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REGULAR ARTICLE

OPTIMIZATION OF CELLULASE-FREE XYLANASE PRODUCED BY A POTENTIAL THERMOALKALOPHILIC *PAENIBACILLUS* SP. N_1 ISOLATED FROM HOT SPRINGS OF NORTHERN HIMALAYAS IN INDIA

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ABSTRACT

Hot spring bacteria are found a novel source of highly active xylanase enzyme with significant activity at high temperature. Among bacteria, *Paenibacillus* sp.N_1 isolated from hot water spring of Manikaran, H.P., India showed highest 24.60 IU.ml^{-1} of cellulase-free xylanase on Reese medium. Growth conditions including medium, incubation time, pH, temperature, inoculum size, aminoacids, carbon sources, nitrogen sources and additives that affect the xylanase production by *Paenibacillus* sp.N_1 were studied sequentially using the classical "change-one factor at a time" method. The optimal cultivation conditions predicated from canonical analysis of this model were achieved by using basal salt medium on 3rd day, pH 9.0, temperature 50ºC with inoculum size of 12.5%, phenylalanine as aminoacid, xylose as carbon source, $(NH_4)_2HPO_4$ as nitrogen source and Tween 20 as detergent added with an approximate yield of 52.30 IU.ml^{-1} escalating the over level of xylanase production by 113.38%. A rare combination of all characters i.e. thermoalkalophilic nature and high units of cellulase-free xylanase produced from a new *Paenibacillus* sp.N_1 make it of special industrial interest.

Keywords: thermophilic, cellulase free xylanase, hot spring, submerged fermentation

INTRODUCTION

Enzymes of industrial interest are routinely being explored in various microbial hosts to increase the yield, to satisfy the needs of both the manufacturer and the end user **(Berquist et al., 2002)**. Thermostable enzymes have been isolates mainly from thermophilic organisms and have found a number of commercial applications because of their overall inherent stability **(Kohilu et al., 2008)**. 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 **(Akmar et al., 2011).** Adaptation of extremophiles to hot environments, production of heat-stable enzymes from thermophiles and hyperthermophiles, structure and function relationships of thermoenzymes (heat-tolerant enzymes) lead to biotechnological and industrial application of thermostable enzymes **(Eicher, 2001)**. Thermophiles are reported to contain proteins which are thermostable and resist denaturation and proteolysis **(Kumar and Nussinov, 2001)**. Xylanases are the endoactive enzymes which are generally produced in the medium containing xylan and also containing xylanase hydrolysate as the carbon source and attack the xylan chain in a random manner, causing a decrease in degree of polymerization of the substrates and liberating shorter oligomers, xylobiose and xylose **(Butt et al., 2008).** They are glucosidase (O-glucosidase hydrolase, EC 3.2.1.x) which catalyse the endohydrolysis of 1,4-β-D-xylosidic linkages in xylan **(Singh et al., 2010)**. Cellulase free xylanase are of paramount significance in some of industries viz. paper and pulp industries to avoid hydrolysis of the cellulose fibres **(Haltrich et al., 1996).** Treatment with xylanase at elevated temperature disrupts the cellwall structure, facilitates lignin removal in the various stages of bleaching of paper. Therefore xylanase must lack cellulolytic activity. Thermostable xylanase active at alkaline pH are of great importance for application in many important industries viz. pulp and paper industry to decrease the consumption of chlorine chemicals **(Khandeparker and Bhosle, 2006).**

The objective of our study was to explore the potential of alkalo-thermophilic bacteria isolated from hot water springs of Himachal Pradesh, India and to improve the yield of cellulase-free xylanase produced from it by optimizing different environmental parameters affecting its activity.

MATERIAL AND METHODS

Isolation of thermophilic bacteria

Hot water samples were collected from the three different thermal springs of Himachal Pradesh i.e. Tattapani (Distt. Mandi), Vashist (Distt. Kullu) and Manikaran perched on the right bank of the roaring Paravati river, situated at an altitude of 1760 m and located in Distt. Kullu, Himachal Pradesh, India. Two method of isolation were adopted i.e. by direct method which water sample were serially diluted and spread on Glucose Yeast (HIMEDIA, Mumbai)and by enrichment method in which water samples were inoculated in modified thermos broth containing 0.8% tryptone, 0.4% yeast extract, 0.2% NaCl and 1.0% glucose (w/v) and broth was incubated at 50ºC for 3 or 5 days and samples were serially diluted and streaked on GYM (pH 9.0) and plates were incubated at 50ºC for 24 h. The colonies so obtained were further subcultured and pure lines were established and maintained on the same medium.

