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Bioprospecting Thermophilic Microorganisms from Hot Springs of Western Himalyas for Xylanase Production and its Statistical Optimization by Using Response Surface Methodology

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Hot spring bacteria are found a novel source of highly active xylanase enzyme with significant activity at high temperature. There has been a great interest in microbial xylanases due to their numerous uses in industrial applications, such as biobleaching of pulp and most notably the conversion of lignocellulosic materials into fermentable substrates for production of economical and environmentally attractive biofuels. In this study, thermophilic xylanase (EC 3.2.1.8) producing microbes were screened from diverse thermal sites such as Manikaran and Tattapani of western Himalyas. Among the isolates, 13 were selected for interested enzyme production (xylanase). Only single isolate namely TP 28 showed maximum xylanase activity at 60p C. Isolate TP 28 was confirmed as *Bacillus sp*. on the basis of colony morphology, stained preparation, biochemical tests as well as 16S rRNA sequencing and showed 99% similarity to *Bacillus aestuarii* (Accession No. AB062696). This is the first study in which we had reported that *Bacillus sp*. which has 99% similarity to *Bacillus aestuarii* is able to produce xylanase. Growth conditions including temperature, pH, incubation time, carbon sources, nitrogen sources were studied sequentially using the classical "change-one factor at a time" method. After optimizing all the parameters this xylanase producing isolate showed 10-fold increase in enzyme activity. Response Surface Methodology was used to generate the process model for obtaining optimal conditions for selected nitrogen sources, carbon sources and metal ion for maximum xylanase production and showed 10% increase in enzyme activity. Thermophilic and alkaline nature of TP28 are of great importance for converting lignocellulosic agricultural waste products into fermentable sugars for production of biofuel. Thermoalkalophilic nature of xylanase produced from a new *Bacillus sp*. make it of special industrial interest.

Key words: Xylanase, *Bacillus*, 16S rRNA**,** Thermoalkalophilic, Biofuel, Response Surface Methodology.

Thermostable enzymes have been isolated mainly from thermophilic organisms and have found a number of commercial applications because of their overall inherent stability[1]. Enzymes from these microorganisms also have got special attention since these enzymes are resistant to extreme pH values and chemical reagents in comparison to their mesophilic homologues[2]. Thermophiles are reported to contain proteins which are thermostable and resist denaturation and proteolysis[3]. Lignocellulolytic microorganisms are ubiquitous in nature and can be isolated from plant residues such as agricultural waste products, or from hot spring environments where organic carbon is available. Members of the bacterial genera *Anoxybacillus* and *Bacillus* have been shown to secrete a variety of lignocellulolytic enzymes such as cellulases[4] and xylanases[5, 6, 7].

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These organisms produce extracellular enzymes to depolymerize hemicellulose, lignin, and cellulose present in the biosphere for a source of carbon and energy, and their xylanases are of great interest for industrial applications converting lignocellulosic agricultural waste products, which are very abundant, into fermentable sugars to generate carbon neutral liquid fuels [8,9] as well as decreasing the amount the biologically detrimental chlorine necessary for biobleaching processes[10, 11]. Xylan (hemicelluloses) is easily found in solid agricultural and agro industrial residues, as well as in effluents released during wood processing[12]. For hydrolyzation of xylan, xylanases are the key enzymes and its role was recorded by Hopper-Seyler 100 year back[13]. Xylanases are consisted of glycosidases (*O*-gloycoside hydrolases) which catalyze endohydrolysis of 1, 4-â-D- xylosidic. Hence, this play significant role in biopulping wood, treating animal feed as well paper and pulp industry.

The hydrolysis of pulp bound hemicelluloses releases the lignin in the pulp, reducing the amount of chlorine required for conventional chemical bleaching and minimizing the toxic, chloroorganic waste. Therefore xylanases from alkalophilic bacteria have been studied widely[14]. The need for pH and temperature readjustment could be greatly reduced by the use of thermostable alkaline xylanases for enzyme assisted pulp bleaching, thus offering enormous technical and economic advantages[15]. The xylanase production by microorganisms is strongly influenced by many factors, such as nutritional sources[16, 17] and cultivation condition[18, 19]. Conventional methods for optimal culture conditions based on the classical method of 'one-variable-at-a-time' bioprocess design in which, one inde-pendent variable is studied while fixing all others at a specific level, may be effective in some situations, but may fail to consider the combined effects of all involved factors and lead to misleading results and inaccurate conclusions[20]. Recently, many statistical experimental design methods have been employed in optimization. Among them, response surface methodology (RSM) is the one suitable for identifying the effect of individual variables and for seeking the optimum conditions for a multivariable system efficiently[21, 22]. RSM is a collection of mathematical and statistical techniques widely used to determine the effects of several variables and to optimize different biotechnological process[23]. In present work, production of xylanase from TP 28 (*Bacillus sp.* SC-2014) using different carbon sources, nitrogen sources and metal ions was studied. A systematic and sequential optimisation strategy was adopted to enhance the production of xylanase from *Bacillus sp.* SC-2014.

