SJIF 2012: 2.545

UIF 2013: 1.075

Received on:

15th September 2015

Revised on:

17th September 2015

Accepted on:

17th September 2015

Published on:

1st October 2015

Volume No.

Online & Print

11 (2015)

Page No.

23 to 26

PRODUCTION OF XYLANASE ON NATURAL SUBSTRATES BY *BACILLUS PUMILUS*(mtcc 9861)

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ABSTRACT:

Xylan is the most abundant non cellulosic polysaccharide present in both hardwoods and annual plants and accounts for 20–35% of the total dry weight in tropical plant biomass. Xylans are linear homopolymers that contain D-xylose monomers linked through *β*-1, 4–glycosyl bonds. Xylanase (E.C 3.2.1.8) degrades xylan by cleaving *β*-1,4 glycosidic linkages randomly, and the resultant products such as xylose and xylo-oligosaccharides like xylobiose are industrially useful in various applications spanning from biofuels to various environmental applications. Microorganisms are the rich sources of xylanases, produced by diverse genera and species of bacteria, actinomycetes,and fungi. Several species of *Bacillus* secrete high amounts of extracellular xylanases. The present study was aimed at producing xylanase using various natural substrates as raw materials by*Bacillus pumilus* obtained from IMTECH, Chandigarh. The natural substrates such as rice straw, rice husk, wheat husk, bagasse and sawdust after processing were pretreated and inoculated with *B.pumilus* grown under both submerged fermentation (SmF) (2% substrate loading) and solid substrate fermentation (SSF)at standard conditions for 4 days.

Periodical samples analysis from SmF with various substrates for xylanase activity revealed that enzyme synthesis starts at 24h (0.73 U\ml) and reaches maximum at 72h(2.91 U/ml), which is comparable with yields reported for this organisms. Among the various substrates screened, *B.pumilus* produced highest xylanase

activity with wheat husk as substrate followed by rice husk, and rice straw and the least activity was received from saw dust. In case of solid state fermentation the maximum xylanase activity is when bagasse is used as the substrate (3.07 U/ml) and minimum when saw dust is used as the substrate.When we compare both the fermentation the enzyme activity is differing for the substrate at different fermentation.

KEYWORDS: *Xylanase; Submerged fermentation; Solid state fermentation; Bacillus pumilus*.

INTRODUCTION:

There has been growing interest in xylanase production and its application because xylanase is important in the bioconversion of hemicellulose, which is a significant component of lignocellulosic material. Different natural lignocellulasic materials were used as substrate for xylanase production rather than easily metabolizable carbohydrates. Mahjabeen saleem *et al*. (1999) reported that the synthesis of cellulasses and xylanases were induced when grown on medium containing crystalline cellulose and plant raw materials.The cost of carbon source plays another major role in the economics of xylanase production.Hence an approach to reduce the cost of xylanase production is the use of lignocellulosic materials as substrate rather than opting for the expensive pure xtlan(Venkatesh kavya *et al.* 2009.

MATERIAL AND METHODS :

Enzyme production: *Bacillus pumilus* obtained from IMTECH, Chandigarh is used as the microbial culture for the production of enzyme. Different natural lignocellulosic materials e.g. rice straw,rice husk, wheat husk,saw dust and bagasse, were milled and sieved to 20 mesh size. The ground substrates were pretreated with 2% NaOH at room temperature for two hours followed by washing several times with water until neutral and then dried in an oven at 50ºC to obtain a constant weight. In case of submerged fermentation *B.pumilus* was cultivated in medium having the composition in g/100 mL: magnesium sulphate 0.02, dipotassium hydrogen phosphate0.05,potassiumdihydrogen phosphate 0.05, calcium chloride 0.01, yeastextract 0.2. 2 per cent alkali treated substrates were used as carbon source.

The fermentation medium inoculated with *B. pumilus* was incubated in a fermenter at 37ºC for 24 hrs and culture supernatant obtained after centrifugationofculturemedium was used as a extracellular enzyme source.

In case of solid state fermentation the same substrates used as the carbon source and the mineral salt solution added up to 60% water holding capacity. After sterilization the substrates inoculated with *B.pumilus* and kept in fermenter for 24 hrs at normal temperature, 37ºC for 24 hrs.The enzyme assay is same like that of submerged fermentation.

Xylanase assay: To determine the xylanase activity1 mL of an appropriately diluted culture supernatant with 1 ml of 1% solubilised birchwood xylan (Sigma Chemical Co.USA),in phosphate saline buffer 5.5 pH was incubated at 60ºC for 10 min. The reducing sugars liberated were estimated as xylose equivalents by DNS method (Ghose,1987).One unit of the enzyme activity is defined as the amount of enzyme that released one micromole of reducing sugars equivalent to xylose per minute under the assay conditions.

Protein estimation: Protein in the culture supernatant was measured by dye binding method (Bradford, 1976) using bovine serum albumin as standard.

RESULT AND DISCUSSION:

Effect of natural substrates on xylanase production: The effect of agro-industrial substrates on xlanase production was studied rather than using easily metabolizable carbohydrates. Table I shows the levels of xylanase production using various lignocellulosic materials. When *B.pumilus* was grown in fermentation medium containing 0.2% pre-treated wheat husk as carbon souce, 2.91 U/mL of xylanases activity was obtained after 72 h of fermentation at 37ºC. The xylanase activity of 2.73, 2.26, 2.19 were produced when rice husk, rice straw and bagasse were used as the carbon source respectively.

The lesser amount of enzyme activity was produced when saw dust was used as carbon source in the growth medium.

In case of solid state fermentation the maximum xylanase activity produced when bagasse were used as the carbon source, 3.07 U/ml after 72 h of fermentation. The xylanase activity of 3.03,2.12, 2.03 were produced when rice straw , wheat husk and rice husk were used as the carbon sources(Table 2). The lesser amount of enzyme activity was produced when saw dust was used as the carbon source. Mahjabeen saleem *et al*. (1999) reported best production of xylanase when *Bacillus subtilis* grown in the presence of bagasse. M. C. de O. Souza *et al.*1999 reported that *Thermoascus aurantiacus* showing highest activity of xylanase when bagasse was used as thesubstrate by solid state fermentation.

When we comparing the xylanase activity in both the type of fermentation , the maximum activity also differing according to the substrates. In case of submerged fermentation the

maximum activity produced when wheat husk was used as the substrate ,but in case of solid state fermentation the maximum activity produced when bagasse was used as the substrates. The result of our study conclude that bagasse which is inexpensive and cheaply available produce higher amount of acivity among the all these natural substrates. Hence we suggest that bagasse may be a good alternative to the pure xylan for production of xylanase by solid state fermenbtation. Also we can suggest wheat husk in case of submerged fermentation.

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Table I. Effect of alkali treated lignocellulosic materials on xylanase production by *B. pumilus* after 72hours of fermentation at 37°C(Submerged fermentation)

Lligno-cellulosic substrates	Soluble protein(mg/ml)	Xylanase activity(U/ml)
Rice husk	0.010	2.73
Rice straw	0.011	2.26
Wheat husk	0.010	2.91
Bagasse	0.012	2.19
Saw dust	0.011	2.05

Table 2. Effect of alkali treated lignocellulosic materials on xylanase production by *B. pumilus* after 72hours of fermentation at 37°C(solid state)

Lignocellulosic substrates	Soluble protein(mg/ml)	Xylanase activity(U/ml)
Rice husk	0.010	2.03
Rice straw	0.011	3.03
Wheat husk	0.010	2.12
Bagasse	0.012	3.07
Saw dust	0.011	1.27