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RESEARCH ARTICLE

BIOTECHNOLOGY

PRODUCTION AND OPTIMIZATION OF XYLANASE FROM ESTUARINE *BACILLUS CEREUS*

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ABSTRACT

Xylan is the most abundant non cellulosic material present in both hardwoods and annual plants, and accounts for 20-35% of the total dry weight in tropical plant biomass. In this study xylanase producing *Bacillus cereus* colonies from vellar estuarine soil sample was isolated and its phenotypic characteristics were also identified. The optimization of pH, temperature, salinity and substrate concentration were characterized by different assay conditions. Purification of xylanase was carried out by ammonium sulphate precipitation method and determination of its molecular weight by SDS-PAGE. The water and sediment sample collected in vellar area was found to harbor respectively 1.0×10^6 CFU/ml and 1.25×10^7CFU/g xylanase producing strains. The optimum pH, temperature, salinity and substrate concentration were as follows 8.0,30ºc, 0.9% and 2.5%. The molecular weight was 22KDa and 24KDa as determined by SDS-PAGE. This study proved that the vellar estuary is abundant in xylanase producing bacteria.

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KEYWORDS

Xylanase, *Bacillus cereus*, Estuarine soil, Ammonium sulphate precipitation, SDS-PAGE.

INTRODUCTION

Xylanase [E.C.3.2.1.8] is the name given to a class of enzymes which degrade the linear polysaccharide β-1,4-xylan to xylose, thus breaking down hemicelluloses which is a major component of the cell wall of plants. Xylan, the most abundant hemicelluloses containing heteropolysaccharides, consists of a backbone of β-1-4-linked xylopyranose residues with substitution of O-acetyl, arabinosyl and methyl glucoronsyl[1]. In 1985 microbial xylase was isolated by Biely[2]. The xylanases can be obtained from a variety of bacteria [3].

Other microorganisms which produce xylanase and comparing the xylanase against the present invention's xylase, other suitable strains can be found[4]. The xylanase is preferably produced and used in granulate or powder form. As compared with cultures of fungi, the cultures of bacteria according to the invention have the advantage that require fermentation times of only one to two days, whereas culturing times for fungi range from three to five days[5]. Microorganisms are rich sources of xylanase enzymes which are produced by diverse genera and species of fungi, bacteria, actinomycetes and fungi. A bacterial protein with xylanase activity was invented in 1994[6]. Xylanase enzyme exhibits two peptide forms of 53 and 64 KDa escherichia clones expressing the activity[7].Xylanases are useful in several industrial applications. They are extensively used in pre-treatment of forage crops and other lignocellulosic biomass, added to swine and poultry cereal based diets to improve nutrient utilization, flour modification for bakery products and saccharification or agricultural industrial and municipal wastes. Moreover, it is reported that xylanases have been widely used for clarifying fruit juices, wine and also in food processing in combination with celluloses[2] and in improving the nutritional properties of agricultural silage and grain feed.

MATERIALS AND METHODS

(i) Collection of samples:

water and sediment samples were collected from vellar estuary. The surface water samples were collected using presterilized sample bottles allowing enough air space in the bottles to facilitate thorough mixing. Sediment samples were collected using a sterile spatula and aseptically transferred into a sterile polythene bags. Samples were stored at 4°c for further study

(ii) Isolation and identification of bacterial colonies:

The xylanase producing bacterial strains from water and sediment samples were isolated using serial dilution and agar plating technique.

Phenotypic characteristics of bacterial isolate:

(iii) Gram staining:

Gram method was used for staining of bacteria gram (+) cells seemed purple white while gram (-) cells seemed pink or red. Cell morphology was examined. Endospores and motility of the organisms were also examined[8,9].

(iv) Starch hydrolysis:

Transfer a loopful of culture into the starch agar containing medium and perform single line streaking across the centre of the petriplate.. Incubate the

plate at 37°C for 24 hours. After the incubation period the plate was flood with iodine solution. The appearance of clear zone around the microbial growth indicates the positive results[10].

(v) Gelatin hydrolysis:

Under aseptic conditions transfer a loopful of culture to the gelatin containing medium. Incubate it at 37°C for 24 hours and keep the tubes inside the refrigerator at 4°c for at least 4 hours. The presence of liquefaction of gelatin shows positive result [11].

(vi) Lipid hydrolysis and catalase test:

Transfer a loopful of culture into the petriplate containing tributyrin medium. The formation of clear zone around the microbial growth shows positive result[12]. For catalase test incubate the plate at 37°C for 24 to 48 hours. After this hydrogen peroxide was poured on to the colonies. Formation of air bubbles indicates the presence of catalase enzyme[13].

(vii)Caesin hydrolysis:

Skimmed milk agar medium is used for this hydrolysis. Transfer a loopful of culture to the petriplate. Perform single line streaking across the centre of the petriplate. The presence of clear zone formation indicates positive result[14].

(viii) Nitrate reduction:

Prepare tripticase soy nitrate broth tubes transfer a loopful of culture and incubate the tubes for 96 hours at 35°C. Following this add 0.1 ml of the reagent and immediately observe for the sharp color change from orange to red. This shows the positive test result[15].

(ix) Citrate utilization test:

Organisms were inoculated on to the simmon citrate agar slants. The inoculate tubes were incubated at 37°Cfor 24 hours. Color changes occur due the raise of pH. Bromothymol blue from green to blue indicates positive result.

