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Effect of Salt on the Production of Xylanase in some Thermophilic Fungi

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Summary

The fungal isolates was studied for the production of xylanase at different salt level i.e., 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0%. Xylanase was produced by growing the test fungus in Bhat and Maheshwari medium supplemented with 1% xylan as carbon source at pH 6.0. All the fungal isolates showed an increase in their xylanase activity with the increase in salt concentration upto a limit, thereafter it decreased. *Chaetomium thermophile* (1.21 U/mg protein), *Humicola insolens* (2.69 U/mg protein), *Rhizopus stolonifer* (1.25 U/mg protein) showed their highest activity at 1.5% salt level, while *Humicola fuscoatra* (1.25 U/mg protein), *Humicola grisea* (1.29 U/mg protein), *Sporotrichum thermophile* (1.19 U/mg protein) showed the highest activity at 2% salt level. *Mucor* sp (2.79 U/mg Protein) exhibited the highest activity at lower salt level (1%). *Thermoascus aurantiacus* (1.26 U/mg Protein) showed the double salt optima for xylanase activity, one at 1.5% and the other at 2.5% salt level.

Keywords: Salt, Xylanase, Thermophilic Fungi

Introduction

Microorganism are present in all ecological niches and play an important role in decomposition of all substances (wheat straw, sugarcane bagasse, rice straw, xylan and other related materials) excepting non-degradable ones. The evolutionary process has exerted a pressure that resulted in production of molecules with substrate adapted biocatalytic activity. Molecular stability, efficient intracellular processing and extra cellular enzymatic mechanism are major functional changes in their transport out of the cell. Biocatalysts are enzymes, able to catalyze reactions under mild conditions at normal pH and pressure, most of these are found typically in filamentous fungi and are also produced as recombinant, heterologously expressed proteins [1].

The fungi are not degenerate organisms [2] but fungi are progressive, ever changing and evolving rapidly in their own way. They are capable of becoming readily adapted to every condition of life. Some fungi possess exceptional ability to grow at high temperatures, i.e., upto 60^0C are called as thermophiles [3].

Hemicellulose is a highly branched, heteropolymer, noncrystalline in nature and readily hydrolyzed. It is made up of pentose (D-xylose, L-arabinose) and hexoses (D- galactose, D-mannose, L-rhamnose, L-facose and organic acids such as D-glucuronic acids xylan. Polymer xylose is the major component of hemicellulose and most abundant plant polysaccharide in plant materials after cellulose.

Xylan is one of the major component of hemicellulose. It constitutes the 35% of hemicellulose in both hardwood and softwood. Hemicellulose consist of common backbone composed of β (1-4) linked xylosyl residues, but differ in the side chains, attached to it [4]. The side chain is heteropolymer, substituted with varing amount of α-L- arabin of uranose and glucuronic acid [5, 6]. Therefore, complete hydrolysis of hemicellulose require several degrading enzymes in addition to key enzymes Endoxylanase and β-xylosidase. The farmar act on xylan to produce small oligosaccharides and the latter hydrolyses dimmers and trimers (xylobiose and xylotriose) respectively to xylose and other monomeric sugar [7].

Xylanase are of potential importance in the bioconversion of lignocellulosic materials to sugar, alcohol and other useful products [8, 9].

Keeping these aplications in the mind,the present investigation has been carried out on some thermophilic fungal isolates from city waste.

Materials and Methods

Microorganism

Some common fungus isolated from city waste were used in this study i.e. *Chaetomium thermophile, Humicola grisea, Humicola fuscoatra, Humicola insolense, Mucor sps, Rhizopus stolonifer, Sporotirichum thermophilie, Thermoascus aurantiacus,* These isolated were encountered from city waste, by using the yeast powder soluble starch medium (pH 7.0) at 45ºC. They were tested for their ability to produced xylanase at different temperature.

Effect of Salt on the Production of Xylanase

To study the effect of salt on xylanase production, different isolates were grown on Bhat and Maheshwari's medium [10] with different concentration of sodium chloride (NaCl) 0.0 to 3.0% at a interval of 0.5 each. Isolates were incubated at optimum temperature, pH & day of production.

Enzyme, activity was evaluated as given above at 50^{0}C and pH 5.5.

Enzyme preparation

Enzyme was prepared by growing the test fungus in enriched glucose di-hydrogen phosphate medium supplemented with 1% xylan as carbon source at pH 6.0. 20 ml of medium was taken into 100 ml Erlenmeyer flask and autoclaved at 15 Lbs pressure. The flasks were incubated with 4 mm disc of freshly grown test fungus. Flasks were incubated at 45 ºC for different days. Flasks were taken out 3rd, 5th and 7th day of incubation. Content was centrifuged at 6000 rpm for 15 minutes and supernatants were used as enzyme.

