrence, Characterization and Potential Uses

Rita de Cássia L. Figueiredo-Ribeiro[1*] • Maria Angela M. Carvalho[1] • Rosemeire A. B. Pessoni[2] • Márcia R. Braga[1] • Sonia M. C. Dietrich[1]

[1] Instituto de Botânica, Seção Fisiologia e Bioquímica de Plantas, CP 3005, 01061-970 São Paulo, SP, Brazil
[2] Universidade Metodista de São Paulo, Fac. Ciências Biológicas e da Saúde, CP 5002, 09735-460 São Bernardo do Campo, SP, Brazil
Corresponding author: * **ritarib@usp.br**

ABSTRACT

The Brazilian cerrado is a biome characterized by a well-defined humid summer and a dry winter, where plant growth is concentrated in certain seasons within the annual cycle. Fructans of the inulin-type are the main reserve carbohydrate in about 60% of the Asteraceae species growing in the cerrado. These soluble carbohydrates have been associated with tolerance to cold and drought and in the adaptation of plants to unfavorable environmental conditions prevailing in the cerrado. The high fructan concentrations found in the cerrado flora and the occurrence of species characterized by inulins with high degree of polymerization among them are of particular importance concerning the production of inulin and derivatives and their industrial use as ingredients of functional foods. The search of efficient fungi isolated from the rhizosphere of Asteraceae for the production of extracellular inulinases, using inulin from the reserve organs of cerrado plants as a carbon source, is of great importance considering the increasing interest in the commercial production of free fructose and other inulin derivatives.

Keywords: Asteraceae, filamentous fungi, FOS, β-D-fructan fructohydrolase, fructans, fructose syrup, invertase, rhizosphere, stress tolerance, underground organs

CONTENTS

INTRODUCTION

The Brazilian cerrado is a floristically and physionomically distinct savanna vegetation, being the second largest biome of Brazil after Amazonia. It comprises 21 percent of the country land area and is characterized by a pronounced dry season, supporting a unique array of drought- and fire-adapted plant species (Mantovani and Martins 1988). The herbaceous components of the cerrado (76.7%) predominate over shrubby and arboreal vegetation (**Fig. 1A**). Asteraceae is the most representative family in a number of genera and species, and about 60% of them store large amounts of soluble carbohydrates (fructans) in their underground organs, mainly of the inulin-type (Figueiredo-Ribeiro *et al*. 1986; Tertuliano and Figueiredo-Ribeiro 1993).

The interest in studying fructan-containing species is greatly due to their agricultural use and the application of fructans and their derivatives in food and pharmaceutical industry (Ritsema and Smeekens 2003). The scientific interest in fructan metabolism arises partly from its close relationship with sucrose and its peculiar mechanism of synthesis, in which no intermediate sugar nucleotide is formed, from the distribution of the fructan-containing flora, and from its role in the adaptation of plants to environmental stresses (Pollock *et al*. 1996). Therefore, we have focused our studies on the occurrence and distribution of fructan-containing floras and on changes in the contents and composition of these carbohydrates during different physiological processes and under environmental stresses, essential to elucidate the ecophysiological role of fructan metabolism.

Plants from the cerrado, besides representing an alternative for inulin-type fructan production, have been used as source of microorganisms able to produce β-fructofuranosidases (e.g. invertases and inulinases) (Vullo *et al*. 1991; Cordeiro-Neto *et al*. 1997) since the soil adjacent to the roots is an area of intense microbial activity due to the presence of organic material coming from the plants. As a consequence of the increasing commercial interest in the enzymatic hydrolysis of inulin by microbial inulinases and the wide environmental diversity of plants and microbes in the tropics, we also addressed our studies to the isolation of filamentous fungi from the rhizosphere of the Asteraceae species from the cerrado. Special emphasis has been given to the selection of the most efficient microbes for production of inulinases, their characterization, the effects of substrates on their activities, and their relationship with fungal structure and biological properties, aiming to improve the production of free fructose and other inulin derivatives.

