Palm fiber as novel substrate for enhanced xylanase production by isolated *Aspergillus* sp. RSP-6

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Abstract

Among all agro industrial material hydrolyzing enzymes, xylanases gained importance due to their role in production of xylose based products of industrial importance. An isolated fungal strain, *Aspergillus* sp. RSP-6, having potential in xylanase production specific to palm fiber was characterized for enzyme production properties under solid suspend fermentation environments. Effective enzyme production was achieved with palm fiber as carbon source compared to all other tested agro materials and carbohydrates. Among different nitrogen sources, beef extract supported maximum enzyme production. Various fermentation parameters (pH of the medium, particle size and its concentration, incubation time and medium volume in addition to carbon and nitrogen sources) influenced the xylanase production. Maximum enzyme production of 62,480 U/l was observed in the medium of pH 3.0 containing 2% palm material having particle size of 2.8–1.4 mm and 1% (w/v) beef extract in presence of other mineral salts in 144 hours of incubation. Over all, the enzyme production has increased significantly from 20,000 to 62,480 U/l under optimized environment and observed to be constitutive in nature in this fungal strain.

Key words

Agro-material, *Aspergillus* sp., Enzyme, Palm fiber, Solid suspended fermentation, Xylanase

Introduction

In the present biotechnological era, environment friendly technologies are gaining importance in industrial sector. In this context, effective utilization of abundantly available agro industrial wastes for biotechnological production of value added products of different nature such as biofuels, organic solvents, microbial enzymes and other metabolites using microbial strains or biocatalysts edge over conventional chemical methodologies. All photosynthetic materials are made of simple carbohydrates, however present in the form of polymers of cellulose, hemicellulose and lignin and are entangled in complex manner. Hence, their digestion to produce simple monomeric forms requires strong alkali or acidic environment. This chemical hydrolysis method generates the effluents of xenobiotic nature and cause environmental pollution. Use of specific hydrolyzing enzymes provides a better solution. Among different hemicellulosic material hydrolyzing enzymes, xylanases are gaining importance because of their wide application in various industrial sectors especially in bioconversion of hemicellulosic materials to ethanol, xylitol and arabinol in addition to their application in animal feed and different biotechnological applications (1-3).

Xylanases catalyzes hemicellulosic xylan (1, 4) and produce xylose. Xylan is the abundant polysaccharide material available on earth after cellulose. It consists of a â-1, 4- linked D-xylose
backbone with L-arabinose, D-galactose, acetyl, feruloyl, p-coumaroyl and glucuronic acid substitutions as side groups and comprises 20 to 30% dry weight of the plants (2, 3, 5). Several xylanase producing microbial strains have been isolated and their enzyme production potentials evaluated (6-12). It was well documented that each microbial strain’s metabolism, growth and enzyme production pattern differ with the fermentation environment (13-16). Detailed survey of literature revealed that xylanase production pattern depends on the microbial growth pattern and fermentation medium (6, 8). In addition, the biochemical, catalytic and physical properties of produced enzyme also vary and depend on the microbial genetic nature (8). This situation warrants for screening of novel microbial strains with material specific hydrolyzing enzyme production property from exotic environments.

Keeping this in view, several microbial strains having xylanase production potential were isolated from exotic soil samples in our laboratory. One of the isolated fungal strains, Aspergillus RSP-6, was found to be efficient in producing xylanase. It has been characterized for its enzyme production potential under submerged fermentation conditions using palm fibre as carbon source. We report that xylanase enzyme production in this fungal strain is regulated by monomeric sugars and other physiological fermentation parameters. The enzyme production titer improved more than 300% under optimized environment. To the best of our knowledge, this is the first report on production of palm fiber specific xylanase production by microbial strains.

Materials and Methods

Microorganism and culture conditions

A fungal strain, Aspergillus RSP-6 was isolated from the soil samples collected from Rajahmandry palm oil factory waste using xylan (1%) based agar (2%) medium by serial dilution method. The purified strain was maintained in the potato dextrose agar medium slants at 4°C after growth. The microorganism was grown regularly in a medium (pH 5.0) consisting (g, w/v) of yeast extract 0.2%, peptone – 0.2%, ammonium sulphate 0.1%, KH2PO4 – 0.05%, K2HPO4 – 0.05%, NaCl – 0.05% and MgSO4 - 0.05% and incubated at 30°C. Spore suspension of 10⁸ spores/ml was prepared under sterilized conditions and used for further experiments.

Xylanase production studies

Xylanase enzyme production fermentation experiments were performed in 250 ml conical flasks containing 100 ml of fermentation medium supplemented with palm fiber as carbon source unless otherwise mentioned. The flasks were inoculated with 2 ml of spore suspension (1 X 10⁸ spores per ml) and incubated at 30°C in rotary shaker adjusted to 150 rpm. Optimum requirements were determined basing on inoculum concentration of 1.0 to 3.0 ml; pH of 2.0 to 9.0 and incubation period up to 72 hours. Whenever required, the selected carbon and nitrogen sources were supplemented individually at 0.2% level to the basal fermentation (medium with mineral salts) medium before autoclaving. For studying the particle size effect, the fermentation experiments were performed using different size (0.21 – 0.3; 0.3 – 0.71; 0.71 – 1.4 and 1.4 -2.8 mm) particle as substrate. The cell free fermentation broth was used as enzyme source. The samples were collected at predetermined time intervals and subjected to centrifugation at 10000 rpm for 10 min at 4°C. The supernatant was collected and used as source of xylanase. Results reported in this study were averages of triplicate samples.

