KAVAKA 59(2): 40-48 (2023) DOI: 10.36460/Kavaka/59/2/2023/40-48

Production of Extracellular Endo-inulinase from *Fusarium oxysporum* Using Garlic Extract as Substrate for Generation of Fructooligosccharides (FOS)

Hemant Kumar Rawat, Sumat Chand Jain, Naveen Kango*

Enzyme and Molecular Catalysis Laboratory, Department of Microbiology, Dr. Harisingh Gour Vishwavidyalaya, Sagar, Madhya Pradesh - 470 003, India.

**Corresponding author Email: nkango@gmail.com*

(Submitted on May 17, 2023; Accepted on June 04, 2023)

ABSTRACT

Various carbon sources were evaluated for production of inulinase by *Fusarium oxysporum* RS-115. Highest inulinase activity was observed with garlic extract (15.24 nkat/ml) as carbon source. The enzyme activity was 2.8 folds higher than that observed in media containing pure chicory inulin (5.39 nkat/ml). The Fungus showed good growth on a simple medium containing garlic extract (20% w/v) and yeast extract (2%w/v) as carbon and nitrogen source respectively, in 120 h at 30°C and 150 rpm. Among various protein sources tested, yeast extract was found to be the best source followed by peptone (12.15 nkat/ml) and beef extract (9.84 nkat/ml). The enzyme was optimally active at pH (5.0) and 50°C. All metal salts except MnSO4, MgSO4 and FeCl3 were not well tolerated and did adversely affect inulinase activity. Inulinase activity was found reduced significantly in presence of EDTA and PMSF whereas the activity of inulinase was inhibited by pCMB. TLC and HPLC analysis of end products revealed that inulinase hydrolyzed inulin exclusively into fructose and fructooligosacharides. Results suggest that the garlic induced endoinulinase synthesis in F. oxysporum RS-115 and can be utilized as a potential substrate for inulinase production.

Key words: Inulinase, *Allium sativum*, fructoologosaccharides, *Fusarium oxysporum*.

INTRODUCTION

Microbial inulinases are an important class of industrial enzymes, which are usually inducible and extracellular. These β-D-fructan fructanohydrolases (EC 3.2.1.7) are versatile enzymes which are used for the production of various carbohydrate-based products. A number of yeasts, filamentous fungi and some bacteria are reported to produce inulinases (Rawat *et al.,* 2015a; Beroigui *et al.*, 2023). Inulinases are receiving increasing attention due to availability of relatively inexpensive and abundant renewable substrate (inulin) for the production of high fructose syrup (HFS). The production of fructose syrup from inulin or inulin rich materials is a major area of applications of inulinases. Fructose is emerging as a healthy and safe alternative sweetener than sucrose, which causes problems, related to corpulence, cariogenicity, atherosclerosis and diabetes (Vandamme and Derycke, 1983; Kango, 2008). Fructooligosaccharides constitute one of the most popular functional food components because of their bifidogenic and health promoting properties. Inulooligosaccharides have very similar structure and functionalities to fructooligosacharides whose beneficial effects on humans and animals have been well characterized as functional sweeteners. Increasing importance of inulooligosaccharides has prompted researchers to focus upon strains that produce endoinulinase exclusively. Enzymes derived from microorganisms like *Aspergillus phoenicis*, *A. japonicus*, *A. niger*, *Fusarium oxysporum*, *Penicillium rugulosum*, and *Arthrobacter* sp. have been reported to produce FOS from sucrose (Housseiny, 2014; Rawat *et al.,* 2015b; Kamble *et al.,* 2019). Apart from being a low calorie sweetener, fructose has other important applications such as in the production of ethanol, acetone and butanol, gluconic acid, sorbitol and fructo-oligosaccharides etc. (Kango and Jain, 2011).

Industrial applications of this enzyme, however, would only be feasible if it were available in large quantities at a competitive price. Production of inulinase is affected by medium components and type of the organism used for fermentation. Various carbon sources like fructose, sucrose, purified inulin have been examined for production of this enzyme (Chen *et al.*, 2022; Rawat *et al.*, 2021). To compensate the high cost of inulin various plant materials like Jerusalem artichoke, dahlia (*Dahlia pinnata*), chicory, kuth roots, etc. have also been used for the production of inulinases (Singh and Singh, 2010, Kango and Jain, 2011). Majority of the plant materials investigated so far either are costly or have a limited availability. Majority of plants materials investigated so far either are costly or have a limited availability. Moreover, it is a

judicious alternative for utilization of abundant inulin occurring in a wide range of plants such as asparagus, dahlia, onion, dandelion and garlic. *Allium sativum* is commonly known as garlic, and belongs to Alliaceae family. The garlic plant's bulb is the most commonly used part of the plant. It contains 9-16% (fresh weight) inulin in its bulb. It is a very commonly used spice in India and world. Commonly, the scaling up probability for the production of an enzyme is based on high yielding microorganism and cheap raw materials (Singh and Bhermi, 2008; Rawat *et al.,* 2017). Among the microbial strains used for inulinase production, those of *K. marxianus* and *A. niger* are the most commonly used ones (Singh *et al.,* 2007; Kango, 2008). In present study production and properties of inulinase by *F. oxysporum* RS-115 were examined on various carbon sources including crude garlic extract.

