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Isolation of Xylan degrading enzyme from *Trichoderma spp.*

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The use of hemicellulolytic enzymes has recently attracted considerable interest as a substitute for chlorine chemicals in pulp bleaching in view of the environmental concerns. The cellulase free xylanase from microorganisms has been isolated and tested for bleaching activity, giving rise to a new concept of biobleaching. In this aspect, the present study aims to check the tolerance of *Trichoderma spp.*, a biological controlling agent, in perceptive to the production of xylanase enzyme. The strains were isolated from the environmental soil samples taken from the effluent treatment area. The organisms were subjected to growth at various pH conditions. The resistant strains T-1 and T-2 to a different pH were further isolated and grown by on selective xylan-agar medium. Maximum growth of the organism was found at 48h under submerged condition in xylan containing enriched medium. The organisms produce an extra cellular xylanase that had a low molecular weight and optimal working temperatures. Further proposed studies include the bleaching activity of the enzymes and comparison of the industrial application with the commercially available enzymes. The trials can also be carried out in paper processing industry and the bleaching sequences can be analyzed for efficacy of bleaching and pre bleaching.

Key Words: Solid state Fermentation, Submerged Fermentation, *Trichoderma spp*, Xylanases

Enzymes are the catalytic cornerstone of metabolism and as such are the focus of intense worldwide research, not only in the biological community, but also with process designers/engineers, chemical engineers and researchers working in other scientific fields. Since ancient times, enzymes have played a central role in many manufacturing processes, such as in the production of wine, cheese, bread, modification of starch etc. The latter half of the twentieth century saw an unprecedented expansion in our knowledge of the use of microorganisms, their metabolic products, and enzymes in a broad area of basic research and their potential industrial applications (Beg *et al.,* 2001). Xylanases (1,4-P-D-xylan xylanohydrolase; EC 3.2.1.8) are hemicellulases that hydrolyze xylan, which is a major constituent of the hemicellulose complex. **(**Browning, 1963**).** These compounds are present in the cell wall and in the middle lamella of plant cells. This term covers a range of noncellulose polysaccharides composed, in various proportions, of monosaccharide units such as D-xylose, D-mannose, D-glucose, L-arabinose, D-galactose, D-glucuronic acid and D-galacturonic acid. Classes of hemicellulose are named according to the main sugar unit. Thus, when a polymer is hydrolyzed and yields xylose, it is a xylan. Hemicelluloses include mannans, glucans, arabinans and galactans (Whistler and Richards 1970; Viikari *et al.*, 1994; Uffen 1997; Ebringerova

and Heinze 2000). Viikari *et al*. (1986), for the first time, demonstrated the usefulness of xylanase in reducing the consumption of bleach chemicals. Today, the beneficial effects of xylanase have also been commercially demonstrated in the pulp and paper industry *(*Kulkarni *et al., 1999,* Techapun *et al., 2003)*

The interest in xylan degrading enzyme and its application in pulp and paper industries has advanced significance over the past few years. A survey of several microorganisms that produce xylanases that could be purified readily indicated that *Trichoderma spp.* would be a good source of the enzyme. The objective of the present study is to extract and characterize xylanases from *Trichoderma spp*. Isolated from environmental soil samples.

Materials and Methods

Materials used

Potato Dextrose Agar (PDA), Xylan – from Oat spelt, D – xylose, Carboxy Methyl cellulose sodium salt, 2- hydroxy-3,5-dinitrobenzoic acid (DNS), were obtained from Hi Media. The other reagents were of analytical grade whilst the buffers used were freshly prepared and of analytical grade.

Collection of Soil Samples

The soil sample used for the present study to isolate the fungal strains was kindly provided by Srivari Paper Boards, Gobichettypaalayam, India.

Selection of strains

The micro organism used for the study was *Trichoderma spp.* isolated from the environmental soil samples. The isolated strains were grown on Trichoderma Selective Medium (TSM) and stock cultures were maintained on PDA slants.

Production of enzyme

Spores were obtained by culturing the organism at 28°C in sporulating medium (Trisodium citrate -5g, KH_2PO_4 – 5g, NH_4NO_3 – 2g, $(NH_4)_2SO_4$ – 4g, $MgSO_4$ – 0.2g, peptone – 1g, yeast extract – 2g, glucose – 2g and distilled water 1000mL) at pH of 5.5. The sporulating medium was taken as the inoculating medium. A loopful of *Trichoderma spp* was transferred into the Enzyme production medium and Modified Vogel's Medium for enzyme production. These Flasks were incubated at 150 rpm at 28°C for five days.

Harvest of culture

Liquid state cultures were harvested by centrifugation at 10000rpm for 20 minutes at 4°C and the resulting supernatant was called as crude enzyme preparation. The crude enzyme extract was used for the following further assays.

