NOTES ON NATURAL HISTORY

Xylanase Production from Fungi Extracted from Two Distinct types of Crop Producing Soil of Chhattisgarh, India

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Study Area: Raipur, Chhattisgarh, India...
Coordinated: 21° 17' 27.9" N; 81° 43' 40.7" E

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Introduction:

Xylanase is hydrolytic enzymes capable of hydrolyzing polysaccharide -1, 4-xylan in hemicelluloses a component of plant cell wall (Dhiman *et al.*, 2008). Xylans are the second most abundant natural polysaccharide (Collins *et al.*, 2005). It is a complex heteropolysaccharide having a highly branched chain of 1, 4-linked xylanopyranosyl residues (Bastawade, 1992). Xylanases (EC 3.2.1.8) catalyze the hydrolysis of xylan, the major constituent of hemicelluloses found in plant cell wall. (Kulkarni *et al.*, 1999). Xylanases are produced by a diverse group of organisms such as bacteria, algae, fungi, protozoa, gastropods and arthropods (Collins *et al.*, 2005). Concentration for xylanolytic enzymes has increased due to its potential use in several industrial processes such as biopulping and bleaching; bioconversion of lignocellulose; food processing, including clarification of beer, wine, and juice; increasing digestibility of animal feedstock; and bread making (Bastawade, 1992; Vikari *et al.*, 1994; Uffen 1997). An attempt has been taken to extract Xylanase from the soil collected from two diverse types of crop producing land i.e., sugarcane and rice

Species extraction and its enzymatic activity:

Soil samples collected during the month of August from targeted fields existing around Raipur were stored at 4°C. Fungi were isolated from soil samples by serial dilution method and pure culture were stored in Potato Dextrose Agar Media (Waksman, 1927; Jasuja *et al.*, 2013). Fungi species extracted were identified under the compound microscope and by lactophenol cotton blue staining following morphological studies Jones, (1965).

For fungi an initial screening was done in PDA xylan medium by plate assay method using congo red solution. Zone of hydrolysis was measured in centimeters (cm) (Ramanjaneyulu, *et al.*, 2015). When the zone of hydrolysis reached more than 5 cm were selected and further screened in submerged state fermentation (SmF) for the production of xylanase. Erlenmeyer flasks (250 ml) containing 50 ml of liquid MSM medium with 0.5mg of wheat bran as carbon source was inoculated with 1 fungal disc of 8 mm size and

incubated at 28° C for 7 days. 1.5ml aliquot were withdrawn from each flask each day and filtered through Whatman No.1 filter paper. Filtrate was collected and used as enzyme source for the assay of xylanase activity.

For the proper reaction mixture, we followed the process forwarded by Miller, (1959) where using xylose as standard, the amount of xylose liberated was quantified.

One unit of xylanase activity (U) can be defined as the amount of enzyme that liberates 1 µmolar of reducing sugar - xylose per minute under standard assay conditions.

Method optimization for better yield:

Variation of incubation time: wheat bran, sugarcane baggase, spilt green gram (mung daal bran) and rice bran were incubated for 24 hours, 48 hours, 72 hours, 96 hours, 120 hours and 144 hours.

Impact of various substrate: wheat bran, sugarcane baggase, mung daal and rice bran were used separately to get the maximum xylanase activity. The highest production was observed in wheat bran (0.065 mg/ml).

Impact of various temperature: to get the optimum range of temperature, test were done on 25°C, 30°C, 35°C, 40°C, 45°C and 30°C was most efficient (0.048 mg/ml).

Impact of various pH range: to get the best-suited pH medium, tests were done with pH ranging from 5 to 9 and it was best in 5 (0.046 mg/ml).

Impact of various Carbon source: to established best-suited carbon source for maximum yield of xylanase, we used xylose, dextrose, lactose, maltose, starch (Tallapragada & Venkatesh, 2011) and dextrose was the most efficient at 5% concentration (0.056 mg/ml).

Impact of various Nitrogen source: Ammonium nitrate, sodium nitrate, peptone, yeast extract (Tallapragada & Venkatesh, 2011) were used to standardized the maximum xylanase activity and the peptone yield maximum output (0.051 mg/ml).

Impact of Shaking / Non- Shaking during incubation: among Shaking and Non Shaking incubation employed at 28°C for 7 days, the shaking conditions for 96hr gave the most satisfactory result.

We identified ten isolates belonging to four genera

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Figure-1: Impact of Time and Substrate on Enzyme Activity

Figure-2: Impact of Temperature on Enzyme Activity

Figure-3: Impact of different pH on Enzyme Activity

Figure-4: Impact of different Carbon sources on Enzyme Activity

Figure-5: Impact of Nitrogen Sources on Enzyme Activity

Figure-6: Impact of Shaking & Non-Shaking Condition on Enzyme Activity

namely *Aspergillus*, *Trichoderma*, *Fusarium* and *Penicillium* from the targetted soil samples. *Aspergillus niger* was found to be the most potent one. In primary screening two isolates viz. *Aspergillus niger*, *Penicillium* exhibited more than 5cm zone of hydrolysis (5.8 cm and 5.3 cm respectively).

Gupta *et al.* (2009) found 16 fungal isolates with zone of hydrolysis ranging between 3.4 to 4.8 cm. *Penicillium oxalicum* was opted for producing xylanase on plate assay method by Mutheszhilan *et al.* (2007). Tallapragada & Venkatesh, (2011) selected *Aspergillus niger* as xylanase producer.

Conclusively the baseline of our study could be the beneficiary in Xylanase production which plays an important role in increasing the nutritional value of feed ingredients, reduces the cost of feed as well as maintains or improves animal performance. Further, fibres of feed ingredients hamper digestibility of poultry, which can be overcome by the use of xylanase.

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