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Medium optimization of production of xylanase by solid state fermentation from *Brevibacillus borstelensis* – MTCC 9874 isolated from soil sample of eastern Nepal

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ABSTRACT

The main aim of this study was to optimize production medium in solid state fermentation for production of xylanase using *Brevibacillus borstelensis* MTCC 9874. The organism was isolated from Morang district of Nepal and it was grown for 96 h in five different mineral salt solutions (MMS) with rice husk and MSS-1 was selected as a medium for further study based on xylanolytic activity measured using DNS method. Plackett Burman design (Minitab 15.1) was done with six variables viz. dipotassium hydrogen phosphate, rice husk, sodium chloride, magnesium sulphate, sodium carbonate and calcium chloride. The result showed that dipotassium hydrogen phosphate and rice husk were significant factors for xylanase production (> 95% confidence levels). Full factorial Centre composite design (CCD) was used to optimize the two significant factors. Response surface and contour plot were used to locate the optimal value of the two factors. There was 279.88% increase in xylanolytic activity after optimization of the medium. Study of effect of temperature on xylanolytic activity showed that maximum xylanolytic activity (6.58±1.1 IU/mL) was found at 60 °C. Optimum pH was found to be 7.6 (Xylanolytic activity = 6.81±2.32 IU/mL). Thermal stability study showed that the enzyme has a good stability at 60 °C (95.62%). Lineweaver – Burk plot showed that the enzyme has V_{max} and K_m values 0.1075 µg/mL .min and 1427.63 µg/mL respectively.

Keywords: Xylanase, Plackett-Burman design, Response surface methodology, DNS method solid state fermentation

INTRODUCTION

Xylan is the most abundant non-cellulosic polysaccharide in hard wood (20–35%) and soft wood (8%), which constitutes approximately one third of all renewable organic carbon sources on earth (Asha Poorna and Prema, 2007). It is composed of a backbone chain of β-1,4-linked xylosyl residues and short side chains of arabinosyl, glucoronosyl and acetyl residues (Kalogeris, 2000). Its enzymatic hydrolysis requires endo-β-1,4-xylanase (β-1,4-D-xylan xylanohydrolase, EC 3.2.1.8) that cleaves glycosidic bonds to produce xylooligosaccharides and β-1,4-D-xylosidase (β-1,4-xyloside xylohydrolase, EC 3.2.1.37), responsible for the final breakdown of small xylooligosaccharides into xylose (Nascimento *et al.*, 2002).

The cost of enzymes is one of the main factors determining the economics of a process. Reducing the costs of enzyme production by optimizing the fermentation medium is the basic research for industrial application (Ghanem, 2000). Several statistical designs have been used to optimize the medium which has several advantages over "Change-in-one-factor-at-a-time" method. In order to make the enzyme applications more cost effective at industrial level, its production using low cost substrates such as agro-wastes has been recommended and use of the agro-wastes has been possible by solid-state fermentation (SSF), which is closer to natural system and has proved to be more efficient in producing certain enzymes and metabolites (Asha Poorna and Prema, 2007).

Xylan-degrading enzymes have many important practical applications in various industrial processes, including the modification of cereal-based foodstuffs, improving the digestibility of animal feedstocks and delignification of paper pulp. Eco-friendly applications in paper/paper product manufacture and recycling, in textile manufacture, in baking, in the release of aroma and anti-oxidant molecules, and in the production of biopharmaceuticals, which are targeted at both selective and extensive modification of xylans, have provided an increased impetus to identify and obtain new xylanases with different specificities and properties (Ryan *et al.*, 2003).

The objective of this study was to optimize medium for production of xylanase by solid state fermentation (SSF) and its purification. In order to improve characterized enzyme production, 'change–one-factor-at-a-time" was replaced by Plackett-Burman design (PBD) followed by Response surface methodology (RSM) because of drawback such as unreliable results and inaccurate conclusion, expensive and time-consuming for large number of variables (Kuhad *et al.*, 1998). In order to know the industrial potential of xylanase from this organism, the enzyme was purified and characterized.