MATERIALS AND METHODS

Microorganism

Fusarium oxysporum RS-115 (NFCCI 2429) was isolated from rhizosphere soil of garlic. Identity of this isolate was confirmed at National Fungal Collection of India, Agharakar Research Institute (ARI), Pune. The culture was grown on Czepak Dox agar at 28°C and maintained at 4°C on the slants of the same media.

Substrates and chemicals

Inulin (from chicory), fructose, Dinitrosalicycilc acid and corn steep liquor were obtained from Sigma chemical co., U.S.A. Precoated silica gel plates UV_{254} were obtained from Merck, Germany. Inulin containing substrates were obtained from a local horticultural nursery and local market.

Preparation of raw inulin extract

100 g of the each vegetal substrate were washed in running water and crushed in a blender with 500 ml of distilled water. The slurry obtained was allowed to stand for sedimentation of particulate matter. Afterwards, it was filtered through muslin cloth and the filtrate was used in media formulation.

Enzyme production

50 ml of each vegetal extract was supplemented with 2% (w/v) yeast extract as N-source. Production media with pure inulin (Chicory root, Sigma) was prepared as described by Skowronek and Fiedurek (2003). Erlenmeyer flasks (150 ml) containing 50 ml aliquots of medium were autoclaved (20 min, 121°C) and inoculated with mycelial discs cut from 7 days old culture of *F. oxysporum* RS-115. Flasks were incubated at 30°C on a rotary shaker (150 rpm). Flasks were withdrawn at regular interval of 24 hrs and clear culture filtrate was obtained by centrifugation. All the experiments were carried out in triplicate and mean values ± SD are reported as results.

Effect of nitrogen sources

The effect of different nitrogen sources including peptone, beef extract, yeast extract, corn steep liquor and casein (organic N-sources,) and $NaNO_3$ was studied by incorporating 2% (w/v) organic and 1% (w/v) of inorganic N- source in garlic extract medium**.**

Enzyme assay

0.2 ml of appropriately diluted enzyme (culture filtrate) was added to 1.8 ml of inulin/sucrose (1% w/v dissolved in 200 mM sodium acetate buffer, pH 5.0) and incubated at 50°C for 15 min. After incubation, total reducing sugars liberated from inulin were measured by adding 3 ml DNS reagent and boiling in a water bath for 5 min (Miller 1959). Samples were allowed to cool and their absorbance was read at 540 nm. Invertase activity was measured using sucrose solution (1% w/v dissolved in 200 mM sodium acetate buffer, pH 5.0). One nanokatal (nkat) of inulinase/ invertase activity was defined as the amount of enzyme which produced 1nano mol of fructose/glucose per second under the assay conditions as described above.

Partial purification of enzyme

Precooled enzyme samples (culture filtrates) of fungi obtained after filtration were centrifuged at 8000 g for 30 minutes. The pellet, if any, was discarded and the clear cell-free supernatant thus obtained was used for bulk precipitation of proteins. Double volume of chilled ethanol (2:1 v/v) was added to the cell free culture filtrate. The mixture was stirred for another 30 minutes to allow equilibration of solvent and protein. It was then centrifuged at 9000g for 15 minutes at 5°C and the supernatant was decanted. The precipitate was air dried, resuspended in 10 ml of distilled water and was used as enzyme sample.

Effect of temperature and pH on inulinase activity

The effect of temperature was determined by incubating 0.2 ml of suitably diluted enzyme and 1.8 ml of inulin (1% w/v in 200 mM sodium acetate buffer, pH 5.0) for 15 min at different temperatures. The effect of pH on inulinase activity was determined by incubating 0.2 ml of suitably diluted enzyme sample in 1.8 ml of (1% w/v) inulin dissolved in different buffers (0.2 M sodium acetate buffer: pH 4.0 and 5.0; 0.1 M phosphate buffer: pH 6.0, 7.0 and 8.0; 0.1 M Tris–HCl buffer: pH 9.0, 0.1 M glycine-NaOH buffer: pH 10). The reaction mixture was incubated at 50°C for 15 min.

Effect of metal ions and protein inhibitors on enzyme activity

The effect of different metal ions and protein inhibitors on inulinase activity was examined by incubating various metal salts and protein inhibitors with enzyme in 200 mM sodium acetate buffer (pH 5.0) at 30°C for 1 h. The enzyme activities remaining after incubation were determined under assay conditions.

Thin layer chromatography

The end products of enzyme reaction were visualized using thin layer chromatography as described by Kango (2008). 200 µL of undiluted enzyme (culture filtrate) was added to 200 µl of inulin (5% w/v in 200 mM NaAc buffer, pH 5.0) and was incubated at 50°C. Aliquots of 3 µL were withdrawn different interval and spotted on TLC plate. Plates were developed with the solvent system containing isopropyl alcohol: ethyl acetate: water (2:2:1 by volume). Sugar spots were developed with reagent containing 0.5% α-naphthol and 5% conc. sulfuric acid in absolute ethanol and by heating the plate at 100°C for 10 min. Fructose, kestose and nystose were used as sugars standards.