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Invited Mini-Review

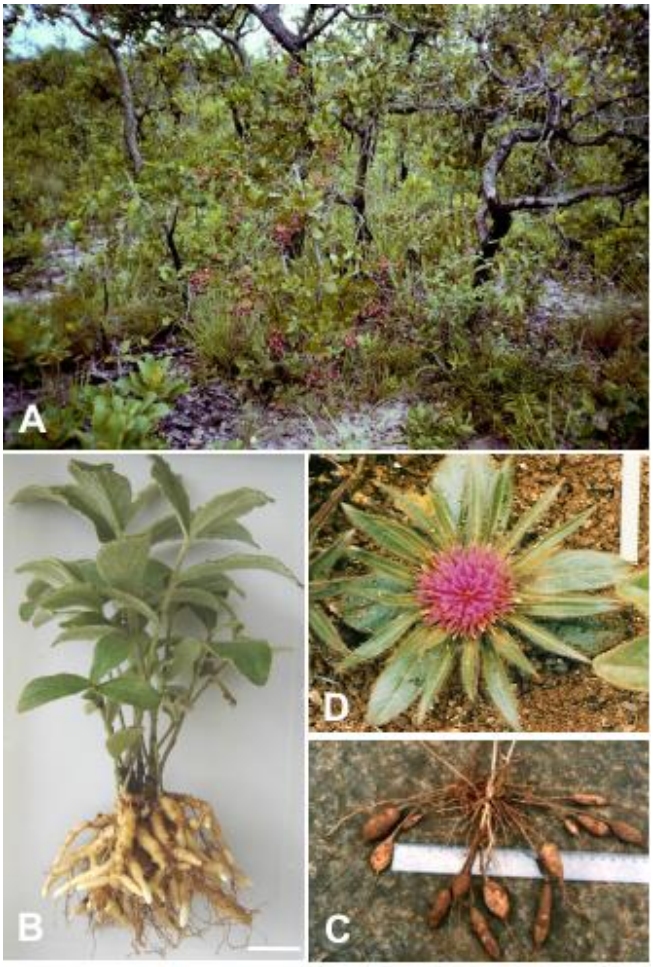

Fig. 1 General view and native herbaceous species from the cerrado. A restricted cerrado area at Moji-Guaçu, SP, Brazil (**A**), *Vernonia herbacea* (Vell.) Rusby (**B**), *Viguiera discolor* Baker (**C**) and *Gomphrena macrocephala* St Hil. (**D**). Bars = 1 cm (**B**); 10 cm (**D**).

OCCURRENCE AND ROLE OF FRUCTANS

Fructans are fructose-based oligo- and polysaccharides that occur as the main reserve carbohydrate in about 15% of flowering plants (Hendry and Wallace 1993) including the economically important and highly evolved families Asteraceae and Poaceae. These carbohydrates are built up by the successive addition of fructose residues with various linkages to three trisaccharides: 1-kestose (1-F-fructosyl sucrose), 6-kestose (6-F-fructosyl sucrose) and neokestose (6-G-fructosyl sucrose), which are synthesized by linking a fructose residue to one of the three primary hydroxyl groups of sucrose. Based on these trisaccharides and the type of fructosyl-fructose linkages, five structurally different types of fructans can be distinguished in higher plants: 1) inulin, a linear molecule typically found in Asterales, contains β (2-1) fructosyl-fructose linkages based on the trisaccharide 1-kestose; 2) levan, also linear and characteristic of the Poales, contains β (2-6) fructosyl-fructose linkages based on 6-kestose; 3) mixed linkage and branched fructans with terminal glucose residues, found in the Poales; 4) 2,1-linked fructans based on neokestose, found in asparagus, onion (Liliales), and some members of the Poales; and 5) 2,6-linked fructans based on neokestose, found in some members of the Poales (**Fig. 2**).

The presence of fructans in Asteraceae has been well documented for floras exhibiting seasonal growth patterns in temperate regions (Pollard and Amuti 1981) and in a subtropical restricted area of the Brazilian cerrado, in which herbaceous plants are subjected to periods of drought in winter (Figueiredo-Ribeiro *et al*. 1986; Tertuliano and Figueiredo-Ribeiro 1993). The Asteraceae is the most representative family, amounting to approximately 17% of the local angiosperm flora in a restricted cerrado area at Moji-Guaçu (22°35′S and 47°44′W), SP, Brazil. Of these, *Vernonia* and *Eupatorium* are the most abundant genera, comprising 5% and 4.5%, respectively (Mantovani and Martins 1988). A screening of the occurrence of soluble carbohydrates in underground organs of herbaceous species, reported the predominance of fructans of the inulin type as reserve compounds in approximately 60% of the Asteraceae growing in this area (Figueiredo-Ribeiro *et al*. 1986; Tertuliano and Figueiredo-Ribeiro 1993). Thirty-five herbaceous species of Asteraceae representing 6 tribes were analyzed and the presence of fructans was detected in 19 species among Eupatorieae, Heliantheae and Vernonieae. Spherocrystals of inulin were histologically detected in approximately 80% of all the examined species. The underground organs studied varied in water content, fructan concentration and tissue localization of the spherocrystals of inulin (**Fig. 3**). Thin layer chromatography revealed the presence of the homologous inulin series in all species although the proportion of the various members of the series varied among them (Tertuliano and Figueiredo-Ribeiro 1993). Of all the analyzed species, *Vernonia herbacea* (Vernonieae) and *Viguiera discolor* (Heliantheae) (**Fig. 1B, 1C**) stood out for their high concentrations of fructans, which reaches as much as 80% of the underground reserve organ on a dry mass basis (Carvalho and Dietrich 1993; Isejima and Figueiredo-Ribeiro 1993). Differently from all inulin-containing species of Asteraceae from the cerrado, the presence of a high degree of polymerization (DP) levan-type fructan in *Gomphrena macrocephala* (**Fig. 1D**), an Amaranthaceae, was described for the first time (Vieira and Figueiredo-Ribeiro 1993) and the molecular structure was later confirmed (Shiomi *et al*. 1996).