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MATERIALS AND METHODS

Microorganism

All the isolates (202) were subjected to xylanase production tests by primary screening using xylan agar plate and congo red (Cordeiro *et al.*, 2002). Among them, 9 isolates showed xylanolytic activity ratio more than 3 and were subjected to secondary screening by submerged fermentation. Among nine isolates, *Brevibacillus borstelensis* MTCC 9874 showed maximum xylanolytic activity. It was isolated from Eastern Sugar Mills, Amadeva, Morang district, Eastern Nepal. Its characterization was done using 16s rRNA analysis at Institute of Microbial Technology (IMTECH), Chandigadh, India. Isolates were maintained at 4-8 °C on half nutrient agar slants.

Substrates in SSF

Rice husk, rice straw and wheat straw were taken as substrates separately for SSF. Rice and wheat straws were cut into small pieces and were kept in hot air oven at 60±10 °C for an hour before use.

Mineral salt solutions

Five mineral salt solutions with following contents were used for selection of a mineral salt solution (Table 1) (Virupakshi *et al.*, 2005).

Table 1: Different mineral salt solution composition used for selection of moisturizing agent

Mineral salt solution (MSS)	K_2HPO_4 (g/L)	NaCl (g/L)	$MgSO_4 \cdot 7H_2O$ (g/L)	$CaCl_2$ (g/L)	Na_2CO_3 (g/L)
MSS-1	6	6	0.4	0.2	10
MSS-2	4	4	0.2	0.1	8
MSS-3	2	1	0.1	0.05	5
MSS-4	2	1	0.05	0.03	5
MSS-5	1	0.5	0.05	0.02	5

Solid state fermentation

Each dried substrate (10 g) was kept in Erlenmeyer flask (250 mL) and mineral salt solution (20 mL) of different composition were kept in each flask. All the flasks were sterilized at 105 kg/cm^2 for 45 min. Sodium carbonate solution was sterilized separately and was added into the sterilized flask under aseptic condition. Inoculum (48 h old) of *Brevibacillus borstelensis* (4 mL) was added aseptically into the flask followed by shaking gently. The flasks were kept in incubator humidified with sterile water (55 °C) for 96 h and the best among the five was selected based on xylanase activity assay measured using DNS method.

Xylanase activity assay

Xylanase activity was assayed using hydrolyzing xylan from birchwood (Sigma, USA). Liberated reducing sugars were quantified by dinitrosalicylic acid (DNS) method (Miller, 1959). One IU/mL is expressed as 1 microgram of xylose formed per mL per min. Each experiment was done in triplicate.

Experimental Design

Plackett-Burman design

Plackett Burman experimental design with six variables viz. dipotassium hydrogen phosphate, sodium chloride, magnesium sulphate, calcium chloride, calcium carbonate and rice husk in solid state fermentation (SSF) was performed using Minitab 15.1 to screen the nutrients that were significantly affecting xylanase production. The six factors were screened in fifty three experimental run. The enzyme activity assay was carried out in triplicate and average of it is reported as response/enzyme activity (Table 2).

Each factor was examined at two levels: –1 for low level and +1 for high level and centre point were run to evaluate the linear and curvature effects of the variables based on PBD (Armstrong NA, 2006). The design is based on the first-order polynomial model:

$$Y = \beta_0 + \Sigma \beta_i x_i \quad (1)$$

Where Y is the response (enzyme activity), β_0 is the model intercept, β_i is the linear coefficient and x_i is the level of the independent variables. This model does not describe interaction among variables and is used to screen the significant factors that influence response/enzyme activity (Wejse *et al.*, 2003).

The factors significant at 95% level ($p < 0.05$) were considered to have significant effect on xylanase production from the regression analysis of variables.

