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Production and optimization of xylanase from newly isolated *Paecilomyces variotii* GYHSP1 SASTRA

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Abstract: Xylanase enzyme, which is of commercial importance, is produced using newly isolated fungal strain from soil sample collected from agricultural fields in Thanjavur by solid state fermentation.. The fungal strain was identified as *Paecilomyces variotii* based on microbiological and molecular characterization with accession number KC960561. Various parameters such as carbon source, nitrogen source, moisture content, initial pH and fermentation time were optimized. With wheat bran as carbon source and yeast extract as nitrogen source, optimum growth of organism and best yield of Xylanase is observed. The maximum enzyme production was obtained at the following condition: moisture content of 1:1.5, initial pH 7.0 and fermentation time of 7 days. The maximum enzyme activity was achieved at pH 7.0 and temperature 60°C. Under above optimized condition, the enzyme yield reached 20.6 U/mg.

Keywords : Xylanase, *Paecilomyces variotii,* Solid state fermentation, optimization, wheat bran.

Introduction

Lignocellulosic biomass is the potential feed stock for the production of value added industrial products. It comprises about 50% world biomass and less expensive and easily available. Usually utilization of these materials for the production purpose also solves the problem of environmental pollution[1].

Xylan are hemicellulosic polysaccharides which forms the structural components of cell wall. Xylans are formed by xylose subunit which is linked together by β-(1→4)-glycosidic linkages. The major macromolecular cell wall components of wood are cellulose, hemicelluloses and lignin. Lignin composition and proposition varies in softwood and hardwood[2].

Hydrolysis of xylan is a tedious process because chemical hydrolysis of xylan may leads to the production of hazardous byproducts. Therefore for specific and complete hydrolytic degradation of xylan requires xylanolytic enzymes. Xylanase is of more important because of its direct involvement in glycosidic bond cleavage[3]. Xylanases are used in various industrial applications.[4]

In the present, a fungal strain was isolated from soil and it was screened for xylanase production by solid state fermentation. The production of xylanase was optimized by using various agro residues as carbon source.

Materials and Methods

Fungal strain isolation and characterization

The soil samples were collected from nearby agricultural fields Thanjavur, Tamil Nadu, India. The soil sample was serially diluted and plated on MEX xylan agar medium. Xylanase producing strain was isolated based on zone of clearance on MEX xylan agar plate. It was further confirmed by growing in xylan agar (DX) media containing Beech wood xylan 1%, Yeast extract 0.5% ,$NaNO_3$ 0.1% ,KH_2PO_4 0.1%, $MgSO_4.7H_2O$ 0.03% and was subculture for 72h at 37°C. The purified strain was stored at 4°C in potato dextrose (PDA) agar slant.

Strain identification

The isolated sample is subjected to morphological and molecular characterization to identify the speech for further studies. The molecular characterization was done by 16S rRNA sequence (Bhat Biotech).

Fungal staining:

The clean glass slide was taken and smear preparation was done by placing a drop of water on the slide, then mixing with loop of culture by means of circular motion. Then the smear was gently flooded with lacto phenol cotton blue and allowed to stand for 1 minute. The smear is observed oil immersion microscope.

Sequence analysis:

The genomic DNA of fungal is extracted using genomic DNA extraction kit and amplification was performing using universal premier. Forward primer: primer: 5'- TCCGTAGGTGAACCTGCGG-3' and Reverse Primer: 5'- TCCTCCGCTTATTGATATGC-3'. Data sequenced were aligned and dendrograms were generated. The comparison of uptime Sequences were done with non-redundant NCBI database by using BLASTN and similar sequence were found by the E score. CLUSTAL W2 is used to generate phylogram using MEGA5 software.

Production of extra cellular Xylanase

Xylanase production fungal were grown in SSF using basal liquid medium fungal growth and enzyme production were monitor the collecting sample at fixed time intervels. The collected sample was centrifuged at 10,000 rpm for 15 min at 4°C and the cell free supernatant was used as crude source of xylanase enzyme.

Assay for xylanase

The crude Xylanase collected from the above step, is assayed for xylanase activity by DNS method[5] where 0.5% beech wood xylan in 20mM sodium citrate buffer (pH 7.0) was used as substrate. The absorbance was measured at 540nm.

Optimization studies

Optimization of xylanase production is done with respect to fermentation period, initial pH, carbon source, organic and inorganic nitrogen source, different moisture level, and impact of temperature and pH on enzyme activity.