Another aspect of the fructan accumulation refers to the size distribution of molecules present in the tissues. Changes in total amounts and in size distribution of molecules have been described in different phenological phases or developmental stages (Carvalho and Dietrich 1993; Isejima and Figueiredo-Ribeiro 1993; Vieira and Figueiredo-Ribeiro 1993; Portes and Carvalho 2006). Approximately the same mass of fructose is detected in each separable oligosaccharide component, or else, the molar abundance declines with increasing size (Edelman and Jefford 1968). Among all the Asteraceae studied, *V. discolor* is the species accumulating the highest DP fructan, with the mean molecular mass throughout the phenological cycle varying between 21 and 28 kDa (Isejima and Figueiredo-Ribeiro 1993). Similar values of mean molecular mass were also found for other species of the tribe Heliantheae from the cerrado (Tertuliano and Figueiredo-Ribeiro 1993).

Several authors have studied the role of fructans in cold and drought tolerance (Pontis and Del Campillo 1985; Puebla *et al*. 1997; Konstantinova *et al*. 2002). It was suggested that fructans play a primary role as an osmotic regulator (Spollen and Nelson 1994). As stated by Hendry and Wallace (1993), the theories of low temperature survival and osmotic regulation come together in one aspect, the seasonality of plant growth. In fact, fructan-containing species are most abundant in areas where growth is concentrated in certain seasons within an annual cycle, for example in regions showing a seasonal pattern of rainfall. Fructan-accumulating plants grow in arid and very warm regions in Mexico, e.g., *Agave* spp. (Agavaceae) (Mancilla-Margalli and Lopez 2006) and in the Antarctic, e.g., *Deschampsia antarctica* (Poaceae) and *Colobanthus quitensis* (Cariophyllaceae), the only two angiosperms that have colonized the Antarctic islands (Bravo *et al*. 2001).

Evidence for the involvement of fructan in drought tolerance was experimentally presented by work with transgenic plants of tobacco and sugar beet capable of synthesizing levan-type fructans. These plants showed enhanced drought tolerance when compared to wild type plants (Pilon-Smits *et al*. 1995, 1999). Since membranes are the primary targets of both freezing and desiccation injury in cells, it was hypo-