Central composite design, response surface methodology and statistical analysis

Significant factors that contribute in xylanase production were further optimized by RSM using Minitab 15.1 (Bocchini *et al.*, 2002; Wejse *et al.*, 2003). Two level full factorial central composite design with 4 star points (α=1.41421) and a centre point with one

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Table 2: Plackett-Burman design showing six variables with coded values along with the observed results for xylanase production.

Run Order	K_2HPO_4 (g/L)	NaCl (g/L)	$MgSO_4 \cdot 7H_2O$ (g /L)	$CaCl_2$ (g/L)	Na_2CO_3 (g/L)	Rice husk (g)	Xylanase activity (IU/mL)
1	10(1)	10(1)	1(1)	0.10(–1)	20(1)	5(–1)	2.13
2	10(1)	10(1)	1(1)	1(1)	20(1)	20(1)	9.37
3	4 (–1)	4(–1)	0.10(–1)	1(1)	6(–1)	20(1)	4.04
4	10(1)	10(1)	1(1)	1(1)	6(–1)	5(–1)	4.18
5	4 (–1)	10	0.10(–1)	1	6(–1)	5(–1)	2.08
6	4 (–1)	10(1)	0.10(–1)	1(1)	20(1)	20(1)	3.25
7	10(1)	10(1)	0.10(–1)	1(1)	6(–1)	20(1)	8.23
8	4 (–1)	4(–1)	0.10(–1)	0.10(–1)	20(1)	20(1)	4.23
9	4 (–1)	10(1)	1(1)	1(1)	20(1)	5(–1)	2.00
10	7(0)	14.14(2.38)	0.55(0)	0.55(0)	13(0)	12.50(0)	3.21
11	10(1)	4(–1)	1(1)	0.10(–1)	6(–1)	5(–1)	4.07
12	4 (–1)	10(1)	1(1)	1(1)	6(–1)	20(1)	4.14
13	–0.14 (–2.38)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	1.86
14	10(1)	4–1)	0.10(–1)	0.10(–1)	6(–1)	20(1)	8.98
15	7(0)	7(0)	–0.52 (–2.38)	0.55(0)	13(0)	12.50(0)	3.22
16	10(1)	4(–1)	1(1)	0.10(–1)	20(1)	20(1)	9.61
17	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	4.70
18	7(0)	7(0)	0.55(0)	1.62(–2.38)	13(0)	12.50(0)	1.94
19	4(–1)	4(–1)	1(1)	0.10(–1)	20(1)	5(–1)	1.76
20	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	3.28
21	4(–1)	10(1)	0.10(–1)	0.10(–1)	20(1)	5(–1)	10.34
22	4(–1)	10(1)	1(1)	0.10(–1)	20(1)	20(1)	4.75
23	10(1)	10(1)	0.10(–1)	1(1)	20(1)	5(–1)	4.30
24	10(1)	4(–1)	0.10(–1)	0.10(–1)	20(1)	5(–1)	4.81
25	7(0)	7(0)	0.55(0)	–0.52 (–2.38)	13(0)	12.50(0)	1.94
26	10(1)	10(1)	0.10(–1)	0.10(–1)	20(1)	20(1)	9.92
27	4(–1)	4(–1)	1(1)	1(1)	20(1)	20(1)	4.82
28	10(1)	10(1)	1(1)	0.10(–1)	6(-1)	20(1)	9.00
29	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	–5.34 (–2.38)	1.86
30	7(0)	7(0)	1.62(2.38)	0.55(0)	13(0)	12.50(0)	3.70
31	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	3.27
32	4(–1)	4(–1)	1(1)	1(1)	6(–1)	5(–1)	2.69
33	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	2.99
34	4(–1)	4(–1)	0.10(–1)	1(1)	20(1)	5(–1)	2.00
35	14.14(2.38)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	5.07
36	7(0)	7(0)	0.55(0)	0.55(0)	–3.65 (–2.38)	12.50(0)	3.52
37	7(0)	–0.14(–2.38)	0.55(0)	0.55(0)	13(0)	12.50(0)	3.70
38	4(–1)	4(–1)	0.10(–1)	0.10 (–1)	6(–1)	5(–1)	1.86
39	10(1)	4(–1)	1(1)	1(1)	20(1)	5(–1)	4.12
40	10(1)	10(1)	0.10(–1)	0.10 (–1)	6(–1)	5(–1)	3.94

