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Enhancement of Inulinase Production from Chicory and Fenugreek Rhizosphere Soil

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Abstract: The chicory and fenugreek rhizosphere soil were used for isolating two fungal species like *Penicillum purpurogenum* and *Aspergillus niveus*, tested for the inulinase enzyme activity. When compared to fungal species isolated from fenugreek rhizosphere, fungal species isolated from chicory rhizosphere produced more inulinase. The activity of inulinase was tested with different parameters such as UV light, pH, temperature and incubation period. In general *Penicillum purpurogenum* showed more inulinase production than *Aspergillus niveus*. After 15 min of UV exposure at pH 4.0 and at temperature 50°C, an increase in inulinase content was observed in 5 days incubation.

Key words: *Penicillum purpurogenum* · *Aspergillus niveus* · rhizosphere · inulinase · incubation period · temperature · pH · UV radiation

INTRODUCTION

Study of medicinal plants is one of the essential and interesting branches of medicine. Man is dependent on plants as they play a vital role in sustainence of life. Herbal medicines have occupied an important position in India than the other countries of the world [10]. The importance of plant derived drugs are potential source of purified biochemicals and also as a source of herbal medicines [2]. The term rhizosphere is defined as the soil volume adjacent to the roots and influenced by them [3]. It represents an area of intense microbial activity [4] in which the organic nutrients coming from the roots favour the development of microorganisms [5]. These nutrients are originated by the deseamation of cells and of exudates such as sugars, organic acids, amino compounds as well as other substances released by the roots [6, 7]. Thus, the rhizosphere of plants that accumulate carbohydrates in the roots, as well as the plant material in decomposition are common source of a microbiota able to produce useful metabolites for industry [8-10].

Inulinase producing microorganisms selected by plating techniques [11] from the rhizosphere of plants like whose root contain inulin, *Taraxacum officinarum* [10], *Helianthus tubexosus* L. [12], *Cichorium intybus* L., *Dahlia pinnata* and *Helianthus annus* L. [13], members of Asteraceae. The inulinase (2,1-b-D-fructanohydrolase EC 3.2.1.7) hydrolyses the inulin into pure fructose, being an excellent alternative for the production of fructose syrups. This sugar is used by the food and beverage industries, besides sweeteners and shows several advantages in comparison to sucrose, being less cariogenic, highly soluble and hygroscopic and therefore, less prone to form crystals; has low calory content and does not cause arterioscelerosis. Furthermore, fructose may be used by diabetic patients and mask the bitter taste of saccharin [14-17]. Inulinases has also been employed for kidney disease diagnosis [18]. With the ever increasing need of inulinase, it is necessary either to isolate different types of microorganisms producing inulinase or to enhance the yield of inulinase in the already available organisms [19]. The present study was aimed to find out the inulinase activity of *Penicillum purpurogenum* and *Aspergillus niveus* cultures isolated from chicory and fenugreek *rhizosphere* soil and also to find out the effect of UV light, pH, temperature and incubation period on the activity of inulinase.

MATERIALS AND METHODS

The *Penicillum purpurogenum* and *Aspergillus niveus* sp used in the present study were isolated from rhizosphere of chicory and fenugreek [17, 19]. The rhizosphere samples (25 g) were suspended in 225 ml of sterilized distilled water (1:10 dilution) and subsequently 10 ml of this suspension was added into 990 ml of

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sterilized distilled water. Petridishes containing the Sabouraued agar medium [20] plus chloraphenicol (100 mg l^{-1}) and Bengal Rose (50 mg l^{-1}) were inoculated with 1 ml of the 1:1000 diluted soil suspension. The plates were kept at room temperature (25±2°C) and the growth of the colonies was accompanied upto 72 h. Fragments of the individual colonies were transferred separately to the same medium containing 50 mg l^{-1} of chloranphenicol. The strain was identified after growth on Czapek Agar by observing its macroscopic characteristics (colour, texture, apperance and diameter of the colonies) and microscopic characteristics (Microstructures) according to Raper and Fennell [21].

Inoculum preparation: *Penicillum purpurogenum* and *Aspergillus niveus* were subcultured on the PDA (potato dextrose agar) and incubated at 30°C for 72 h. Spores from the slants were suspended in sterile 0.85% saline containing 0.01% tween 80 to obtain 2.0106 spore ml^{-1}. For all the experiments 0.5 ml of this suspension was used.

Biomass determination: The mycelical mass of *Penicillum purpurogenum* and *Aspergillus niveus* were collected by filtration (Whatman paper No. 1) of the culture medium. The biomass was determined after washing the mycelial mass with distilled water and dried at 105°C overnight untill constant weight attained according to Warcup [22].

