



Isolation and identification of thermostable and alkali stable xylanase producing *Bacillus subtilis* DB1 from soil

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ABSTRACT

Microbes are the chief source of enzymes; microorganism produces extracellular as well as intracellular enzymes. Extracellular enzymes are economically feasible than intracellular enzymes. Extracellular enzymes are commonly employed in large scale production. Enzymes from microbes are more interested than the plant and animal. Xylanase is one of the enzymes that can be utilized in food, biofuel, paper and animal feed industries.

In this study xylanase producing bacteria was isolated from the soil at Gangakhed region and was isolated on Oat Xylan Media. The isolate was identified on the basis of its morphological and biochemical properties. These properties of the novel isolate were compared to known bacteria *Bacillus subtilis* by referring to Bergey's manual of systematic bacteriology (Vol 2). This bacterium is labelled as *Bacillus subtilis* DB1 due to variation in some biochemical properties. This novel bacteria *Bacillus subtilis* DB1 is Gram-positive rod and able to produce 2322 U/ml of xylanase enzyme at 50°C, PH 8.

INTRODUCTION

Enzymes are biocatalyst and used in food, pulp and ethanol industries. Enzyme industries commercialized the production of lipase, amylase, protease etc. Xylanase (E.C.3.2.1.8) catalyze the hydrolysis of xylene, the chief constituent of hemicellulose occur in the plant cell wall (Kulkarni et.al (1999). Enzymes are more attracting to biofuel, paper and food industry because xylanase enzymes break down the hemicellulose of plant and generate simple molecules (Collin 2005). It is an account for approximately one-third of all renewable organic carbon on the earth (Parade et.al 1996). Xylan in hardwood accounts for about 20 to 30% of total biomass (H. Shete and C.N. Khobragde 2010). Xylan forms an interface between lignin and cellulose through β 1-4 glycosidic bond. Bacteria, fungi and actinomycetes are reported to produce xylanase (A.R.jadhav 2012). In this study, Xylanase producing bacteria was isolated on Oat Xylan medium containing Xylan as a sole source of carbon. The composition of the medium was (g.L⁻¹). Oat flour; 1.0 gm, NH₄NO₃: 0.2 gm; KH₂PO₄ 0.1 gm, MgSO₄.7H₂O; 0.1 gm, CaCl₂:0.01gm, Distilled Water 100ml , PH 7.The inoculated plate plates were incubated for 7 days at 30° C. The zone of clearances was recorded and selected the Xylanase produces culture for further study.

MATERIALS AND METHODS

Collection of soil sample

The Soil sample was collected at Gangakhed region (located in Maharashtra, India) in a sterile plastic bag and preserved. About 10 gm of soil was collected

Isolation of Efficient Xylanase producer

Xylanase producing microorganisms were isolated by applying resistance spore selection technique as well as by using a selective medium containing Xylan as the primary source.

0.1 gram soil was inoculated in 10 ml sterile distilled water and subjected to heat shock at 80°C for 10 min. This was then diluted as 10⁻² and 10⁻³ with sterile water. The 0.1 ml supernatant of each dilution was poured and spread over Xylan yeast extract agar medium, the plate were incubated at 30°C for 24 hours to select the fast-growing xylanase producing organism. The efficient xylanase producer was selected based on the formation of a clear zone of xylene hydrolysis around the colony. The selected colony were then transferred onto Oat Xylan agar medium, in which Oat Xylan was used as principal carbon source and ammonium sulphate was the nitrogen source. The plate was incubated at 30°C for 24 hours, colonies developed on plates, among these the colony which produce a remarkable zone of clearance was selected and isolated as OXA slant for further study.

Identification of xylanase producing organism

Isolated xylanase producing organism was identified on the basis of morphological and biochemical properties. These properties of the isolate were compared with known similar stain *Bacillus subtilis* by referring Bergey's manual of systematic bacteriology second edition.