RESULTS AND DISCUSSION

Effect of substrates

In the present study *F. oxysporum* RS-115 was able to utilize entire test C-sources for its growth. Growth on garlic extract was maximum of the medium was also noticed. Infusion prepared from garlic was found to support maximal inulinase production (15.24 nkat/ml) as compared to pure inulin and other substrates (**Figure 1**). The results of this experiment showed inherent ability of test strains to utilize inulin-containing substrates of different origins for

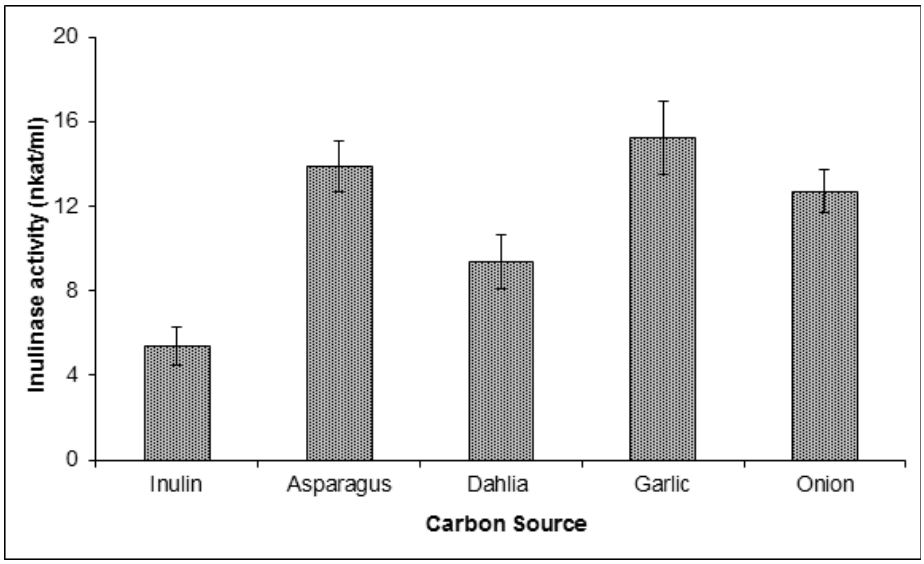

Figure 1: Effect of carbon source on inulinase production by *Fusarium oxysporum* RS-115. Culture condition: 30°C, 150 rpm; 120 h. Results represent mean ± S.D. of three experiments.

inulinase production. The results are very important in terms of utilization of non-conventional and locally available inulin containing substrates (e.g. asparagus, dahlia) to obtain an enzyme of commercial value. Sharma *et al.* (2006) have used garlic powder for production of exoinulinase by *Streptomyces* sp. and have observed that enzyme yield (0.554 IU/ml) was 1.6 folds higher as compared to pure inulin as a carbon

sources. Gupta *et al.* (1989) and have reported production of inulinase by *Fusarium oxysporum* using *Cichorium intybus* root extract. Singh and Bhermi (2008) have evaluated root tubers of *Asparagus officinalis* for production of exoinulinase by an indigenous strain *K. marxianus* YS-1 strain and have observed that enzyme yield (8.42 IU/ml). Recently, a perennial herb dandelion (*Taraxacum officinale*), which belongs to *Asteraceae*, and stores inulin, has been reported as a competitive substrate for inulinase production by *Aspergillus niger* (Kango, 2008). Tubercles of yacon (*Polymnia sanchifolia*), also a member of Asteraceae, have been reported as an inexpensive substrate for inulinase production from *Kluyveromyces marxianus* (Cazetta *et al.*, 2005). In the present study, compared to pure inulin as a carbon source, about 2.8-folds higher activity was obtained with garlic. Recently, Rawat *et al.* (2015b) have used dahlia tubers, asparagus and dandelion root extract for production of inulinase by various microorganism and observed higher inulinase activity on asparagus root 11.3 (U/ml) as compared to other crude inulin form. Nakamura *et al.* (1997) reported 9.9 U/ml of inulinase production by *Penicillium* sp. TN-88 using inulin as carbon source. Trivedi *et al.* (2012) have indicated use of low-cost substrates such as wheat bran and corn steep liquor in the production of inulinase by newly isolated *Aspergillus tubingensis* CR16.

Many microbial preparations of inulinase possess remarkable invertase activity accompanying the inulinase activity. Their catalytic activity is described in terms of I/S ratio which represents ratio of the activity of enzyme preparation on inulin and sucrose (Vandamme and Derycke 1983). I/S ratios (0.45-0.81) were observed on all the carbon sources (**Table 1**). Large variation in I/S ratios has been noticed in case of *A. niger* grown on various C-sources (Kango, 2008). The ratios in the range of 0.02-7.9 have been reported in literature (Moriyama *et al.*, 2002).