MATERIALS AND METHODS

Collection of Sample

Soil samples were collected as per standard soil sampling procedure[24] from different thermal sites Manikaran and Tattapani of western Himalyas. Manikaran having altitude and temperature of 1760m and 80p C. Tattapani having altitude and temperature of 665m and 60p C respectively. Samples were kept in the research laboratory of Shoolini University for further screening.

Isolation and Identification

One gram of collected soil sample was inoculated for enrichment in xylan containing medium. The culture was enriched at 60°C for 48 hrs. In a volume of one ml enriched culture was spread on screening agar and further incubated at 60°C for 48 hrs and plates were observed for colony characteristic. To visualize the hydrolysis zone, the plates were flooded with an aqueous solution of 0.1% Congo red for 15 min[25]. To visualize clear zones formed by xylanase positive strains the plates were destained using 1M NaCl solution. Positive and better zone producing strain was chosen and used for further studies. Positive colony from these xylan containing agar plates were sub cultured on Berg's mineral salt medium. These plates were used as master plate. The selected isolates were confirmed on the basis of colony morphology, stained preparation, biochemical tests as well as molecular method (16S rRNA).

Molecular characterization based on 16S rRNA gene

Selected bacterial isolate was further identified at genomic level using 16S rRNA technique. Genomic DNA was extracted and purified, and its purity was assessed. PCR amplification was done from the genomic DNA by using forward and reverse primers i.e 8-27f (59-

AGAGTTTGATCCTGGCTCAG-39) and 1492r (59-TACGGYTACCTTGTTACGACTT-39). Based on the sequence BLAST phylogenetic tree was established.

Xylanase Assay

Xylanase activity was measured by dinitrosalicylic acid method described by Miller, 1959 by using D-xylose as standard. The enzyme assay was carried out at 60p C using 0.5% (w/v) xylan (50mM Acetate buffer, pH 6.0) as substrate. The colour intensity was measured at 550 nm using spectrophotometer (Systronic, Ahemdabad). The One unit (U) of xylanase is defined as amount of enzyme that releases 1µmol/ml/min under the assay conditions.

Optimization of temperature, pH and incubation time for xylanase production

Many conditions influence microbial metabolic activity and their effects were analyzed. The effect of temperatures ranging from 30-80°C and pH varying from 5.0–10.0, on xylanase production was studied. Effect of incubation time on enzyme production by selected isolate was studied by incubating at optimized temperature and pH for 24, 48, 72, 96 and 120 hrs time intervals. The cultures were proceeding for xylanase activity[26].

Selection of optimum nitrogen source, carbon source and metal ions for maximum xylanase production

The effect of carbon sources (xylan, xylose, carboxymethylcellulose, glucose, fructose, galactose, sorbitol, sucrose, maltose and lactose) on xylanase production was studied. The effect of different nitrogen sources (Beef, peptone, yeast, KNO_3, NH_4NO_3, NH_4Cl, NH_4SO_4, urea and tryptone) and metal ions (Cd^{2+}, Cu^{2+}, Al^{2+}, K^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+} and Co^{2+}) was also studied. Classical experimental method was done in which only one variable be changed at a time to determine its effect. Among these, best carbon source, nitrogen source and metal ion was therefore selected for further experiments. Experiments were performed at various combinations of 'high' (H) and 'low' (L) values of the process variables and analysed for their effects on the response of the process.

Experimental design and data analysis

The concentrations of carbon source, nitrogen source and metal ions were optimized by using Response Surface Methodology (RSM). The value of the dependent response was the mean of two replications. Second-order polynomial coefficients were calculated and analysed using 'Design Expert' software.

Optimization of reaction conditions

The reaction incubation temperature, pH optima of alkaline buffer (Glycine-NaOH) and thermostability of xylanase was studied. Thermostability of xylanase was determined by the pre-incubating enzyme at different temperature ranges from 40p C to 80p C for time interval 1, 2, 3, 4 and 5hrs.

