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EFFECTS OF GAMMA RADIATION ON INULINASE PRODUCTION BY *Aspergillus terreus*

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Abstract

This work aims to study the effect of gamma radiation on the inulinase production by the fungus Aspergillus terreus. The fungus was screened for the ability to produce enzymes from other strains belonging to the Collection of Microorganisms of Industrial Importance of the National Institute of Chemical-Pharmaceutical Research and Development. Subsequently, the fungi were irradiated at 3 doses (500, 1000 and 1500 Gy), using the vegetative form of the fungi. A fermentation assay for enzyme production was made using the DNS method. The best enzyme activity was 770.7 U/L, obtained from the media with orange peels. The use of gamma radiation increased the production of enzymes compared to tests without radiation. Statistically, the best dose of radiation was 1000 Gy.

Key words*: Aspergillus sp., inulinase, radiation, orange peel.*

INTRODUCTION

Recently, inulinases have received much attention as they can be widely applied to hydrolyze inulin for the production of fuel ethanol, fructose, and fructo-oligosaccharides (Gao et al., 2009). The various oligosaccharides derived from inulin also find their application in the medical and dietary sector. High fructose syrup and fructooligosaccharides are two major industrial applications of inulinases. The industrial production of short-chain fructo-oligosaccharides and inulo-oligosaccharides is expanding rapidly due to the pharmaceutical importance of these compounds (Guimaraes et al., 2007; Maria Rosa Vela Sebastiao Fernandes and Bo Jiang, 2013). Inulinases are a group of hydrolases which target on the β-2,1 linkage of inulin and hydrolyze it into fructose and glucose. Inulinases can be divided into endo-inulinase and exo-inulinase. The endo-inulinase hydrolyzes the internal linkages in inulin to produce inulotriose, inulotetraose, and inulopentaose as the main products. The exo-inulinase hydrolyzes inulin into fructose and glucose, then, the formed fructose and glucose can be further fermented into ethanol by specific microorganisms (Li et al., 2013). Inulinases can be secreted by a variety of microbes including fungi, yeasts, and bacteria (Gavrailov and Ivanova, 2014; Ram Sarup Singh and Kanika Chauhan, 2016; Yun et al., 1997; Nirobol et al., 2012). Among them, *Aspergillus* and *Kluyveromyces* strains are generally preferred choices for commercial applications (Zhang et al., 2004). Recently, many studies have been conducted using inulinase from *Aspergillus* for enzymatic hydrolysis of inulin (Gill et al., 2006; Sirisansaneeyakul et al., 2006). Some efforts have been made to enhance enzyme activity of *Aspergillus* such as transgene expression (Zhang et al., 2004) and coculture with other species (Ge et al., 2009).

Artificial mutations are mutations that are experimentally induced using a wide range of mutagens. Mutagens are classified by nature in three categories: physical, chemical, and biological. Chemical or radiation-based mutagenesis (eg UV and gamma) are approaches that have the potential to generate gain of function mutations. In our study we have been working on obtaining mutants by ionizing radiation on the strain of *Aspergillus terreus*. Gamma radiation was use in order to increase inulinase productivity.

MATERIALS AND METHODS

The *Aspergillus terreus* microorganism is part of our Culture Collection of Microorganism of Industrial Importance, a newly isolated microorganism. The ionized radiation treatment was achieved using 3 different doses, 500, 1000 and 1500 Gy, conducted with the support of a team from IFIN-HH. The reagents (organic

solvents, analytical reagents and mineral salts) used for research were purchased from Merck and Sigma-Aldrich.

Obtaining mutants to increase bioproductivity of inulinases

After ionizing radiation treatment of the *Aspergillus terreus* strain ICCF 262, the fungus in liquid suspension was seeded on agarized media with 1% inulin content (1% inulin, 0.3% malt extract, 0.3% yeast extract, 0.5% peptone, 2% agar). Isolated colonies were selected and passed to the inoculum phase and then to the bioprocess to observe the differences in enzymatic activity of the inulinases.

Culture media and cultivation conditions

Preinoculum phase. The 17 mutated *Aspergillus terreus* strains that were tested, were cultivated on solid agar medium, PDA (potato dextrose agar). They were incubated in optimal development conditions: 24-48 h / 28 - 29°C.

Inoculum phase. The preinoculum tubes were washed with 2 ml sterile inoculum medium and inoculated into 500 ml Erlenmayer flasks containing 100 ml of liquid medium.

The bioprocessing phase was performed according to the classical scheme, in optimal temperature conditions (28-29ºC), initial pH 6.5 and biosynthesis time (3 or up to 7 days), for the development of tested microorganisms, but also for the induction of inulinase production. The inoculum volume was 2% using 500 ml Erlenmayer vials with 100 ml of liquid medium (shaking 220 rpm). The main sources of carbon in the media were the different sources of inulin (laboratory grade inulin and orange peel (agro-food waste)) along with the other components indispensable to the development of microbial life: nitrogen sources (corn extract, peptone, ammonium salts, yeast extract) and secondary nutrients (potassium phosphate, magnesium sulphate, sodium chloride, citric acid).

The *Aspergillus terreus* strain ICCF 262 is grown on inoculum medium with the following composition: 1% glucose, 0.3% malt extract, 0.3% yeast extract, 0.5% peptone, for 24 h and then grown for accumulation of inulinase, on biosynthesis medium with the following composition: 2% yeast extract, 0.3% NH_4NO_3, 0.4% $(NH_4)_2HPO_4$, 0.1% KH_2PO_4, 0.05% $MgSO_4$. The fermentation conditions were 28-29°C, initial pH 6.5, agitation on a rotary shaker with 220 rpm and 2 cm agitator eccentricity for 7 days.

