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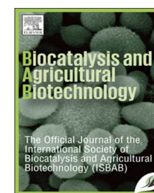

Isolation, characterization and purification of xylanase producing bacteria from sea sediment

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ABSTRACT

Xylan is the most important natural hemicellulose which has several industrial applications such as food, textile, bleaching of cellulose pulp, seed germination, degumming and agro waste treatment. Micro-organisms play a major role in the production of xylanase enzyme. In the present research, an attempt has been made to produce the xylanase enzyme from bacterial strains. The sea sediment was collected from Kovalam beach, Chennai (*Latitude*: 8.4004° North, *Longitude*: 76.9787° East) and serial dilution was done. The serially diluted sample was plated on xylan agar medium. The isolates were characterized and identified based on morphological, biochemical and physiological characters. The zones of hydrolysis for twelve xylan utilizing bacterial isolates were obtained. Of the 12 bacterial isolates, the species obtained from the serial dilution of 10^{-8} showed higher activity. The single colonies from the xylan agar plate was isolated and grown in nutrient broth supplemented with xylan in shake flask. The culture medium was centrifuged and the supernatant was used as crude enzyme. The enzyme partially purified to homogeneity by a combination of Ammonium-sulphate precipitation and dialyzed using culture supernatant as crude enzyme. The activity of the enzyme xylanase was assayed by DNSA method. The enzyme was optimally active at temperature 55 ºC and pH 9.0. The enzyme showed 95%, 90%, and 85% thermal stability at 55 °C, 60 °C and 65 °C. The enzyme was stable over a broad pH range of 8.0–10.0.

1. Introduction

Nowadays, xylanase enzyme plays a major important role in the industrial products, in the field of food, paper, pulp, animal feed and biofuel. The combination of xylanase enzyme with glucanase, amylase, cellulose and pectinase has potential applications for the production of bakery foods, biscuits and wafers (Moteshafi et al., 2016; Polizeli et al., 2005; Xin and He, 2013; Beg et al., 2001). There has been much mechanical enthusiasm for xylanase: as a supplement in creature nourish; for the fabricate of bread, sustenance and beverages; for the maker of materials; for the dying of cellulose mash; and for xylitol generation. Utilization of xylanases could enormously enhance the general financial matters of preparing lignocellulosic materials for the age of fluid powers and chemicals. Xylanases are glycosidases (E.C.3.2.1.8) which is an important hemicelluloses hydrolytic enzyme that degrades xylan and xylo-oligosaccharides and xylose. Different kinds of xylanase enzymes such as endo-β-1,4-xylanase (E.C.3.2.1.8), β-xylosidase (E.C.3.2.1.37), α-L-arabinofuranossidases (E.C.3.2.1.55), α-D-glucuronidases (E.C.3.2.1.139), acetylxylanesterases (E.C.3.1.1.72) and series of enzymes were responsible for the degradation of the polymer main chain. Amongst that, β-1,4-xylanase plays an important role in the cleavage of glycosidic bonds to produce short chain xylooligosaccharides (Li et al., 2010; Battan et al., 2008; Ratnadewi et al., 2016). Xylan is the most abundant hemicellulose, consisting of a homopolymeric back bone chain of 1,4-linked β-D-xylopyranose units, including short chains O-Methyl-D-glucuronyl residues (Lan et al., 2008; Karaoglan et al., 2014). The xylanolytic enzymes from different sources such as plant cell wall, bacteria and fungi, especially from microorganisms, have been studied to understand their physical and biochemical characteristics (Sunna and Antranikian, 1997). Nowadays, xylan-degrading enzymes have been reported to be produced by a variety of bacteria species such as *Bacillus pumilus* (Poorna and Prema, 2007), *Acinetobacter junii* (Lo et al., 2010), *Cellulomonas uda* (Rapp and Wagner, 1986), *Bacillus mojavensis* (Kallel et al., 2015) and fungi species such as Aspergillus niger (Karunakaran et al., 2014), *Paecilomyces thermophile* (Lan et al., 2008), *Trichoderma longibrachiatum* (Azin et al., 2007), *Thermomyces lanuginosus* (Li et al., 2005), *Pichia stipitis* (Haan and van Zyl, 2003) and possesses a range of industrial and environmental applications. Although bacterial xylanase was reported to have higher value of optimum pH which is beneficial from the view of applying in bio

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bleaching process, xylanases produced from bacteria usually has higher enzyme activity (Subramaniyan and Prema, 2002). The xylanase enzyme can be classified as endo - and exo – xylanases. Several microorganisms produce multiple xylanases, each of the enzymes may have a specialized function in the degradation of the complex polysaccharides and those specialized functions of individual xylanases may be useful for applications in human consumption, animal feed, and the paper industry. Evolution in nature may give rise to strains producing enzymes with different properties including their thermo stability. The cellulose-free xylanases are active at high temperature and pH is gaining importance in pulp and paper industry. The xylanases are useful in bioconversion of lignocellulosic to fuel and chemicals, to improve silage for better digestion by ruminants, to improve quality of detergents, and also used for clarification of juices, in flour improvements for bakery products and in controlling environmental hazards through bio pulping. Only a few xylanases are reported to be active and stable at alkaline pH and high temperature. In view of this, there is a need to search for new sources of xylanases and their characterization, especially with extremophilic properties. Bacterial xylanases are profitable when contrasted with contagious xylanases. Stability and movement in alkaline range, high thermosatbility, low cellulase action, more prominent animosity towards xylans because of high persistence of sugar spaces, and capacity to specifically solubilize arabinoxylan make bacterial xylanases amiable to particular modern application. A large portion of the accessible bacterial xylanases has just few of these properties which make modern procedures to depend much on costly and risky synthetic procedures. The mechanical procedure completed at raised temperature has many focal points including enhanced solvency of substrate, diminished thickness in response blend, and lessened danger of microbial defilements. It is of awesome enthusiasm to investigate more xylanases that are warm steady. The main objective of the present study is the isolation of bacterial strain from marine environment and production, purification of the xylanase enzyme. Generally, the marine bacterium is dynamic at soluble pH 9 – 12 and ideally dynamic at temperature of 40 °C. The marine xylanases have particular properties like cold adjustment and haloalkalitolerance. The frosty dynamic xylanases have high action at low temperatures (2–30 °C). The secretion of recombinant proteins desired for purification purposes by avoiding contamination by intracellular proteins (Macauley-Patrick et al., 2005). The marine xylanases can have some unique properties however there are very few studies on marine xylanases. A marine isolate produced large amounts of thermophilic and halotolerent enzyme. It has been accepted that suitability of an enzyme for an industry application depends upon the enzymes homogeneity.

