RESEARCH ARTICLE

Characterisation of exo-inulinase concentrates from newly isolated thermophilic Bacillus strains – Bacillus sp. SG113 and Bacillus sp. SG115

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ABSTRACT

Enzyme concentrates with inulinase and invertase activity were derived from strains *Bacillus sp*. SG113 and *Bacillus sp*. SG115. The enzyme concentrate from strain *Bacillus sp*. SG113 has two distinct temperature optima – 50°C and 65°C for inulinase activity and 55°C and 65°C for invertase activity. Both activities are 100% sustainable for an hour at 60°C and 65°C. The inulinase activity of the enzyme concentrate from strain *Bacillus sp*. SG115 has two pH optima – pH 5.0 and pH 9.0, whilst invertase activity has only one – pH 6.0. The enzyme is stable at pH 7.0 (inulinase activity) and pH 6.0 (invertase activity).

The concentrate from strain *Bacillus sp*. SG113 hydrolyses inulin completely for 60 minutes at 60°C and 65°C. The invertase in the concentrate hydrolyses saccharose completely at 65°C, and partially (50%) at 70°C. Inulin extracts from topinambour, onion and garlic are almost entirely hydrolysed. The enzyme from strain Bacillus sp. SG115 hydrolyses to a lesser extent the studied inulin extracts and it was observed that hydrolysis was effective for 40 minutes. Thereafter the amount of reducing sugars does not increase, presumably due to product inhibition.

TLC data suggests that enzymes from strain *Bacillus sp.* SG113 and strain *Bacillus sp*. SG115 completely degrade inulin, onion and garlic extracts, whilst only partially degrading raffinose, with fructose being the end product in all cases, which proves their exo-activity. However, the above-mentioned enzymes do not hydrolyse melezitose.

Key words: inulinase, *Bacillus,* thermophile, hydrolysis, inulin

Introduction

Fructose and fructooligosaccharides are rapidly becoming a fundamental factor in the pharmaceutical and food industries on the grounds of their positive effect on bifidobacterium and the fact that they are derived from sources high in dietary fiber.

Conventional fructose production is based on degradation of starch with α-amylase and amyloglucosidase, followed by treatment with glucose isomerase, which catalyses the conversion of glucose into fructose as much as 45% fructose, 50% glucose and 8% other various oligosaccharides are generated in the process.

Fructose and fructooligosaccharides can be derived from inulin, which is a fructan - a polymer of fructose molecules, linked with fructose or glucose residues from a saccharose molecule. Inulins differ from one another based on the level of polymerization, which results in different functional characteristics (Kierstan, 1978; Zittan, 1981). The level of polymerization depends on the plant source, the weather conditions and the amount of time in which the product has been stored after it had been harvested, etc. (Chi et al., 2009, 2011; Neagu (Bonciu) & Bahrim, 2011). Plants use inulin as a means to store energy in the form of carbohydrates and it can be found in their roots and rhizomes. Plants with a high amount of inulin include onion, Jerusalem artichoke, chicory, leeks, banana, dahlia, as well as dandelion, garlic, burdock, purple salsify, yacón and the European goldenrod (Solidago virgaurea) (Pandey et al., 1999; Singh & Gill, 2006; Kango, 2008). The largest quantities of low and high molecule weight fructans have been found in topinambour (Jerusalem artichoke) at about 75% - 85% of the dry mass, with low molecular mass inulin being the bigger fraction (Bagaoutdinova et al., 2001). Inulin can be extracted industrially from chicory roots, as well as topinambour rhizomes (Hoebregs, 1997; Frank & Leenheer, 2002; Rhee et al., 2002; Stolzenburg, 2005).

Inulinases catalyse the hydrolysis of inulin with inulinooligosaccharides, fructose and glucose being the main

products of the reaction (Neagu (Bonciu) & Bahrim, 2011). These enzymes, depending on their mechanism of action and the place where they split the β-2,1 glycosidic bond in the inulin molecule, can be divided in two groups: exo-inulinases and endo-inulinases (Ertan et. al., 2003; Kango & Jain, 2011).

The exo-inulinases (EC 3.2.1.26; β-D-fructofuranosidase) and (EC 3.2.1.80; fructan-β-fructosidase) cut off the fructose residue on the nonreducing end of the inulin molecule, as well as the fructose residue in saccharose and raffinose (Onodera & Shiomi, 1988; Uchiyama, 1993; Ohta et al., 2002). In contrast, the endoinulinase (EC 3.2.1.7; β-fructanfructanohydrolase) interacts with the intramolecular bonds (Cairs, 2003; Kango & Jain, 2011; Pouyez et al., 2012; Chen et al., 2013). However, it shows no invertase activity (Nakamura et al., 1997). This enzyme is responsible for the partial hydrolysis of the polymer into fructooligosaccharides, such as inulotriose, inulotetraose, inulopentaose (Rocha et al., 2006; Pouyez et al., 2012; Flores et al., 2016).