Table 1: Relationship between inulinase and invertase activity

Carbon source	Inulinase (nkat/ml)	Invertase (nkat/ml)	*I/S* ratios
Inulin (chicory)	5.39 ± 0.91	11.98 ± 0.79	0.45
Asparagus root powder	13.89 ± 1.21	18.74 ± 1.11	0.74
Dahlia tuber extract	9.38 ± 1.89	13.64 ± 1.12	0.69
Garlic bulb extract	15.24 ± 1.83	27.70 ± 1.29	0.55
Onion bulb extract	12.7 ± 1.07	15.64 ± 1.03	0.81

Results represent mean ± S.D. of three experiments.

Table 2: Effect of metal salts and protein inhibitors on activity of inulinase of *Fusarium oxysporum* RS-115

Compounds	Concentration	Relative activity (%)
Control	-	100.00 ± 2.98
$MgSO_4$	1mM	70.23 ± 2.26
$ZnSO_4$	1mM	66.86 ± 1.02
$BaCl_2$	1mM	66.07 ± 2.95
$MnSO_4$	1mM	102.97 ± 2.15
$CaCl_2$	1mM	54.16 ± 2.52
$CuSO_4$	1mM	63.88 ± 3.12
$FeCl_3$	1mM	76.19 ± 2.71
$HgCl_2$	1mM	32.53 ± 1.32
PMSF	1mM	73.71 ± 2.89
pCMB	1mM	53.71 ± 1.97
EDTA	1mM	81.85 ± 2.48

Results represent mean ± S.D. of three experiments.

Effect of incubation period

Furthermore, the investigate the influence of incubation period, the fermentations were carried out for 120 hrs under 150 rpm. The maximum inulinase activity was obtained on the fifth day of cultivation and asparagus extract with a pH shift from 6.2 to 7.2, on garlic yeast extract medium (**Table 3**). Ayyachamy *et al.* (2007) have found that the pH of the culture medium in all carbon sources was maintained in the range from six to eight, except for inulin. The pH of inulin containing medium increased to nine at the end of incubation.

Table 3: Production of inulinase by *Fusarium oxysporum* RS-115 on garlic yeast extract medium. Results represent average of three independent experiments.

Time (h)	Inulinase (nkat/ml)	pH	Biomass (g/50 ml)
0	0	6.2	0
24	3.2	6.4	0.09
48	7.4	6.7	0.13
74	10.3	6.8	0.16
96	14.1	6.9	0.19
120	17.2	7.1	0.23

Effect of nitrogen sources

Yeast extract was found to be the best nitrogen source to be used in conjunction with garlic extract for inulinase production followed by peptone (**Figure 2**). Complex nitrogen sources were better than inorganic nitrogen source because of free acids liberated in the medium after utilization of ammonium ions, which cause acidic conditions. These cause a drift in medium pH which decreases microbial growth and accordingly inulinase production. Kango (2008) also found yeast extract to be the best N-source in media containing dandelion roots. While meat extract (Singh *et al.*, 2007), peptone (Singh and Bhermi, 2008) and corn steep liquor (Viswanathan and Kulkarni, 1995) have also been reported to be better N-source.

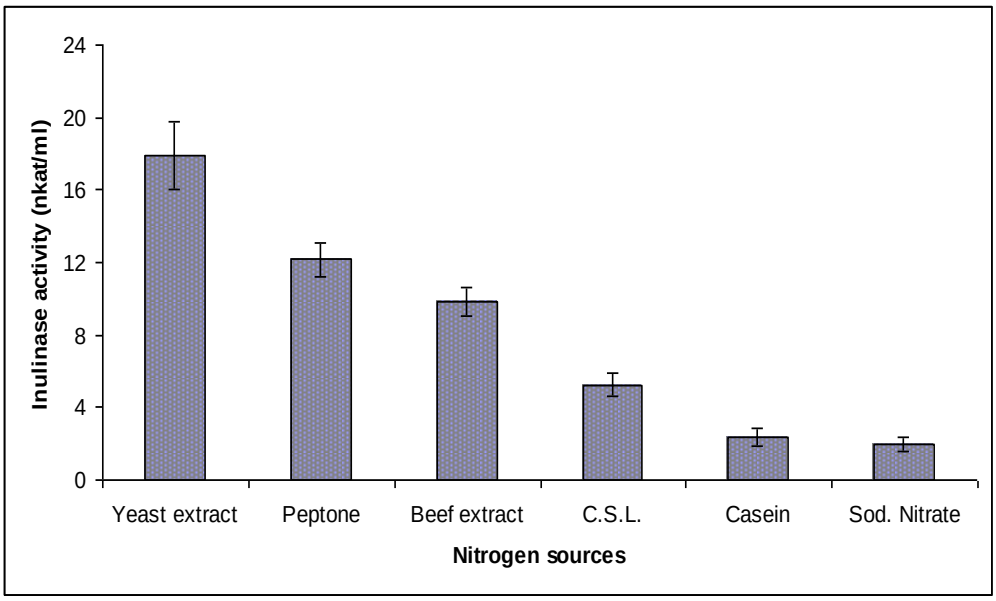

Figure 2: Effect of nitrogen source on inulinase production by *Fusarium oxysporum* RS-115 Media contained garlic extract (20% w/v) + nitrogen source (2% w/v). Results represent mean ± S.D. of three experiments.