(x) Screeing of xylanase producing bacterial isolate:

Xylanase producing bacteria were screened by primary and secondary screening methods.

(xi) Determination of xylanase activity and enzyme kinetics:

Xylanase activity and kinetics were determined by DNS method[16].

(xii) Optimization of culture conditions for enzyme production:

Xylase production was studied at different pH (3-10), temperature (25, 30 and 35°c), salt concentration (0.1 to 1.5%) and carbon sources (xylan-05%-3.0%, glucose 0.5%-3.0%) and assayed for production at several incubation periods[17].

(xiii) Determination of protein concentration:

The protein concentration of the crude enzyme was determined by Lowry's method[18].

(xiv) Purification of xylanse:

The partial purification of xylanase was carried out by ammonium sulphate (80%) precipitation[19].

(xv) SDS-PAGE:

The enzyme sample was mixed with equal amount of sample buffer and heated at 95°c for 5 minutes. Then the samples were loaded into the wells and allowed for electrophoresis at 50v initially. After the dye front has reached the end of the stacking gel, the voltage was increased to 100v and proteins were allowed to migrate through resolving gel [20].

RESULTS

1. Xylanase Production and biochemical characteristics

Optimization of cultivation conditions variables that had a significant impact on xylanase production was necessary. Xylose standard values were obtained by DNS Method. Figure1 illustrates the xylanase

production and table 1 illustrates the biochemical characteristics of xylanase. The optimum pH, temperature, salinity and substrate concentration were as follows 8.0, 30°c, 0.9%, and 2.5%.

Xylanase production

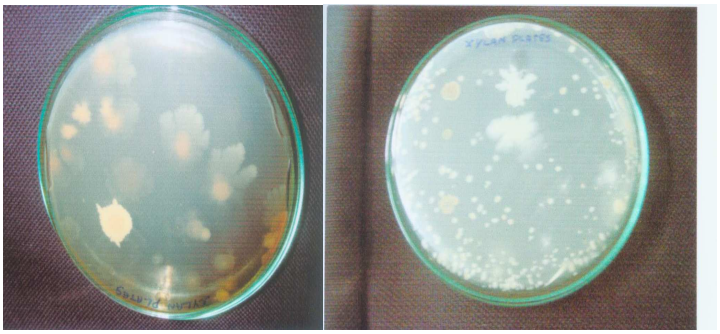

Fig 1

Illustrates the xylanase production in 10^{-3} water sample and 10^{-4} sediment sample obtained from vellar estuarine.

characteristics of xylanase

Table 1

Showing the biochemical characteristics of Xylanase producing organism.

Gram Reaction	+
Shape of the cell	Rod
Spore	+
Motility	+
Starch hydrolysis	+
Gelatin hydrolysis	+
Fat	+
Casein hydrolysis	+
Catalase	-
Nitrate reduction	+
Citrate utilization test	+

2. ***Analysis of SDS – PAGE***

The partially purified xylanase was electrophoresed by SDS-PAGE, it showed single band with a molecular weight of 22 KDa, when compared with with molecular weight standard. Figure 2 shows the electrophoresis of xylanase.

SDS - PAGE

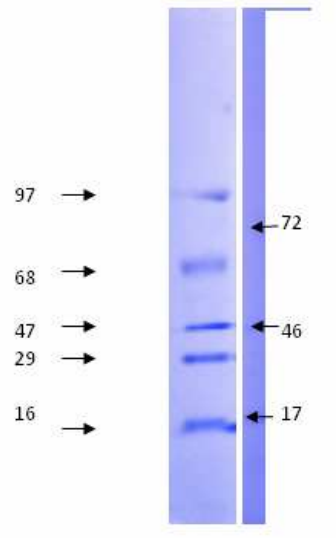

Fig 2
SDA-PAGE showing Xylanase havin molecular weight of 22KDa.

3. Kinetic determinations:

The crude enzyme (xylanase) produced from bacterial strains showed quite different K_m and V_{max} values for the same substrate under the conditions studied. Substrate concentration is one of most important factors which determine the velocity of enzyme reactions.

Table 2
Summarized Kinetic parameters of the Xylanase.

Monad Kinetics	**Value**
μmax	0.7580 hr^{-1}
Ks	0.5146 g/l
M.M.Kinetics	Value
V max	3.795 hr^{-1}
Km	0.99 g/l

DISCUSSIONS

The present study is on the isolation of a xylanase producing bacterium from Vellar estuary with luxuriant growth of mangrove trees and characterization of enzyme from it. The water and sediment samples collected in this area were found to harbour respectively 1.0 x 10^6 CFU / ml and 1.25 x 10^7 CFU/g xylanase producing strains. Among them 7 strains were selected in the primary screening. The most potential strain was identified in the secondary screening procedures. It was identified as *B.cereus* using standard manual. When the strain was optimized against varying pH (3.0 - 10.0), salinity (0% - 1.5%), temperature (25°C – 40°C) and substrate concentration (0.5-3%), pH 8.0, 30°C, 1% salinity, 2.5% of xylose were found to be ideal for xylanase production. The molecular weight of xylanase was 22kDa as determined by SDS – PAGE.

CONCLUSION

Thus this study has proved, that the Vellar estuary is abundant in xylanase producing bacteria and on proper strain improvement the *B.cereus* strain used in the present study may be used for industrial production of xylanase.

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