Screening of isolates for cellulase-free xylanase-production

Xylanase assay

Selected isolates were quantitatively assayed by growing them in Reese medium (HIMEDIA, Mumbai) at 50ºC at 120 rpm. Xylanase activity in the culture broth was assayed in triplicates. 0.2 ml of crude enzyme was mixed with 0.3 ml of citrate buffer (pH 5.0) and 0.5 ml of xylan solution (kept overnight at 37ºC in citrate buffer pH 4.0, centrifuged and clear suspension was used) and incubated at 45ºC for 10 min and then reaction was terminated by adding 3 ml of Dinitrosalicylic acid **(Miller, 1959).** The absorbance (Thermo electron spectrometer) was measured against the control at 540 nm, using xylose as a standard.

Cellulase assay

The activities of total cellulose i.e. filter paper activity, endoglucanase and β-glucosidase were determined using standard methods of FPase and CMCase **(Reese and Mendel, 1963)** and β-glucosidase **(Berghem and Petterson, 1973).**

One international unit (IU) of enzyme activity represents µmoles of xylose/glucose/p-nitrophenol released ml of enzyme per min.

Protein content of culture filtrate was also determined by Folin-Ciocalteu reagent using Bovine Serum Albumin (BSA) standard **(Lowry et al., 1951).**

The best bacterial strain showing maximum xylanase activity without any cellulase synthesis was selected for optimization studies.

Identification of hyperenzyme producing isolate

Morphological studies

Characteristics of selected bacterial colonies were observed according to colony color, elevation, margin and by using differential staining method .

16S rRNA technique

Selected bacterial isolate was further identified at genomic level using 16S rRNA technique. PCR amplification was done from the genomic DNA by using forward and reverse primers i.e 16SF (5'AGAGTTTGATCCTGGTCA3') and 16SR (5'TACCTTGTTACGACTT3'). The translated nucleotide sequence was then analyzed for similarities by BLASTN tool (www.ncbi.nim.nih.gov:80/BLAST).

Optimization of process parameters

The optimization of the growth conditions was carried out based on stepwise modification of the governing parameters for xylanase production by using Miller method **(Miller, 1989)**.

Effect of media

The nutritional requirement of the selected bacterial isolates was determined by adding various nutritional supplement media i.e. Basal salt medium BSM **(Dhillon et al., 2000)**,

Nakamura medium NM **(Nakamura et al., 1995)**, Emerson medium EM **(Garg et al., 2009)**, Trypton glucose yeast medium TGY **(Garg et al., 2009)**, Xylan medium XM **(Cacais et al., 2001)** and Reese medium RM **(Singh et al., 2010)**. Xylanase activity was assayed as mentioned earlier.

Effect of incubation time

Flask containing 50 ml of production medium showing highest enzyme (i.e Basal medium) were inoculated with 5% seed culture and incubated at 50ºC with constant shaking. Following incubation for various time interval (1, 2, 3, 4,5, 6 days). The culture filterate was centrifuged at 12,000 rpm for 15-20 min and xylanase activity was assayed by method of **Miller (1959)**.

Effect of pH

The pH of optimized media was set at different levels such as 4, 5, 6, 7, 8, 9, 10 and activity of xylanase was determined after incubation of 3 days at 50ºC under constant shaking at 120 rpm.

Effect of temperature

Erlenmeyer flask each containing 50 ml of optimized medium was seeded and incubated at a temperature range varying form 30, 35, 40, 45, 50, 55ºC for 3 days under optimized pH condition. After incubation xylanase was extracted and assayed.

Effect of inoculum size

Culture flasks each containing basal salt medium were inoculated at a level of 2.5%, 5%, 7.5%, 10%, 12.5%, 15% (v/v). The enzyme was extracted from each set following an incubation of 72 h at 50ºC. Xylanase assay was performed to quantify the enzyme.

Effect of aminoacids

To determine the effect of amino acids for xylanase production. The optimized fermentation medium was supplemented with different amino acids i.e. phenylalanine, arginine, glutamic acid, tryptophan, alanine and cysteine individually at a concentration of 10 $\mu g.ml^{-1}$ in 50 ml of optimized medium and xylanase activity was measured individually.

Effect of different carbon sources

Various carbon sources including xylose, arabinose, mannose, sucrose, dextrose, arabinose, and rice straw at a concentration of 1% added in each of the flask containing optimized medium (50 ml). Enzyme production was measured after 3 days at 50ºC by Miller's method **(Miller, 1959)**.

Effect of different nitrogen sources

Different nitrogen sources i.e. peptone, yeast extract, beef extract, tryptone, casein, diammonium hydrogen phosphate, diammonium dihydrogen phosphate, sodium nitrate, sodium nitrite, ammonium nitrate and ammonium carbonate were used at a concentration of 0.2%. All the flasks were incubated at 50ºC at 120 rpm. The enzymes was extracted and assayed for activity on 3^{rd} day of incubation.

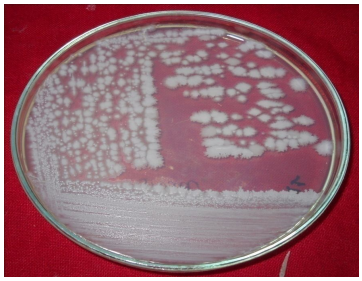
Effect of detergents

Various detergents like tween 20, tween 80, Sodium dodecyl sulphate, polyethylene glycol, PEG 2000 at a concentration of 10 $\mu g.ml^{-1}$ were added in optimized medium and their effect on xylanase production was estimated after incubation at 50ºC for 3 days under constant shaking conditions. The enzymes was then extracted by centrifugation at 12,000 rpm for 15-20 min and assayed for activity by using Miller's Method **(Miller, 1959)**.