Effect of Fermentation time

To analysis the optimum fermentation time, the process was performed using 5 g wheat bran as carbon source. Solid state fermentation was performed at room temperature for 9 days**.** Enzyme activity was determined at 24 h interval.

Effect of initial medium pH

The initial pH of medium for enzyme production was determined by varying pH from 3.0 to 9.0 using various buffers (10mM Citrate buffer pH 3.0 – 5.0, 10mM phosphate buffer pH 6.0-7.0, 10mM Tris HCl pH 8.0, 10mM carbonate-Bicarbonate buffer pH 9.0) and organism cultured for 6 days at room temperature and the crude enzyme was extracted and activity was determined.

Effect of carbon source

Different carbon source such as wheat bran, boiled rice bran, unbilled rice bean, saw dust, rice straw, cassava bagasse were used to study affiance of different carbon source and the enzyme activity of the crude xylanase extracted after 6 days of fermentation was determined.

Effect of nitrogen source

Different organic (ammonium sulphate, yeast extract, peptone, beef extract) and inorganic (sodium nitrite, potassium nitrate, urea) nitrogen source were tested and the most suitable source of nitrogen for xylanase production was determined with respected to enzyme activity of crude xylanase which is extracted after 6 days of fermentation.

Effect of moisture level

The impact of moisture on the production of xylanase was studied by differing the moisture ratio between 30% and 80%. The moisture ratio is calculated with respect to liquid added.

Effect of pH on enzyme activity

The dependency of enzyme activity on pH was studied by incubating xylanase enzyme at various pH ranges i.e.3.0-9.0 using following buffers: 10mM Citrate buffer pH 4.0 – 5.0, 10mM phosphate buffer pH 6.0-7.0, 10mM Tris HCl pH 8.0, 10mM carbonate-bicarbonate buffer pH 9.0. Enzyme activity was assayed using DNS method [5]. The enzyme stability at various pH was estimated by storing enzyme in different pH range for 2 h and the sample was assayed at a regular interval of 30 min.

Effect of temperature on enzyme activity

To determine the optimum temperature for improved xylanase activity and to evaluate thermal stability of xylanase, the enzyme was incubated at varying reaction temperature from 30° to 90°C. The corresponding enzyme activity was noted at 30 min time interval.

Results and Discussions

Isolation and Identification of Fungal Strain

Detection of fungi with lactophenol blue solution enables the specimen to be stained and stand out well against the light blue background.The isolated strain, labeled as GYHSP1SASTRA, collected from soil sample of agricultural field, Thanjavur, shows promising xylanase activity and resulting zone of clearance in xylan plate assay(Fig. 1) as it produced large amount of xylanase.

Fig 1 Xylanase test assay

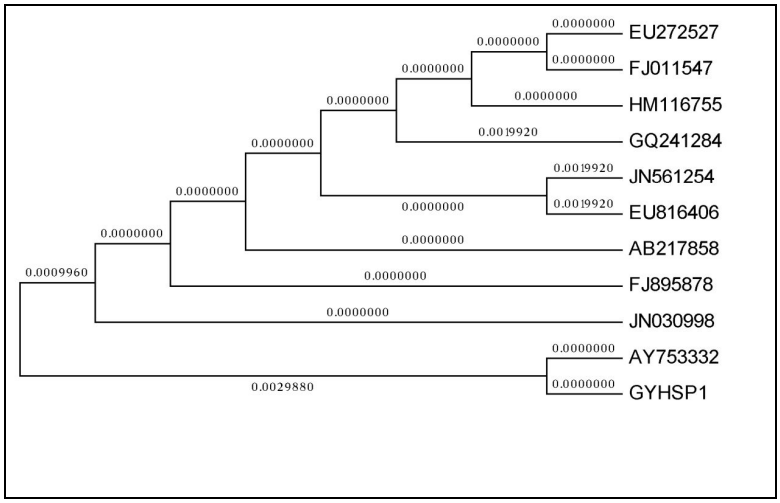

Fig 2 Phylogenetic tree

Characterization was performed and the strain GYHSP1 SASTRA was explored as *Paecilomyces variotii* through molecular characterization done by 16S rRNA sequencing, performed at Bhat Biotech and deposited in GenBank with accession number KC960561.The phylogenetic tree has been constructed for the isolated strain *Paecilomyces variotii* and is shown in fig 2.

Effect of Fermentation Time

Xylanase activity reached highest value (93.7 U/g) after 7 days of incubation and started to decline on further incubation (Fig 3). Xylanase from *Paecilomyces variotii* were growth-associated, progressing after 6 days, and the enzyme production appeared closely same upto 7th day.