Production of xylanase

Enrichments of xylanase producers were carried out at 50°C, PH 8 and enzyme production was carried out by maintaining the same parameter and Oat Xylan media was used. 1 ml inoculum was used to ferment 50 ml Oat Xylene Media. Fermentation was carried out using the stationary technique for 72 hours at 50°C PH 8.

After fermentation, the fermented broth was filtered to remove bacterial growth using a centrifuge at 8000rpm for 20 minutes twice. The resulting supernatant was collected and used as a crude enzyme for xylene assay.

Radial diffusion assay

The xylene production was detected by radial diffusion assay. The cell-free extract of the crude enzyme was used for assay. The OXM agar plate was used for the assay. The OXM agar plate was prepared and agar cup was bored using 8 mm cork borer. The cup was filled with the crude enzyme extract and incubated for 1 hour at normal room temperature. The plate was then flooded with 0.5% congored solution and zone of clearance was observed.

Observation and result

Isolation and identification of xylanase producer bacterial species

Morphological properties of the isolate

The isolated bacteria were shown distinct colony on Oat xylene medium and Gram stain conformed as Gram positive bacillus (fig 1 and 2). The colony of isolated bacteria was red-tinted dark centered and having diffusible pigment around it. The isolate shows mucoid colony. Other morphological properties are tabulated in table 1.

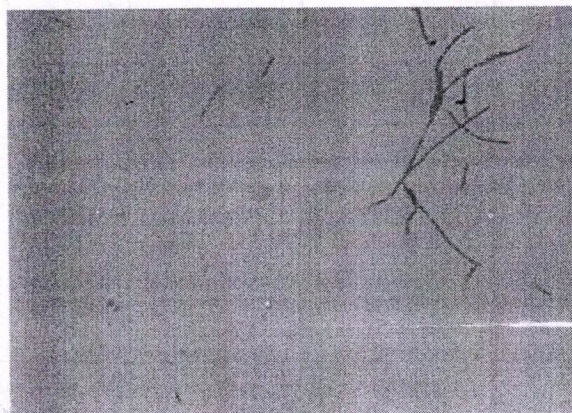


Figure 1. Gram staining of *Bacillus subtilis* DB1

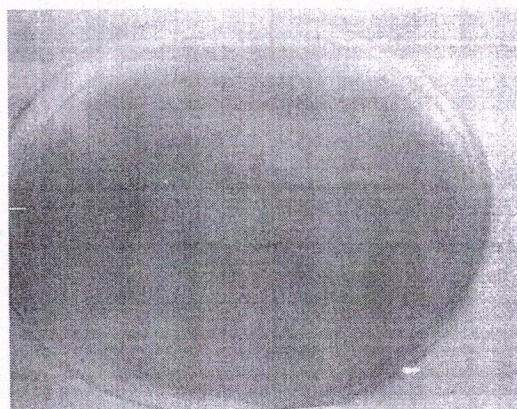


Figure 2: Growth of Xylanase producer on Oat Xylan Agar



Biochemical Properties of the isolate

Biochemical properties of the novel isolate are tabulated in the table and were compared with *Bacillus subtilis*. The biochemical properties such as catalase, citrate utilization, and nitrate reduction test are found similar with *Bacillus subtilis*, whereas sugar fermentation test such as Arabinose, mannose, glycerol, maltose are not detected in the novel strain, hence on this basis the isolate labeled as *Bacillus subtilis* DB1 (table 2).

Xylanase production:

The novel isolates produced xylanase in Oat Xylan media at 50°C PH 8 within 72 hours and the enzymes assay detected enzyme production, the production of enzyme was recorded as 2322 U/ml.

DISCUSSION

The world population is about 7 billion and it will cross 10 billion by the year 2030. The constantly increasing population demands more food and technical material like pulp. The increasing enzyme technology provided the best supportive technology for many industrial processes. The global market of enzyme-based product is expanding (C.N, Khobragde 2010). The extracellular enzyme from microbes is more feasible than intracellular enzymes. Xylanase is an extracellular enzyme used to hydrolyzed hemicellulose to generate simple molecules, than can be used in bioethanol production (D.B.Chavan and C.N. khobragade 2017). Xylanase constitutes a major group of hydrolytic enzyme EC 3.2.1.8 which provide a wide range of industrial application.