2. Materials and methods

2.1. Sample collection

Soil samples were collected from Kovalam beach, Chennai (*Latitude*: 8.4004° North, *Longitude*: 76.9787° East). The samples were collected in sterile plastic bags, brought to the Environmental laboratory, SSN College of Engineering and were processed within 2 h.

2.2. Chemicals and equipment

Xylan agar medium, nutrient broth, Basal salt solution medium, DNS reagent and ammonium sulphate was purchased from Ranbaxy Fine Chemicals Ltd, Mumbai. All the chemicals were used for analytical grade. Fresh deionized water was used during the experimental work. The pH of the solution was measured by using pH meter (HI 98107; Hanna Equipment Private Limited, Mumbai, India). The sterilization process in the present study was carried out using Manish autoclave (Manish Scientific Instruments, Chennai, India). The inoculation and sub-culturing process was done under specified Laminar Air Flow (Model No. D-0284, Dolphin Instruments and Equipments, Chennai, India).

2.3. Isolation of xylanase producing bacteria

Approximately one gram of sample was weighed and suspended in 100 mL of sterile distilled water. Then serial dilution was done up to ten dilutions. From each dilution 0.1 mL of samples were plated on xylan agar medium. All the plates were incubated at 37 °C for 2–3 days. The morphologically distinct colonies from dilutions 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} were collected and further streaked onto the same medium to obtain pure colonies. The obtained pure colonies were sub cultured in xylan agar medium for further purpose.

2.4. Screening of xylanase producing bacteria

The colonies found on the plates of serial dilution 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} were transferred onto another xylan agar plates which were again incubated at 37 °C for 2 days. Single colonies producing clear zone formation in the above mentioned plates were selected. A total of 12 bacterial isolates were obtained from the soil. Of the 12 bacterial isolates, the species obtained from the serial dilution of 10^{-8} showed higher activity. The Xylanase producing species was identified on the basis of their physiological, morphological and biochemical characteristics. The colonies found by zone formation on the plate 10^{-8} were screened and spreader on a new agar plate. The strain was stored and maintained on nutrient agar plates at 4 °C. A loop full of culture from the plates of colonies were inoculated into the nutrient broth containing (g/L): peptone-5.0, yeast extract - 6.0, KNO_3 - 1.0, KH_2OPO_4 - 1.0, $MgSO_4$ - 0.1, xylose - 5.0 followed by incubation at 37 °C for overnight under shaking at 200 rpm. The xylanase was harvested by centrifugation at 10,000 rpm for 20 min at 4 °C. The cell free supernatant was used as crude enzyme.

2.5. Xylanase production

One mL of pre-grown culture was inoculated into 100 mL of the BSS medium (Basal Salt Solution). The trace metal solution has the following composition H_3BO_3 - 2.85 g, $MnCl_2 \cdot 7H_2O$ - 1.80 g, $FeSO_4 \cdot 7H_2O$ - 2.49 g, Na Tartarate - 1.77 g, $CuCl_2$ - 0.03 g, $ZnCl_2$ - 0.02 g, $CoCl_2$ - 0.04 g, $Na_2MoO_4 \cdot 2H_2O$ - 0.02 g, distilled water – 1000 mL. The pH of the medium was adjusted to 9.0 using 1 N NaOH. The culture was incubated on a shaker incubator 100 rpm maintained at 50 °C for 72 h. After the prescribed time interval, the samples were withdrawn from the incubator and allowed for centrifugation at 4 °C at 10,000 rpm for 10 min. The supernatants were analyzed for xylanase activity.

2.6. Assay of xylanase activity

The xylanase activity was examined by the 3,5-dinitrosalisylic acid (DNS) method by measuring the amount of reducing sugars liberated from xylan and using a calibration curve for D-xylose. Xylose was used as the standard. Assay mixture (0.5% xylan - 0.5 mL, phosphate buffer - 50 mM, pH – 7.0 and enzyme- 1.5 mL) was incubated at 50 °C for 15 min and then 1.5 mL of DNS reagent was added to terminate the reaction. Test tubes containing reaction mixture were stoppered and kept in boiling water bath for 10 min and then cooled to room temperature. The absorbance was recorded against reagent as blank at 575 nm keeping enzyme as control. The one unit of xylanase activity was defined as the amount enzyme required producing 1 μM of xylose released in 1 min.

2.7. Partial purification of xylanase enzyme

The cell free supernatant was used as crude enzymes. The crude xylanase was precipitated with 40% saturation of ammonium sulphate and allowed to settle overnight at 4 °C and then centrifuged at

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