Measurement of xylanase activity

Xylanase activity was determined using modified Bailey method (19). In this, 0.1 ml of
the enzyme solution was added to 1.9 ml xylan solution (1 %, w/v). (Xylan solution was prepared by dissolving the xylan in 50 mM citrate buffer (pH 5.0) and incubated at 50 °C for 30 min). The reaction was terminated by adding 2 ml of dinitrosalycilic acid and the contents were boiled for 5 minutes and diluted the volume to 10 ml with distilled water. The final solution absorbance was read at 540 nm using UV–Visible spectrophotometer (Xploral, XP2001) and the xylanase activity was calculated using xylose standard curve. One unit of xylanase activity was defined as 1 µmole of xylose liberated min⁻¹ ml⁻¹ of enzyme.

**Results and Discussion**

**Influence of agro material on xylanase production**

The role of different agroindustrial waste materials impact on xylanase production by isolated *Aspergillus* sp. RSP-6 was evaluated by supplementing the selected material individually at 2 % (w/v) to the fermentation medium and subsequent estimation of xylanase activity in cell free broth during fermentation. The xylanase enzyme production data presented in Fig. 1 revealed that all selected agroindustrial materials support the growth of this isolated fungal strain and production of xylanase enzyme. The enzyme production pattern however, varied with the incubation time. Maximum enzyme production was noticed at 5th day of incubation and further increase in fermentation time resulted in reduction of xylanase activity. Similar variation of enzyme yield was also found in all fermentation experiments (Fig 1). Xylanase production yields varied from 1000 to 2000 U/ml (Fig 1) depending on the type of agro industrial waste materials. Among all selected materials, palm fiber supported the maximum enzyme production and minimum was noticed with pea nut meal and red gram husk as carbon source. Such medium component variation dependent enzyme production was reported in literature in variety of microbial strains (5, 14-17, 21). Hence, further enzyme improvement studies with this isolated fungal strain were aimed with palm fiber material as carbon supplement.

**Effect of particle size on xylanase production**

The influence of palm fiber particle size on xylanase enzyme production was presented in Fig 2. Maximum enzyme production (2200 U/ml) was observed with fermentation experiment supplemented with 2.8 - 1.4mm and any variation in particle size adversely affected the enzyme production (Fig 2). This may be due to effective support provided by this size particle for attachment of fungal strain and subsequent alteration in microbial metabolism. This is further confirmed based on the literature report that microbial metabolism varies under free and immobilized/ partial immobilized environments under similar fermentation environments (22). In fact, better microbial growth associated product production was reported in literature in case of microbial biofilm formation environments compared to free cell fermentation. Such type of microbial xylanase enzyme production variation with different size of particle in solid suspended fermentation was not noticed in the literature, however, particle size dependent microbial product production variations under solid state fermentation was reported (14, 20).
To understand the influence of concentration of the particle size on enzyme production, further experiments were performed with variation of solid material supplementation in the medium. Variation of solid material quantity did affect the enzyme production pattern and maximum (2500 U/ml) being observed with 2% (w/v) material compared to 1 and 3% (w/v) material (Fig 3). This may be attributed to the fact that higher quantity of solid material (3%, w/v) supplementation may provide better support for attachment of microbial strain but nutrient mass transfer may be affected during fermentation as reported in various solid state fermentations (14, 15). However, in case of 1% (w/v) supplemented environment, the suspended solid material may not be sufficient enough to provide support for microbial attachment. This data further suggest that optimization of particle size and its distribution in the suspended fermentation medium are the important parameters to achieve economic xylanase production with isolated Aspergillus sp. RSP-6.

**Influence of medium pH on xylanase enzyme production**

pH of the medium is one of the regulatory parameters during fermentation (18, 23, 24). Hence, the influence of pH of the medium on xylanase production by this fungal strain was studied by supplementing with 2% (w/v) of 2.8 – 1.4 mm size palm fiber particles into the fermentation medium. Optimum enzyme production of 2900 U/ml was noticed at pH 3.0 (Fig 4). Further analysis of the enzyme production data denoted that xylanase enzyme production did not vary much in the acidic pH range of 2 – 6.0 and drastically reduced at neutral pH and no enzyme production was noticed in alkaline medium of pH 8.0 (Fig 4).
Effect of RPM on xylanase production

The enzyme production results at different RPM indicated that xylanase production varied with RPM during fermentation. Maximum enzyme production yields (3150 U/ml) was achieved with culture incubated at 150 rpm and variation of 50 rpm in either sides of this resulted in reduction of enzyme values (Fig 5). More than 30% reduction was observed at 100 rpm which may be attributed to the fact that at lower rpm, mass transfer may be the limiting factor for fungal growth. Increase of rpm from 150 to 200 resulted in approximately 15 % reduction of enzyme production values (Fig 5). Such data suggest that the microbial metabolism is regulated by the mass transfer behaviour of the system and optimum production values could be achieved with 150 rpm fermentation environment. Any variation in rpm disturbs the equilibrated interaction between fungal metabolism related xylanase production and its interaction with fermentation medium components. Similar results were reported with other microbial strains where rpm mediated metabolic production variation was noticed (5, 13, 14, 16-18).