RESULTS

Isolation and identification

Out of 200 isolates, total 13 isolates were screened for xylanase production at 60p C. The appearance of the clear zone around the colony after the addition of Congo red solution was strong evidence that the bacteria produced xylanase in order to degrade xylan. A single isolate namely TP-28 showed maximum xylanase activity at 60p C. This isolate was selected for further optimization studies. On Berg's mineral salt medium, the colonies of *Bacillus sp.* were two mM in diameter with creamy- white colour, entire margin, opaque and they appears gram positive under microscopic field. A isolate TP 28 confirmed as *Bacillus sp.* on the basis of colony morphology, microscopic examination of stained preparation, Biochemical test and 16S rRNA. Isolate TP28 showed 99% similarity to *Bacillus aestuarii* (Accession number AB062696). The genomic DNAs of selective isolate were extracted by Invitrogen DNA extraction kit and extracted DNAs after testing for the quality suitable for PCR amplification. The DNAs were stored at -20°C until their use in experiment. Genomic DNAs was used as templates for PCR amplification of 16S rRNA. A band of approx.1500 base pairs (bps) of the amplicons was observed in agarose gel (Fig.2). Phylogenetic tree was generated (Fig.3). Database search and comparisons were done with the BLAST search using the National Center for Biotechnology Information (NCBI) database. The nucleotide sequences of the amplicon were submitted to NCBI and have been assigned accession number KF922470 and named *Bacillus sp.*SC-2014.

Optimization of Temperature, pH and incubation time for xylanase production

The fermentation temperature appeared to have a dramatic effect on xylanase production. *Bacillus sp.* SC-2014 produced maximum xylanase activity at elevated temperature of 50ºC, while displaying minimum activity at 30ºC (Fig.4a). Optimum temperature range obtained in the present study clearly reflects strong thermophilic nature of *Bacillus sp*. SC-2014. The effect of pH on xylanase production has been presented in (Fig.4b) showing optimum pH for xylanase production at 9.0. Time course experiments indicated that the enzyme production reached its peak after 72 hrs. Xylanase production was acceptable still 72 hrs but declined thereafter (Fig.4c).The increase in xylanase activity during later stages in the medium might be due to the release of small amounts of xylanase from the aged cells, entering into autolysis. Further incubation did not increase xylanase yield due to the depletion of available nutrients to microorganism or due to the proteolysis, but it resulted in a decline.

The influence of supplemented carbon sources to the medium on xylanase production was assessed. The data indicated that xylanase production by *Bacillus sp*. SC-2014 was significantly higher than others when xylose (0.820 U/ml) was added as a source of carbon into the medium. The optimization step thus conducted in the presence of xylose as the best enzyme inducer and optimum carbon source for xylanase production (Fig4d).

Among the nine nitrogen sources tested beef extract was found to exhibit the maximum enzymatic activity. Similarly, yeast extract, peptone and tryptone also showed relatively higher activity, however lower than that of beef extract (Fig4e). Potassium nitrate and ammonium chloride showed very low enzymatic activity. In the present study, it was found that beef extract and yeast extract are good supplements for the xylanase production. *Bacillus sp.* SC-2014 showed highest xylanase production when the growth medium was supplemented with beef extract. The effect of metal ions such as Cd^{2+}, Cu^{2+}, Al^{2+}, K^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+} and Co^{2+} was also studied. Metal salts had a profound effect on the production of xylanase. Among the different metal salts tested, Fe^{2+} and Co^{2+} enhanced xylanase production from *Bacillus sp.* SC-2014. Cu^{2+}, Mg^{2+}, Mn^{2+} and K^{2+} showed relatively higher activity but lower than that of Fe^{2+} and Co^{2+}. Cd^{2+} and Al^{2+} strongly inhibited

Table 1. ANOVA for Response Surface Quadratic Model: Analysis of variance table

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	1.73	9	0.19	10.13	0.0006	significant
A-Xylose	0.40	1	0.40	21.33	0.0010	
B-Beef	0.029	1	0.029	1.54	0.2430	
C-Metal ions	0.11	1	0.11	5.93	0.0351	
AB	7.200E-003	1	7.200E-003	0.38	0.5513	
AC	0.068	1	0.068	3.61	0.0865	
BC	0.12	1	0.12	6.34	0.0305	
A2	0.048	1	0.048	2.54	0.1420	
B2	0.23	1	0.23	11.98	0.0061	
C2	0.021	1	0.021	1.11	0.3177	
Residual	0.19	10	0.019			
Lack of Fit	0.14	5	0.028	3.03	0.1246	not significant
Pure Error	0.047	5	9.400E-003			
Cor Total	1.92	19				

Table 2. Model fitting values

No.	Model Terms	Values
1.	Coefficient of the variation	20.08
2.	R-Squared	0.9012
3.	Adj R-Squared	0.8122
4.	Pred R-Squared	-0.2108
5.	Adeq Precision	9.624

xylanase production (Fig.4f). Optimizing all parameters this xylanase producing isolate, *Bacillus sp.* SC-2014 showed (1.02 U/ml) 10 fold increase in enzyme activity as compared to initial enzyme activity (0.102 U/ml). Xylose as best carbon source, Beef extract as best nitrogen source and Fe^{2+} as best metal ion enhanced the xylanase production and was selected for further experiments.