Isolation of microbial inulinase

After fermentation, the supernatant was separated by centrifugation at 8000 rpm, 4°C for 20 minutes, in a centrifuge (Hettich - Germany).

Measurement of enzyme activities

The inulinase activity was assessed as follows: 0.1ml of crude enzyme solution was mixed 0.9 ml of 2% inulin in acetic buffer 0.1M at pH 5.5. The sample was incubated at 50°C for 15 min. The reducing sugars were determined by the dinitrosalicylic acid (DNSA) method (Miller, 1959). One activity unit is defined as the amount of enzyme required to produce one micromole of reducing sugar per minute under assay conditions.

RESULTS AND DISCUSSIONS

To optimize the yield of inulinase production, we monitored both the evolution of cell growth by determining optical density, dry biomass, final pH value, and the enzymatic activity of extracellular inulinases.

Following the treatment with ionizing radiation, 17 mutant strains were tested to monitor the increase in inulinase bioproductivity (Figure 1, Figure 2 and Figure 3).

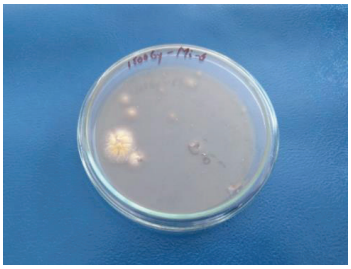

Figure 1. Mutants of *Aspergillus terreus* strain after gamma radiation treatment - 1500 Gy

It can be seen from Table 1 that five strains (6, 7, 8, 10 and 17) had the highest activity; these 5 strains were selected for bioproductivity testing in case of agro waste as C source (orange peel). The mutant strains G1, G2 and G5 (see Table 2) had the highest enzymatic activity.

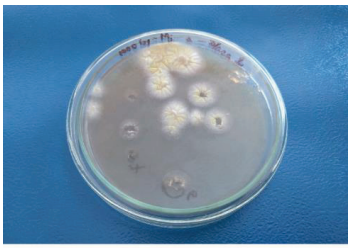

Figure 2. Mutants of *Aspergillus terreus* strain after gamma radiation treatment - 1000 Gy

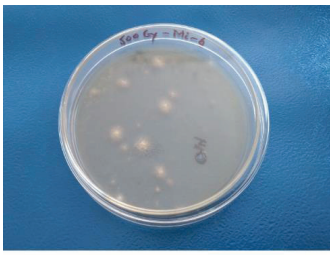

Figure 3. Mutants of *Aspergillus terreus* strain after gamma radiation treatment - 500 Gy

Table 1. Study to determine the bioproductivity of inulinases with pure inulin as C source (values after 72 h of bioproces)

Strain n (isolated mutants)	Final volume (mL)	pH	Enzymatic activity (U/ml)
1	45	3.51	0.21
2	47	3.36	0.19
3	47	4.47	0.22
4	36	3.92	0.19
5	56	2.92	0.06
6	**48**	**3.84**	**0.48**
7	**55**	**2.69**	**0.58**
8	**50**	**3.13**	**0.59**
9	42	3.24	0.24
10	**36**	**4.67**	**0.65**
11	38	3.32	0.05
12	47	3.23	0.19
13	43	3.39	0.21
14	37	4.0	0.25
15	54	3.50	0.23
16	36	3.25	0.14
17	**38**	**4.79**	**0.39**

Table 2. Study to determine the inulinases bioproductivity of the mutant strains with agro-food waste (orange peel) as C-source (values after 72 h of bioprocess)

Strain n°	pH	Enzymatic activity (U/ml)
G1	**2.8**	**0.64**
G2	**2.68**	**0.77**
G3	2.03	0.13
G4	1.93	0.49
G5	**2.39**	**0.55**

CONCLUSIONS

Research in the production and application of FOS and IOS is gaining momentum due to several health benefits and biofunctional properties of these compounds. Prebiotics are produced by crops such as chicory and Jerusalem artichoke. However, FOS can be synthesised *in vitro* from precursors such as sucrose using fructosyltransferase enzymes. Furthermore, IOS can also be produced from the enzymatic hydrolysis of inulin under controlled conditions. The main drawback of the production process is the low yields of FOS. It is therefore crucial to explore other methods such as molecular methods to improve the efficiency of the enzymes involved in the synthesis of FOS and IOS. More research on the efficacy and mode of action of prebiotics is critical to harness maximum benefits from the preparation and consumption of these oligosaccharides.

Aspergillus terreus strain was chosen to be the most important in terms of bioproductivity of inulinases on different nutrient substrates.
The optimization of the biosynthesis conditions of selected strains following screening of enzymatic activities was carried out by diversifying the carbon source using inulin and agro-alimentary by-products (orange peel). The highest inulinase activity was achieved with the mutant strain G2 (0.77 U/mL), grown on orange peel as a inulin source.
These values are comparable to the values reported in the literature for the *Aspergillus niger* strain (Rawat et al., 2015; Fawzi 2011; Cruz et al.,1998, Singh and Chauhan, 2016).
It is noted that the bioproductivity of the strains is improved with the use of substrate from agro-food waste.

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