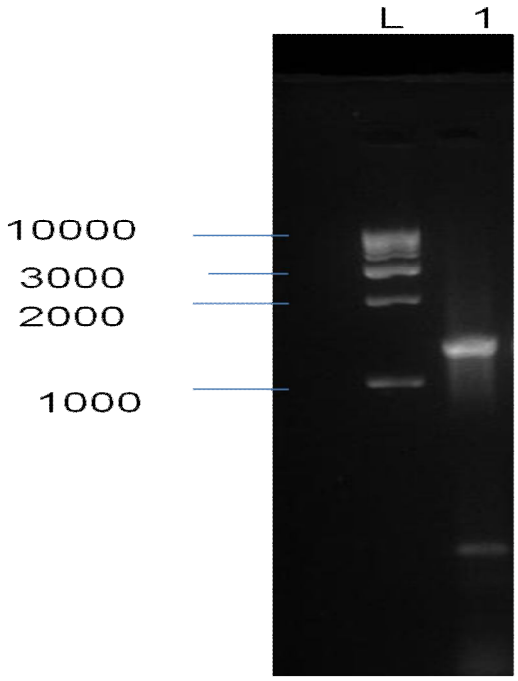
RESULTS AND DISCUSSION

Identification of xylanase producing thermophilic bacteria

The selected bacterial strain N_1 was creamish in colour having irregular form, flat elevation and erose margin (Picture 1) It was gram positive in nature with coccobacilli in shape and had been identified as *Paenibacillus* sp.N_1 using (16S rRNA) PCR technique (Picture 2).



Picture 1 Cellulase-free xylanase producing *Paenibacillus* sp.N_1 isolated from hot spring.



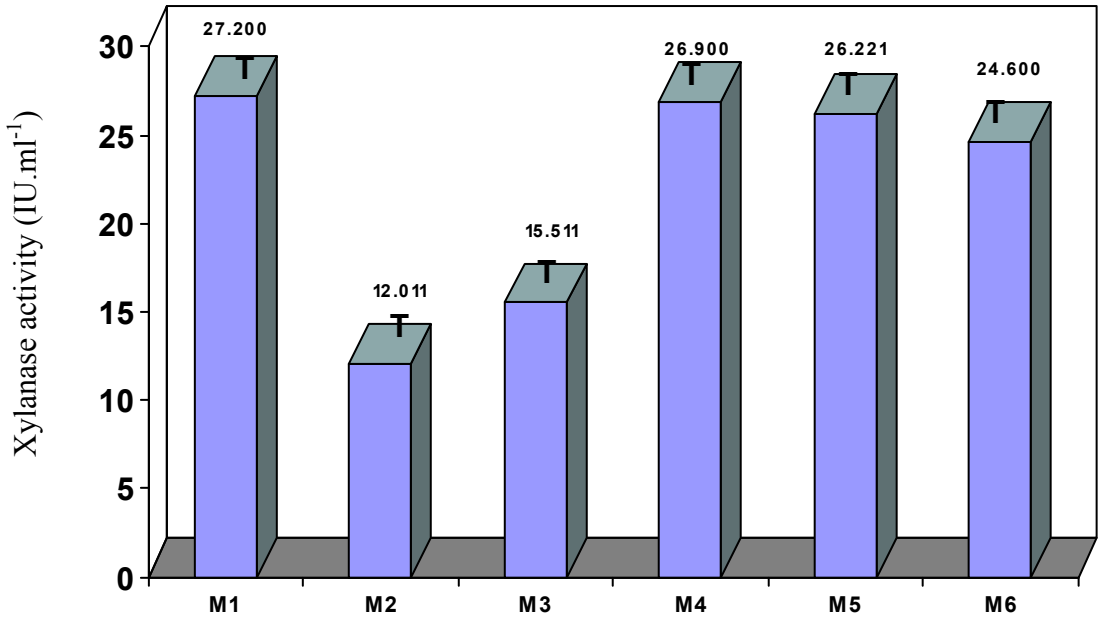
Picture 2 PCR product of bacterial genomic DNA

Paenibacillus sp.N_1 showing maximum xylanase activity i.e. 24.60 $IU.ml^{-1}$ was also assayed for its cellulase activity i.e. FPase 0.0062 $IU.ml^{-1}$, CMCase 0.016 $IU.ml^{-1}$, β-glucosidase 0.00 $IU.ml^{-1}$, thus indicating its cellulase free nature.

