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Utilization of rice and wheat waste for production of extra cellular 1-4-beta-D- endo xylanase by *Bacillus brevis*

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A B S T R A C T

The xylanase biosynthesis is induced by xylan. For the cost effective production of endo-xylanase, an enzyme of immense industrial importance, xylanase production from *Bacillus brevis* was studied. The high xylan content in some of the wastes like rice bran, wheat bran, rice straw and wheat straw makes them an accessible and cheap source of inducers. Culture medium for xylanase biosynthesis at shake flask has been optimized. Xylanase activity was measured by DNSA methods using birchwood xylan (Sigma-Aldrich,USA) as standard substrate. The substrate optimization process was analyzed using agro-waste concentration from 0.5-10% (w/v). Maximum xylanase activity was observed in media supplemented with 2% of agro-waste. Wheat bran was found to be the best producer for xylanase production out of other substrates tried out; viz. Rice bran, wheat bran, rice straw and wheat straw; with xylanase activity 39 IU/ml. Optimum incubation temperature of *B. brevis* xylanase was found to be 60 °C at pH 7.0.

Introduction

Xylan is the major component of the hemicelluloses of plant cell walls in which β-1, 4-linked D- xylose residues constitute the backbone of xylan. After cellulose, xylan is the most abundant renewable polysaccharide in nature, accounting for 20-30% of the dry weight of woody tissue. In order to maximize the hydrolysis of lignocellulasic residues by cellulose enzyme, synergistic action of xylanase is also need for effective degradation of xylan and combined action of xylanase, β-xylosidase and xylan de-branching enzyme is required. Endoxylanase hydrolyze β-1,4-xylosidic linkages of xylan. The rate of xylooligomers hydrolysis by exoxylanase increases with the increase in its chain length. Endoxylanases are produced by a

number of microorganisms. Xylanase production depends on pH, temperature, time of incubation and substrate. With the help of its enzymes, that works synergistically to break the plant cell wall microorganisms has the capacity to use lignocellusole as substrate for their growth by breaking the plant cell wall by substrate de-polymerization (Siqueira *et al*., 2010; Moreira *et al*., 2012).

Globally, an estimated amount of 650 million tons wheat bran and 354 million tons wheat straw is produced annually (Bathia *et al*., 2012; Knob *et al*., 2014). Total biomass of wheat bran can be estimated at 150 million tons, which is mainly used in the feed industry (Pruckler *et al*., 2014). The annual global generation of rice straw is 685 million tons and about 60% of the mass produced by rice crop is rice straw (Lim *et al*., 2012). Generally it is removed from the fields at harvest time and is subjected to open field burning leading to serious environmental and health issues (Sarkar and Aikat 2012; Shaife *et al*., 2012). Looking to the large biomass produced by the wheat and rice crops the present study was done to study the utilization of rice and wheat crop wastes for production of industrial enzyme-xylanase by *Bacillus brevis* so, that the waste of these crops could be used for enzyme production.

Materials and Methods

Micro-organisms

Bacillus brevis strain was grown on minimal media at 37°C and stored at 4°C. A homogeneous suspension was obtained from 72 hrs old cultures.

Substrates

Natural lignocelluloses (agro-industrial wastes), namely wheat straw, wheat bran, rice straw and rice bran, were procured locally, air dried, pulverized to 100 mesh size and utilized as substrates for xylanase production.

Xylanase production on different substrates

Erhlenmeyer flasks (250 ml) containing 5 g lignocellulose, supplemented with agro-waste substrate were autoclaved at 121°C for 15 min. After cooling they were inoculated with culture broth and incubated at 37°C for 48 hrs.

Enzyme extraction

The suspended slurry was filtered and centrifuged at 10, 000 rpm for 10 min. The clear filtrate thus obtained was used for enzyme assay. Each batch was prepared in triplicate and average values were obtained.

Enzyme study

Xylanase activity was estimated by the dinitrosalicylic acid method described by Gawande and Kamat (1998). One unit of xylanase activity was defined as the amount of enzyme that produced one micromole of xylose or glucose equivalent per minute under the assay conditions, respectively (pH 7.0, 55 °C and 5 min).

Total extra cellular protein was measured by Lowry's method using bovine serum albumin (BSA) as a standard. Optimum temperature of xylanase was carried out in the temperature range of 25-90 °C in phosphate buffer, pH 7.0.

Optimum pH was determined by measuring xylanase activity at optimum temperature using sodium acetate (pH 3.0-6.0); phosphate buffer (pH 6.5-8.0) and Tris buffer (8.5-9.5) for various pH ranges. Thermal stability was studied by calculating

the enzyme activity at 60 C for after a fixed time interval of 20 min for 3 hours.