Xylanase Activity

Xylanase activity was assayed according to Bastawde Method [11]. Assay mixture consisted of 0.20 ml of enzyme and 1.8 ml of xylan (1% as substrate) in 0.05 M citrate phosphate buffer, pH 5.5. This was incubated at 50ºC for 30 min. 1 ml of reaction mixture was taken from above preparation after incubation and 1 ml distilled water was added to it, shaked well, the reaction was terminated by addition of 3ml of freshly prepared DNS reagent. Reducing sugar released as xylose equivalents was measured by Miller method [12].

A unit of activity was expressed as amount [13] and specific activity (U/mg Protein) of enzyme was calculated according to [14].

Results

Effect of salinity on the production of xylanase

The results of xylanase activity at different salt level are presented in table-1 at their optimum temperature, pH and optimum day of production.

Table-1: Effect of salt on the xylanase production* in some thermophilic fungi from city waste Bareilly

Name of fungi	Salt Concentration (%)						
	0	0.5	1	1.5	2	2.5	3
Chaetomium thermophile	1.02 ± 0.0057	1.12±0.0115	1.18 ± 0.0230	1.21± 0.0115	1.09 ± 0.0115	1.0± 0.0346	1.0± 0.0288
Humicola grisea	1.17± 0.0120	1.22± 0.011547	1.22± 0.0173	1.25± 0.0202	1.29± 0.0115	1.21± 0.0260	0.99± 0.0080
Humicola fuscoatra	0.80 ± 0.0491	0.94± 0.0144	1.04± 0.0145	1.12± 0.0173	1.25± 0.0202	0.92± 0.0230	0.88± .0260
Humicola insolens	2.49± 0.01154	2.58± 0.0190	2.62± 0.0173	2.69± 0.0115	2.60±0.0173	2.46± 0.0288	2.11± 0.0057
Mucor sp	2.73± 0.0115	2.75± 0.01453	2.79± 0.0173	2.65± 0.0115	2.56± 0.0202	2.50± 0.0260	2.38± 0.0145
Rhizopus stolonifer	1.08±0.0173	1.13± 0.0088	1.19± 0.0173	1.25± 0.0115	1.20± 0.0173	1.12± 0.0230	1.09± 0.0202
Sporotrichum thermophile	0.51±0.0145	0.68± 0.0202	0.72± 0.0173	0.92± 0.0115	1.19± 0.0202	1.01± 0.0433	0.88± 0.0233
Thermoascus aurantiaeus	0.88±0.0517	0.96± 0.0115	1.21± 0.0173	1.26± 0.0202	1.22± 0.0088	1.26± 0.0230	1.11± 0.0230

*Produced in Bhat and Maheshwari medium (1987) at optimum temp. & pH.
**Activity at 50^{0}C and pH 5.5
S.A. = Specific Activity (U/mg protein)

All the fungal isolates showed an increase in their xylanase activity with the increase in salt concentration upto a limit, thereafter it decreased. *Chaetomium thermophile* (1.21 U/mg protein), *Humicola insolens* (2.69 U/mg protein), *Rhizopus stolonifer* (1.25 U/mg protein) showed their highest activity at 1.5% salt level, while *Humicola fuscoatra* (1.25 U/mg protein), *Humicola grisea* (1.29 U/mg protein), *Sporotrichum thermophile* (1.19 U/mg protein) showed the highest activity at 2% salt level. *Mucor* sp (2.79 U/mg Protein) exhibited the highest activity at lower salt level (1%). *Thermoascus aurantiacus* (1.26 U/mg Protein) showed the double salt optima for xylanase activity, one at 1.5% and the other at 2.5% salt level (Figure-1.

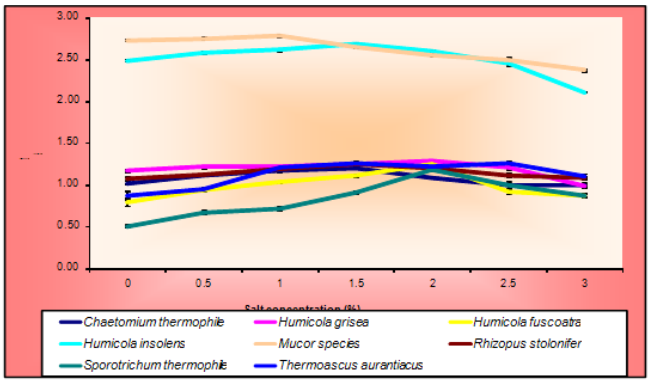

Fig-1: Effect of salt on the xylanase production

Discussion and Conclusion

During the present investigation the effect of salt on the producton of xylanase was studied. The maximum level of xylanase was found at different salt concentrations for different tested fungal isolates. *Mucor* sp. exhibited the highest activity at 1%; *Chaetomium thermophile*, *Humicola insolens, Rhizopus stolonifer* at 1.5%; *Humicola fuscoatra* at 2% salt concentration, whereas *Thermoascus aurantiacus* showed the double salt optima for xylanase, one at 1.5% and another at 2.5% salt level. The similar results were also found by [15]. The salt treatment also promote the swelling of fibers, beyond water soluble dimensions, by anmonolysis of intermolecular esterbonds, thereby allowing for increased enzymatic penetration into the fine structure of the cell wall [16].

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