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41	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	3.13
42	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	30.34(2.38)	8.34
43	10(1)	4(–1)	0.10(–1)	1(1)	6(–1)	5(–1)	4.33
44	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	3.83
45	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	3.62
46	10(1)	4(–1)	1(1)	1(1)	6(–1)	20(1)	10.02
47	7(0)	7(0)	0.55(0)	0.55(0)	29.65(2.38)	12.50(0)	5.21
48	10(1)	4(–1)	0.10(1)	1(1)	20(1)	20(1)	9.84
49	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	4.04
50	7(0)	7(0)	0.55(0)	0.55(0)	13(0)	12.50(0)	4.12
51	4(–1)	10(1)	1(1)	0.10(–1)	6(–1)	5(–1)	1.78
52	4(–1)	10(1)	0.10(–1)	0.10(–1)	6(–1)	20(1)	7.18
53	4(–1)	4(–1)	1(1)	0.10(–1)	6(–1)	20(1)	7.67

Table 3: Full factorial central composite design matrix for two variables in five settings in real and coded units (parenthesis) and response of xylanase activity.

Run Order	K_2HPO_4 (g/L)	Rice husk (g)	Xylanase activity (IU/mL)		Residuals
			Observed	Predicted	
1	17.5 (0)	30 (0)	10.400	9.647	0.7540
2	6.89 (–1)	30 (–1)	10.094	9.881	0.2130
3	28.11 (0)	30 (0)	10.441	10.282	0.1590
4	17.5 (+1)	30 (–1)	10.237	9.647	0.5900
5	17.5 (–1)	30 (+1)	8.225	9.647	–1.4220
6	17.5 (–2)	44.14 (0)	10.278	9.600	0.6780
7	25 (0)	20 (+2)	8.041	7.861	0.1800
8	17.5 (0)	30 (0)	9.226	9.647	–0.4210
9	10 (0)	20 (0)	8.205	8.063	0.1420
10	17.5 (+1)	30 (+1)	10.145	9.647	0.4980
11	17.5 (+2)	15.66 (0)	6.183	6.487	–0.3050
12	25 (0)	40 (–2)	10.033	10.547	–0.5150
13	10 (0)	40 (0)	9.226	9.779	–0.5530

replication resulting in a total of 13 experiments were used to optimize the chosen key variables for xylanase productivity in SSF. All the experiments were done in triplicate and average of enzyme activity is reported in Table 3. Second-order polynomial equation was fitted to correlate the relationship between variables and response (xylanase activity) in order to predict optimal point (Kalogeris *et al.*, 1998). The equation is:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i + \sum_i \sum_j \beta_{ij} X_i X_j \quad (2)$$

Where Y is the predicted response, k is the number of factor variables, β_0 is the model constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, β_{ij} is the interaction coefficient.

Minitab 15.1 was used to evaluate analysis of variance (ANOVA) to determine significance of each term in the equation fitted and to estimate goodness of fit in each case. Based on experimental results, response surface was drawn to show individual and cumulative effects of variables and the mutual interaction between them.

Purification of enzyme

Crude enzyme was precipitated using 80 % saturation of Ammonium sulphate. The precipitate was dissolved in phosphate buffer pH 6.8 and was dialysed. A column (50 cm x 26.63 mm) with Sephadex G-100 was saturated with phosphate buffer pH 6.8. Dialysed enzyme (50 µL) and phosphate buffer pH 6.8 (50 µL) were loaded in the column. The velocity of liquid movement in the column was maintained at 12 mL/h. Phosphate buffer pH 7.6 was maintained above top surface of the Sephadex G-100 layer to prevent the column from becoming dry. Each four drops from the column was collected separately. Then eluted sample (0.1 mL) and xylan (20 mg/mL; 0.9 mL) were incubated at 55 °C for 30 min. Xylanase activity was determined for crude enzyme, ammonium sulphate precipitation and elution through sephadex G-100 column by DNS method.