Results and Discussion

Selection of agricultural waste for xylanase production

Effects of various kinds of agricultural wastes such as wheat straw, wheat bran, rice straw and rice bran on xylanase production were compared in suspension culture. Maximum xylanase production was obtained from the wheat straw medium (Fig. 1).

The optimum concentration of wheat straw for endoxylanase production were found to be 2% (w/v) (Fig 2), further increase of which in the media reduced the enzyme production probably due to the adverse effect of higher load of nutrient supplements present in these substrates or as a result of hindrance of mass transfer of oxygen by higher amount of solid substrate.

Temperature and pH optimization

Temperature optimum of xylanase was determined by varying the temperature from 30 °C to 90 °C. Maximum activity was observed at 60 °C while (Fig. 3). The pH of reaction mixtures were varied from 4.0 to 9.0. Maximum enzyme activity was observed at pH 7 (Figure 4).

Effect of incubation time on xylanase activity: Incubation time optimization studies were done by measuring the xylanase activity at different time intervals at 60 °C. Optimum time of incubation was found 5 min (Fig. 5) as after that no significant change in the enzyme activity was observed.

Thermal Stability: Thermal stability of xylanase was studied and more than 90 % activity was retained up 60 min (Fig. 6). Half life of the enzyme was calculated as 155 min.

Recently, many efforts have been made to convert lignocellulosic residues in to some valuable products. Due to this, a number of lignocelluloses have been evaluated for their use in xylanase production. However, looking into the variability of waste composition as well the optimization of the production process, larger studies are required to study the utilization of agro waste for lab scale to piolet scale or industrial scale for xylanase production (Knob *et al*., 2014).

Horikoshi and Atsukawa in 1973 published first report on xylanase from alkaliphilic bacteria (Goswami and Pathak, 2013). A broad pH optimum ranging from 6.0 to 8.0 was exhibited by the purified enzyme of *Bacillus* sp. C-59-2. Xylanase multiplicity in *Bacillus* spp. suggested that these bacteria produce two types of xylanases: one is basic (pH 8.3-10.0) with low molecular weight (16-22 kDa) and the other is acidic (pH 3.6-4.5) with high molecular weight (43-50 kDa). Many of the xylanases produced by alkaliphilic organisms such as *Bacillus* sp. (Okazaki *et al*., 1984) and *Aeromonas* sp. 212 (Okoshi *et al*., 1985) with optimum growth at pH 10 showed remarkable stability at pH 9-10. The enzymes from *Bacillus* sp. TAR-1, C-125 (Nakamura *et al*., 1994, Honda *et al*., 1985), *Bacillus* sp. NTU-06 (Wang *et al*., 2010) *Bacillus arseniciselenatis* DSM 15340 (Kamble and Jadhav, 2012) and alkaliphilic *Bacillus* sp. (NCL-86-6-10) (Balakrishna *et al*., 1992) were optimally active at pH 9-10.

Wheat straw has extensively used as a carbon source for xylanase production (Narang *et al*., 2001; Sanghvi *et al*., 2010; Liao *et al*., 2012; Panday and Gupte, 2012).