Influence of medium volume on xylanase production

In most of the aerobic fermentations, medium volume plays a vital role and observed to be influencing the microbial growth and subsequent metabolite production in Bacillus circulans (14), Candida tropicalis (5), Amycolatopsis mediterranei (21) and Lactobacillus delbrueckii (25). Keeping this in view, the impact of medium volume (ml) in 250 ml conical flasks on xylanase production during palm fiber suspended fermentation was evaluated. The data presented in Fig 6 suggested that the fermentation medium does influence xylanase production pattern of the selected fungal strain. Maximum enzyme production (3550 U/ml) was noticed with 75 ml of medium in 250 ml conical flask. More than 25% of enzyme production variation was noticed with the variation of medium volume from 75 to 125 ml (Fig 6). Such difference may be assigned to the medium volume dependent variation of aeration during fermentation.

Fig 5: Effect of RPM on xylanase production by Aspergillus sp RSP-6

Fig 6: Influence of the medium volume on xylanase production by Aspergillus sp RSP-6
Effect of different carbon sources on xylanase production

Carbon source is one of the essential constituents of the microbial fermentation medium which has major role overall cellular growth and metabolism. Different carbon sources (glucose, xylose, fructose, maltose, mannose, galactose, arabinose, lactose and glycerol) impact on xylanase production was studied and compared with palm fiber material as carbon source. It was noticed that the enzyme production was not influenced by easily metabolizable carbohydrates in this fungal strain as it is evidenced by the fact that none of the selected carbon sources improved the xylanase production compared to palm fiber as carbon source (Fig 7). Such data reveal that the enzyme production in this fungal strain is constitutive in nature and not regulated by any of the selected carbon sources. This could be the major advantageous character of this fungal strain, for bio-hydrolysis of the agro industrial material and its subsequent use as substrate for biotransformation of xylose for production of value added products like xylitol in a multi-stage fermentation process. This is because; hydrolysis of the hemicellulosic material is always associated with small fraction of glucose production which may hinder the biotransformation process of xylose to xylitol using microbial strains (5).

Effect of different nitrogen sources on xylanase production

The impact of inorganic (ammonium sulphate, ammonium nitrate, ammonium chloride, sodium nitrate, potassium nitrate and urea) and complex nitrogen (yeast extract, peptone, beef extract, typtone, soya bean meal, corn steep liquor and pea nut meal) sources was evaluated on xylanase production by Aspergillus sp. RSP-6, by supplementing the 0.2 % of each selected nitrogen source individually in the fermentation medium before autoclaving. Among all nitrogen sources studied, yeast extract supported the maximum enzyme production followed by beef extract and pea nut meal (Fig 8). Though all selected inorganic nitrogen sources supported the enzyme production but not as efficient as yeast extract.

In order to understand the influence of concentration of nitrogen source on enzyme production, xylanase production pattern was studied in fermentation experiments supplemented with different concentration of yeast extract, beef extract and soya bean meal ranging from 0.2 to 1.4 %. It was noticed that enzyme production values varied with type of selected nitrogen sources and its concentration (Fig 9). Maximum enzyme production (6248 U/ml) was noticed with 1 % (w/v) beef extract as
nitrogen source. A gradual increase in enzyme production was observed with increase in these nitrogen sources concentrations up to 1% level and further increase resulted in decrease of enzyme values. Among selected three different complex nitrogen sources, soya bean meal and beef extract were found to be the least and best preferred sources for xylanase production by *Aspergillus* sp. RSP-6, respectively.

![Fig 9: Effect of selected nitrogen sources at different concentration on xylanase production by *Aspergillus* sp RSP-6](image)

Over all, the present investigation of xylanase production by *Aspergillus* sp. RSP-6 under solid suspended fermentation revealed that palm fiber is the best preferred source of carbon. The enzyme production is constitutive in nature and not influenced by easily metabolizable microbial carbon sources. Different fermentation parameters such as pH of the medium, particle size of the material, quantity of the solid material, aeration levels, carbon and nitrogen sources and their concentration regulate the fungal metabolism related xylanase production. Xylanase production has improved more than 300% upon optimization of various fermentation conditions. Further fermentation process engineering of this microbial strain would help in improved enzyme production. This is the first report of its kind on production of xylanase under solid suspended fermentation environment.

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**References**