Inulinases have different catalytic properties (molecular mass, pH optimum, temperature optimum, thermal and pH-stabilities), which highly depend on their microbial origin. Overall, inulinase activity (I) is correlated with invertase activity (S) and the enzymatic complex can be characterised by the relation I/S (or S/I). When this relation I/S is higher than 10-2 (or S/I<50) the enzymatic complex has predominantly inulinase activity. In the case of predominant invertase activity the ratio I/S is lower than 10-4 (or S/I from 1600 to 2800) (Belamri et al., 1994; Neagu (Bonciu) & Bahrim, 2011). Inulinase is a multifunctional enzyme and can be subjected to catabolite repression (Vandamme & Derycke, 1983). It can be used to derive a high fructose syrup, and in the production of ethanol, acetone, butanol, pullulan, gluconic acid, sorbitol and inulooligosaccharides (Mughal et al., 2009).

The industry's need for stable enzymes which can retain their properties in extreme conditions has shifted the scientific interest towards extremophiles. The production of hydrolases, including inulinases, from thermophiles can be characterised by: 1. Brief fermentation, due to the rapid growth of thermophiles; 2. Low viscosity of the growth medium at high temperatures, which makes aeration and mass exchange easier; 3. Reduced risk of microbial contamination; 4. Higher substrate solubility; 5. Thermostability of the enzyme molecule; 6. Suitability for use in the food industry, determined by the thermophiles and their products' nonpathogenic nature.

The aforementioned advantages have led to our interest in the isolation of thermophilic inulinase producers from hot springs in Bulgaria, the development of effective methods for the production, purification and characterization of the properties of the synthesized inulinases.

Materials and Methods

Bacterial strain isolation

The strains were isolated from thermal water and mud samples with temperatures 63-70°C and pH 7.7-8.2 from the Rupite region, South-West Bulgaria. Five milliliters from the samples were mixed with 5 ml isolation medium and incubated at 37°C and 50°C for 48 h for enrichment. After that the suspensions were heated at 80°C for 10 min for isolation of *Bacillus* strains (Sneath, 1986). After chilling, 5 ml of these suspensions were mixed again with 5 ml isolation medium and cultivated for 48 h at 37°C and 50°C. Then the samples were serially diluted, prior to plating 30 µl on meat agar (1.5% (w/v), Oxoid) containing inulin for isolation of single colonies. Plates were incubated at 37°C and 50°C for 3 days. Pure colonies were obtained by repetitive dilution streaking on peptone-yeast extract agar with additional inulin (0.2%, w/v) as a carbon source, which helped in the selection of colonies with inulinase enzyme activity. The active cultures were transferred several times on the same agar medium, and then individual colonies were isolated. The strains were screened for exo-inulinase production.

Inulinase assay

The culture medium was centrifuged at 4000 rpm for 15 min and the supernatant was used as the inulinase source. Inulinase activity was measured by determination of the reducing sugars released from substrate inulin by DNS-method (Miller, 1959). The reaction mixture contained 100 µl substrate inulin (from Dahlia tubers, Fluka, Buchs, Switzerland; 20 g/l, phosphate buffer pH 7.0) and 100 µl enzyme solution. After incubation at 60°C for 20 minutes, the reaction was stopped by 200 µl DNS-reagent. Reducing sugars were determined by calibration curve obtained using a standard solution of fructose (Scharlab S.L., Spain). One unit of inulinase activity was defined as the amount of enzyme that liberates one µmol of fructose per minute under the assay conditions.

Invertase assay

Invertase activity was determined under the conditions described above with saccharose (sucrose) (Scharlab S.L., Spain; 20 g/l in phosphate buffer, pH 7.0) as substrate. A calibration curve was obtained using an equimolar standard solution of glucose and fructose. One unit of invertase activity was defined as the amount of enzyme that hydrolyzes 1 µmol of saccharose per minute under the assay conditions.

Determination of protein, cell growth

Total protein content in enzyme solution was measured by the Bradford method (1976), using bovine serum albumin as standard.

The culture growth was determined by the absorbance at 650 nm.

Enzyme concentrate preparation

The active cultural liquid was centrifuged at 4000 rpm for 20 min to remove the bacterial cells. The supernatant was two-fold concentrated and partially purified at room temperature, a pressure of 0.2 MPa and flow rate 330 l/h through pilot ultrafiltration unit with a polyacrylonitrile membrane with 10 kDa pores, followed by concentration using an ultrafiltration unit Amicon 50 (Amicon Corp., USA, membrane filter pore size 10 kDa). Afterwards, the concentrate was lyophilized (Lyovac GT2, Leybold-Heraeus) and diluted in 0.025 M Tris-HCl buffer to protein concentration of 2.0 mg/ml.

Enzyme characterization

The effect of pH on the partially purified inulinase was investigated by measuring the enzyme activity at 50°C at the pH range 4.0 to 6.0 (0.1 M acetate buffer), 7.0 (0.1 M phosphate buffer) and 8 to 10.0 (0.1 M Tris-HCl buffer). For the pH stability determination, aliquots of 0.5 ml of enzyme plus 0.5 ml of the same buffer in the mentioned pH range were maintained at 4°C for 2 h and the residual activity was estimated. The optimum temperature was determined by measuring the enzymatic activity in 0.1 M phosphate buffer, pH 7.0 in the temperature range from 40°C to 70°C. For the thermal stability determination of the inulinase, a reaction medium composed of 0.5 ml of enzyme solution and 0.5 ml of 0.1 M phosphate buffer with a pH value of 7.0, was maintained at 60, 65 and 70°C and the residual activity was measured as described in the enzyme assay.