Optimization of parameters for xylanase production

Effect of different media

Paenibacillus sp.N_1 showed maximum growth in Basal Salt medium i.e. 27.20 $IU.ml^{-1}$ supplemented with different nutrients i.e. NH_4SO_4 0.6%, yeast extract 0.9%, KH_2PO_4 0.3%, NH_4Cl 0.1%, NaCl 0.05%, $MgSO_4$ 0.01% $CaCl_2$ 0.1%, pH 7.0 at 50ºC for 5 days of incubation. Xylanase produced in BSM was statistically higher than other media used in the present study (Fig. 1). Highest xylanase production using defined medium may be due to the presence of nitrogen, carbohydrate and other compounds in adequate quantity that could be utilized easily by the growing isolate thus enhancing the cell ability to produce xylanase enzyme **(Basar et al., 2010).** Magnesium chloride and calcium chloride as a growth supplements seemed to have promoted extracellular xylanase production. Sodium chloride present in the medium probably helped in maintaining the osmotic balance of the medium while magnesium sulfate was a cofactor for a variety of metabolic reaction. Basal salt medium contains ammonium sulfate and ammonium chloride as its major ingredients and both being rich source of nitrogen might have exerted positive influence for highest xylanase production as compared to other media used **(Briggs et al., 2005).**



Legend: M1= Basal salt medium, M2= Nakamura medium, M3= Emerson medium, M4= Tryptone glucose yeast medium, M5= Xylan medium, M6= Reese medium

Figure 1 Effect of media on xylanase production from *Paenibacillus* sp. N_1 under submerged fermentation

Similarly a thermoalkalophilic *Arthobacter* sp.MTCC5214 had also shown optimal extracellular xylanase production in modified Basal Salt medium **(Khandeparker and Bhosle, 2006).** Similarly maximum xylanase production by *Bacillus circulans* i.e. 55.00 $IU.ml^{-1}$ has been found in basal salt medium as reported by **Dhillon *et al*. (2000).**

Effect of incubation time

Xylanase activity was highest (29.46 $IU.ml^{-1}$) after 3 days of incubation and declined on further increasing the time. Statistically enzyme produced on 3rd day was found significantly higher than others (Table 1). 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 **(Flores et al., 1997).** Xylanase produced by *Paenibacillus* sp.N_1 was growth-associated, reaching to maximum after 72 h at exponential peak and enzyme production remained more or less the same up to 96 h. Maximum production of xylanase is observed by **Wahyuntri *et al*., (2009)** in a culture incubated at 50ºC, pH 7.0 at 72 h by *Bacillus* sp.AQ-1. While *Bacillus* SSP-34 produced

maximum xylanase activity (38.00 IU.ml^{-1}) when grown for 96 h **(Subramaniyan et al., 2001).**

Table 1 Effect of incubation time on xylanase production from *Paenibacillus* sp. N_1

Incubation Time (Days)	Protein conc. (mg.ml^{-1})	Xylanase activity (IU)
1	0.960	*25.521 **(26.516)
2	1.211	27.441 (23.470)
3	**1.340**	**29.461 (21.980)**
4	1.222	28.453 (23.320)
5	1.100	27.200 (24.720)
6	0.390	15.312 (39.230)
CD $_{0.05}$	0.167	0.177
S.E. (difference of mean)	0.079	0.081

Legend: * IU: µmoles of reducing sugar released / min / ml of enzyme.

** Value in parentheses depict specific activity i.e. enzyme activity/ mg of protein.

Effect of pH

The effect of pH on xylanase production has been presented in Table 2. Showing optimum pH for xylanase production at 9.0 (31.86 IU.ml^{-1}). Each microorganism holds a pH range for its growth and activity with optimum value around this range. pH influences many enzymatic systems and the transport of several species of enzymes across the cell membrane (**Subramaniyan and Prema, 2002**). If cultivation of the organisms is carried out at an unfavourable pH, it may limit the growth and consequently xylanase production by substrate inaccessibility. 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 addition to pulp bleaching **(Jain, 1995).** Some industries such as laundry detergents, leather and paper industries require alkaline and thermostable enzymes **(George et al., 2001).** Industrially desirable characterstics like thermostability and alkalophilic nature are main requirements for any potentially commercially important enzyme in industries. Maximum yield of xylanase has

also been reported at pH 9.0 by *Bacillus* sp. **(Anuradha et al., 2007)** and an alkalophilic *Bacillus* strain MK001 **(Kapoor et al., 2008).**

Table 2 Effect of pH on xylanase production from *Paenibacillus* sp.N_1

pH	Protein conc. (mg.ml^{-1})	Xylanase activity (IU)
4	0.981	*20.312 **(24.750)
5	1.200	24.00 (20.081)
6	1.311	25.502 (19.771)
7	1.340	29.461 (21.980)
8	1.480	30.311 (14.440)
9	**1.670**	**31.860 (19.390)**
10	0.970	24.501 (25.56)
CD $_{0.05}$	0.162	NS
S.E.(difference of mean)	0.078	3.171

Legend: * IU: µmoles of reducing sugar released / min / ml of enzyme.