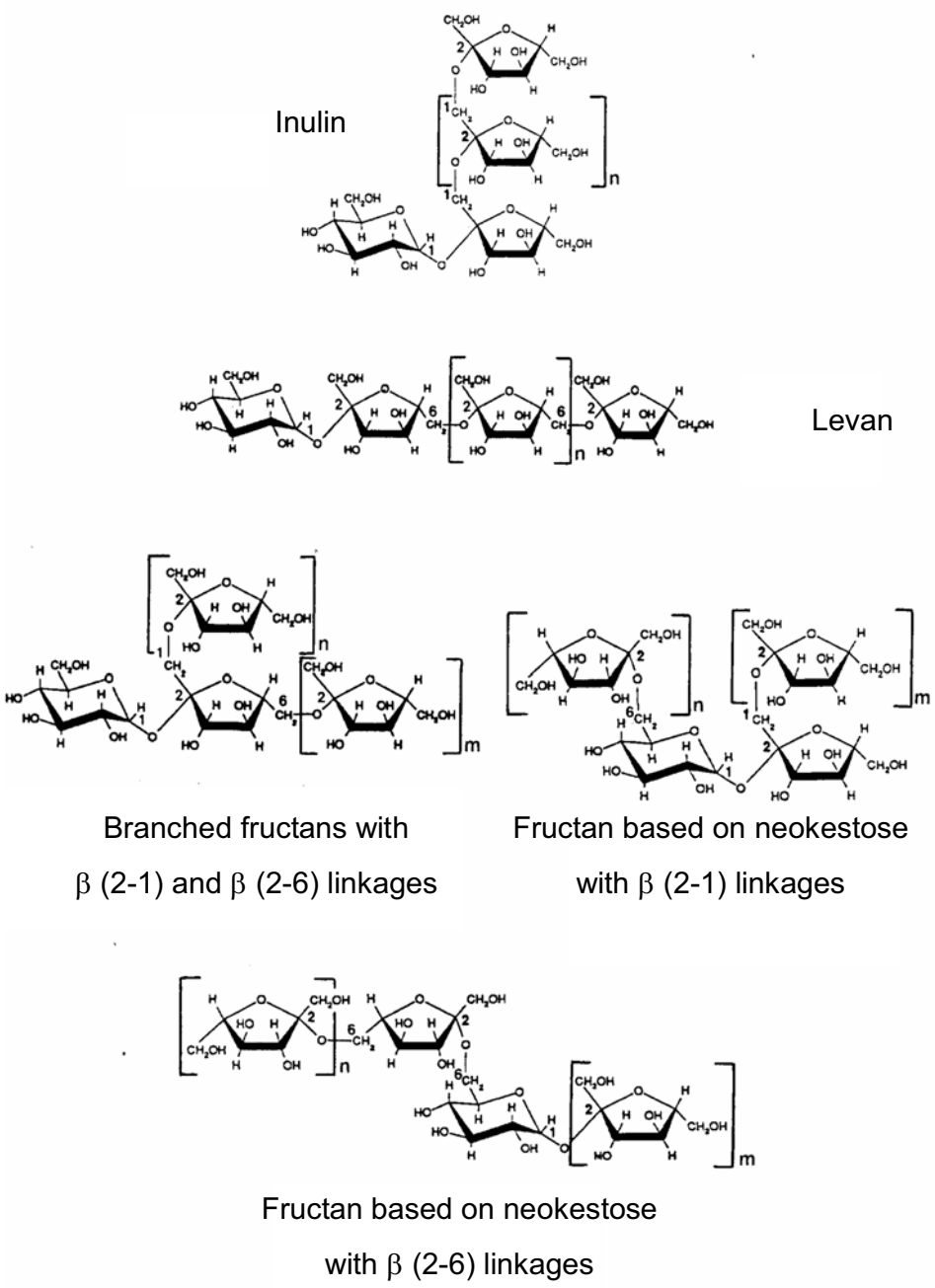

Fig. 2 Structures of different types of fructans. Modified from Carvalho and Figueiredo-Ribeiro 2001.

thesized that fructans have a direct interaction with the stabilization of the membranes under these stressing conditions (Demel *et al*. 1998). Further studies revealed that both levan and inulin-type fructans have an interaction with membrane lipids, supporting the hypothesis that fructans can have a membrane-protecting role in plants during drought (Vereyken *et al*. 2001).

The ability to accumulate readily accessible sugars, such as fructans, instead of starch may be of considerable advantage in cerrado plants, not only as a reserve compound but also to overcome unfavorable environmental conditions. As such, the cerrado and its fructan containing flora fit the hypothesis on the role fructan plays in drought tolerance considering that the dry season in the cerrado lasts for at least three months and coincides with the low temperatures of winter. Experiments of water suppression with *V. herbacea* showed that water deficit is associated with changes in plant growth and in fructan metabolism, as seen by the reduction of leaf number and area, and by the increase in free fructose and sucrose in plants submitted to different watering frequencies (Dias-Tagliacozzo *et al*. 2004). It was shown that while the water content in the soil declined, the water content in the rhizophores (underground cauline systems that originate adventitious roots and axillary buds) remained practically unchanged for the first 30 days of water suppression due partly to a decrease in water potential. After 30 days of water suppression, plants presented higher contents of fructo-oligosaccharides and -polysaccharides. More recent studies of water suppression with *V. herbacea* showed that increases in the activities of both enzymes involved in fructan synthesis (sucrose:sucrose fructosyltransferase-SST and fructan:fructan fructosyltransferase - FFT) early in the period of water suppression contributed to the increase in fructan contents, whereas later increases in free fructose were associated to increases in fructan deploymerization (MAM Carvalho, unpublished).

MICROBIAL INULINASES AND INVERTASES FROM THE CERRADO

Inulinases and invertases (β-fructosidases) are enzymes with β-D-O-fructofuranosyl fructohydrolase activity, capable of transferring β-D-O-fructofuranosyl residues (or their derivatives) from carbohydrates to water to form free D-fructose. Some β-fructosidases exhibit endohydrolase activity and catalyse the formation of β-D-fructo-oligosac-