Enzyme Activity Assay

Xylanase was assayed by measuring the release of reducing groups from a xylan substrate. The enzyme activity was measured by modifying the method described by Khanna and Gauri (1993), using a 0.1 M sodium citrate buffer with pH 5. One ml of 1 % xylan solution (in 0.1 M, pH 5 sodium citrate buffer) and 2ml of enzyme were added to the reaction tubes and incubated at 40°C and the amount of reducing sugar in the reaction tubes was measured using the DiNitrosalicylic Acid Method (DNS) described by Miller (1959). The absorbance was read at 550 nm using UV spectrophotometer. The amount of reducing sugar was calculated from the standard curve based on the equivalent xylose. One unit of xylanase activity is defined as 1µmol of xylose equivalent produced/min under the assay conditions.

Effect of Carbon Source on xylanases production

The *Trichoderma spp* was grown in Vogel's Medium with different Carbon Sources like Glucose, Xylose and arabinose to check their effect on the expression of xylanases.

Effect of time factor on xylanase production

The time course of xylanase production by the *Trichoderma spp* in vogels

medium containing 1% xylan as sole carbon source was assayed from cell supernatant at 50˚C and pH 5 for every 24 hours.

Results and Discussion

Growth of the culture

Trichoderma spp. T-1 and T-2 were used in this study. EPM was used for the growth of fungi. However a comparatively better growth was obtained in the modified voegels media for the sporulation and enzyme production.

Optimum pH for Xylanase Production

The optimum pH for the growth of *Trichoderma spp.* and xylanase production was found to be 5.5 for T-1 and 5.7 for T-2.. Hence the pH of the medium was adjusted to in accord for the growth of the *Trichoderma spp.* in Vogel's medium in all subsequent experiments (Fig. 1).