Effect of UV light, pH and temperature on inulinase activity: Inoculum having 2.0106 spore ml^{-1} of *Penicillum purpurogenum* and *Aspergillus niveus* strains were prepared in physiological saline solution and 10 ml of spore suspension was poured at the centre of 9 cm wide agar plates and spread with the help of a sterile spreader following exposure to UV light (60 w) for 5-25 min at a distance of 15 cm from the UV source and pH (2-6) and each petriplate was wrapped in black paper and incubated at different temperature (30-70°C) and pH (2-6). After 4 days of incubation, number of colonies was counted and petriplates having 1.0 % survival rate were used for inulinase study. After selecting well separated colonies, each colony was picked up with the help of a sterile loop and transferred to fresh PDA slants and incubated at 50°C for 4 days. After 4 days of growth, spore suspension was prepared as described earlier and inoculum containing 10 spores /ml were inoculated in 250 ml Erlenmeyer flasks containing 50 ml PDA supplemented with 1% inulin (w/v). The flasks were incubated in rotatory incubator shaker (Sunsine, India) at 200 rpm for 9 days at 50°C. The culture broth was centrifuged at 8000 rpm for 20 min at 4°C and supernatants were stored at 20°C for enzymetic assays according to Miller [23].

Statistical analysis: The effect of UV light, pH, temperature and incubation period on the activity of inulinase were monitored at regular intervals. Data of three independent experiments represented by 5 replicates from each experiment were subjected to statistical analysis (Mean ± SE) and New Duncan's Multiple Range Test [24].

RESULTS AND DISCUSSION

Penicillum purpurogenum and *Aspergillus niveus* strains were screened for the inulinase activity under different treatments. The incubation period influenced inulinase production and maximum inulinase activity was observed at 5th day after culturing (Table 1). The *Penicillum purpurogenum* cultures exhibited more inulinase activity than *Aspergillus niveus* especially the cultures isolated from rhizosphere of chicory (Table 1). A 2.5 total higher inulinase activity was observed in *Penicillum purpurogenum* and *Aspergillus niveus*. Further, in order to increase inulinase productivity, present study has tried with different pH, temperature and UV treatment on *Penicillum purpurogenum* and *Aspergillus niveus* (Tables 2-4). The *Penicillum purpurogenum* cultures incubated at 50°C were produced more inulinase than the cultures incubated at 30, 40, 60, 70°C at 5th day of incubation (Tables 1 & 2). The results are in accordance with [10, 25, 26] works with *Aspergillus* sp, best inulinase activity produced by fungi

Table 1: Effect of incubation period on inulinase activity in the fungi isolated from the rhizosphere of fenugreek and chicory

Fungal species	Incubation period (Days)	Inulinase activity (U ml^{-1}) Fenugreek	Chicory
P. purpurogenum	3	08.00±0.42^{e}	09.00±0.64^{f}
	4	11.00±0.76^{b}	12.00±0.79^{c}
	5	12.00±0.87^{a}	13.55±0.99^{a}
	6	09.00±0.62^{d}	10.00±0.76^{e}
	7	08.00±0.52^{e}	09.00±0.63^{f}
A. niveus	3	06.00±0.42^{g}	07.00±0.63^{h}
	4	10.00±0.88^{c}	11.00±0.74^{d}
	5	11.50±1.02a^{b}	13.00±0.88a^{b}
	6	08.55±0.62d^{e}	10.00±0.76^{e}
	7	07.70±0.74^{ef}	08.00±0.56^{g}

Each value represents the mean ± standard error (S.E.) of five replicates per treatment in three repeated experiments. Means within a row followed by the same letters are not significant at p=0.05 according to DMRT

Table 2: Effect of Temperature on inulinase activity in the fungi isolated from the rhizosphere of fenugreek and chicory

Fungal species	Temperature (°C)	Inulinase activity (U ml^{-1}) Fenugreek	Chicory
P. purpurogenum	30	09.00±0.52^{g}	10.00±0.86^{f}
	40	12.00±0.88^{c}	13.00±0.97b^{c}
	50	14.00±0.96^{a}	14.55±0.99^{a}
	60	10.50±0.77^{a}	11.00±0.89^{e}
	70	09.00±0.62^{g}	10.00±0.88^{f}
A. niveus	30	07.00±0.74^{h}	08.00±0.62^{h}
	40	11.00±0.66^{d}	12.00±0.77^{d}
	50	13.00±1.02^{b}	13.50.±0.87^{b}
	60	10.00±0.94^{ef}	11.22±0.75^{de}
	70	08.88±0.74^{gf}	09.00±0.56^{g}