** Value in parentheses depict specific activity i.e. enzyme activity/ mg of protein.

Effect of temperature

The fermentation temperature appeared to have a dramatic effect on xylanase production. *Paenibacillus* sp.N_1 produced maximum xylanase activity (31.86 IU.ml^{-1}) at elevated temperature of 50ºC, while displaying minimum activity at 30ºC (6.23 IU.ml^{-1}) as is depicted in Table 3. Optimum temperature range obtained in the present study clearly reflects strong thermophilic nature of *Paenibacillus* sp.N_1. 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 **(Kumar and Nussinov, 2001).** Specialized proteins such as chaperonins are produced to refold the protein to their native form and restore their function **(Everly and Alberto, 2000),** Cell membrane of thermophiles is made up of saturated fatty acids. Thermal stability of xylanase is an important property due to its potential application in several industrial processes, use of such enzymes has been expected to greatly reduce the need for pH and temperature adjustments before the enzyme

addition. **Sharma and Bajaj (2005)** demonstrated the stability of *Streptomyces* sp. CD3 at 50ºC after incubation for 3 days. Similarly *Bacteroides* sp. strain P1 also grew rapidly and produced maximum xylanase at 50ºC **(Ponpium et al., 2000)**.

Table 3 Effect of temperature on xylanase production from *Paenibacillus* sp.N_1

Temperature(ºC)	Protein conc. (mg.ml^{-1})	Xylanase activity (IU)
30	0.560	*6.320
		**(11.251)
35	0.640	9.331
		(14.530)
40	0.931	15.302
		(16.452)
45	1.119	24.711
		(22.071)
50	**1.670**	**31.860**
		(19.390)
55	1.257	25.000
		(19.881)
$CD_{0.05}$	0.171	1.030
S.E. (difference of mean)	0.078	0.476

Legend: * IU: µmoles of reducing sugar released / min / ml of enzyme.

** Value in parentheses depict specific activity i.e. enzyme activity/ mg of protein.

Effect of inoculum size

The influence of the inoculum size on xylanase production was assessed by altering the amount of inoculum added. The flasks were incubated at optimum fermentation temperature 50ºC for 3 days and were analyzed for xylanase activity. The inoculum added initially had a direct effect on xylanase production from *Paenibacillus* sp.N_1. An increased inoculum size up to 12.5% raised xylanase titres (35.85 IU.ml^{-1}) (Table 4). Enzyme activity was found maximum at optimal level because at this point equilibrium was maintained between the inoculum size and availability of the substrate. A decline in enzyme yield beyond threshold point might be due to

Table 4 Effect of inoculum size on xylanase production from *Paenibacillus* sp.N_1

Inoculum size (%)	Protein conc. ($mg.ml^{-1}$)	Xylanase activity (IU)
2.5	0.810	*11.361
		**(14.062)
5	0.821	14.000
		(15.000)
7.5	0.890	14.162
		(15.911)
10	1.670	31.860
		(19.390)
12.5	**2.241**	**35.850**
		(15.981)
15	0.820	12.162
		(14.821)
CD $_{0.05}$	0.177	1.037
S.E.(difference of mean)	0.081	0.476

Legend: * IU: µmoles of reducing sugar released / min / ml of enzyme.

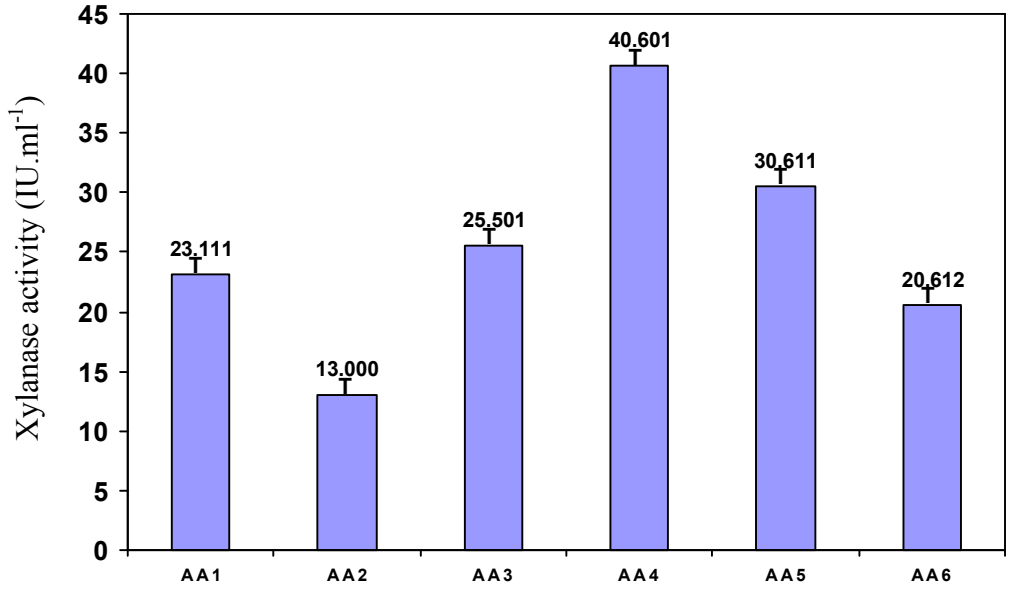
** Value in parentheses depict specific activity i.e. enzyme activity/ mg of protein.