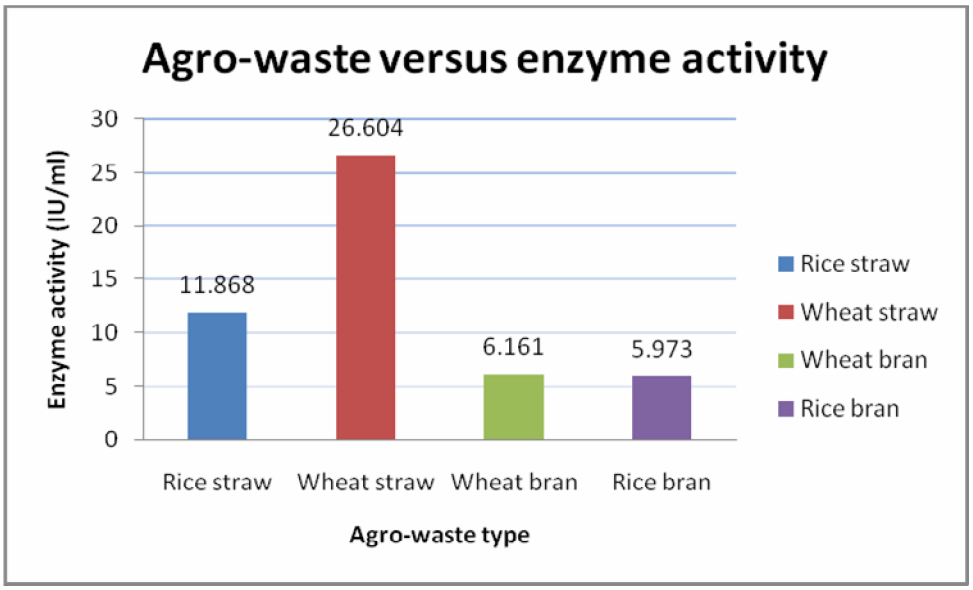

Fig.1 Substrate utilization for xylanase production

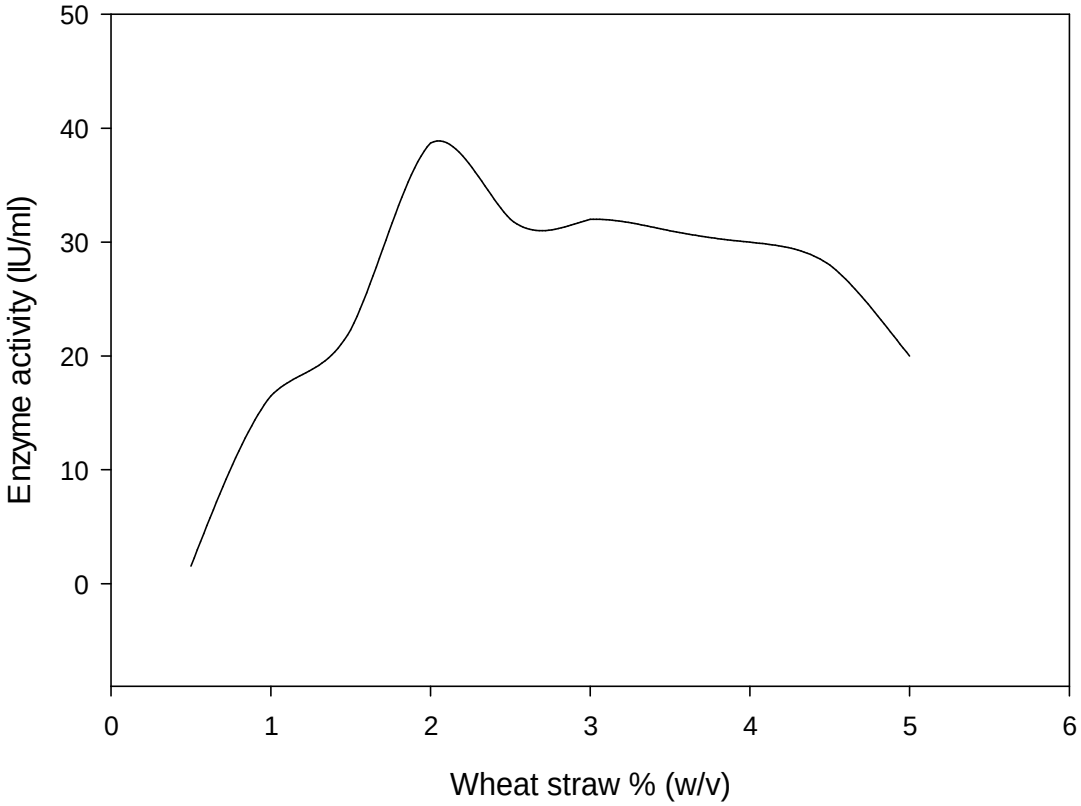

Fig.2 Substrate concentration optimization for xylanase production

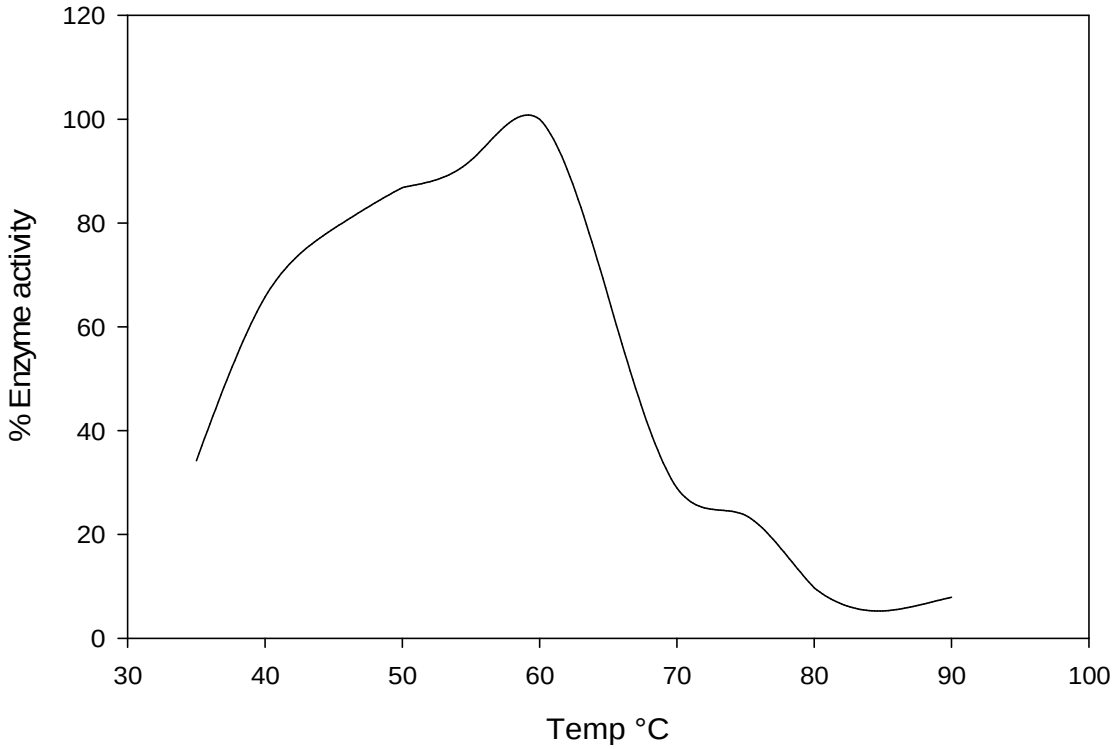

Fig.3 Incubation temp Optimization

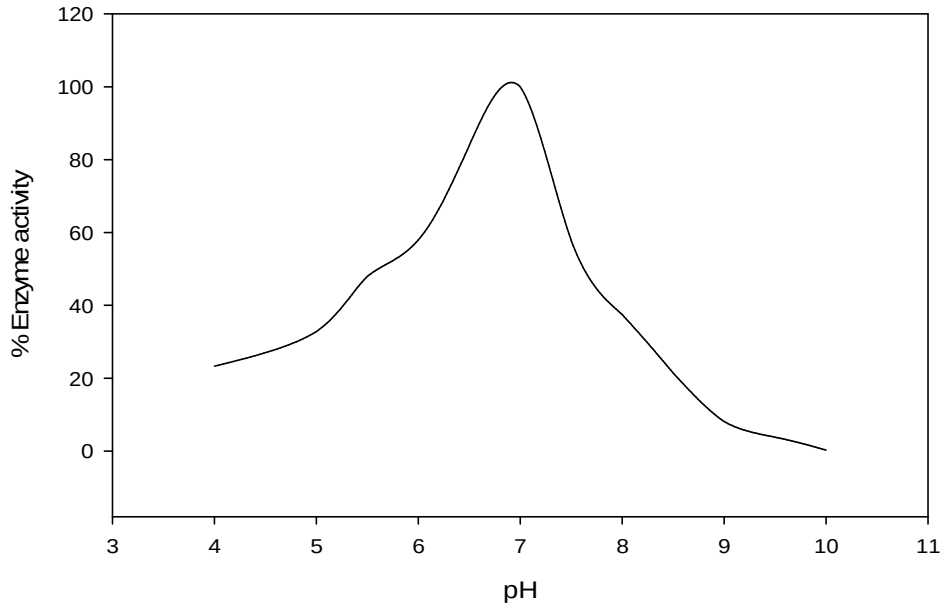

Fig.4 pH Optimization

Presence of high xylan content in wheat straw makes it cheap inducer source for xylanase production (Michelin *et al*., 2012).

Xylanase production has been reported from many Bacillus spp but we did not find any report claiming xylanase production by *Bacillus brevis* using agro waste. Sorror *et al*., (2013) reported xylanase production by *T. ressei* F418 using rice straw as carbon source. Xylanase from fungal sources has a great attraction due to high level of production and thermal stability but generally they suffer from undesirable cellulase activity.

In our study, we found that the *Bacillus brevis* xylanase is free from cellulose activity and xylanase production process could be optimized for enzyme production for industrial applications. The interesting part of this study is that the enzyme was stable in a broad range of temperatures (45-90°C) and showed good thermal stability at 70°C with half life of 2 h and these features makes it suitable for industrial applications in bleaching process of kraft pulp.

Conclusion

In the present study, we studied rice and wheat wastes for xylanase production. Out of four different types of agro waste (rice straw, wheat straw, rice bran and wheat bran) used in the present study; wheat straw was found to be best source for xylanase production by *B. brevis*.

Produced enzyme was active at high temperature, which is a prerequisite for its application as bleaching agent for bio-bleaching the kraft pulp. Xylanase showed good thermal stability for more than one hour with half life of more than two hours. This feature could help in developing the enzyme based bleaching of pulp by the pulp and paper industry.

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