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Isolation and Characterisation of Alkalophilic Xylanase Producing Bacteria Ms-2-1 from Marine Water Sample

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Abstract

A novel marine bacterium was isolated from Pichavaram mangrove forest located near Chidambaram, Tamil Nadu and designated as MS-2-1. The morphological, cultural, physiological characteristics as well as 16S rRNA gene sequence-based phylogenetic analysis confirmed the taxonomic affiliation of MS-2-1 as *Marinobacter Aquaeolei*.

Keywords: Xylanase, *Marinobacter*, Genomic DNA

1. Introduction

Ligno cellulosic biomass represents one of the biggest renewable energy resources and can be exploited not only for the production of energy, speciality chemicals, nutrient-rich animal feed, biofuel and other high-value products [1] but can also offers environmental and strategic advantages [3]. Xylan is the major hemicellulosic constituent of Lignocelluloses and is composed of a backbone of 1-4 linked D-Xylopyranose units and short chain branches including O-acetyl, Larabinofuranosyl, and D-glucuronosyl residues [2]. The complexity of xylan requires an action of multiple xylanases with overlapping yet different specificities to affect complete hydrolysis.

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Microbial xylanases (1, 4-D-xylanxylanohydrolase, EC 3.2.1.8) are the preferred catalysts for xylan hydrolysis due to their high specificity, mild reaction conditions, negligible substrate loss, and side product generation. Xylanases have got potential applications in a wide range of industries viz. pulp and paper, animal feed, bakery, textile. Hence, the search for novel bacterial strains that produce xylanases which withstands hostile industrial conditions, specifically extremes of temperature and pH is important. Particularly, bacterial strains that possess additional desirable features like high specific activity, high turnover number, prolonged stability, no cofactor requirement, and overall robustness deserves merit.

2. Methods

2.1 Isolation of Xylanolytic Bacteria

In order to isolate xylanolytic bacteria, soil samples were collected from a coastal soil sediment sample collected from the Bay of Bengal. To isolate extracellular xylanase producing bacteria, soil suspension was obtained by shaking 1g of soil in 100 ml saline water. Ten-fold dilutions of soil suspension were plated onto Xylanase agar, incubated for 3 days at 30˚C, and examined for clearing zone around the bacterial colony due to xylanolytic activity. The colonies showing xylanolytic activity on xylanase agar plate were selected and pure cultures were established by streaking isolated colonies on a nutrient agar plate.

2.2 Molecular Phylogeny of Xylanolytic Bacterium

2.2.1 Extraction of Genomic DNA

Total genomic DNA was extracted as described [2]. For checking the purity and concentration of DNA, 10 µl of DNA sample was mixed with 990 µl of TE buffer and the absorbance was read at 260 and 280 nm in a UV-Visible spectrophotometer Ultraspec 4300 pro (Amersham Bioscience, Hong Kong).

2.2.2 16S rRNA Amplification, DNA Sequencing and Editing

Amplification of 16S rRNA gene from the genomic DNA of the xylanolytic bacterium was done by using universal primer set,

forward primer fD1 (5′-GAGTTTGATCCTGGCTCA-3′) and reverse primer rP2 (5′-ACGGCTACCTTGTTACGACTT-3′) [5].

2.2.3 Phylogenetic Tree Analysis

The reference sequences required for comparison were downloaded from the NCBI database using the site (http://www.ncbi.nlm.nih.gov/Genbank). All the sequences of 16S rRNA of *Marinobacter* genus aligned using the multiple sequence alignment programme CLUSTAL V developed by [7]. The aligned sequences were then checked for gaps manually, arranged in a block of 250 bp in each row and saved as molecular evolutionary genetic analysis (MEGA) format in software MEGA v2.1.

2.3 Optimisation of Conditions for Xylanase Production

2.3.1 Time Course Induction

The growth of bacteria and the production of Xylanase were determined in xylan fermentation broth at 30˚C for 60 h with a rotation of 160 rpm. The culture samples were collected at every 12 hours and culture turbidity (i.e. OD at 600 nm) was measured to record the growth.