Inulinase action pattern

Aliquots of 0.5 ml of the partially purified enzyme were incubated, separately in 2.0 ml of raffinose solution (Sigma-Aldrich, USA), sucrose (Scharlab S.L., Spain), melezitose (Sigma-Aldrich, USA), inulin (Jerusalem artichoke, Sigma-Aldrich, USA), garlic and onion extracts and extract from flour of Jerusalem artichoke tubers, in a final concentration of 1.0% in 0.1 M phosphate buffer, pH 7.0, at 50°C. The profile of the enzymatic reaction was followed by thin layer chromatography (TLC).

Thin-layer chromatography (TLC)

TLC was performed on Silica gel 60 pre-coated plates (Merck, Darmstadt, Germany, 25 x 25 cm). A mixture of n-propyl alcohol/ethyl acetate/water (7:1:2, v/v/v) was used as a developing solvent. Sugars were detected by spraying of the air-dried plates with staining reagent containing ethanol/acetic acid/sulfuric acid/anis aldehyde (9:0.1:0.5:0.5, v/v/v/v). Carbohydrates were revealed after heating for 10 min at 120°C and were visualized as dark green spots. Fructose, glucose, raffinose, melezitose, inulin, saccharose in concentration 2.5 mg/ml (Sigma, USA) were used as standards. Garlic and onion extracts and extract from flour of Jerusalem artichoke tubers were also tested.

Preparation of onion and garlic extract

Two kilograms of the bulbs or cloves were peeled and chopped, then heated up to 90°C with 2 liters of distilled water. The slurry obtained was allowed to cool down and to stand for sedimentation. Afterward, it was filtered through muslin cloth and the filtrate was used in media formulation.

Preparation of Jerusalem artichoke flours from tubers

Jerusalem artichoke (Helianthus tuberosus L.) tubers were collected from several farms. The tubers were washed, peeled and cut, then were dried at 80°C to constant weight. The dried tubers were grounded mechanically and the resulting flours were used in the next experiments without fractionation. The inulin present in the tubers and the soluble carbohydrates were extracted with a steam jet (twice for a 10 min).

Results and Discussion

Bacterial strain isolation

The strains of Bacillus sp. SG113 and SG115 were isolated from thermal water and mud samples with temperatures 63-70°C and pH 7.7-8.2 from the region of Rupite, South-West Bulgaria. From the samples were isolated three thermophilic bacterial strains with inulinase and invertase activity by enrichment culture, growing on a medium containing inulin as the sole carbon and energy source. They were determined to be thermophilic since they could grow at 50°C, but not at 37°C (Table 1).

Table 1. *Isolation of thermophilic inulin-degrading bacterial strains and activity on inulin and sucrose.*

Strain	Growth at		Enzyme activity (U/ml) on		Saccharose/inulin ratio (S/I)
	37°C	50°C	Inulin*	Saccharose**	
Bacillus sp. SG113	-	+	0.79	2.50	3.16
Bacillus sp. SG115	-	+	0.91	2.75	3.06
Bacillus sp. SG6411	-	+	0.05	0.21	4.20

Legend: * - Statistical significance ($p<0.01$). Data are mean value ±SD from 0.01 to 0.03, n=5; ** - Statistical significance ($p<0.05$). Data are mean values ±SD from 0.02 to 0.06, n=5.

Table 2. *Partial inulinase purification from the strain Bacillus sp. SG113.*

Stage	Volume (ml)	Total protein (mg)	Total inulinase activity (U)	Specific inulinase activity	Yield (Y%)	Purification (fold)
Supernatant	2000	1700.0	1600.0	0.94	100	1.0
Enzyme concentrate 1	998	1160.1	1092.8	1.27	68.3	1.35
Enzyme concentrate 2	200	344.5	1021.7	2.97	63.8	3.16

According to Belamri et al. (1994), the inulin degrading enzymes are characterized by the S/I relation, which is lower than 50 for inulinases and between 1600 and 2800 for invertases. It is evident from Table 1 that the saccharose/inulin (S/I) relation ranges from 3.06 to 4.2, thus proving that the enzymes from the isolated strains are inulinases. The thermophilic strains *Bacillus* sp. SG113 and *Bacillus* sp. SG115 were selected for further analyzes.

Characterization of the enzyme concentrate from Bacillus sp. SG113

After the first concentration, the inulinase was purified 1.35-fold and the process was characterized by extensive losses – over 30% (Table 2). In comparison, after the second concentration, the losses were only 7%. However, taking in consideration the initial loss, the total yield was only 63.8%. Thus the inulinase was purified 3.16-fold and the specific inulinase activity reached 2.97 U/mg. The specific invertase activity increases from 2.94 U/mg protein (supernatant) to 3.97 U/mg protein (enzyme concentrate 1), and 9.29 U/mg protein after the second concentration (enzyme concentrate 2).

Temperature optimum and temperature stability

The temperature optima and thermostability of inulinases in the enzyme concentrate were studied. Inulinase and invertase activity were also analysed.

