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Production and characterization of thermostable xylanase and pectinase from *Streptomyces* sp. QG-11-3

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Streptomyces **sp. QG-11-3, which produces a cellulase-free thermostable xylanase (96 IU ml^{-1}) and a pectinase (46 IU ml^{-1}), was isolated on Horikoshi medium supplemented with 1% w/v wheat bran. Carbon sources that favored xylanase production were rice bran (82 IU ml^{-1}) and birch-wood xylan (81 IU ml^{-1}); pectinase production was also stimulated by pectin and cotton seed cake (34 IU ml^{-1} each). The partially purified xylanase and pectinase were optimally active at 60°C. Both enzymes were 100% stable at 50°C for more than 24 h. The half-lives of xylanase and pectinase at 70, 75 and 80°C were 90, 75 and 9 min, and 90, 53 and 7 min, respectively. The optimum pH values for xylanase and pectinase were 8.6 and 3.0, respectively, at 60°C. Xylanase and pectinase were stable over a broad pH range between 5.4 and 9.4 and 2.0 to 9.0, respectively, retaining more than 85% of their activity. Ca^{2+} stimulated the activity of both enzymes up to 7%, whereas Cd^{2+}, Co^{2+}, Cr^{3+}, iodoacetic acid and iodoacetamide inhibited xylanase up to 35% and pectinase up to 63%; at 1 mM, Hg^{2+} inhibited both enzymes completely.** *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 396–402.

Keywords: xylanase; pectinase; thermostable; *Streptomyces* sp

Introduction

Xylanases, a repertoire of hydrolytic enzymes, facilitate complete hydrolysis of xylan; thus, they have gained considerable attention because of their application in biobleaching of kraft pulps [35], fiber modification, extraction of coffee, plant oils and starch and in the improvement of nutritional properties of agricultural silage and grain feed [4,16]. Xylanases have been reported from bacteria [16,32], fungi [32,36], actinomycetes [2,34] and yeasts [22]. The polygalacturonases or pectinases degrade pectins or pectate by the hydrolysis of α-1, 4-glycosidic linkages [5] in a random way and thus have extensive use in the clarification of fruit juice [1,5] and the extraction of oils from vegetable and citrus peels [27]. The microbial sources of pectinases are bacteria [8,12], fungi [1,27], actinomycetes [6,7] and yeasts [5].

A potential application of xylanolytic and pectinolytic enzyme systems is in the removal of xylan from kraft pulp fibers [35], and in the processing of plant fibers such as flax, hemp and ramie [6,8,31]. Pectinases are believed to play the lead role in this process since 40% of the dry weight of plant cambium cells is pectin. There is high hemicellulose content in the phloem of cambium cells, hence xylanases may also be involved [36]. Replacement of slow natural retting by treatment with a mixture of enzymes could become a new fiber liberation technology. Both pectinases and xylanases play a significant role in the debarking process, which is the first step in the processing of wood [36].

In the present investigation, we report the production and characterization of a thermostable alkaline xylanase and a thermostable pectinase from a newly isolated *Streptomyces* sp. QG-11-3.

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Materials and methods

Microorganism and culture conditions

A protease- and cellulase-negative (Table 1) strain of *Streptomyces* sp. QG-11-3 was maintained on actinomycete isolation agar plates (pH 8.0) containing (g l^{-1}) sodium caseinate, 2; asparagine, 0.1; sodium propionate, 4; K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.1; $FeSO_4.7H_2O$, 0.001; and agar, 20. In liquid-batch fermentations, xylanase and pectinase production were studied in two different physiologically optimized media: minimal medium and Horikoshi medium [19]. The minimal medium (pH 8.0) used for xylanase and pectinase production contained (g l^{-1}) xylan/pectin, 2.5; NaCl, 2.5; K_2HPO_4, 7; KH_2PO_4, 3; $MgSO_4.7H_2O$, 0.1; $(NH_4)_2SO_4$, 1; soil extract (v/v), 2 ml. Horikoshi medium (pH 8.0) used for production of enzymes contained (g l^{-1}) xylan/pectin, 5; peptone, 5; yeast extract, 5; KH_2PO_4, 1; $MgSO_4.7H_2O$, 0.1. Production medium (50 ml) in a 250-ml baffled flask was inoculated with 2% of a 24-h-old seed culture and incubated at 37°C with shaking (200 rpm). The levels of xylanase and pectinase production, extracellular protein content, dry weight of cell biomass and pH change during the growth of the organism were monitored for up to 96 h.