2.3.2 Xylanase Activity Assay

Xylanase activity was determined by monitoring xylan hydrolysis and production of reducing sugar from xylan. Xylanase activity was measured by the DNS (3,5-dinitrosalicylicacid) method [6] through the determination of the amount of reducing sugar liberated from Xylan solubilised in 10 mm Tris buffer (pH 8.0).

2.3.3 Optimisation of Temperature for Xylanase Production

Optimisation of temperature for xylanase production was determined by growing the strain MS 2-1 in starch broth at different temperatures (25˚C, 30˚C, 35˚C and 40˚C) for 60 hours and xylanase production was monitored at every 12 hours intervals over a 60 hours fermentation period through assay of xylanase activity.

2.3.4 Optimisation of pH for Xylanase Production

Optimisation of pH forxylanase production was determined by growing the strain MS 2-1 in xylan broth of different pH using Appropriate Buffer, Acetate Buffer (pH 5.0), Phosphate Buffer (pH 6.0), Tris Buffer (pH 8.0), and Glycine-NaOH Buffer (pH 9.0 and pH 10.0) at 30˚C for 60 hours and Xylanase production was monitoredat every 12 hours intervals over a 60 hours fermentation period through assay of xylanase activity.

2.3.5 Optimisation of Different Substrates for Xylanase Production

Optimisation of carbon sources for xylanase production was determined by growing the strain MS 2-1 in xylan broth containing different substrates (sucrose, CMC, xylan and starch) at 30˚C for 60 hours and xylanase production was monitored at every 12 hours intervals over a 60 hours fermentation period through assay of xylanase activity.

2.3.6 Optimisation of Different Nitrogen Sources for Xylanase Production

Optimisation of nitrogen sources was done by growing the strain MS 2-1 in xylan broth containing different nitrogen sources (Ammonium Nitrate, Potassium Nitrate, Urea, Yeast Extract, Ammonium Chloride, Peptone, Yeast Extract + Peptone and Tryptone) at 30˚C for 60 hours and xylanase production was monitored at every 12 hours intervals over a 60 hours fermentation period through assay of xylanase activity.

3. Results

3.1 Isolation of Xylanolytic Bacterium

The xylanolytic bacterium designated, MS 2-1 was isolated from a coastal soil sediment sample collected from the Bay of Bengal. Xylanolytic activity of strain MS 2-1 was determined by the formation of a clear zone around the bacterial colony on xylanase agar plate (Fig 1).

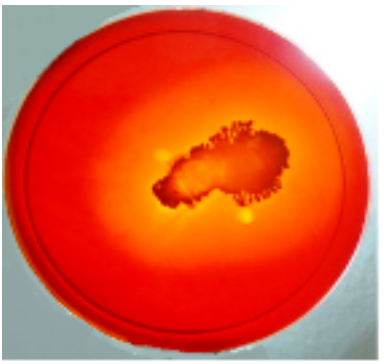


Figure 1: Xylanolytic activity of strain MS 2-1 as indicated by the clear zone around the bacterium on xylanase agar plate

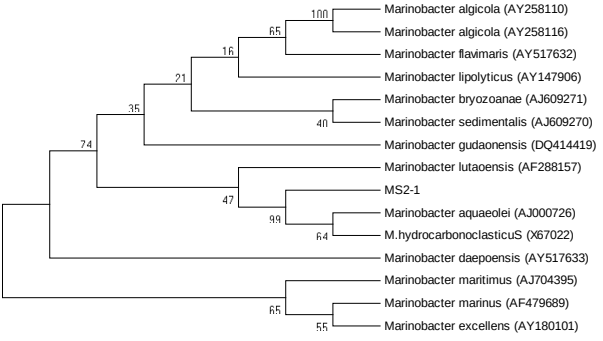
3.2 Molecular Phylogeny Analysis of Atrain MS 2-1

The isolation of genomic DNA, 16S rRNA gene amplification, sequencing, and phylogenetic tree analysis were done.