Purified enzyme was lyophilized and stored at 4 – 8 °C in refrigerator. Protein estimation of the enzyme was done by Lowry method (Lowry *et al.*, 1951).

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Partial Characterization of the enzymes

Effect of pH on xylanase activity

To determine the optimum pH for enzyme activity, the samples were assayed at various pH viz. 4, 5, 6, 6.6, 7, 7.6, 8 and 9 by DNS method after exposing the samples for 10 min at 55 °C (Kalogeris *et al.*, 1998).

Effect of temperature on xylanase activity

To determine the optimum temperature for enzyme activity, the samples were assayed at various temperatures viz. 40, 45, 50, 55, 60, 65, 70, 75 and 80 °C by DNS method after exposing the samples for 10 min in phosphate buffer (pH 7.6).

Determination of thermal stability of the enzyme

The crude enzyme (1 mL) was incubated at different temperature (30, 40, 50, 60, 70, 80, 90 and 100 °C) for 10 min. Then xylan (100 µL; 20 mg/mL) was added to the crude enzyme samples and kept at 60 °C for 10 min. Residual xylanase activity of each sample was measured by DNS method (Sa-Pereira, 2002; Xiong, 2004).

RESULTS AND DISCUSSION

Identification of organism

The isolate was sent to Institute of Microbial Technology, IMTECH, Chandigadh, India, for its identification. They have conducted taxonomic studies as well as 16S rRNA gene analysis (1393 bp).

On the basis of their taxonomic studies and 16s rRNA analysis, IMTECH identified it as *Brevibacillus borstelensis*. It was deposited in IMTECH with accession number MTCC 9874. So far there are no reports on the production of xylanase from *Brevibacillus borstelensis.* This strain is important as it can produce thermophilic and alkali-tolerant xylanase.

Enzymatic production

Maximum xylanolytic activity among five medium after SSF for 96 hours was seen in MSS-1 (Table 4). Thus, this medium was chosen for medium and condition optimizations study.

Table 4: Xylanolytic activity Mineral salt solutions with different substrates (Rice husk, Rice straw and Wheat straw) after SSF of 96 h.

Medium	MSSRH1	MSSRH2	MSSRH3	MSSRH4	MSSRH5
Xylanolytic activity	–0.17885	1.521154	0.876923	0.175	1.275
Medium	MSSRS1	MSSRS2	MSSRS3	MSSRS4	MSSRS5
Xylanolytic activity	10.38269	0.157692	-0.26923	8.598077	–0.33462
Medium	MSSWS1	MSSWS2	MSSWS3	MSSWS4	MSSWS5
Xylanolytic activity	0.021154	0.509615	0.792308	1.478846	1.213462

MSSRH: Mineral salt solution with Rice husk; MSSRS: Mineral salt solution with Rice straw and MSSWS: Mineral salt solution with wheat straw.

Plackett-Burman Design

The result of PBD showed that dipotassium hydrogen phosphate and rice husk were significant factors for xylanase production. Sodium chrloride, Magnesium sulphate, sodium carbonate and calcium chloride were found insignificant.

Therefore, the quantity of all the ingredients of medium which were insignificant in production of xylanase was kept constant in subsequent experiments. The model equation for xylanase production neglecting insignificant variables is as follows:

$$Y_{activity} = 36.5 + 16X + 32.4Y \quad [3]$$

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Where, $Y_{activity}$ = Enzyme activity, X = K_2HPO_4 and Y = Rice husk

Central Composite Design and statistical analysis

The result of enzyme activity when *Brevibacillus borstelensis* was incubated for 96 hours as per 13 experiment of CCD showed that maximum enzyme activity (10.4 IU/mL) was found in run 1 in which 17.5 g/L and 30 g of K_2HPO_4 and rice husk were used respectively (Table 3).