Toshiyoshi (1987) isolated xylanase producer *Vibrio* from the bottom mud of coastal sea in fukuka prefecture, Japan. Narayan rao and Belaudin (2004) isolated *Bacillus* sp from soda lake with optimum PH 9 for growth.

During the present research work, the wonderful isolate *Bacillus subtilis* DB1 proved very efficient xylanase producer.

Xylan Hydrolysis,

Xylan is a major polysaccharide of the plant cell wall, and the hydrolysis of Xylan is very essential to get a refined product as a filterable beer, ethanol, and instant coffee or leavened dough of bread industry.

Many microorganisms show the ability to hydrolyze Xylan was reported by many workers, *Aspergillus Trichoderma* and penicillin (Nariko et.al 1991). The fungal isolate SATU was reported to produce about 14.24U/ml of xylanase enzyme. (Anjum et.al 2017). Youngs, Ghasemi et.al (2014) reported a newly isolated Xynolical sphindobacterium strain saH-05 was reported to produce maximum enzyme production 40U/ml in wheat brane medium with 0.9 % ammonium sulphate at 37°C after 24 hours. Rajeeva Gaur et.al (2015) reported that bacterial strain *Bacillus omertis* able to produce xylanase about 3768 U/ml using Birchwood Xylan and beef extract at 55°C PH 7 within 48 hour of incubation.

Anuradha et.al (2007) isolated three *Bacillus* sp on oat spelt Xylan agar medium and she also proved oat spelt Xylan as the best substrate for getting high enzyme yield.

The isolate identified as *Bacillus subtilis* and labelled as *Bacillus subtilis* DB1 due to variation in some biochemical properties when compared with known *Bacillus subtilis* strain using Bergey's manual records of known bacteria. This novel bacteria is thermophilic and alkalophilic and produce 2322U/ml xylanase which is stable at 50°C PH8.

CONCLUSION

The DB1 isolate, identified as *Bacillus subtilis* and labelled as *Bacillus subtilis* DB1 due to variation in some biochemical properties and its ability to withstand at high temperature and PH 8, the isolated strain *Bacillus subtilis* DB1 can produce a significant amount of xylanase under-maintained condition using oat Xylan media with no specific additive. The important thing is that the enzyme from this naturally isolated strain is thermostable and alkali stable. This bacterium can be used in the production of xylanase enzyme

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Table 1. Morphological and cultural characters of isolated *Bacillus subtilis* DB1 on oat xylene medium

S/N	Name Of Character	Result
1	Size in MM	2.5
2	shape	circular
3	margin	entire
4	colour	Red tinted
5	opacity	opaque
6	Elevation	Raised wrinkle
7	consistency	Mucoid
8	Gram character	Gram positive
9	motility	Motile

Table 2. Sugar fermentation Biochemical properties of isolated *Bacillus subtilis* DB1 compared with *Bacillus subtilis*

SN	Name of properties	<i>Bacillus subtilis</i>	<i>Bacillus subtilis</i> DB1
1	glucose	+	+
2	Arabinose	+	-
3	lactose	+	-
4	Mannose	+	-
5	Xylose	+	-
6	maltose	+	-
7	glycerol	+	-
8	Gas	-	-
9	citrate	+	+
10	catalase	+	+
11	gelatinase	+	+
12	Nitrate reduction	+	+
13	Methyl red	-	+
14	Indole production	-	-
15	Oxidase	+	+
16	Amylase	+	-
17	Phenyl alanine Deaminase	-	-
18	Xylose	+	+++
19	Tyrosine	-	-

Legands-

- 1) Production of enzymes or sugar fermentation +
- 2) No production of enzyme or sugar fermentation -

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