According to the results displayed in Figure 1, the enzyme concentrate has two temperature optima: 50-65°C for inulinase activity and 55-65°C for invertase activity. The data show that the enzyme concentrate’s maximal inulinase activity and maximal invertase activity are at a temperature of 65°C. The results shown in Figure 1B and 1C prove the thermal stability of the inulinase at temperatures between 60°C and 65°C for over 60 minutes. Half-life at 70°C does not exceed 15-18 minutes.

pH optimum and pH stability

The pH optimum and pH stability interval of the enzyme concentrate are shown in Figure 2A and 2B. The inulinase in the concentrate is stable at pH range 5.0-6.0 (Figure 2B), while the pH optimum is at pH 6.0 (Figure 2A).

The invertase activity’s pH optimum is 6.0-7.0 (Figure 2A). However, the invertase activity is most stable at pH 4.0 (Figure 2B).

Inulinase activity towards different substrates

The hydrolysis of inulin, saccharose, topinambour, onion and garlic extracts with enzyme concentrate with inulinase and invertase activity from strain *Bacillus* sp. SG113 (Figure 3A, 3B and 3C) was studied. Complete hydrolysis of inulin was observed after 60 minutes at temperatures of 60°C and 65°C (Figure 3A). Partial hydrolysis was observed at 70°C, which correlates to the low thermostability of inulinase at the said temperature (half-life 15-20 minutes).

The invertase in the enzyme concentrate hydrolyses 100% of saccharose at 65°C and 50% at 70°C (Figure 3B).

All three inulin extracts – from topinambour, onion and garlic were almost completely hydrolysed by the enzyme concentrate from strain *Bacillus sp*. SG113 (Figure 3C).

The products from the hydrolysis with the enzyme concentrate with inulinase and invertase activity from strain *Bacillus sp*. SG113 were also analysed with thin-layer chromatography (data not shown). The enzyme can partially hydrolyse raffinose. Fructose was not detected after hydrolysis of melezitose. The sole product of inulin hydrolysis was fructose. Fructooligosaccharides were not detected after the initial 20 minutes.

The results verify the typical exo-inulinase action, which consists of removing fructose units from the fructose end of the inulin molecule, regardless of its origin. The generation of fructose as the only product of inulin hydrolysis and its release from saccharose and raffinose proves the exo-active nature of the studied enzyme.

Sharma and Gill (2007) purified extracellular exo-inulinase from *Streptomyces* sp. and proved that the enzyme is indeed an exo-inulinase, since the only product of inulin hydrolysis was fructose. The results from studies on inulinases from *Bacillus polymyxa* (Kwon et al., 2003), *A. awamori* (Arand et al., 2002) and *Geobacillus stearothermophilus* (Tsujimoto et al., 2003) are similar.

Characterization of the enzyme concentrate from Bacillus sp. SG115

After the first concentration, the inulinase was purified 1.28-fold (Table 3). The process was accompanied by big losses – approximately 35%. The losses after the second concentration were little – 7%. Consequently, the inulinase was partially purified 3.49-fold with 60.5% yield and specific inulinase activity 3.77 U/mg. Specific invertase activity

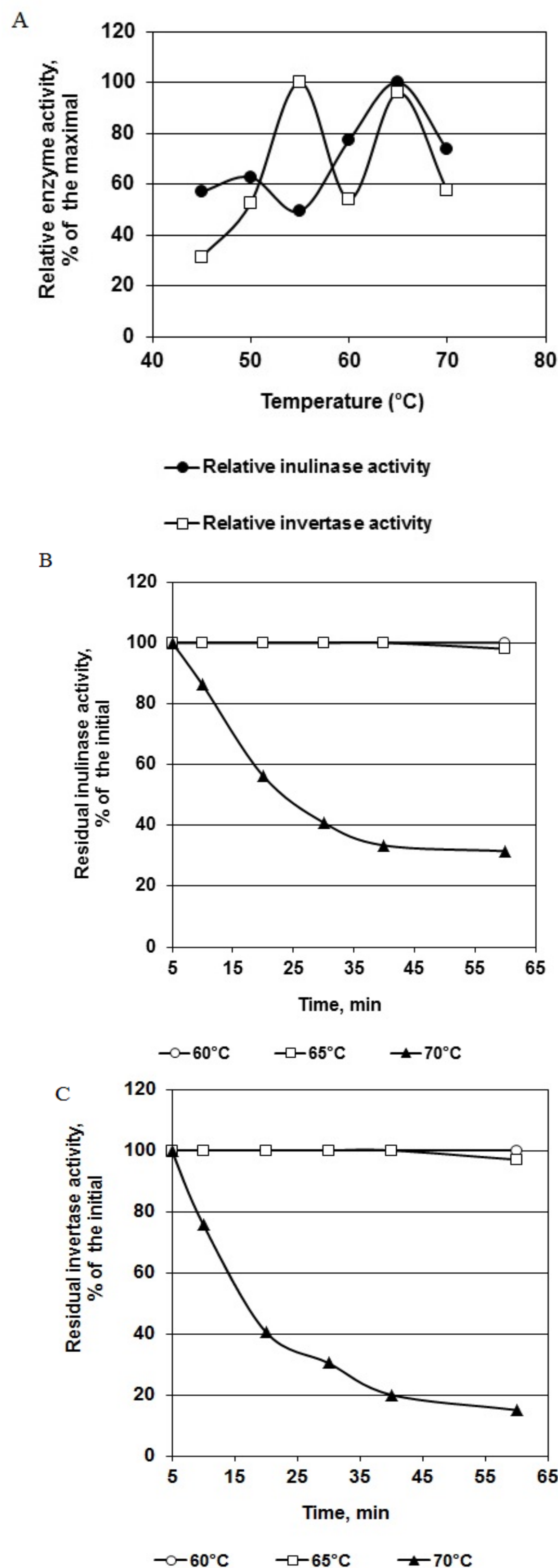

Figure 1. *A: Temperature optimum of inulinase and invertase activity of enzyme concentrate, derived from the strain Bacillus sp. SG113; B and C: Thermostability of enzyme concentrate with inulinase (B) and invertase (C) activity from strain Bacillus sp. SG113*