Optimization of reaction conditions

Effect of reaction incubation temperature

Temperature plays a very crucial role in expressing the activity of any biological system. The reaction was performed at the temperature range of 30°C to 80°C. The optimum reaction incubation temperature for xylanase activity was found to be 50°C (Fig.4g). Similar finding was observed in the work of Knob and Carmona,

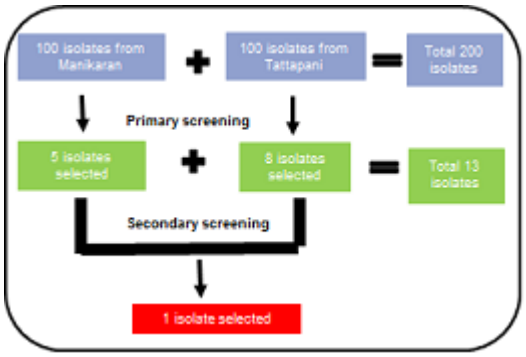


Fig.1. Diagrammatic representation of isolation of bacterial strain

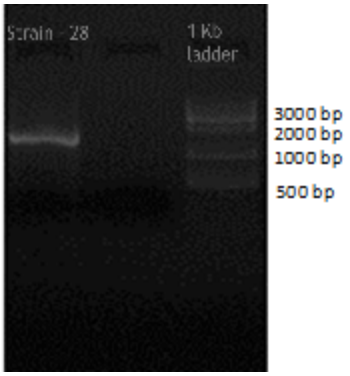
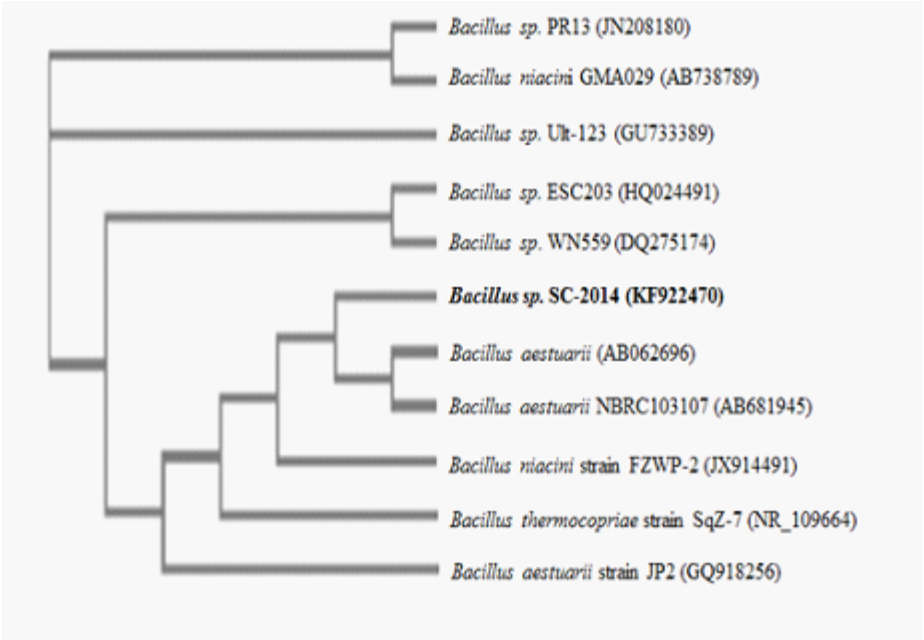


Fig. 2. Gel of strain TP28 showing amplification of 16s DNA amplicon. Lane 1 indicated band of amplicon with expected size of 1500bp, lane 2 showing negative result while lane 3 showing 1 kb molecular marker



BMolecular phylogenetic tree deduced from the sequence of 16S rRNA *of Bacillus sp.* SC-2014 with respect to the closely related sequence available in Gene Bank

reported 50°C as optimum temperature for xylanase from *Penicillium sclerotiorum*.

Effect of pH on Enzyme activity

The most important parameter which affect the enzyme activity is pH of buffer. The effect of pH on enzyme activity was determined by several pH values (pH 8.0-11.0) with 50mM glycine-NaOH buffer and reaction was carried out at 50°C for 10 minutes. Enzyme retained its activity at pH 11.0 but maximum enzyme activity was at pH 9.0 (Fig.4h)

Thermostability

Thermal stability is an interesting enzymes property due to the great industrial importance. The enzyme was preincubated at different temperatures and its activity was assayed. Enzyme was stable at 60p C but fairly stable at 40°C and 50°C after 2 hrsof incubation (Fig.4i). As the temperature was increased, the xylanase activity decreased considerably.