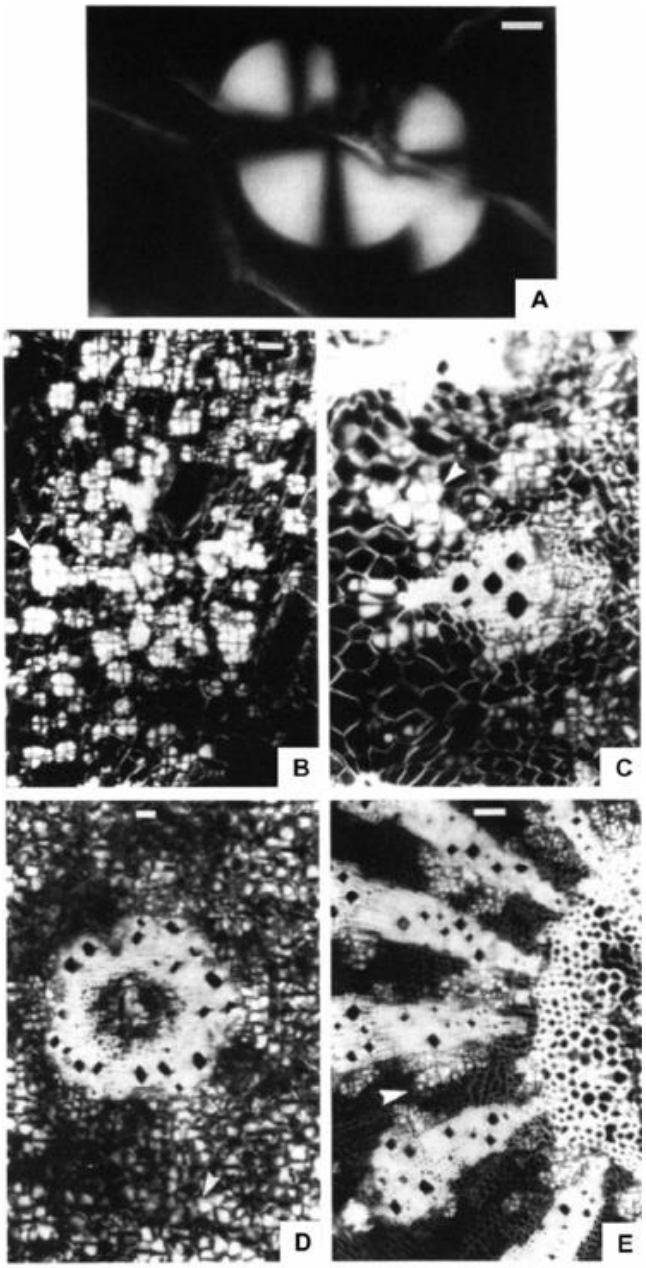

Fig. 3 Spherocrystals of inulin in Asteraceae species from the cerrado. Distribution in parenchyma cells of reserve tissues (**A, B, D**) or in association with vascular tissues (**C, E**) in transverse sections of tuberous roots of *Isostigma peucedanifolium* (**A, B**) and *Viguiera robusta* (**C**) and in thickened roots of *Eupatorium chlorolepis* (**D**) and *Mikania oblongifolia* (**E**). Bars = 100 μm; except (**A**) = 20 μm. Reproduced from Tertuliano and Figueiredo-Ribeiro (1993), with kind permission from New Phytologist.

charides (Akimoto *et al*. 2000).

The enzymes showing β-fructosidase activity differ in their substrate specificity. Their natural substrates include sucrose and its derivatives, sucrose-6-phosphate, raffinose, stachyose, and oligo- and polyfructans. The enzymes hydrolyzing polyfructans are generally called fructanases (EC 3.2.1.65) and include inulinases and levanases, splitting inulin (β-2,1 fructan) and levan (β-2,6 fructan), respectively (Naumov and Doreshenko1998). Microbial inulinases can be divided into exo- and endo-acting enzymes depending on the mode of action on inulin. Exoinulinases (β-D-fructan fructohydrolase, EC 3.2.1.80) split the terminal β-2,1 fructofuranosidic linkages present in sucrose, raffinose, and inulin. In contrast, endoinulinases (2,1-β-D-fructan fructan hydrolase, EC 3.2.1.7) are specific for inulin and hydrolyse the internal linkages of the polymer to release inulotriose, -tetraose, and -pentaose, as the main products (Akimoto *et al*. 2000).

Invertases (β-D-fructofuranosidase fructohydrolase, EC 3.2.1.26) preferentially hydrolyse sucrose, but they can also break inulin with less efficiency (Naumov and Doreshenko 1998; Laloux *et al*. 1991). Microbial invertases and inulinases are considered digestive enzymes, being generally secreted to the environment in an opportunistic fashion. It is believed that the mechanisms of protein secretion and glycosylation have been conserved throughout evolution and those that operate in yeast and other eukaryotes are also of major importance in filamentous fungi (Wallis *et al*. 1997). Many, if not all, of these secreted fungal macromolecules are glycoproteins, which have attracted increasing research interest as a valuable source of industrial enzymes.

The main source of a microbiota able to produce useful inulinases and invertases is the rhizosphere. It includes the soil volume adjacent to the roots that is influenced by them and favours the development of microorganisms (Lynch 1990). Soil samples of the rhizosphere of *Dahlia pinnata* and *Taraxacum officinale* from Argentina, and of *Vernonia herbacea* from the Brazilian cerrado were screened as source of inulinase-producing microbes. From 30 bacterial strains, three were capable of degrading inulin, being *Bacillus subtilis* 430A selected as the best producer of extracellular inulinases (Vullo *et al*. 1991). Using inulin as the sole carbon source, Cordeiro Neto *et al*. (1997) isolated 50 filamentous fungi from the rhizosphere of the native Asteraceae species from the Brazilian cerrado *Calea platylepsis*, *Vernonia cognata*, *Vernonia herbacea*, *Viguiera discolor*, and *Viguiera aff-robusta*. Forty-one fungal species were able to metabolise inulin as well as sucrose and 6 of them produced inulinases, the highest activity being detected in *Fusarium solani* and *Penicillium janczewskii*.