increases from 2.8 U/mg protein (supernatant) to 3.58 U/mg

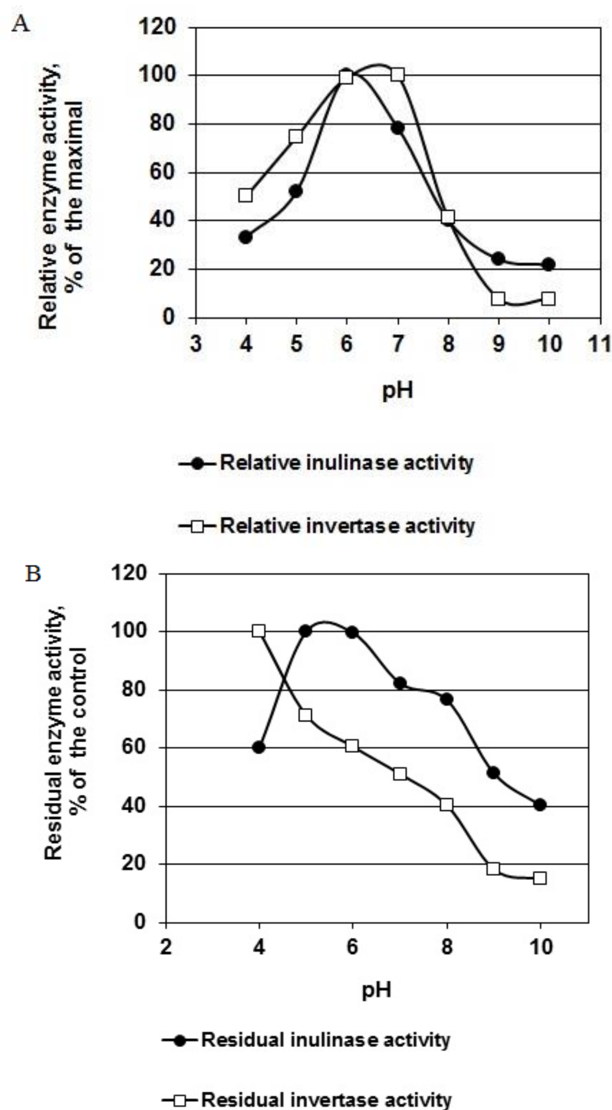

Figure 2. *A: pH-optimum of inulinase and invertase activity from enzyme concentrate from strain Bacillus sp. SG113; B: pH-stability of inulinase and invertase activity from enzyme concentrate from strain Bacillus sp. SG113.*
Chlorophyll mutation in an M2 plant.

protein in enzyme concentrate 1 and 10.56 U/mg protein in enzyme concentrate 2.

Temperature optimum and thermostability

Temperature optima for inulinase and invertase activity of enzyme concentrate from strain *Bacillus sp*. SG115 is 65°C (Figure 4A). Thermostability of inulinase activity and invertase activity at 70°C is 17 and 45 minutes, respectively. Both activities are 100% sustainable for 1 h at 60°C and 65°C (Figure 4B and 4C).

The thermostability of this enzyme bears certain similarities to that of *Bacillus stearothermophilus* (Belamri et

al., 1994) and is higher in comparison to other bacterial inulinases. For example, the exo-inulinase from *Bacillus sp.* LCB41 sustains only 70% of its initial activity after 10 min treatment at 60°C (Alais et al., 1987). Meanwhile, the exo-inulinase from *Arthrobacter* sp. has 50%