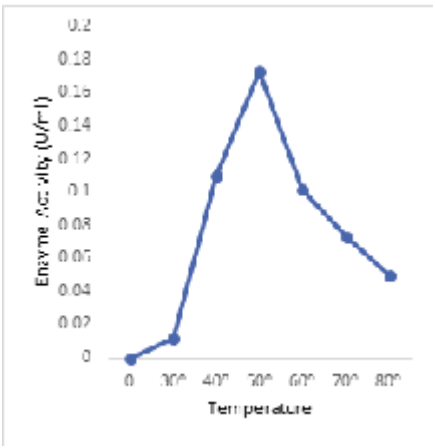


Fig. 4a. Effect of temperature on xylanase production by TP28 (*Bacillus sp.* SC-2014). The isolated bacterium shows optimum enzyme production at temperature 50p C.

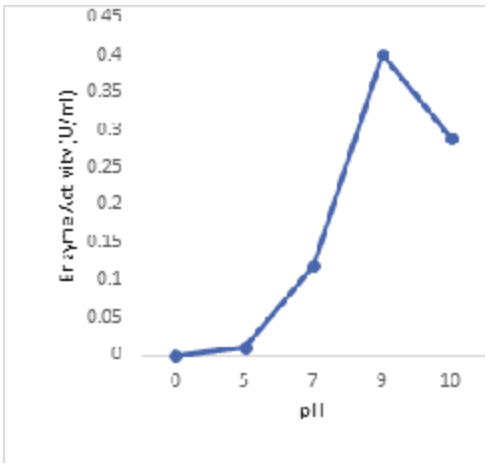


Fig. 4b. Effect of pH on xylanase production by *Bacillus sp.*SC-2014 shows optimum enzyme production at pH-9.0.

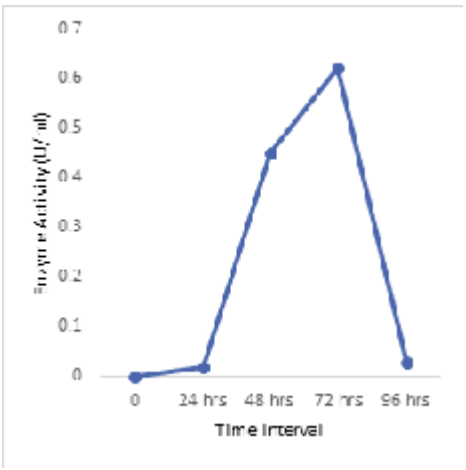


Fig. 4c. Effect of incubation time on xylanase production by *Bacillus sp.* SC-2014 shows optimum enzyme production at 72 hrs

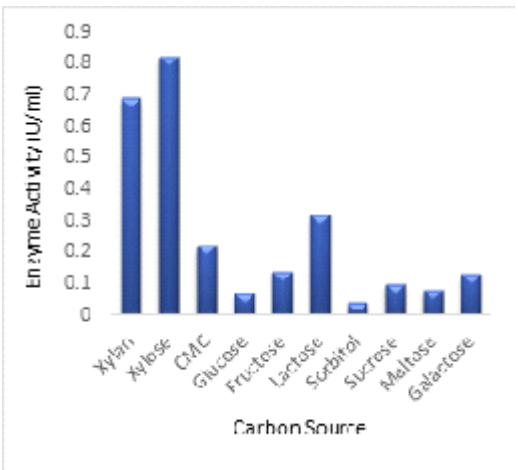


Fig. 4d. Effect of different carbon sources on xylanase production by *Bacillus sp.* SC-2014. The isolated bacterium shows optimum xylanase production in xylose as carbon source

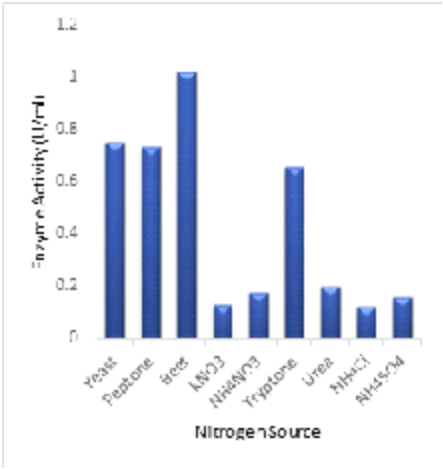


Fig. 4e. Effect of different nitrogen sources on xylanase production by *Bacillus sp.* SC-2014.The isolated bacterium shows optimum xylanase production in beef extract as nitrogen source

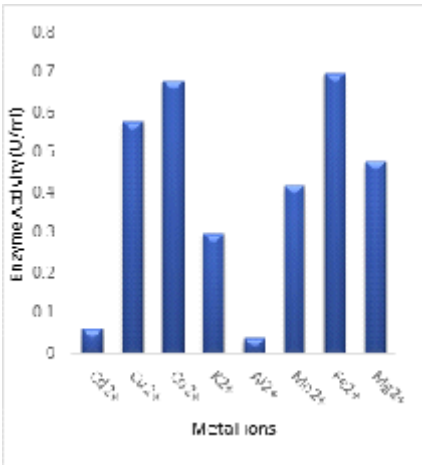


Fig. 4f. Effect of different metal ionsxylanase production by *Bacillus sp.*SC-2014. The isolated bacterium shows optimum xylanase production in FeSO4 as metal ion source

