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Scale up of Xylanase Production by Submerged Fermentation from *Brevibacillus Borstelensis* (MTCC 9874) and Digestibility Assessment of Crude Xylanase in Wheat

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Abstract

The main objective of this study was to scale up xylanase production from isolated soil microbe (*Brevibacillus borselensis*; the Microbial Type Culture Collection (MTCC) 9874) in a bioreactor and check digestibility of this crude enzyme in animal feed (wheat). Scale up was done from lab production Xylanolytic activity in bioreactor is greater than flask method due to more controlled condition in bioreactor which is not possible in flask method. Digestibility of crude enzyme was checked in wheat and was found that 16 (15.797) times digestion of wheat animal feed will be increased if it is supplemented with crude xylanase produced in bioreactor in the given condition. Although, pure enzymes are very expensive, this may not be affordable for farmers. Crude enzymes can be produced locally, or it will be very cheap and of low cost as it is present in the crude form thus, it is considered of a great value to farmers to increase their productivity and change their socio-economic condition

Keywords: Animal feed, Crude enzyme, Feedstock, Safe microbes.

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Competing interest: The authors have declared that no competing interest exists.

Introduction

Generally recognized as a safe microbe, many reports have already described the advantages of using the genus bacillus as an industrial workhorse for extra-cellular enzyme production (Helianti et al., 2016). Bacillus is the largest genus that is used to produce extracellular xylanase.

Mainly there are two types of hemicelluloses; xylan and glucomannan. Xylanase is the xylanolytic enzymes. The applications of xylanase are in white biotechnology, such as pulp bleaching, bioconversion of lignocellulosic materials into fermentative products, improvement of digestibility of animal feedstock (Haddar et al., 2012). Xylan has a complex structure consisting of β-1, 4 linked xylose residues in the backbone though side chains can be broken down by other enzymes backbone will be broken down only by xylanase. Thus, complete breakdown of xylan present in animal feed is not possible without xylanase as it is not present in digestive system of animal. Thus, complete digestion is impossible until animal feed is pretreated or supplemented with crude xylanase (Langhout et al., 1997, Danicke et al., 2001, Muszynski et al., 2019). Use of animal feed without treatment is wastage of large portion of animal feed as it cannot be digested by animals themselves. Therefore, the objective of this study is to scale up production of crude xylanase using soil microbe in a bioreactor maintaining the controlled condition those were optimized in rotary shaker incubator and test the possibility to use crude enzyme in animal feed to enhance digestibility.

Materials and Methods

Ⅰ. Microorganism:

Brevibacillus borstelensis (MTCC 9874) was isolated from soil sample from Eastern Sugar Mill, Biratnagar, Nepal in 2010 and it was deposited and preserved in the Microbial Type Culture Collection (MTCC), Chandigadh, India. The organism was retrieved as a lyophilized powder from MTCC in a vial in 2019.

Ⅱ. Regrowth of microbe:

The tip of the vial was broken, and 5 ml of nutrient broth was added and left overnight in a refrigerator (0-4 °C) as per instruction by MTCC. The vial content was shaken, and 1 ml was added in 50 ml nutrient broth in volumetric flask (250 ml). Then it was shaken and incubated in an incubator at 37 °C for 24 hours and this was used as seed for fermentation.

As the microbe was deposited and preserved in MTCC for more than 8 years, the organism was preserved for long time, there is a possibility of loss of enzyme production due to mutation during multiple times of sub-culture. It was isolated and confirmed production of xylanase more than 8 years before. Thus, the organism retains xylanase production or not was tested taking 50 ml nutrient broth with 1% xylan (Xylan from birchwood, Himedia, India) as a substrate. Fermentation was carried out in a rotary shaker incubator for 6 days (37 °C; 100 rpm). Production of xylanase was confirmed by a well-known method; the dinitrosalicylic acid (DNS) method. The fermentation conditions were maintained as per optimization carried out before (Data is not shown here) (Budhathoki et al., 2009, Budhathoki et al., 2011) . This experiment confirmed that this microbe still retains xylanase production. Then scale up of xylanase was carried out in bioreactor (Fermac 200, Electrolab, UK) (Fig. 1).

Ⅲ. Scale up experiment:

Fermentation scale up was done from a rotary shaker incubator (Flask method; 50 ml nutrient agar broth) to 1000 ml of nutrient broth in a bio reactor (Fermac 200,

Electrolab, UK). For validation, scale up was done 20 times. Fermentation in bioreactor was carried out at 200 rpm and at 7.6 pH. The pH was adjusted by bioreactor using 1M HCl and 1M NaOH solution automatically. These parameters were set as per optimized condition in lab scale production (Budhathoki et al., 2009, Budhathoki et al., 2011). For fermentation temperature, though optimized temperature was 60 °C in lab scale fermentation, temperature set in bioreactor was 50 °C because the maximum temperature that can operate bioreactor was 50 °C (Ghio, Insani et al., 2016), thus 50 °C was set in bioreactor instead 60 °C. Higher temperature and higher pH was set in fermentation to confirm that xylanase produced is thermophilic and alkali-tolerant which are very important for industrial applications. After 6 days of fermentation, the liquid content of bioreactor was collected, and down streaming was started with separation of solids and liquid by centrifugation using cooling centrifuge (-4 °C; 10000 rpm). Then supernatant (crude xylanase) was collected and stored in refrigeration until further use.