Effect of pH and temperature on enzyme activity

The optimum pH for inulinase activity was found to be 5.0 (**Figure 3a**). Optimal pH 5.0 has also been reported for inulinase activity from *A. niger* NK-126 (Kango, 2008). However, *Fusarium oxysporum* displayed optimal activity for purified inulinases at 5.5-6.5 (Kaur *et al.*, 1992). Derycke and Vandame (1984) reported maximum inulinase activity at 4.3-4.4 for *Aspergillus niger*.

To determine the optimum temperature for enzyme activity, reactions were performed at various temperatures (30-90°C) at pH 5.0 for 15 min. Inulinase showed optimal activity at 50°C (**Figure 3b**). Temperature optima of 50°C have also been reported for *K. marxianus* and *A. niger* NK-126 (Jain *et al.,* 2011, Kango, 2008). Kaur *et al.* (1992) have reported optimum temperature 35-45°C for inulinases of *Fusarium oxysporum.* While optimum temperature has been reported as 60°C (Cazetta *et al.,* 2005) and 70°C (Singh *et al.,* 2007) for inulinase activity for *Kluyveromyces marxianus*.

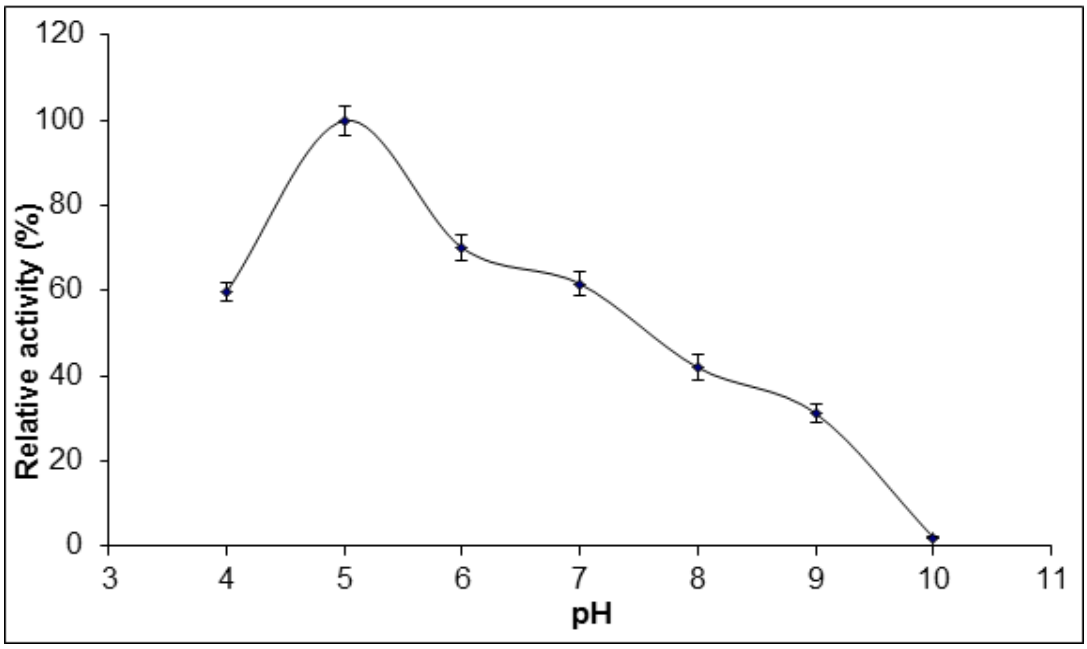

Figure 3a: Effect of pH on activity of inulinase of *Fusarium oxysporum* RS-115.