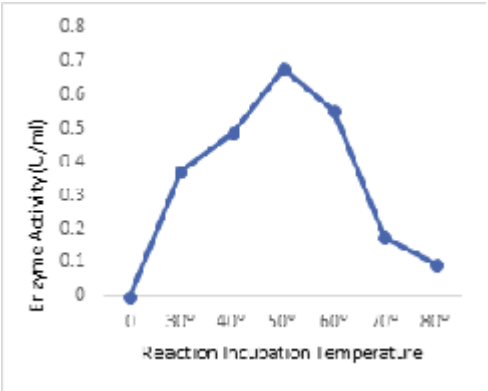


Fig. 4g. Optimization of enzymatic reaction incubation temperature. The recovered enzyme xylanase from TP28 (*Bacillus sp.* SC-2014) shows maximum enzyme activity at reaction incubation temperature 50p C

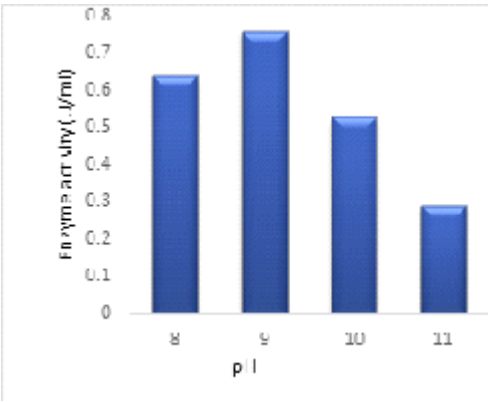


Fig. 4h. Effect of pH on enzyme activity by *Bacillus sp.*SC-2014. The isolated bacterium shows optimum xylanase activity at pH 9.0

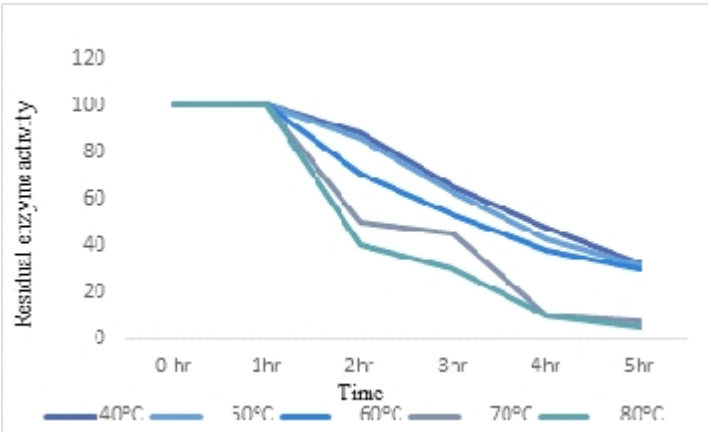


Fig. 4i. Thermostability of xylanase recovered from isolated strain Tp28 (*Bacillus sp.* SC-2014)

Optimization of enzyme production by RSM

The second-order polynomial equation was used to correlate the independent process variables with xylanase production. The second order polynomial coefficient for each term of the equation was determined through multiple regression analysis using the Design Expert. After regression analysis, the second order response model was obtained (Table 1). The Model F-value of 10.13 implies the model is significant. There is only a 0.06% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, C, BC, B2 are significant model terms.

Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 3.03 implies the Lack of Fit is not significant. Non-significant lack of fit is good.

A negative "Pred R-Squared" implies that the overall mean is a better predictor of our response than the current model (Table 2)."Adeq Precision" measures the signal to noise ratio. A

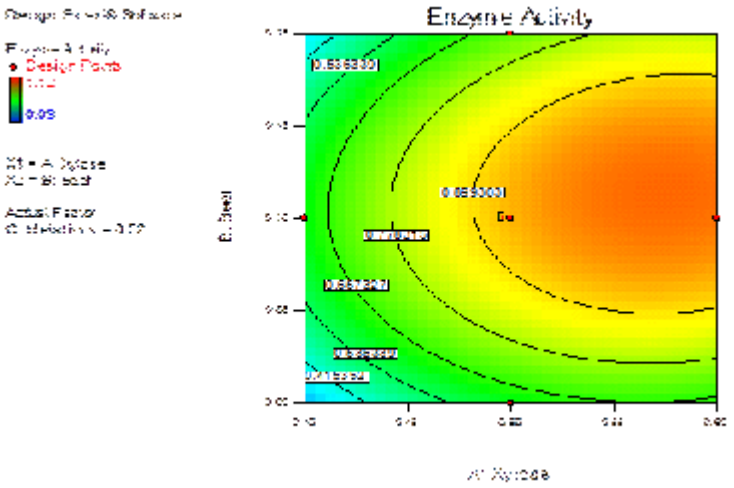


Fig. 5. Contour plot showing the effect of the central composite design (CCD) experiment for xylanase production by *Bacillus sp.* SC-2014