Minitab 15.1 was used to find out quadratic mathematical model for uncoded values [Equation 4]:

$$Y_{activity} = 1.68218 - 0.213301X + 0.534309Y + 0.00386256X^2 - 0.00801420Y^2 + 0.00323393XY \quad [4]$$

Where, $Y_{activity}$ = Enzyme activity, X = K_2HPO_4 and Y = Rice husk

The model (equation 4) did not show lack of fit (p = 0.706) as shown in Table 6. The model can explain 76.83% (R^2 = 76.83%) of the variation in xylanase activity and can predict 29.05% (R^2 predicted = 29.05%) of the xylanase activity (Table 5).

Response surface to estimate dependent variable, xylanase activity, over independent variables, K_2HPO_4 and Rice husk concentrations as per equation (4) is shown in Figure 1. The surface was curved in structure with the change in K_2HPO_4 concentration showing that K_2HPO_4 (25 g/L) is showing peak xylanase activity where as rice husk (40 g) has shown maximum xylanase activity. Contour plots obtained from Response surface methodology suggested optimized concentrations of xylan

Table 5: Results of regression analysis of the full factorial Central Composite Design (CCD).

Term	Coefficient	SE Coefficient	T- statistics	*p*-value
Constant	9.6467	0.3629	26.5840	0
K_2HPO_4 (X)	0.1418	0.2869	0.4940	0.6360
Rice husk (Y)	1.1005	0.2869	3.836	0.0060
K_2HPO_4× K_2HPO_4 (X^2)	0.2173	0.3076	0.706	0.5030
Rice husk × Rice husk (Y^2)	-0.8014	0.3076	-2.605	0.035
K_2HPO_4 × Rice husk	0.2425	0.4057	0.5980	0.569

Standard deviation of error term in the model, S = 0.811411; Sum of squares of the prediction errors, PRESS = 14.1147; R^2 = 76.83; R^2 (predicted) = 29.05% & R^2 (adjusted) = 60.29%

Table 6: Analysis of variance (ANOVA) for the model regression representing xylanase activity.

Source	DF	Sequential SS	Adjusted SS	Adjusted MS	F-ratio	p-value
Regression	5	15.2861	15.2861	3.0572	4.6400	0.0350
Linear	2	9.8499	9.8499	4.9250	7.4800	0.0180
Square	2	5.2009	5.2009	2.6004	3.9500	0.0710
Interaction	1	0.2353	0.2353	0.2353	0.3600	0.5690
Residual error	7	4.6087	4.6087	4.6087		
Lack of fit	3	1.2460	1.2460	0.4153	0.4900	0.7060
Pure error	4	3.3627	3.3627	0.8407		
Total	12	19.8948				

Where, DF: Degree of freedom; SS: Sum of square; MS: Mean square

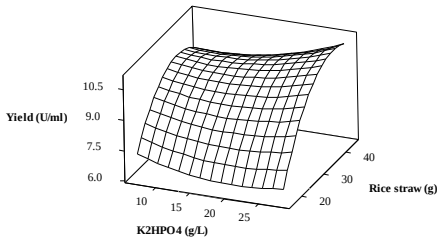

Figure 1: Response surface described by equation 4 which represents xylanase activity (IU/mL) as a function of K_2HPO_4 and rice husk concentration).

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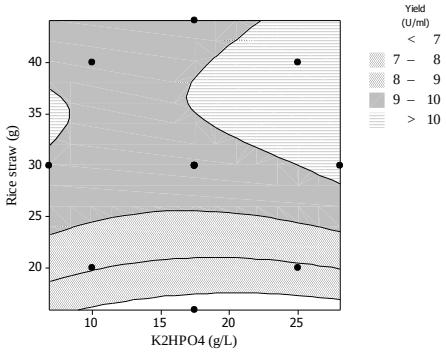

Figure 2: Contour plot of xylanolytic activity (IU/mL) vs. K_2HPO_4 (g/L) and rice husk (g).