Table 3: Effect of pH on inulinase activity in the fungi isolated from the rhizosphere of fenugreek and chicory

Fungal species	pH	Inulinase activity (U ml^{-1}) Fenugreek	Chicory
P. purpurogenum	2	09.50±0.57^{ef}	10.22±0.74^{fg}
	3	14.44±1.02^{a}	15.00±1.04^{a}
	4	12.50±0.78^{c}	13.00±0.88^{c}
	5	10.66±0.76^{d}	11.17±0.74^{e}
	6	10.00±0.75^{de}	10.75±0.71^{ef}
A. niveus	2	07.42±0.52^{h}	09.00±0.62^{h}
	3	13.85±0.86^{ab}	14.55±0.98^{ab}
	4	09.52±0.82^{e}	12.00±0.79^{d}
	5	10.00±0.88^{de}	10.65±0.74^{f}
	6	08.55±0.74^{g}	09.00±0.66^{h}

Table 4: Effect of UV exposure on inulinase activity in the fungi isolated from the rhizosphere of fenugreek and chicory

Fungal species	UV exposure (min)	Inulinase activity (U ml^{-1}) Fenugreek	Chicory
P. purpurogenum	5	10.00±0.88f^{g}	22.55±0.66^{d}
	10	12.05±0.92d^{e}	25.50±0.78^{bc}
	15	15.55±1.04^{a}	35.50±1.22^{a}
	20	14.00±0.96^{bc}	27.60±0.94^{b}
	25	12.05±0.82^{de}	22.05±0.71^{de}
A. niveus	5	08.00±0.65^{h}	10.12±0.52^{i}
	10	10.55±0.82^{f}	12.22±0.87^{h}
	15	14.55±0.88^{b}	17.16±1.22^{f}
	20	12.44±0.82^{d}	15.52±0.77^{fg}
	25	09.22±0.77^{g}	10.99±0.67^{hi}

Each value represents the mean ± standard error (S.E.) of five replicates per treatment in three repeated experiments. Means within a row followed by the same letters are not significant at p=0.05 according to DMRT

was observed at pH range between 4.0 and 7.0 and temperature around 45°C and 50°C. Vanddamme and Derycke [10] obtained higher inulinase activity at pH 4.4 and 4.6 at temperature 55°C and 56°C. Baysal *et al.* [27] found maximum activity at 56°C and at pH 4.0-4.5 at 60°C some species of *Aspergillus* produce inulinases with maximum activity at higher temperatures 60°C. Above 55°C could inactivate some inulinases produced by fungi [10]. In our study inulinase production increased at pH 3 and at 50°C which are found as optimum conditions and were used to study the inulinase production in mutated strains. Similar to our results on extracellularly produced inulinase in *Penicillum purpurogenum* and *Aspergillus niveus* possesses higher thermostablitty by maintaining 95% residual activity after I h incubation at 60°C and it shows maximum inulinase activity at 50°C [19, 28, 29]. Hence improved strain of this study, which has maintained thermostablity, must be a significant one for industry where inulin hydrolysis is carriedout in higher temperature (about 60°C) to prevent microbial contamination and also because of higher solubility of inulin at elevated temperature.

The *Penicillum purpurogenum* and *Aspergillus niveus* were screened after 5-25 min UV treatment (Table 4) and inulinase profile was studied in these mutants for 7 days after culturing. The *Penicillum purpurogenum* cultures exhibited more inulinase activity than *Aspergillus niveus* especially the cultures isolated from rhizosphere of chicory (Table 4). Among these mutants, colonies were selected as the representative samples for inulinase study and further tested in 250 ml culturing medium on a rotatory shaker. The inulinase activity of the investigated mutants was substantially higher in comparison to the parent strain but a significant enhancement was observed in 15 min UV treated cultures (Table 4). The results of the present study supported by several findings [19, 28-30], who have observed 2-fold increase in inulinase activity after UV mutagenesis. This makes it possible to utilize *Penicillum purpurogenum* and *Aspergillus niveus* mutants for industrial production of enzymes. Further improvements may be achieved by optimizing the culture conditions in bioreactors such as oxygen supply which or cannot be controlled in a flask culture. In conclusion, there seems to be increased ability to synthesize inulinase among selected mutants with high enzymatic activity. Aditionally, better adaptation of these mutants to abiotic stresses can affect fungal growth and enzyme productivity. The rational for the procedures used are direct rather than pleiotropic. The advantage gained in direct screening is to reduce in a very specific way the number of cultures isolated from the plates, which would normally require testing of productivity via shake flask culture.

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