disturbing mass substrate ratio as well as formation of the thick suspension and improper mixing of the substrate in shake flask **(Osmojasola et al., 2008)**. Higher number of microorganism restrict microbial activity due to nutrient limitations whereas a lower amount of inoculation causes lower number of cells in the production medium thus producing lesser enzymes **(Nagar et al., 2010)**. Bacterial strains including *B.licheniformis* A99 and *Bacillus pumilus* ASH 7411 produced highest xylanase when used at 15% inoculum level **(Battan et al., 2006)**.

Effect of amino acids

Xylanase production was measured in the presence of several amino acids at a concentration of 20 mg in 100 ml of broth. Cysteine (25.50 $IU.ml^{-1}$) and tryptophan resulted in maximum enzyme titres i.e. 40.60 $IU.ml^{-1}$ (Figure 2). Statistically xylanase production from tryptophan was significantly higher than others. Amino acid enhances the growth rate as well as improves protein synthesis because they may directly or indirectly be absorbed by the cell.

The enhancement in the xylanase yield in the presence of tryptophan could be due to the presence of indole functional group as the oxidation dissimilation of tryptophan is catalyzed by special of enzymes **(Spaepen et al., 2007).** An optimal production of xylanase is observed in the medium supplemented with leucine and tryptone by *Bacillus pumilus* strain under the submerged fermentation **(Kapoor et al., 2008).**

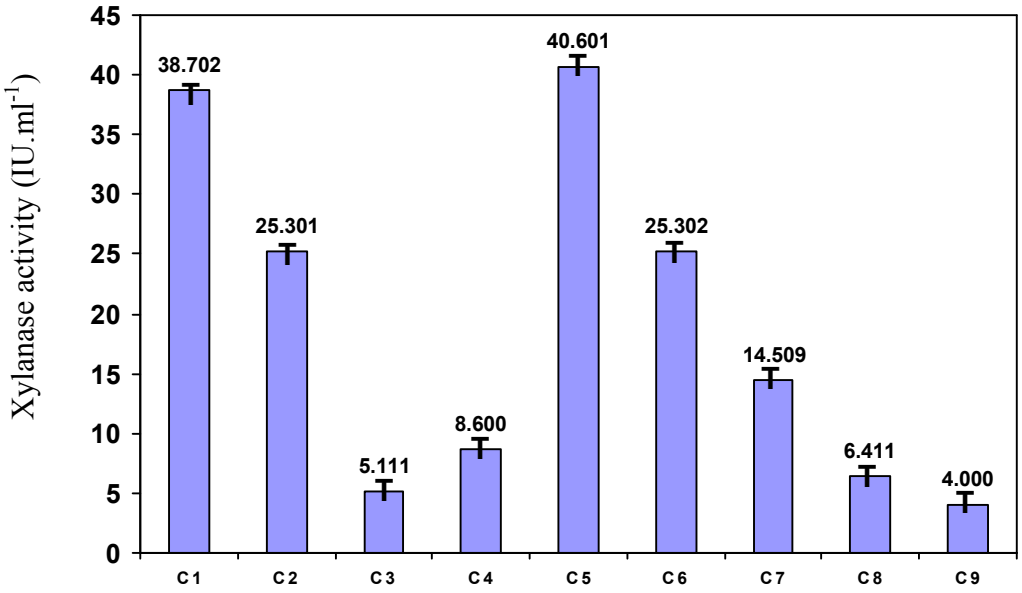


Legend: AA1= Phenylalanine, AA2= Alanine, AA3= Cysteine, AA4= Tryptophan, AA5= Arganine, AA6= Glutamic acid

Figure 2 Effect of amino acids on xylanase production from *Paenibacillus* sp.N_1 under submerged fermentation

Effect of carbon sources

The influence of supplemented carbon sources to the medium on xylanase production was assessed. The statistical analysis of the data indicated that xylanase production by *Paenibacillus* sp.N_1 was significantly higher than others when xylose (40.60 $IU.ml^{-1}$) was added as a source of carbon into the medium followed by arabinose (38.70 $IU.ml^{-1}$) as shown in Figure 3. 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 **(Satyanarayan, 2007)**.