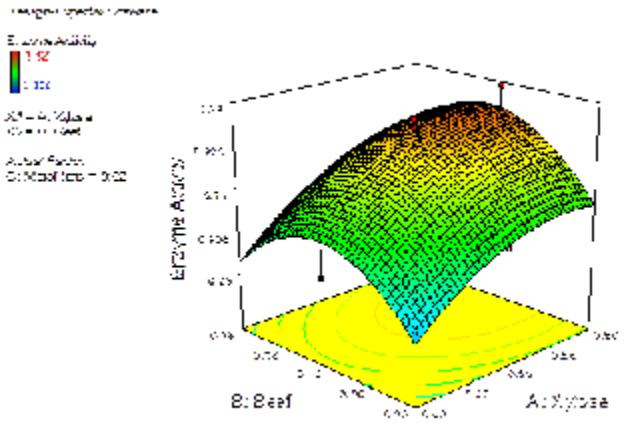


Fig. 6. Three dimensional (3D) response surface plot of the CCD experiment for alkali-stable xylanase production by *Bacillus sp.* SC-2014

ratio greater than 4 is desirable. A ratio of 9.624 indicates an adequate signal.This model can be used to navigate the design space.

To investigate the interaction among the variables and to determine the optimum concentration of each factor for maximum xylanase production by *Bacillus sp.* SC-2014, contour plot and three-dimensional response surfaces were plotted on the basis of the model equation. The effects of varying the concentration of xylose, yeast extract and metal ions are shown in Fig.5 and 6, which demonstrates that the response surfaces for three combinations were similar to each other.

DISCUSSION

The importance of xylanases is not confined to the paper and pulp industry and there are other industries with equal importance of applicability. Potential applications of xylanases also include bioconversion of lignocellulosic material and agro-wastes to fermentative products, clarification of juices, improvement in consistency of beer and the digestibility of animal feed stock [27]. Application of xylanase in the saccharification of xylan in agrowastes and agrofoods intensifies the need of exploiting the potential role of them in biotechnology. The selected bacterial strain TP28 has been identified as gram positive *Bacillus sp.* on the basis of colony morphology, stained preparation, biochemical tests, 16S rRNA sequencing. It exhibited 99% similarity to *Bacillus aestuarii*. A band of expected size of 1500 base pairs (bps) of the amplicons was observed in agarose gel. The nucleotide sequences of the amplicon were submitted to NCBI and have been assigned accession number KF922470 and named *Bacillus sp.* SC-2014. The selected isolated strain TP28 (*Bacillus sp.* SC-2014*)* shows maximum enzyme production at temperature 50p C at pH 9.0. Xylanase activity was highest after 72 hrs of incubation and declined on further increasing the time. Maximum production of xylanase by *Bacillus* sp.AQ-1 was reported in a culture incubated at temperature of 50ºC at pH 7.0 for 72 hrs[28]. A decline in enzyme activity afterwards may be because of proteolysis or due to depletion of nutrients available to the isolate, causing a stressed microbial physiology resulting in an inactivation of enzyme[29]

For xylanase from *Bacillus sp.*SC-2014, appreciable activity was recorded in the temperature range of 30-70p C. The temperature of 50p C was found most favourable for enzyme activity. Optimum temperature range obtained in the present study clearly reflects strong thermophilic nature of *Bacillus sp.*SC-2014. The enzyme produced from *Bacillus sp*. SC-2014 retained its activity at pH 11 but maximum enzyme activity was at pH 9.0. The enzyme showed optimum pH and temperature of 9.0 and 50°C, indicating the alkalophilic and thermostable nature of xylanase. However, pH optima for xylanases isolated from many bacteria was mainly reported in neutral pH range. Xylanase isolated from *Bacillus* sp. K-1 and *Bacillus* sp. No.C-125 exhibited an optimum pH of 6.0[30]. Xylanase from *B. stearothermophillus* has an optimum pH of 6.0-7.0[31], while xylanases of *B. subtilis*[32] and *B. agaradhaerens* [33] have optima pH of 5.6. Only a few xylanases isolated from bacteria such as *Enterobacter* sp. MTCC 5112[34] and *Staphylococcus* sp.[35] have an optimum pH of 9.0. The first alkaline xylanase produced by *Bacillus sp.* strain 41M-1 which had optimum temperature and pH of 50p C and 9.0 respectively[36].