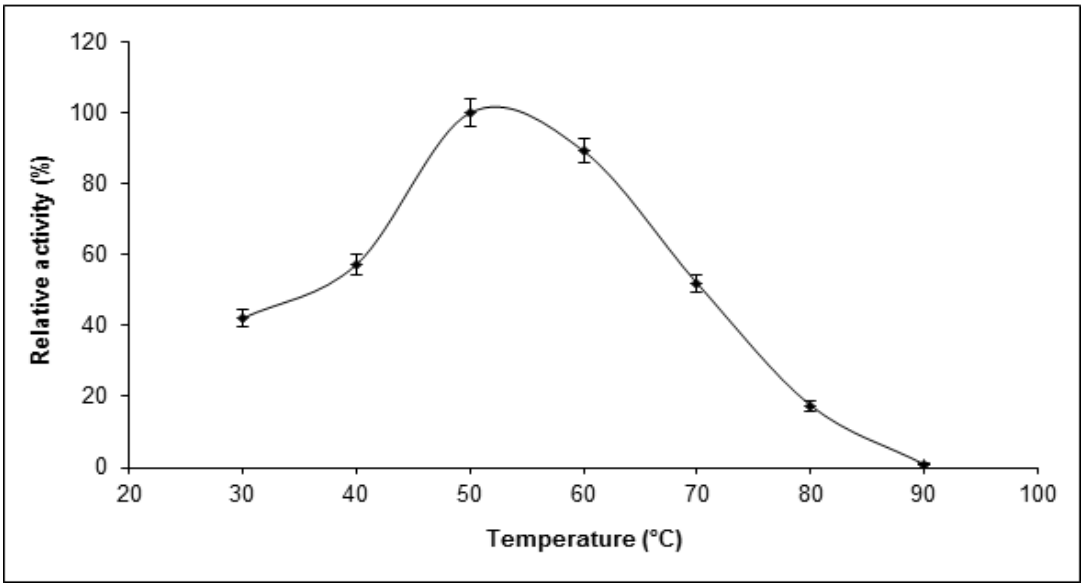

Figure 3b: Effect of temperature on activity of inulinase of *Fusarium oxysporum* RS-115.

Effect of metal ions and protein inhibitors on enzyme activity

The effect of various salts and protein inhibitions on inulinase activity was investigated at a concentration of 1 mM (**Table 2**). *F. oxysporum* RS-115 was slightly increased in presence of Mn^{2+}. Inulinase activity was found reduced significantly in the presence of Fe^{3+}, Mg^{2+}, Cu^{2+}, Ca^{2+} and Zn^{2+} ions and it was inhibited by Hg^{2+}. Singh *et al.* (2007) have also reported Mn^{2+} and Ca^{2+} to increase the enzyme activity by 2.4 and 1.2 folds,

respectively while Hg^{2+}, Ag^{2+} completely inhibited the activity of *K. marxianus* YS-1 inulinase. The strong inhibitory effect observed with Hg^{2+} suggested presence of some -SH- groups in the protein. This has been earlier observed for other microbial inulinases also (Sharma *et al.*, 2006). *Aspergillus fumigatus* exoinulinase (type II) was completely inhibited in the presence of 5 mM Hg^{2+} and Fe^{2+} whereas K^{+} and Cu^{2+} enhanced its activity (Gill *et al.*, 2004). Ba^{2+} and Ca^{2+} were found to stimulate the enzyme activity while Cu^{2+}, Fe^{3+}, Hg^{2+} were recorded as strong inhibitors in case of *Alternaria alternata* inulinase (Hamdy, 2002). Inulinase from *F. oxysporum* RS-115 was found reduced significantly in presence of EDTA (81.85 %) and PMSF (73.71) whereas the activity of inulinase was inhibited by pCMB.

Thin layer chromatography

Thin layer chromatography was used for qualitative analysis of the products of inulinase action. Inulinases obtained from various medium showed liberation of fructose from chicory inulin indicating only endoinulinase activity (**Figure 4**). The random attack by inulinolytic enzyme yielded fructose as the major product. However, it also showed formation of traces of F2 and kestose and nystose equivalents after 2 hours of incubation. Kango (2008) has found endoinulinases by *A, niger* NK-126 and endoinulinase from *Penicillium purpurogenum* produced F_3, F_4 and F_5 oligosaccharides (Onodera and Shiomi, 1988) while endoinulinase of *Penicillium* sp. TN-88 liberated only F_3 (Nakamura *et al.*,, 1997).