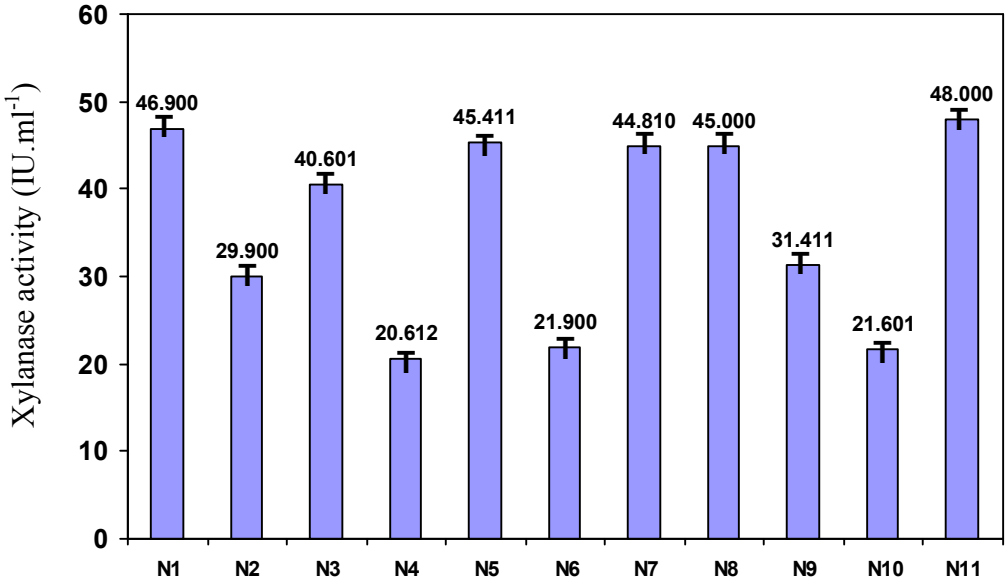
Legend: C1= Arabinose, C2= Mannose, C3= Sucrose, C4= Dextrose, C5= Xylose, C6= Lactose, C7= Rabinose, C8= Fructose, C9= 1% rice straw

Figure 3 Effect of carbon sources on xylanase production from *Paenibacillus* sp. N_1 under submerged fermentation

Carbon sources are essential elements for microorganisms during the period of growth and metabolism **(Nagar et al., 2010)**. **Gupta and Kar, (2008)** reported stimulation of xylanase production by xylose from thermophilic *Bacillus* sp. under submerged fermentation. Moreover **Kapoor *et al.* (2008)** observed that maximum xylanase production from *Bacillus pumilus* MK001 in the medium containing xylose as a source of carbon.

Effect of nitrogen sources

Xylanase production was measured in the presence of several organic and inorganic nitrogen sources as a substrate at a concentration of 1.0%. Many organic nitrogen sources such as yeast extract and beef extract resulted in higher enzyme titres i.e. 40.60 $IU.ml^{-1}$, 46.90 $IU.ml^{-1}$ respectively while inorganic compounds such as $NaNO_3$ and $NaNO_2$ produced 44.81 $IU.ml^{-1}$,45.00 $IU.ml^{-1}$ of xylanase. Maximum increase was observed in $(NH_4)_2HPO_4$ resulting in enzyme production of 48.00 $IU.ml^{-1}$ as shown in Figure 4. Inorganic nitrogen i.e. $(NH_4)_2HPO_4$ consists of ammonium ions and phosphoric acid. Ammonium salts have enhanced the growth rate as well as improved the protein expression by mediating ammonium assimilating enzymes **(Wang et al., 2009)**. Different reports are available in literature citing the influence of different amino acids on enzyme synthesis viz. the best nitrogen source for xylanase production by *Bacillus circulans* AB16 occurred in the medium containing tryptone as nitrogen source as observed by **Dhillon *et al.* (2000)**. In contrast maximum production of xylanase by *Bacillus mojavensis* called AG137 is observed when medium is supplemented with mixture of 1% yeast extract and tryptone as a nitrogen source **(Sepathy et al., 2011)**.



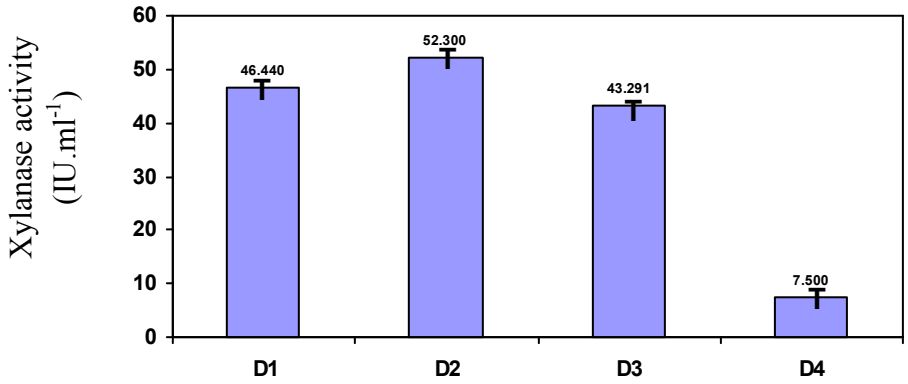
Legend: N1= Beef extract, N2= Peptone, N3= Yeast extract, N4= Casein, N5= Tryptone, N6=Ammonium nitrate, N7= Sodium nitrate, N8= Sodium nitrite, N9= Ammonium carbonate, N10= Diammonium dihydrogen phosphate, N11= Diammonium hydrogen phosphate