IV. Crude xylanase and digestibility:

One hundred gram of wheat was taken in two beakers (500 ml each), 350 ml of crude enzyme was added in one beaker and 350 ml of sterile distilled water was added in the other beaker containing 100 grams of wheat. Wheat contains xylan and it is excreted by animals as the animals do not have endogenous xylanase in their digestive system. Digestibility of wheat can be improved by breaking down xylan using crude xylanase (Langhout, Schutte et al., 1997, Danicke, Kluge et al., 2001, Muszynski et al., 2019). All beakers were kept for 6 days with intermittent stirring with glass rod in room temperature, liquids were collected from each and xylose content or xylanolytic activity was measured by DNS method (Fig. 2). More xylose content indicates better digestibility of wheat which is commonly used in animal feed.

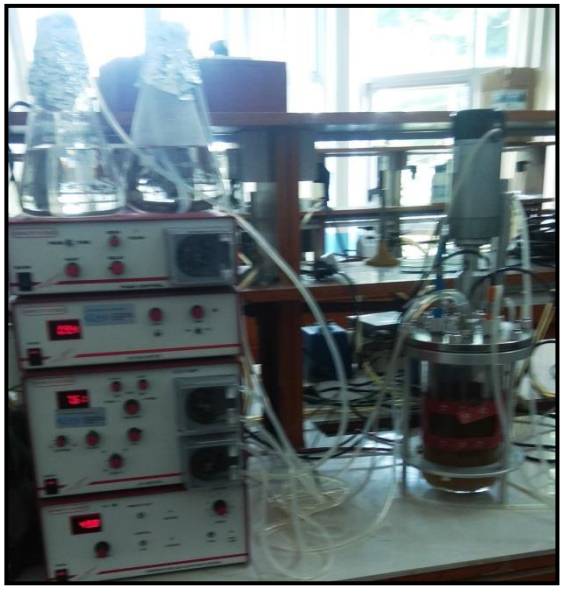

Fig. 1. Submerged Fermentation in Bioreactor (Fermac 200, Electrolab, UK).

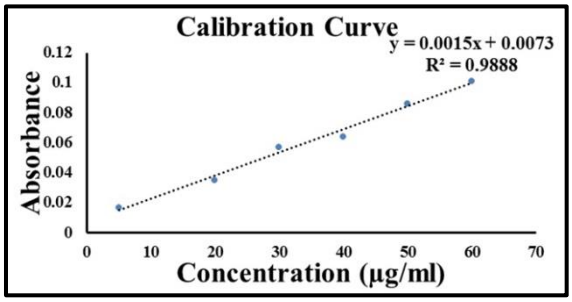

Fig. 2. Calibration curve drawn with xylose gor the dinitrosalicylic acid (DNS) method.

Results

Scale up of production of xylanase was carried out from flask method (50 ml) to bioreactor (1000 ml). So it was 20 times more than lab scale production and lab scale xylanolytic activity was found to be 56.19 µg/ml/day and bioreactor xylanolytic activity was found to be 63.75 µg/ml/day in both the cases xylan (from birch wood) was used as a substrate (0.5%). Wheat (100 g) supplemented with 350 ml of crude

enzyme showed xylanolytic activity 260.22 µg/ml/day whereas wheat (100 g) supplemented with 350 ml sterile distilled water showed xylanolytic activity of 16.47 µg/ml/day. This showed crude xylanase supports in digestibility of wheat 16 (15.797) times more if supplemented with crude enzyme before feeding animals. Crude xylanase greatly helps in digestion of wheat in animal feeds is found elsewhere (Karimi and Shokrollahi, 2013, Sa et al., 2013, Guo et al., 2014, Gonzalez-Ortiz et al., 2017).

Discussion

Generally, scale up is carried out 5 or 10 times of lab scale production but in this study, we carried out 20 times the volume of lab scale (from 50 ml flask method to 1000 ml bioreactor). Further studies can be done on optimization of parameters of bioreactor such as rotation per minute of paddle, microbes' seed volume, temperature of the bioreactors, and oxygen saturation in bioreactor to optimize production of xylanase. Xylanolytic activity was more in bioreactor (56.19 µg/ml/day) than flask method (2.34 µg/ml/day) which could be due to more control of parameters such as pH by auto control using inbuilt peristaltic pump in bioreactor, Oxygen saturation of 100 % which may not be in flask method.

Digestibility of wheat is increased almost 16 times after supplemented with crude xylanase which could be due to breakdown of xylan back bone in presence of the enzyme. (Walia et al., 2017).

Conclusion

This study concluded that crude xylanase is very important for animal feed for better digestibility of xylan containing animal feed such as wheat though the enzyme has multipurpose applications in paper and pulp, cloth industries to pharmaceutical purposes. This can enhance or uplift socio-economic status of farmers as it will reduce animal feed wastage due to indigestion.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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