Xylanase from *Bacillus* sp. showed optimum pH and temperature of 9.0 and 50 °C, indicating the alkalophilic and thermostable nature of xylanase [37]. The use of alkaline xylanase has special advantage in industry as it allows direct enzymatic treatment of the alkaline pulp and thus avoids cost of incurring as well as time. Alkaline active xylanase also has potential application in many industries in addition to pulp bleaching[38]. Industrially desirable characterstics like thermostability and alkalophilic nature are main requirements for any potentially commercially important enzyme in industries. In the present study, optimum temperature and pH was 50p C and 9.0 which indicate the possible industrial use of this enzyme.

The influence of supplemented carbon sources to the medium on xylanase production was assessed. Xylanase production by *Bacillus* sp.SC-2014 was significantly higher when xylose was added into the medium as carbon source (Fig. 4d). Some of the carbon sources used in the medium supports good growth of isolate as well as good enzyme synthesis, while others may lead to good

growth with reduced enzyme synthesis[39]. Carbon sources are essential elements for microorganisms during the period of growth and metabolism[40]. Gupta and Kar[41] reported stimulation of xylanase production by xylose from thermophilic *Bacillus* sp. under submerged fermentation. Moreover Kapoor *et al*., (2008)it was observed that maximum xylanase production from *Bacillus pumilus* MK001 in the medium containing xylose as a source of carbon[42]. To study the effect of nitrogen source on production of xylanase using xylose as carbon source, various nitrogen sources such as beef extract, peptone, yeast extract, KNO_3, NH_4NO_3, NH_4Cl, NH_4SO_4, urea and tryptone were tested. All the nitrogen sources except KNO_3 and NH_4Cl, enhanced xylanase production. Maximum xylanase production from *Bacillus sp.* SC-2014 was observed by using beef extract as the nitrogen source. The organic nitrogen sources such as yeast extract, beef extract, peptone and tryptone had already been reported for supporting good xylanase production[43]. Different metal ions are clearly influencing the production of xylanase from *Bacillus sp.* SC-2014. Studies on the influence of different metal ions were carried out using Cd^{2+}, Cu^{2+}, Al^{2+}, K^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+} and Co^{2}. The xylanase production was met with drastic reduction with $Cd^{2+}$and Al^{2+}. According to our study, all the metal ions except $Cd^{2+}$and Al^{2+} enhanced the xylanase production but highest xylanase production occurred with Fe^{2+}. These results are somewhat familiar with the xylanase of *Trichoderma sp*. MS 2010[44].

Stability of the enzyme was the most important factor in studying characteristics. Thermostable microorganisms are the potential sources of thermostable enzymes. Thermophiles can tolerate high temperature by using increased interaction than non-thermotolerant organisms, because of the presence of hydrobhobic, electrostatic and disulphide interaction[3]. In our study, the enzyme was stable upto 60p C. The xylanase from *Bacillus sp.*SC-2014 (TP28) retained 88.5% activity at 40°C after 2 hours while at 50°C only marginal decrease was found. The enzyme was sensitive to 70°C, retaining 50 % after 2 hours of incubation. Similar results were also obtained for xylanase of *B. stearothermophillus* T-6[31].

Currently, there is growing acceptance of the use of statistical experimental designs in biotechnology to optimize culture medium components. Many studies have reported satisfactory optimization of xylanase production from microbial sources using a statistical approach[45]. RSM was employed to optimize a fermentation medium for the production of xylanase by *Bacillus sp.*SC-2014 at pH 9.0. Production of xylanase from *Bacillus sp.* SC-2014 using different carbon, nitrogen spurces and metal ions was firstly studied by conventional method and was selected for further experiments. A systematic and sequential optimisation strategy was adopted to enhance the production of xylanase by using a face-centre central composite design (FCCCD) of RSM. The use of RSM has increased the production of xylanase from *Bacillus sp*. SC-2014 by 10- fold.

CONCLUSION

Xylanases reported by other workers had temperature optima of 60p C and pH optima of below 9.0[31, 46].While in present study, the optimum temperature and pH for enzyme was 9.0 and 50p C which indicate the possible industrial use of this enzyme. Using RSM, it was possible to model the individual and interactive effects of media components on production of xylanase. Optimization of media components by RSM effectively enhanced xylanase production by 10-fold. The major character of *Bacillus sp.*SC-2014 has aroused interest because of its extreme tolerance to high pH and temperature.

From all the data obtained in present study we conclude that TP28 strain (*Bacillus sp.*SC-2014) showed highest activity at temperature of 50p C at pH 9.0. This data indicates that this xylanase from *Bacillus sp.* SC-2014 exhibits favorable potentials for application in industrial scale.

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