Changes in the carbon source or micronutrient composition of the culture medium can affect growth, sporulation, colony morphology, and polysaccharide storage in microorganisms. Inulinase production is also affected by the composition of the medium and by the carbon source (Grootwassink and Hewitt 1983). A variety of carbohydrates have been used for enzyme production (**Table 1**), although inulin and sucrose are the most common ones (Pandey *et al*. 1999).

Penicillium janczewskii, originally isolated from the rhizosphere of *Vernonia herbacea*, grows rapidly on medium containing sucrose or inulin as carbon source (Pessoni *et al*. 1999). However, maintenance of *P. janczewskii* on inulin medium induces secretion of proteins with higher inulinase activity when compared to those secreted by the fungus growing on sucrose-containing medium. Also, the thickness of the hyphae and cell walls, revealed by Scanning (SEM) and Transmission Electron Microscopy (TEM), and the cell wall content of chitin, and 3-linked glucans were influenced by the carbon source (Pessoni *et al*. 2005). Since hyphae of the fungus grown on inulin were more fragile than those cultivated on sucrose, and were more easily damaged, one can speculate that differences in hyphae thickness and in wall strength might be associated with higher inulinase secretion when the fungus grows on inulin.

The purification of invertases and inulinases produced by fungi, yeast and bacteria is performed using conventional methods of centrifugation and/or ultrafiltration, salt or solvent precipitation, followed by column chromatography (Pandey *et al*. 1999). In *P. janczewskii*, three different forms of extracellular β-fructofuranosidases (invertase or inulinase) were purified from filtrate of cultures growing for 12 days in liquid medium containing sucrose or inulin from *Vernonia herbacea* as carbon sources. Enzyme purification performed through precipitation with ammonium sulphate, anion-exchange, hydrophobic interaction and gel permeation chromatographies allowed to isolate two peaks of protein, which coincided with the detection of inulinase and invertase activities. These enzymes were thermostable, with an optimum temperature around 60°C and pH between 4.0 and 5.5. The apparent molecular mass (M*r*) of both proteins was 80 kDa as determined by gel permeation chromatography on Superose 12HR. This was confirmed by SDS-PAGE that showed a single Coomassie-stained protein band.

Table 1 Substrates used for inulinase production.

Substrate	Microorganism	Reference
Glucose	*Kluyveromyces marxianus*	Parekh and Margaritis 1986
Fructose	*K. fragilis*	Parekh and Margaritis 1986
Sucrose	*Aspergillus niger*	Nakamura *et al*. 1995
	Penicillium janczewskii	Pessoni *et al.* 2007
	Kluyveromyces sp. and yeast	Gupta *et al*. 1989
Maltose	*Candida thermoautotrophicum*	Drent and Gottschal 1991
Inulin from various sources	*A. niger*	Öngen-Baysal *et al.* 1994
	Arthrobacter sp.	Viswanathan and Kulkarni 1995
	K. marxianus	Pandey *et al.* 1999
	Candida kefyr	Negoro 1973
	Fusarium oxysporum	Gupta *et al*. 1994
	P. purpurogenum	Onodera and Shiomi 1988
Inulin from *V. herbacea*	*P. janczewskii*	Pessoni *et al.* 2007
Starch	*Panaeolus papollonaceus*	Mukherjee and Sengupta 1985

Table 2 Substrate specificities of inulinases and invertases (mean and standard deviation) of the filamentous fungus *Penicillium janczewskii* isolated from the rhizosphere of Asteraceae from the Cerrado (modified from Pessoni 2002).

Substrate	Relative activity (%)	
	Invertase	Inulinase
Sucrose	100.0 ± 4.3	92.1 ± 6.2
Raffinose	23.9 ± 10.2	92.1 ± 6.0
1-kestose	39.6 ± 9.4	65.6 ± 21.9
Nystose	23.7 ± 2.4	67.7 ± 13.1
Polymnia sonchifolia	106.8 ± 0.9	100.0 ± 19.3
Helianthus tuberosus	3.7 ± 2.5	88.5 ± 10.7
Vernonia herbacea	1.4 ± 0.7	38.7 ± 26.6
Viguiera discolor	12.8 ± 1.4	52.6 ± 8.0
Gomphrena macrocephala	1.5 ± 2.7	4.7 ± 4.8