The total genomic DNA from the strain MS 2-1 was extracted and checked for purity. The concentration was adjusted to 20µg/ml using double distilled water. The 1.5 kb 16S rRNA gene was amplified using the universal primers, fD1 and rP2.

3.3 Molecular Phylogeny Analysis

The 16S rRNA nucleotide sequence of strain MS 2-1 showed homology towards *Marinobacter* species. On the basis of sequence homology (99%) and subsequent molecular phylogenetic analysis of the 16S rRNA, the taxonomic affiliation of strain MS 2-1 was confirmed as *Marinobacter Aquaeolei* (Fig 2).



3.4 Morphological, Physiological and Biochemical Characterisation of Strain MS 2-1

Morphological, physiological and biochemical characteristics of strain MS 2-1 are summarised in Table 1.

Table 1: Phenotypic and biochemical traits of strain MS 2-1

Biochemical test	Inferences
Shape	rod
Gram's staining	+
Citrate utilisation	_
Triple sugar iron	+
Urea test	+
5% NaCl	+
Growth at 42˚C	+
Catalase	+
Oxidase	_
Indole utilisation	_
Nitrate reduction	+
V-P test	
Hydrolysis of substrates	
Starch	+
Carboxymethylcellulose	+
Lipid	_
DNA	+
Proteins	+

+, positive reaction; -, negative reaction

3.5 Optimisation of Condition for Xylanase Production

3.5.1 Time Course Induction of Xylanase Production and Growth of MS 2-1

The optimum growth and Xylanase production by strain MS 2-1 were observed in xylan fermentation broth at 30˚C up to 60 hours. Growth curve analysis revealed that the maximum cell growth and enzyme activity was observed after 24 hours. The xylanase activity appeared to be high at late log phase after 24 hours (Fig 3).

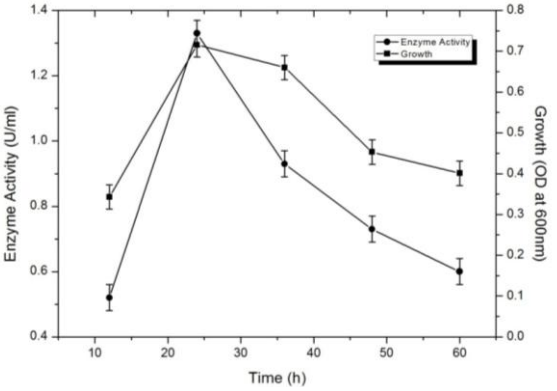


Figure 3: Time course induction of xylanase production (U/ml) (filled circle) and biomass production (filled square) by strain MS 2-1

3.6 Optimisation of Temperature for Xylanase Production by Strain MS 2-1

Growth and xylanase production by strain MS 2-1 was measured in a range of temperature between 25˚C to 40˚C. Optimum growth (OD at 600 nm, 0.417) and maximum xylanase activity (0.8 U/ml) was observed at 30˚C after 24 hours (Fig 4).

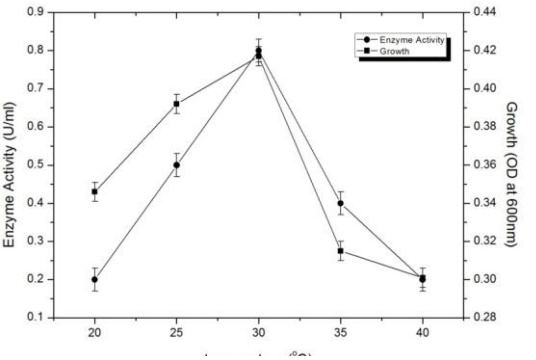


Figure 4: Optimisation of temperature on xylanase production (U/ml) (filled circle) and biomass production (filled square) by strain MS 2-1

3.7 Optimisation of pH for xylanase production by strain MS 2-1

Growth and xylanase production by strain MS 2-1 were measured in a range of pH between 5 to 10. Optimum growth (OD at 600 nm,

0.778) and maximum xylanase activity (2.2 U/ml) was observed at pH 8.0 at 30˚C after 24 hours (Fig 5).