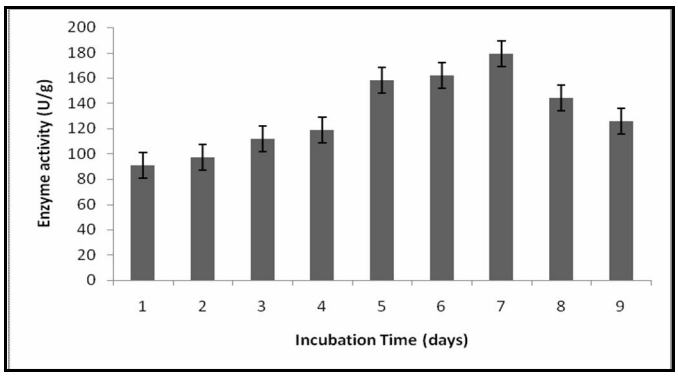

Fig 3 Effect of fermentation time

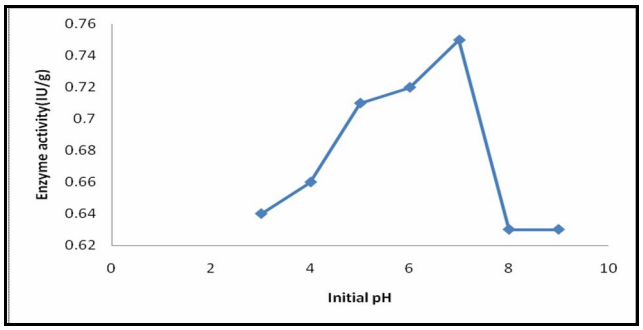

Fig 4 Effect of pH

Effect of pH

Appreciable xylanase enzyme yield was obtained between pH 6.0 and 8.0, expressing highest at pH 7.0(Fig 4).Evidently, Pandya *et al*.,[6] reported the same optimum pH for xylanase production