Figure 4 Effect of nitrogen sources on xylanase production from *Paenibacillus* sp.N_1 under submerged fermentation

Effect of detergents

To induce hyperxylanase production by *Paenibacillus* sp.N_1 using various detergents was tested. Among the various additives, tween 20, added at concentration of 10µml led to maximum xylanase i.e. 52.30 $IU.ml^{-1}$ which was found statistically significant higher than others (Figure 5). Tween 20 a surfactants disrupts non specific binding of enzymes to subtrates and thus exerts a positive effect on desorption and recycling of xylanase **(Kapoor et al., 2008)**.

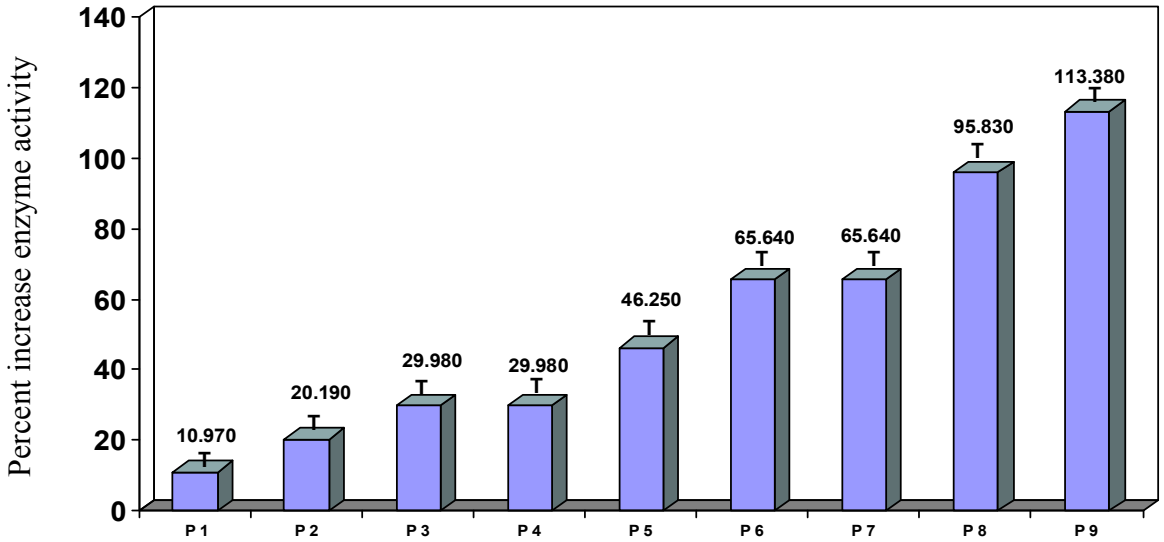
The stimulatory effect of tween 20 on xylanase production could also be due to its favourable effect on cell membrane permeability **(Uma Maheswar Rao and Satyanarayan, 2003)**. In other reports, maximum xylanase production by *Geobacillus thermoleovorans* was observed when 0.1% (v/v) of tween 80 supplemented into the medium **(Sharma et al., 2007)**. Similarly an increase in xylanase activity is observed by *Bacillus* sp. in medium supplemented with tween 80 **(Nagar et al., 2010)**.



Legend: D1= Polyethylene glycol, D2=Tween 20, D3 Tween 80, D4= Sodium dodecyl sulphate

Figure 5 Effect of detergents on xylanase production from *Paenibacillus* sp. N1 under submerged fermentation

The optimization of process parameters such as medium (BSM), incubation time (3 days), pH (9.0), temperature (50ºC), inoculum size (12.5%), aminoacid (tryptophan), carbon source (xylose), nitrogen source $(NH_4)_2HPO_4$, additive (tween 20) have led to an improved xylanase production starting from 24.60 IU.ml^{-1} to 52.39 IU.ml^{-1} and escalating the xylanase yield upto 113.38% (Figure 6).



Legend: P1= Medium, P2= Incubation time, P3= pH, P4= Temperature, P5= Inoculum size, P6= Amino acid, P7= Carbon sources, P8= Nitrogen source, P9= Detergent

Figure 6 An overview of percent increase in xylanase activity from *Paenibacillus* sp. N_1 after optimization of different parameters under submerged fermentation

CONCLUSION

The requirement for large-scale enzyme production in industry is low cost, simple cultivation and minimal amount of downstream processing. These goals can be achieved with a production system in which enzyme is effectively secreted into the cultivation medium. The results obtained in the present study clearly indicate that *Paenibacillus* sp.N_1 which emerged as a combination of rare attributes i.e. alkalophilic as well as thermostable and thus can become a potential strain for cellulase-free xylanase production under submerged fermentation. High unit of cellulase free xylanase production by thermoalkalophilic strain indicates its importance in paper and pulp industry.

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