(25 g/L) while rice husk (40 g) (Figure 2). There was 279.88% increase in xylanolytic activity after optimization of the medium.

Purification of enzyme

Crude enzyme (500 mL) obtained from fermentation followed by centrifugation (-10 °C, 10,000 rpm) for 10 min, was subjected to xylanolytic activity as described earlier. Crude enzyme (500 mL) was 80% saturated with ammonium sulphate and precipitates were dissolved in 400 mL of phosphate buffer pH 7.6. Xylanolytic activity of ammonium sulphate precipitation was measured. Similarly, dialyzed enzyme (0.5 µL) and phosphate buffer pH 7.6 (0.5 µL) were loaded in phosphate buffer pH 7.6 saturated sephadex G – 100 column (50 cm x 26.63 mm). Sample number 18 to 22 showed xylanolytic activity. The samples were mixed and xylanolytic activity of the mixture was measured and showed in Table 7. Other parameters such as total activity, protein, specific activity, yield and purification fold of the enzyme were determined after each step of purification (Table 7).

Kinetic Study

The enzyme kinetics was studied at optimum pH (pH 7.6) and temperature (60 °C) and the results of Michaelis – Menten and Lineweaver-Burk plots are shown in Figures 4 and 5 respectively.

Initial reaction rates were determined at different substrate (xylan from birchwood) concentration ranged from 10 to 90 mg/mL. Reaction rate (xylanolytic activity) vs. xylan concentration showed that the enzyme is obeying Michaelis-Menten kinetics. For the determination of V_{max} and K_m values, the Lineweaver-Burk plot was plotted between the reciprocal values of S and V (Bakir *et al.*, 2001). Lineweaver – Burk plot gave V_{max} and K_m values of 0.1075 µg/mL.min and 1427.63 µg/mL respectively (Figure 4).

Table 7: Summary of purification of xylanase

State of enzyme	Xylanolytic activity (µg/mL×min)	Total activity (µg/min)	Protein Conc (µg/mL)	Total protein (µg)	Yield (%)	Specific activity(Total activity/Total protein)	Purification fold
Crude	10	500	71.28	3564	100	0.14	1
Ammonium sulphate precipitation	14	420	62.12	1863.6	84	0.23	1.4
Elution through Sephadex G-100 column	797.54	159.51	43.96	8.79	31.9	18.15	79.75

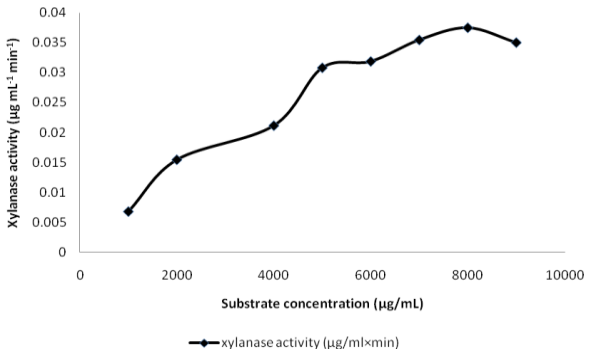

Figure 3: Substrate concentration vs. Xylanase activity to confirm that data is following saturated kinetics.

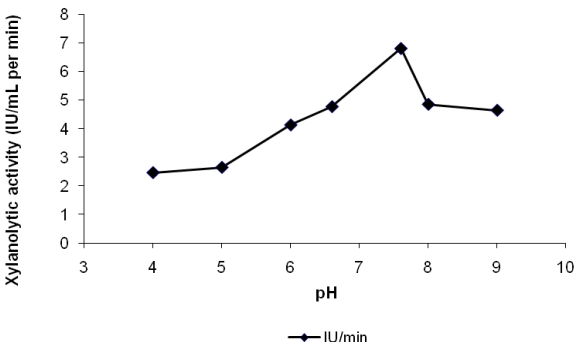

Figure 4: pH vs. xylanolytic activity for determination of optimum pH for maximum xylanolytic activity.