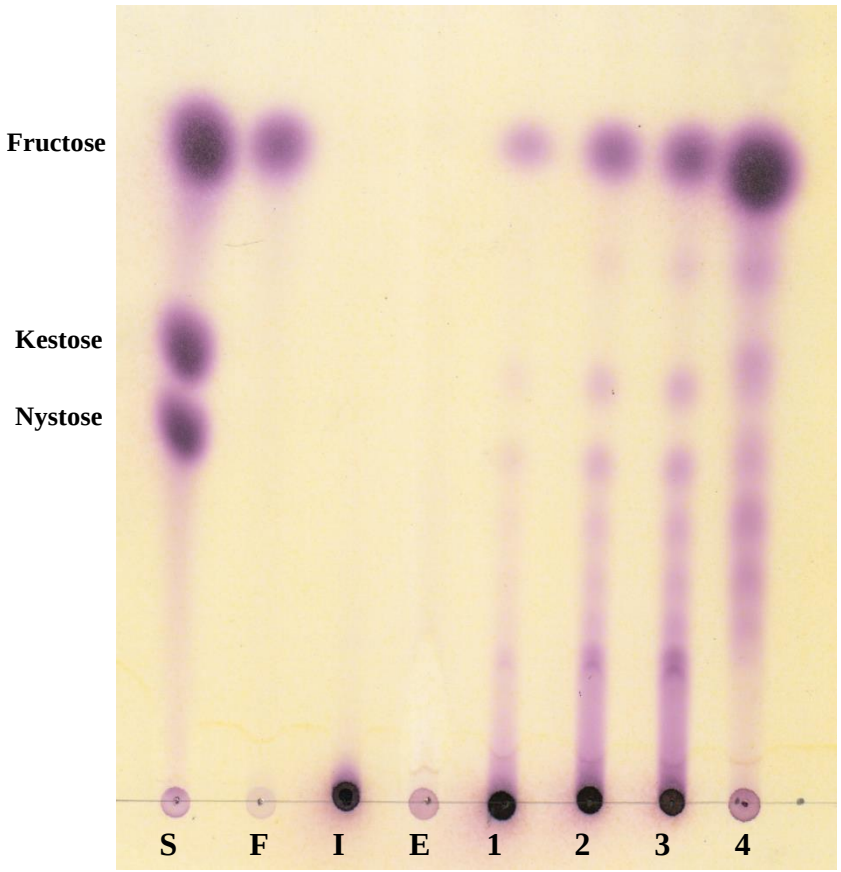

Figure 4: End product analysis of inulinase preparations of *Fusarium oxysporum* RS-115. S, standards (Fructose, kestose and nystose); F, Fructose; I, Dahlia inulin; E, enzyme sample of *Fusarium oxysporum*; Lane 1-4, end product of inulin hydrolysis after 0.5, 2 , 12, and 24 h, respectively.

CONCLUSIONS

In the present study we have found that crude garlic extract can be used as a low-valve substrate for synthesis of endo-inulinase using *Fusarium oxysporum* RS-115. Inulinase thus produced is optimally active at pH 5.0 and 50°C and liberates FOS (kystose, nystose and fructofuranosylnystose) and fructose from inulin. The present work clearly

demonstrates that potential of garlic bulb extract as a source of fructan for FOS generation.

ACKNOWLEDGEMENT

Authors are thankful for Head, Department of Microbiology, Dr. Harisingh Gour University Sagar, M.P. for providing research facility.

CONFLICT OF INTEREST

Authors have no conflict of interest whatsoever.

REFERENCES

Ayyachamy, M., Khelawan, K., Pillay, D., *et al*., 2007. Production of inulinase by *Xanthomonas campestris* pv. phaseoli using onion (*Allium cepa*) and garlic (*Allium sativum*) peels in solid state cultivation. *Letters Applied Microbiology*, **45**: 439-444; doi: 10.1111/j.1472-765X.2007.02222.x.

Beroigui, O., El Ghadraoui, L., Errachidi, F. 2023. Production, purification, and characterization of inulinase from *Streptomyces anulatus*. *Journal of Basic Microbiology*, **63**:427-438. doi: 10.1002/jobm.202200491.

Cazetta, M.L., Martins P.M.M., Monti, R., *et al*., 2005.Yacon (*Polymnia sanchifolia*) extract as a substrate to produce inulinase by *Kluyveromyces marxianus* var. *bulgaricus*. *Journal of Food Engineering* **66**: 301-305; doi: 10.1016/j.jfoodeng.2004.03.022.

Chen, X., Chen X., Zhu L., Liu W., *et al.*, 2022. Efficient production of inulo-oligosaccharides from inulin by exo- and endo-inulinase co-immobilized onto a self-assembling protein scaffold. *International Journal of Biological Macromolecules* **210**:588-599; doi: 10.1016/j.ijbiomac.2022.04.213.

Derycke, D.G. and Vandamme, E.J. 1984. Production and properties of *Aspergillus niger* inulinase. *Journal of Chemical Technology Biotechnology*, **34**: 45-51; doi: 10.1002/jctb.280340108.

Gill, P.K., Manhas, R.K., Singh, P. 2004. Purification and characterization of an exo-inulinase from *Aspergillus fumigatus*. *Applied Biochemstry and Biotechnology*, **117**:19-32.

Gupta, A., Gill, A., Kaur, N., Singh, R. 1994. High thermal stability of inulinases from *Aspergillus* species. *Biotechnology Letters* **16**: 733-734; doi: 10.1007/BF00136480.

Gupta, A.K., Kaur, N., Singh, R. 1989. Fructose and inulinase production from waste *Cichorium intybus* roots. *Biological Wastes*, **29**: 73-77; doi: 10.1016/0269-7483(89)90105-5.

Hamdy, H.S. 2002. Purification and some important characters of extracellular inulinase of *Alternaria alternata* (Fr.) Keissler. *Indian Journal of Experimental Biology*, **40**:1393-1398.

Housseiny, M.M. 2014. Production of an endoinulinase from *Aspergillus niger* AUMC 9375, by solid state fermentation of agricultural waste, with purification and characterization of the free and immobilized enzyme. *Journal of Microbiology*, **52**:389-398; doi: 10.1007/s12275-014-3561-y.

Jain, S.C., Jain, P.C., Kango, N. 2011. Production of inulinase from *Kluyveromyces marxianus* using Dahlia tuber extract. *Brazillian Journal of Microbiology*, **43**:62-69; doi: 10.1590/S1517-83822012000100007.

Kamble, P.P, Suryawanshi, S.S., Jadhav, J.P., *et al.*, 2019. Enhanced inulinase production by *Fusarium solani* JALPK from invasive weed using response surface methodology. *Journal of Microbiology Methods*, **159**:99-111; doi: 10.1016/j.mimet.2019.02.021.

Kango, N, and Jain, S.C. 2011. Production and properties of microbial inulinases: Recent advances. *Food Biotechnology*, **25**: 165-212; doi: 10.1080/08905436.2011.590763.