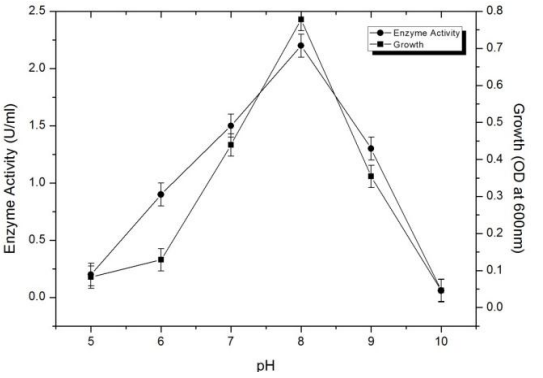


Figure 5: Optimisation of pH on xylanase production (U/ml) (filled circles) and biomass production (filled square) by strain MS 2-1

3.8 Optimisation of Different Substrates for Xylanase Production by Strain MS 2-1

Growth and xylanase production by strain MS 2-1 in xylan broth was measured in a range of substrates such as sucrose, CMC, xylan and starch. When xylan used as a substrate, Optimum growth (OD at 600 nm, 0.719) and maximum xylanase activity (1.9 U/ml) were observed at 30˚C, pH 8.0 after 24 hours (Fig 6).

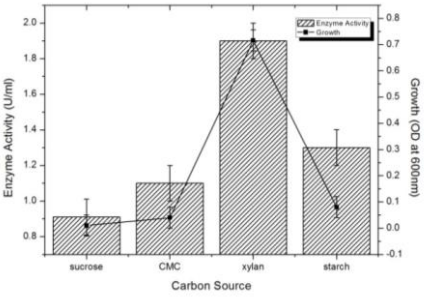


Figure 6: Optimization of different carbon sources on xylanase production (U/ml) (bars) and biomass production (filled square) by strain MS 2-1.

3.9 Optimisation of Different Nitrogen Sources for Xylanase Production by Strain MS 2-1

Growth and xylanase production by strain MS 2-1 in Xylan fermentation broth were measured in a range of organic nitrogen sources such as Urea, Tryptone, Yeast Extract, Peptone, Yeast Extract + Peptone and Inorganic Nitrogen sources such as Ammonium Nitrate, Ammonium Chloride and Potassium Nitrate. After 24 hours incubation at 30˚C, pH 8.0, and 1.5% xylan, yeast extract (0.5%) and peptone (1.0%) showed Optimum Growth (OD at 600 nm, 0.554) and maximum xylanase activity (0.36U/ml) (Fig 8).

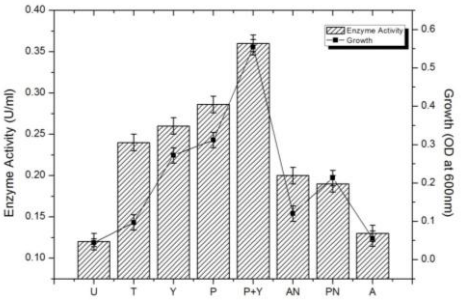


Figure 7: Optimisation of different nitrogen sources on xylanase production (U/ml) (bars) and biomass production (filled square) by strain MS 2-1. U = Urea, T = Tryptone, P = Peptone, Y = Yeast extract, Y+P = Yeast extract and Peptone, AN= Ammonium Nitrate, A = Ammonium Chloride, PN = Potassium Nitrate

4. Conclusion

A potent xylanase producing bacteria was isolated from the marine water sample. Morphological and biochemical tests were done for the identification. Molecular and Phylogenetic tree analysis confirmed the affiliation of MS 2-1 as *Marinobacter Aquaeolei*. Thereafter, the physical and chemical parameters for the production of enzyme were optimised.

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