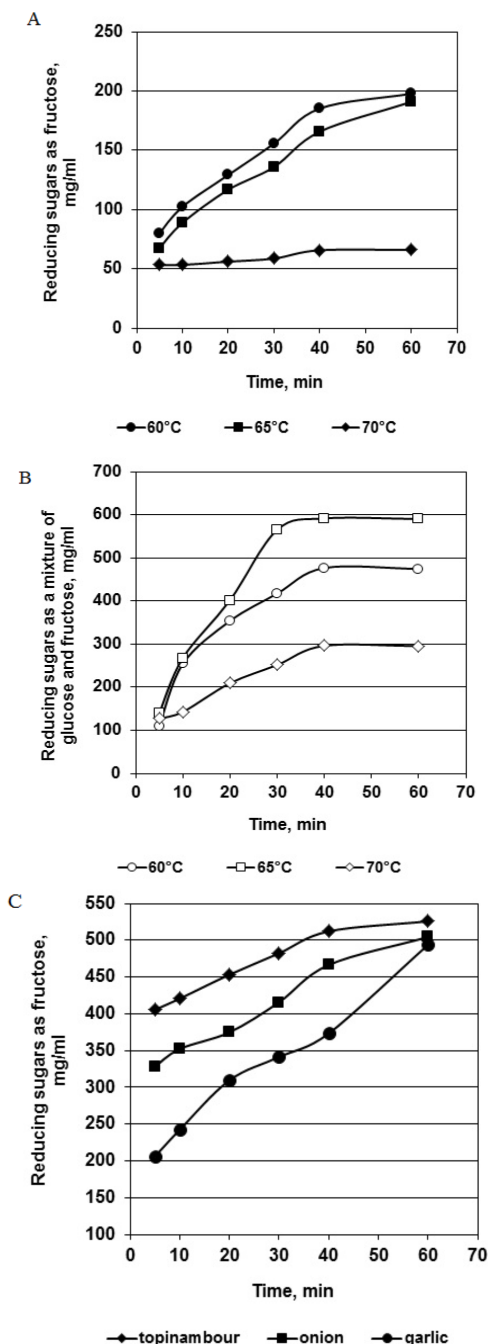

Figure 3. *Hydrolysis of inulin, saccharose, topinambour extract, onion extract and garlic extract. A: Hydrolysis of inulin at three different temperatures; B: Hydrolysis of saccharose at three different temperatures; C: Hydrolysis of topinambour extract, onion extract and garlic extract at 65°C with enzyme concentrate with inulinase and invertase activity from strain Bacillus sp. SG113.*

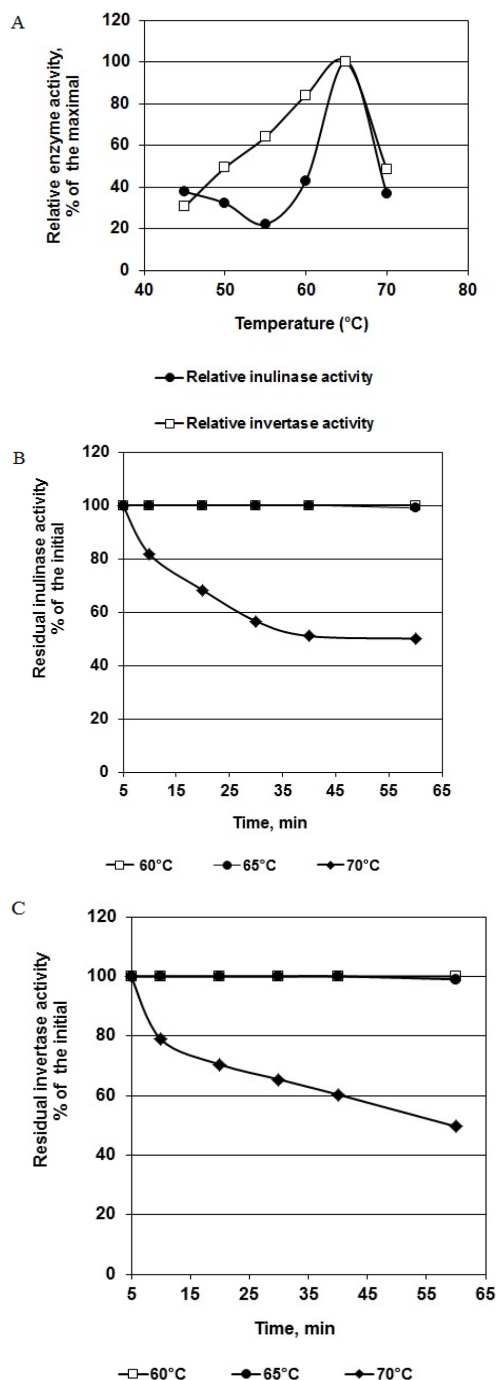

Figure 4. *A: Temperature optimum of enzyme concentrate with inulinase and invertase activity from strain Bacillus sp. SG115; B and C: Thermostability of enzyme concentrate with inulinase activity (B) and invertase activity (C) from strain Bacillus sp. SG115.*

Table 3. *Purification of inulinase from strain Bacillus sp. SG115.*

Stage	Volume (ml)	Total protein (mg)	Total inulinase activity (U)	Specific inulinase activity	Yield (Y%)	Purification (fold)
Supernatant	1600	1408.0	1520.0	1.08	100	1.0
Enzyme concentrate 1	746	715.0	988.8	1.38	65.0	1.28
Enzyme concentrate 2	150	244.0	918.7	3.77	60.5	3.49