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Effect of pH

The effect of pH on enzyme activity was in the pH range of 6.6 to 8 and the results depicted in Figure 4. The figure clearly showed that maximum xylanolytic activity was observed at pH 7.6. Reasonably good enzymatic activity was observed in the alkaline range (7.6 to 9) indicating that it is an alkalophilic enzyme though pH greater than 10 can be encountered during kraft pulp bleaching (Morris, 1997).

Effect of temperature

The results indicated that maximum xylanolytic activity was observed at 60 °C (6.58±1.1 IU/mL). Good enzyme activity was observed from 50 to 70 °C. Thus, it suggests that the enzyme is the most suitable for pulp and paper industries as it has optimum activity at 60 °C (Figure 5).

Thermal stability of the enzyme

The crude xylanase samples were incubated at 40, 50, 60, 70 and 80 °C for a period of 10 min at optimum pH and their residual activities were estimated. The result indicated that the enzyme was relatively stable in between 25 and 60 °C (Figure 6). But at 40, 50, 60, 70 and 80 °C, about 0,8, 1.68, 4.38, 53.1 and 67.24% of original activity were lost respectively. Thermal stability study indicated that the enzyme has a good stability upto 60 °C (95.62%) in comparison with initial xylanolytic activity. Xylanolytic activity was found to be decreasing when temperature was increased beyond 60 °C (Figure 6). It showed that this xylanase is thermostable in comparison with other xylanases found in literature (Ratto *et al.*, 1992; Wang *et al.*, 2003).

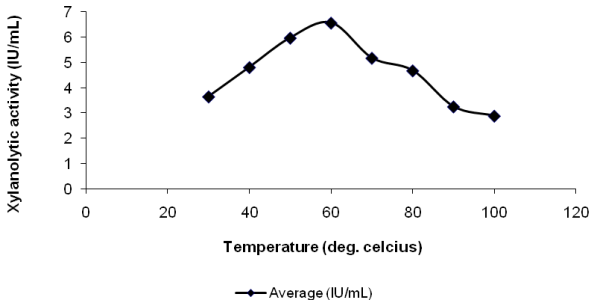

Figure 5: Temperature vs. xylanolytic activity for determination of optimum temperature for maximum xylanolytic activity.

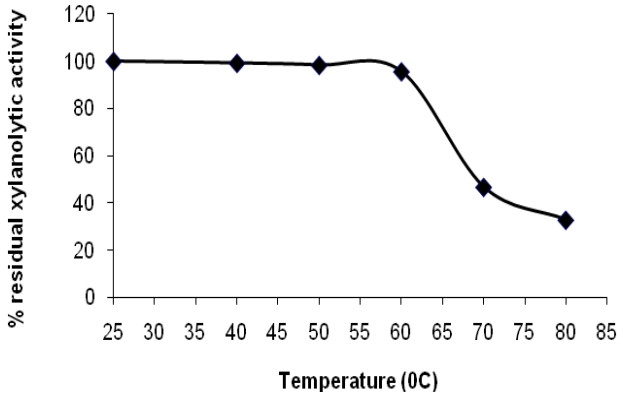

Figure 6: Thermostability study of xylanase.

CONCLUSION

So far there are no reports on the production of xylanase from *Brevibacillus borstelensis.* In the present investigation, we isolated a novel strain of thermophilic *B. borstelensis* which is producing thermostable and alkali-tolerant xylanase. Xylanase was produced by our new isolate *B. borstelensis* and purified. As the enzyme is thermostable and alkali-tolerant, it has a potential application in the industrial processes where high temperature and high pH environments are existed during processing.

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