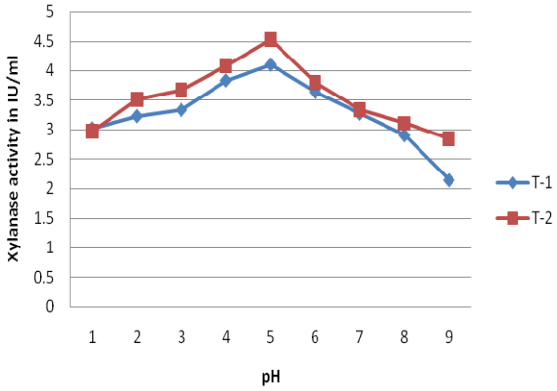

Fig. 1: Effect of initial pH values on Xylanase Production

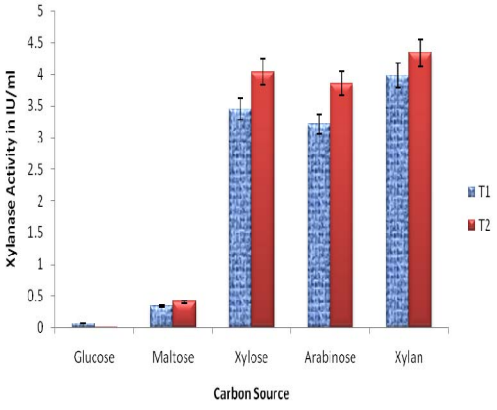

Fig. 2: Effect of Carbon sources on Xylanases Production

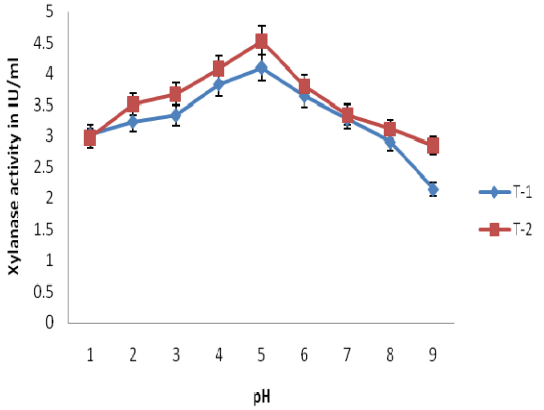

Fig. 3: Effect of pH on xylanase activity

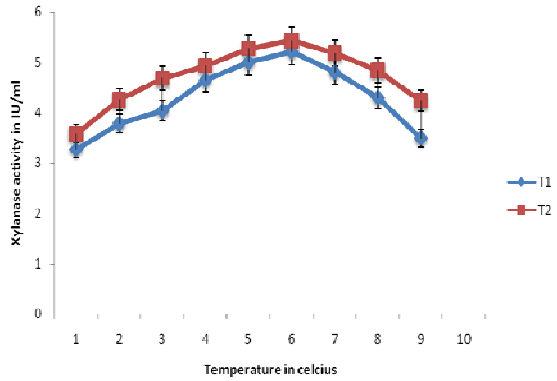

Fig. 4: Effect of Temperature on xylanase activity

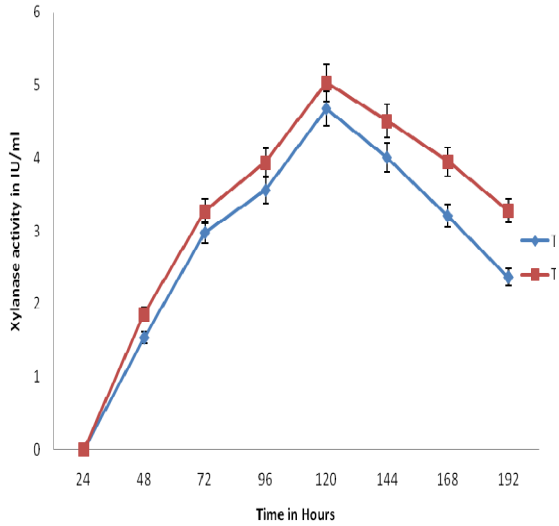

Fig. 5: Effect of time factor on xylanase production

Optimization of xylanase activity

Three different buffers (sodium citrate, sodium acetate and phosphate buffers) were used to evaluate which buffer yielded the maximum xylanase activity by DNS Method with xylose as standard. Maximum activity was observed in the citrate phosphate buffer. This buffer was used for the subsequent experiments.

Effect of carbon source on xylanase production

Effect of different carbon sources on xylanase expression was also studied. It is well established fact that the culture conditions effect significantly the production of xlyan degrading enzymes. The choice of appropriate substrate is of great importance in producing the xylanase in large scale level. These substrates not only serve as the carbon sources but also they are necessary inducing compounds for the growth of the compounds. All the carbon sources were testing 1% (w/v). The production was about to increased (Fig. 2).

Effect of pH and Temperature on crude xylanase activity

The xylanases activity investigation at various pH values revealed that optimum pH for crude xylanases was 5 for T-1 and 5.4 for T-2 (Fig. 3). The xylanases activity investigation at various temperatures revealed that optimum temperature for crude xylanases was 55◦ C for T-1 and 54◦ C for T-2 (Fig. 4)

Effect of time factor on xylanase production

The production of crude xylanases was assayed for different time intervals at 0, 24, 48 and 72 hours respectively. At 120 hours, the production was found to be 4.68 IU/ml for T1 and 5.03IU/ml for T2 (Fig. 5).

The mechanism by which the plant hydrosylates interact with cells and particularly with xylanase production machinery is unclear (Xiong *et al*., 2005). In this regard our result was coinciding from the one reported by Dhillon *et al*., (2005) for *Bacillus circulances*, where an increase in biomass and xylanase were observed. The fast decreases in both xylanase activity and the biomass were surprising and microscopic observation of the fermented broth indicated autolysis (Reys *et al*., 2000). On the other hand, the use of agricultural residues rich in hemicellulose increased xylanase production (Dhillon *et al*., 2000). Contaminating cellulase in xylanase preparation can, under certain conditions resulted in a reduction of fibre strength (Da-silva *et al*., 1994). Xylanase might be considered to be belonging to the wide gap of enzymes which are used as bleaching boosting agent and decreasing the bleaching chemical requirements (Damiano *et al*., 2003).

Xylanase production on an industrial scale is based on a microbial biosynthesis. Filamentous fungi, such as *Aspergillus, Penicillium* and *Trichoderma* (Anzin *et al.,* 2007), have been most extensively studied and have demonstrated a great capability for secreting a wide range of xylanase. There are two possibilities for cultivation of microbial xylanase producing strain, either submerged cultivation or solid-state. Currently, 80–90% of commercial xylanase are produced in submerged culture because it has a higher degree of processes intensification and a better level of automation

Xylanases from some other bacterial strains were also used to increase the brightness. Several aspects of xylanases have stimulated research on the study of biochemical, regulatory and molecular aspects of xylanolytic enzyme systems (Kulkarni *et al*., 1999). In addition xylanases have a significant impact on industrial scale. There will be a need to consistently effective under various operating conditions.

Further studies can be carried out in the same strains. Though the strains were characterized under genus *Trichoderma* species, efforts were not taken to go for

species level identification. Based upon the tests that are to be carried out in the paper processing industry, the effective strain capable of producing the xylanase enzyme can be characterized and cloning studies can be done.

Appreciable quantities of xylan are present in materials released from wood during pulping and pulp processing. It is presently regarded as waste and often is deposited in streams and rivers where it is ecologically harmful. Considerable amounts are also present in agricultural residues. The conversion of xylan to useful products represents part of our efforts to strengthen the overall economics of the processing of the lignocellulose biomass, and also to develop new ways of energy production from renewable resources.

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