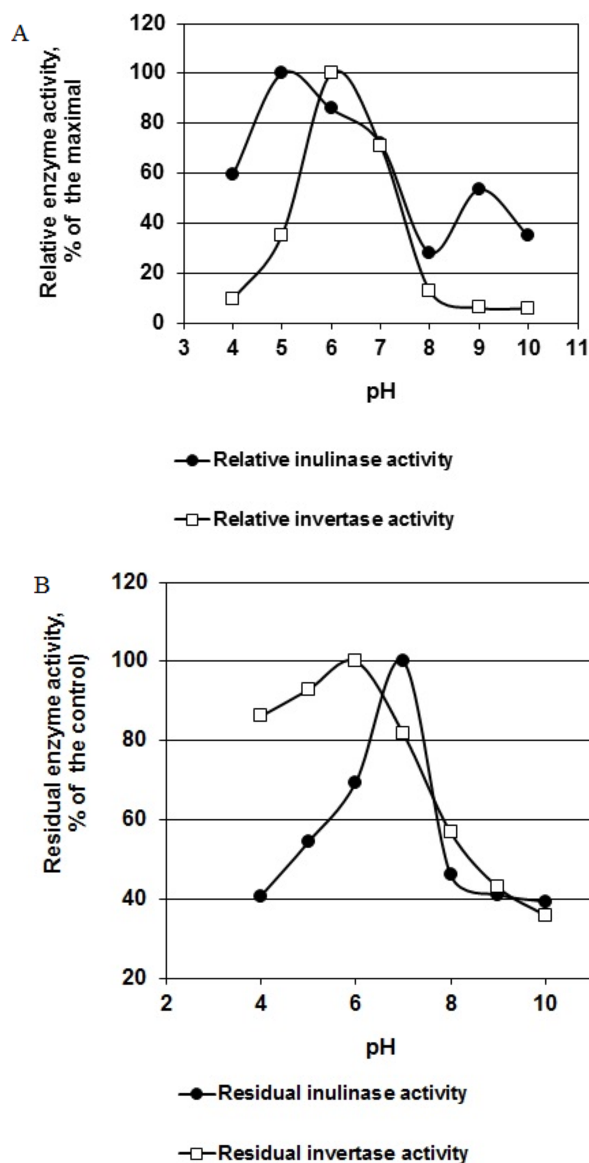

Figure 5. *A: pH-optimum of enzyme concentrate with inulinase activity and invertase activity from strain Bacillus sp. SG115; B: pH-stability of enzyme concentrate with inulinase activity and invertase activity from strain Bacillus sp. SG115.*

activity after a 30 min treatment at 55°C (Elyachioui et al., 1992) and the inulinase from *Clostridium thermosuccinogenes* retains 38% of its activity after a 30 min treatment at 63°C (Drent et al., 1991).

pH optimum and pH stability

Inulinase activity in the enzyme concentrate from strain *Bacillus sp.* SG115 has 2 distinct pH optima – 5.0 and 9.0 (Figure 5), whilst invertase activity's pH optimum is at 6.0. The enzyme is stable at pH 7.0 (inulinase activity) and pH 6.0 (invertase activity). The results for pH and temperature optima are similar to those for other bacterial inulinases (Alais et al., 1987; Drent & Gottschal, 1991; Drent et al., 1991; Elyachioui et al., 1992; Belamri et al., 1994).

Inulinase action on various substrates

TLC data suggest that the enzyme partially degrades raffinose, the product of which process is fructose (Figure 6). Furthermore, it completely degrades inulin, onion and garlic extracts, with the end product again being fructose. It does not hydrolyze melezitose. The generation of fructose as the sole product of the reaction proves the exo-activity of the enzyme, which breaks off fructose units from the fructose end of the inulin molecule, regardless of its origin.

The studied enzyme successfully hydrolyses inulin, saccharose, topinambour extract, onion extract and garlic extract (Figure 7). For 1 h at temperatures of 60°C and 65°C it hydrolyses over 50% of inulin, but only up to 20% of inulin at 70°C, due to the enzyme's sensitivity towards this temperature. Furthermore, the enzyme completely hydrolyses a 60% saccharose solution at optimal invertase activity temperature of 65°C (Figure 7B). In comparison to the

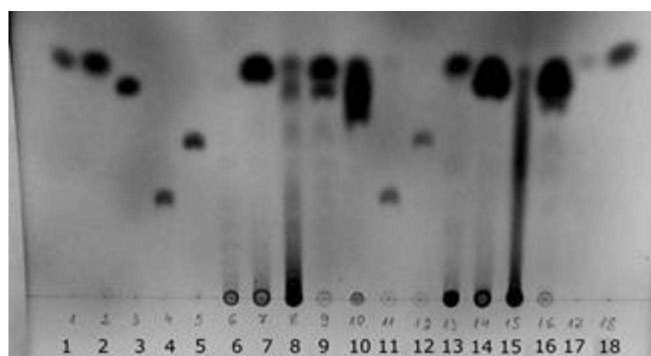

Figure 6. *TLC of reaction products obtained with inulinase from strain Bacillus sp. SG115. Standards: 2.5 mg/ml (Sigma, USA): 1, glucose; 2, fructose; 3, saccharose; 4, raffinose; 5, melezitose; 6, inulin; 14, topinambour extract; 15, garlic extract; 16, onion extract; 17, glucose; 18, fructose.*