Kango, N. 2008. Production of inulinase using tap roots of dandelion (*Taraxacum officinale*) by *Aspergillus niger*. *Journal of Food Engineering*, **85**: 473-478; doi: 10.1016/j.jfoodeng.2007.08.006.

Kaur, N., Kaur, M., Gupta, A.K., *et al.*, 1992. Properties of β- fructosidases (invertases and inulinases) of *Fusarium oxysporum* grown on aqueous extract of *Cichorium intybus* roots. *Journal of Chemical Technology Biotechnology*, **53**:279- 284; doi: 10.1002/jctb.280530308.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry*, **31**:426-428; doi: 10.1021/ac60147a030.

Moriyama, S., Akimoto, H., Suetsugu N, Kawasaki S, *et al.*, 2002.Purification and properties of an exoinulinase from *Penicillium* sp. strain TN-88 and sequence analysis of the encoding

gene. *Bioscience Biotechnology and Biochemistry*, **66**:1887-1896; doi: 10.1271 /bbb.66.1887.

Nakamura, T., Shitara, A., Matsuda, S., Matsuo,*et al.*, 1997. Production, purification and properties of endoinulinase of *Penicillium* sp. TN-88 that liberates inulotrioses. *Journal of Fermentation and Bioengineering*. **84**:313-318; doi: 10.1016/S0922-338X(97)89250-1.

Onodera, S, and Shiomi, N. 1988. Purification and substrate specificity of endo-type inulinase from *Penicillium purpurogenum*. *Agriculture and Biological Chemistry*, **52**:2569-2576; doi: 10.1080/00021369.1988.10869080.

Rawat, H.K., Ganaie, M.A., Kango, N. 2015a. Production of inulinase, fructosyltransferase and sucrase from fungi on low-value inulin-rich substrates and their use in generation of fructose and fructooligosaccharides. *Antony van Leeuwenhoek*, **107**:799-811; doi: 10.1007/s10482-014-0373-3.

Rawat, H.K., Jain S.C., Kango, N. 2015b. Production and properties of inulinase from *Penicillium* sp. NFCC 2768 grown on inulin containing vegetal infusions. *Biocatalysis and Biotransformation*, **33**:61-68; doi: 10.3109/10242422.2015.1018188.

Rawat H.K., Soni H., Treichel H., Kango N. 2017. Biotechnological potential of microbial inulinases: Recent perspective. *Critical Reviews in Food Science and Nutrition*, **57**:3818-3829; doi: 10.1080/10408398.2016.1147419.

Rawat, H.K., Soni, H., Suryawanshi, R.K., *et al.*, 2021. Exo-inulinase production from *Aspergillus fumigatus* NFCCI 2426: The purification, characterization, and immobilization for the application of continuous use for fructose production. *Journal of Food Science*, **86(5):**1778-1790; doi: 10.1111/1750-3841.15681.

Sharma, A.D., Kainth, S., Gill, P.K. 2006. Inulinase production using garlic (*Allium sativum*) powder as a potential substrate in *Streptomyces* sp. *Journal of Food Engineering*, **77**:486-491; doi: 10.1016/j.jfoodeng.2005.06.072.

Singh, R.S., and Singh, R.P. 2010. Production of fructooligosaccharides from inulin by endoinulinases and their prebiotic potential. *Food Technology and Biotechnology*, **48**: 435-450.

Singh, R.S., and Bhermi, H.K. 2008. Production of extracellular exoinulinase from *Kluyveromyces marxianus* YS-1 using root tubers of *Asparagus officinalis*. *Bioresource Technology*, **99**:7418-7423; doi: 10.1016/j. biortech.2008.01.004.

Singh, R.S., Sooch, B.S., Puri, M. 2007. Optimization of medium and process parameters for the production of inulinase from a newly isolated *Kluyveromyces marxianus* YS-1. *Bioresource Technology*, **98**:2518-2525; doi: 10.1016/j.biortech.2006.09.011.

Skowronek M, and Fiedurek J. 2003. Selection of biochemical mutants of *Aspergillus niger* resistant to some abiotic stresses with increased inulinase production. *Journal of Applied Microbiology*. **95(4):**686-92. doi: 10.1046/j.1365-2672.2003.02027.x.

Trivedi, S., Divecha, J., Shah, A. 2012. Optimization of inulinase production by newly isolated *Aspergillus tubingensis* CR16 using low cost substrates. *Carbohydrate Polymers*, **90**:483-490; doi: 10.1016/j.carbpol. 2012.05.068.

Vandamme, E.J., and Derycke, D.G. 1983. Microbial inulinases: Fermentation process, properties and applications. *Advances in Applied Microbiology*, **29**:139-176; doi: 10.1016/s0065-2164(08)70356-3.

Viswanathan, P. and Kulkarni, P.R. 1995. *Saussurea lappa* (Kuth) as a new source of inulin for fermentative production of inulinase in a laboratory stirred fermenter. *Bioresource Technology*, **52**:181-184; doi: 10.1016/0960-8524(95)00023-8.