The values of K_m and V_{max} of the enzyme determined on sucrose were 3.7 x 10^{-4} M and 7.9 x 10^{-2} mmol/min/ml, and on inulin were 6.3 x 10^{-2} M and 2.09 x 10^{-2} mmol/min/ml, respectively. The K_m values for the inulinases varied between 8.11 x 10^{-4} M and 2.62 x 10^{-3} M, lower in inulin than in sucrose, suggesting a higher affinity for the former (Pessoni 2002). These values were lower than those reported for the inulinase obtained from *Bacillus subtilis* also isolated from the rhizosphere of *V. herbacea* (Vullo *et al*. 1991). Properties of invertases and inulinases isolated from *P. janczewskii*, such as pH optimum ranging between 4.5 and 7.0 and optimum temperature around 50°C, have been found for enzymes purified from a number of microorganisms (Ratledge 1994). Therefore, invertase and inulinases produced by *P. janczewskii* grown on sucrose and inulin share several physicochemical characteristics, possibly due to subtle changes in the secreted proteins, mostly related to their affinity to the substrates.

The purified invertase of *P. janczewskii* proved to be specific for β-fructofuranosides, with a high affinity for sucrose, raffinose and oligo-fructans (from roots of *Polymnia sonchifolia* - yacon), but do not hydrolyze polyfructans such as the inulins from *Vernonia herbacea* and *Viguiera discolor* and levan from *Gomphrena macrocephala*. In contrast, inulinases from *P. janczewskii* were capable of hydrolyzing sucrose, raffinose, fructo-oligosaccharides and also inulo-polysaccharides (**Table 2**). HPLC analyses of the products of hydrolysis of different substrates by inulinases suggested a terminal (exo-) mode of action, since only fructose monomers and a small amount of glucose were detected.

POTENTIAL USES OF INULIN AND APPLICATIONS OF MICROBIAL INULINASES FROM THE CERRADO

Fructans are considered prebiotics since they promote the selective growth of beneficial colon bacteria like lactobacilli and bifidobacteria (Cummings *et al*. 2001). Because of the β-configuration of the anomeric C_2 in their fructose monomers, inulin-type fructans resist hydrolysis by intestinal digestive enzymes, being classified as 'non-digestible' carbohydrates and therefore considered dietary fibre. Fructans can also stimulate Ca^{+2} absorption and possibly play a role in preventing colon cancer (Roberfroid 2005). Therefore, there is a growing interest in the use of fructans as health food ingredient alternative for low-calorie sweeteners, dietary fibre and fat substitute (Ritsema and Smeekens 2003). Presently, fructan used in the industry is mainly low DP inulin-type derived from chicory roots. The benefits of these low DP oligosaccharides are that they are promptly fermented in the proximal colon, stimulating beneficial bacteria growth in this part of the colon.

Vernonia herbacea from the Brazilian cerrado is a potential source for inulin production and although it lacks a history as vegetable crop, several studies have focused on mineral nutrition, plant growth and biomass allocation aiming at the increase in rhizophore biomass and inulin production (Teixeira *et al*. 1997; Carvalho *et al*. 1998; Cuzzuol *et al*. 2003, 2005). In a field trial in a cerrado area, the inulin production by plants of *V. herbacea* reached 0.522 ton ha^{-1} after two years of cultivation (Carvalho *et al*. 1998). On a second trial, young, six-month old plants, presented lower fructan concentrations and increases in height, leaf area, dry mass of the aerial organs and rhizophores as treatment with nitrogen increased from 0 to 24 kg N ha^{-1}. At twelve months there was a 70% increase in inulin production (Cuzzuol *et al*. 2003), similarly to the production obtained in the first trial for two year old plants. This showed that the application of 24 kg N ha^{-1} was effective in promoting fructan production in *V. herbacea*.

The use of slow degrading high DP inulin as food additive could allow its beneficial role throughout the whole length of the colon (Roberfroid 2005). According to van de Wiele *et al*. (2007), the slower fermentation rate and the higher prebiotic potency of inulin make it a more interesting compound than oligofructans to beneficially influence the microbial community from both the proximal and distal regions of the colon. In this respect, the recombinant *Viguiera discolor* high DP 1-FFT enzyme (van den Ende *et al*. 2005) could have advantageous biotechnological applications, including the production of high DP inulin for use in both food and non-food industries. Introduction of the gene in chicory, the widely used crop for commercial inulin extraction or in another cerrado species, e.g. *V. herbacea*, could be a good alternative for the production of high DP inulin. Moreover, *in vitro* propagation represents an additional alternative for the commercial production of high DP fructans from *V. discolor* and may be of biotechnological value. Plants of this species propagated *in vitro* and callus originnated from stem nodes presented ratio SST/FFT <1 and high DP inulin-type fructans, similar to those found in plants growing in the cerrado, only in lower concentrations (Itaya *et al*. 2005), a situation that could be overcome by optimization of culture conditions leading to increases in fructan production.