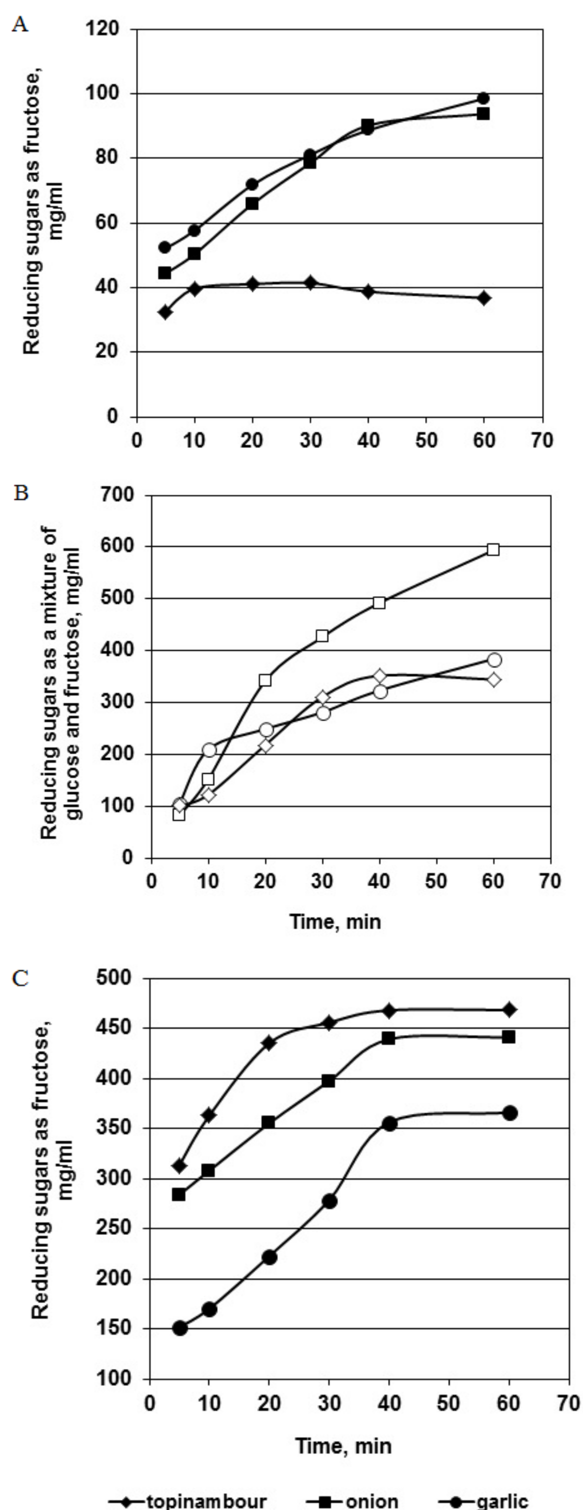

Figure 7. *Hydrolysis of inulin, saccharose, topinambour extract, onion extract and garlic extract. A: Hydrolysis of inulin at three different temperatures; B: hydrolysis of saccharose at three different temperatures; C: hydrolysis of topinambour extract, onion extract and garlic extract at 65°C with enzyme concentrate with inulinase and invertase activity from strain Bacillus sp. SG115.*

inulinase from strain *Bacillus* sp. SG113 (Figure 3C), the enzyme from strain *Bacillus* sp. SG115 hydrolyses inulin extracts from topinambour, onion and garlic to a lesser extent (Figure 7C). It is also noticeable that hydrolysis is active up to the 40th minute and thereafter the amount of reducing sugars does not increase, which could probably be attributed to product inhibition.

In conclusion, two enzyme concentrates with inulinase and invertase activity were derived from strains *Bacillus* sp. SG113 and *Bacillus* sp. SG115 by ultrafiltration.

The enzyme concentrate from strain *Bacillus* sp. SG113 has specific inulinase activity of 2.97 U/mg and specific invertase activity of 9.29 U/mg protein, whilst the concentrate from strain *Bacillus* sp. SG115 had a specific inulinase activity of 3.77 U/mg and specific invertase activity of 10.56 U/mg protein.

The enzyme concentrate from strain *Bacillus* sp. SG113 has 2 distinct temperature optima – 50°C and 65°C for inulinase activity and 55°C and 65°C for invertase activity. This could suggest the presence of two separate forms of inulinase in strain *Bacillus* sp. SG113. The pH-optimum for inulinase activity is at pH 6.0, and the pH-stability is at pH values of 5.0-6.0.

The temperature optima for inulinase and invertase activity in enzyme concentrate from strain *Bacillus* sp. SG115 are the same – 65°C. Thermal stability slightly differs at a treatment temperature of 70°C – half-life for inulinase is 17 min and for invertase – over 45 min. Both activities are 100% sustainable for an hour at 60°C and 65°C.

The inulinase activity of strain *Bacillus* sp. SG115 has two distinct pH-optima – at pH 5.0 and pH 9.0, whilst the invertase activity has only one – at pH 6.0. The enzyme is stable at pH 7.0 and pH 6.0 for inulinase and invertase activity, respectively.

The enzyme concentrate from strain *Bacillus* sp. SG113 completely hydrolyses the inulin substrate for 60 minutes at temperatures of 60°C and 65°C. Partial hydrolysis was observed at 70°C, which is in correlation with the poor temperature stability at this temperature (half-life 15-18 minutes). The invertase in the concentrate completely hydrolyses the saccharose substrate at 65°C and incompletely (up to 50%) at 70°C. All three inulin extracts – from topinambour, onion and garlic, were hydrolysed almost completely. In comparison, the enzyme from strain *Bacillus* sp. SG115 hydrolysed to a lesser extent inulin extracts from topinambour, onion and garlic. The hydrolysis was effective until the 40th minute; afterwards, the amount of reducing sugars does not increase, probably due to product inhibition.

TLC data suggest that the enzymes from strains *Bacillus* sp. SG113 and *Bacillus* sp. SG115 partially degrade raffinose to fructose, and completely degrade inulin and extracts from

garlic and onion to fructose as the sole product of the reaction, which proves their exo-action.

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