Effect of Carbon and Nitrogen Source

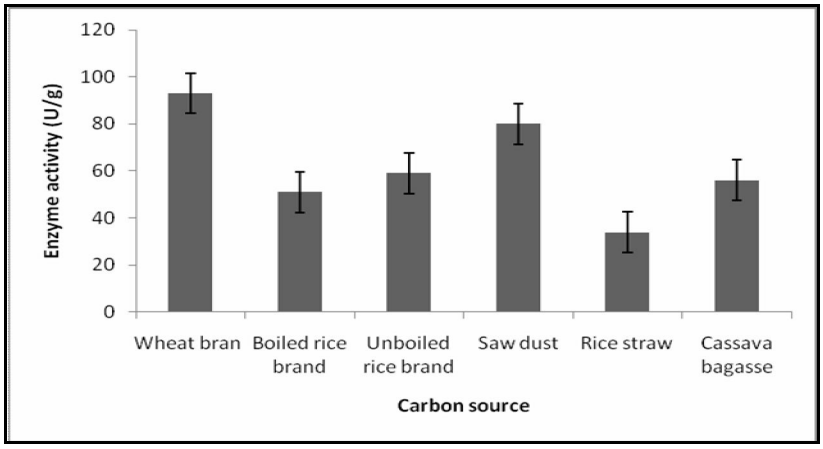

Fig 5 Effect of carbon source

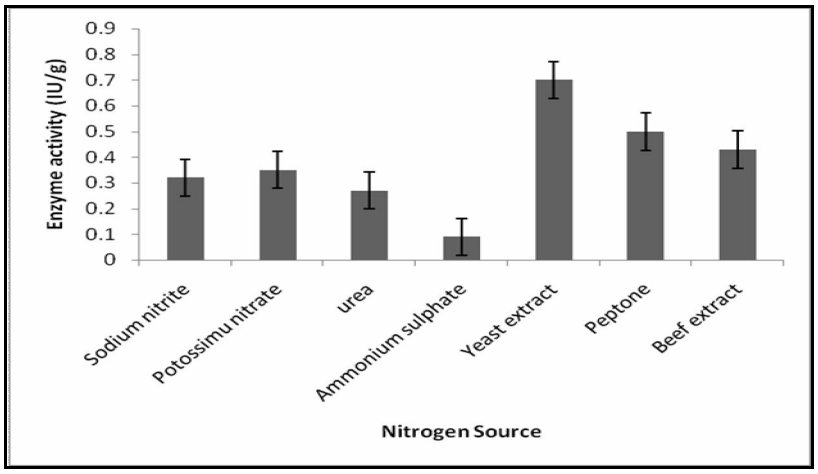

Fig 6 Effect of nitrogen source

On evaluating xylanase activity with respect to different carbon sources, although hemi cellulosic substrates such as boiled rice bran, unboiled rice bran, saw dust, rice straw, cassava bagasse and others are able to promote xylanase production, the best enzyme activity was obtained with wheat bran (Fig 5). Coman *et al*.,[7] and Gowdhaman et al [8,9] has proved that wheat bran as substrate resulted in a increased level of xylanase production. On studying the impact of different nitrogen sources (Fig 6), it indicated that yeast extract possessed highest growth providing characteristics to the organism which resembles the work result of Li *et al*., [10] who stated that yeast extract is the best source of organic nitrogen source.

Effect of Moisture Content

Higher Xylanase yield of 267.8 U/g was achieved at 60% initial moisture content owing to reduced porosity, change in the structure of particle or decreased oxygen transfer at higher moisture and least nutrients diffusion on the substrate, lesser rate of swelling and improved water tension at lower moisture content (Fig 7). Archana *et al*.,[10] also described the same note regarding influence of moisture content with thermophilic organism in solid state fermentation.

Under optimum condition, in solid state fermentation, *Paecilomyces variotii* produced 267.8 U/g of Xylanase, which was greater than the unoptimized basal medium (93.7 U/ml). The enzyme titer in our study achieved commendable value than the value reported by Pandya *et al*. [6].

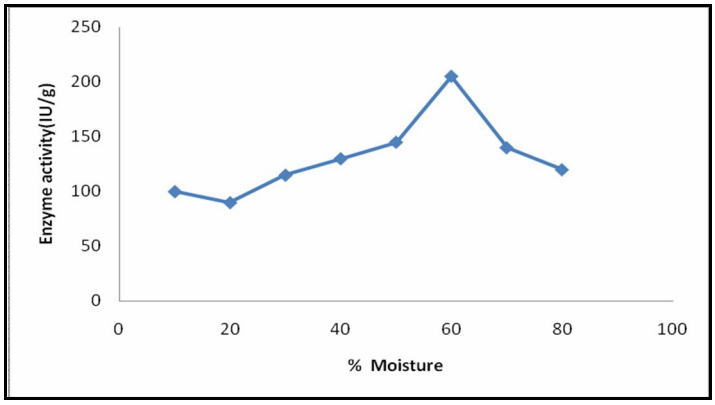

Fig 7 Effect of moisture content

Effect of Temperature

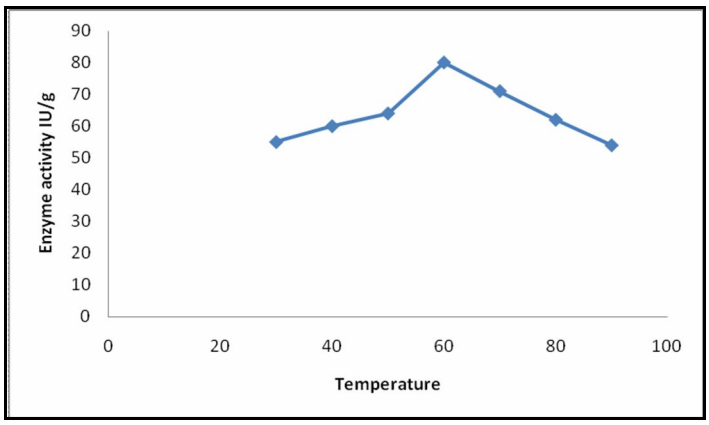

Fig 8 Effect of temperature

The influence of temperature on xylanase activity was evaluated in the scale of 30–90°C (Fig 8). The results spotted that xylanase activity was optimum at 60°C with evident value (80 U/g). Around (54U/g) of xylanase activity still persisted even at 90°C. Lu *et al.,* [11], stated maximum activity of xylanase at 70°C which retained 60% activity at 75°C temperature expressing thermo stability of purified xylanase from *Halorhabdhus utahensis*.

Conclusion

In this work, the feasibility of wheat bran to produce increased quantity of xylanase through solid-state fermentation was investigated and highlighted the isolated new strain, *Paecilomyces variotii* GYHSP1 SASTRA, which is able to produce xylanolytic system, confirmed through xylan plate assay that efficiently hydrolyzes complex wheat bran, the cheap and abundant source of carbon. Maximum xylanase production were achieved with thermal stability of 60^{o}C at 60% moisture content, initial pH 7.0, yeast extract as organic nitrogen sources with fermentation time of 7 days.

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