Another important use of inulin in medicine concerns its application in the determination of kidney function in human beings and in research laboratory animals (Brenner *et al*. 1986). When properly prepared, inulin is physiologically inert and non-toxic at the doses required for physiological investigations. On a comparative study to evaluate the glomerular filtration rate in male Wistar rats, inulin from *V. herbacea* was shown to be similar to the commonly used commercial inulin from *Dahlia* (Sigma) (Dias-Tagliacozzo *et al*. 1996). The effects of inulin from *V. herbacea* in the colon anatomy and lipid metabolism in Swiss mice were evaluated by RAB Pessoni *et al*. (unpublished data). Changes in colon morphometry and mucin secretion, with stimulation of the beneficial sulfomucins and neutral mucins, were observed after a 35-day diet of 10% of inulin. Although no variation in the total cholesterol and triglicerides contents was observed, an increase in the proportion of HDL/LDL was detected.

Free fructose is a safe alternative sweetener for use by diabetics because, unlike glucose, it is metabolized in low concentrations, independently of insulin. Fructose has no active absorption mechanism in the intestinal mucosa. Because of slow absorption, the blood glucose-increasing effect of fructose is lower than after ingestion of most other carbohydrate sources (Uusitupa 1994). Although low levels of fructose can be metabolised in the absence of insulin, its effect on the reduction of glycemia has not been elucidated yet. In the liver, fructose is rapidly taken up by hepatocytes and phosphorylated to fructose-L-phosphate independent of insulin action by the enzyme fructokinase (Doiron *et al*. 1994).

As already mentioned, inulin and fructo-oligosaccharides (FOS) are considered as functional food ingredients (Heyer and Wendenburg 2001) and they affect physiological and biochemical processes in rats and human beings, resulting in better health and reduction in the risk of many diseases (Kaur and Gupta 2002). Previous studies have also demonstrated that these sugars are non-carcinogenic, stimulating the immune system, and reducing the risk of atherosclerosis by lowering the synthesis of triglycerides and fatty acids in the liver and decreasing their level in serum (Kang and Kim 1999; Iizuka *et al*. 2000; Kaur and Gupta 2002; Roberfroid 2005). The production of fructose syrup or FOS from inulin is the major area of application for inulinases.

Conventional fructose production from starch needs at least three enzymatic steps, including α-amylase, amyloglucosidase, and glucose isomerase action, yielding only *ca*. 45% fructose solutions (Vandamme and Derycke 1983; Manzoni and Cavazzoni 1992; Godfrey and West 1996). An alternative to this process is the hydrolysis of inulin by inulinases. Enzymatic production of fructose from inulin involves a single enzymatic step and yields up to 95% fructose. Chemical acid hydrolysis of inulin to fructose displays several drawbacks and reinforces the interest toward the microbial inulinases and their applications (Vandamme and Derycke 1983; Nakamura *et al*. 1995). Moreover, in many cases, inulinases are thermoresistant enzymes, with an average optimum temperature of about 50°C. An elevated temperature optimum seems to be favourable for industrial use to avoid microbial contamination of reactors and to increase the solubility of the substrate (Pandey *et al*. 1999).

Fructose syrup was produced from inulin of *V. herbacea* by hydrolysis with partially purified inulinase from *P. janczewskii* and evaluated with respect to the effect on plasma glucose level in diabetic rats. A decrease of *ca*. 46% of glucose levels in the plasma and no mortality were observed when rats were treated with inulin hydrolysates. The high amounts of inulin stored by *V. herbacea*, the high activity of inulinase secreted by *P. janczewskii* and the absence of mortality of animals in these assays indicate that fructose produced in this way can be a suitable alternative for the commercial production of fructose syrup (Pessoni *et al*. 2004).

The search for new fungal producers of inulinases and the characterization of these enzymes have not only theoretical importance, but also practical interest because they can be used in the synthesis of new fructose-containing oligosaccharides and ultra-high-fructose syrups from inulin, through enzymatic hydrolysis by either the sole action of exoinulinase or the synergic action of exo-and endoinulinases.

CONCLUDING REMARKS

Presently, inulin produced for commercial purposes is mainly obtained from *Cichorium intybus* (chicory). The Brazilian cerrado presents a wide diversity of fructan-producing species that represent alternatives to the main inulin-producing crops. The cerrado plants are well adapted to oligotrophic soils; however, growth and productivity can be increased by treatments with mineral nutrients. Moreover, the production of high DP inulin can be improved by use of the recombinant *V. discolor* 1-FFT enzyme and by *in vitro* culture, as both represent promising materials in view of biotechnological applications. The use of inulin from the Brazilian cerrado plants can be further extended if we consider the utilization of microorganisms associated to the fructan flora capable of producing extracellular inulinases, as the filamentous fungi *P. janczewskii*. The high activity of these enzymes and the high inulin contents in underground reserve organs of the cerrado species are potential sources for the commercial production of free fructose and other inulin derivatives. From a physiological point of view, the study of fructan metabolism of the cerrado flora may also help elucidate the ecophysiological role played by these carbohydrates in the adaptation of plants to nutritional and drought stresses.

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