Partial purification of enzyme

Protein purification was carried out with the enzyme produced in minimal media supplemented with 0.25% w/v each of xylan and pectin, respectively. The cell-free supernatant solutions were saturated with 60% ammonium sulphate, left overnight at 4°C, centrifuged, and the precipitates were dissolved in 100 mM glycine:NaOH buffer (pH 8.6) for xylanase and 100 mM citrate:phosphate buffer (pH 3.0) for pectinase. The enzymes were dialyzed against their respective buffers for 24 h and the dialyzed proteins obtained were treated as the partially purified enzymes.

Table 1 Morphological, physiological and biochemical characteristics of *Streptomyces* sp. QG-11-3

Characteristics	Results
Gram character	+
Morphology	Long branching filaments
Substrate mycelia	Yellow to light brown
Spore/color	+/gray to white
Spore chain morphology	Rectiflexibiles
Metabolism	Aerobe
Catalase	+
Oxidase	−
Melanin pigment	−
Nitrate reduction	+
Citrate utilization	+
Glycerol utilization	−
Phenylalanine deaminase	−
Arginine dihydrolase	−
H_2S production	−
Indole formation	−
Growth temperature range	10–46°C (optimum 37°C)
Growth pH range	5.0–10.5 (optimum 8±0.2)
Hydrolysis profile	
Xylan, pectin, starch, urea, tributyrin, Tween-20, -40, -60, -80, chitin, guar gum	+
Casein, elastin, keratin, gelatin, guanine, tyrosine, hippurate, xanthine, hypoxanthine, tannic acid, carboxy methyl cellulose	−
Growth in presence of inhibitory compounds (% w/v)	
Crystal violet (0.0001), phenol (0.1), phenyl ethanol (0.1, 0.3), potassium tellurite (0.001, 0.01), sodium azide (0.01, 0.02)	−
Sodium chloride (4%, 7%, 10%)	+
Sodium chloride (13%)	−
Utilization of sugars (1% w/v)	
D-glucose, D-galactose, D-ribose, sucrose, D-xylose, D-fructose, L-arabinose	+
L-rhamnose, D-mannose, maltose, D-lactose, trehalose, inulin, mannitol, adonitol, D-sorbitol, erythritol	−
Utilization of nitrogen sources (0.1% w/v)	
L-arginine, L-cysteine, glycine, L-hydroxyproline, DL-methionine, DL-β-phenylalanine, DL-threonine, DL-valine, L-proline, L-asparagine, potassium nitrate	+

Enzyme assays and protein content

Xylanase activity was assayed at 60°C in 100 mM glycine:NaOH buffer (pH 8.6). The pectinase assay was performed at 60°C in 100 mM citrate:phosphate buffer (pH 3.0). The amount of reducing sugars released during the enzyme–substrate reaction was determined by the dinitrosalicylic acid method [26] as described in our previous studies of xylanase [17,18] and pectinase [12]. One international unit (IU) of xylanase or pectinase was defined as the amount of enzyme that liberates 1 μmol of reducing sugar (xylose and galacturonic acid for xylanase and pectinase, respectively) per minute under standard assay conditions. Protein content was determined by Lowry's method [24] using bovine serum albumin as standard.

Chemicals

Birch-wood xylan and citrus pectin were purchased from Sigma Chemical (St. Louis, MO, USA). All other chemicals used were of the highest purity available commercially. Agro residues were purchased locally.

Statistical analysis

Experiments were carried out independently in triplicate and the results given are the mean of the three values. The standard deviations were within 15%.

Results and discussion

Streptomyces sp. QG-11-3 was isolated from a sample of decaying coconut fibers from Goa, India. It was an aerobic, Gram-positive, oxidase-negative and catalase-positive *Streptomyces* with branching filaments from aerial mycelia. Initially, colonies were raised, shining and smooth; later they produced gray spores on actinomycete isolation agar after 2 days of incubation at 37°C. Substrate mycelium was yellow to light brown. The organism grew well in the pH range of 5.0 to 10.5 with an optimum at 8.0. It grew in the temperature range of 10°C to 46°C but did not grow at 50°C. The isolate was screened for xylanase and pectinase production on nutrient agar plates containing 0.5% w/v xylan or pectin. After incubation at 37°C for 48 h, residual xylan was visualized by congo red staining [33]. Pectinase production was visualized by flooding the surface of a pectin–agar plate with 1% cetrimide solution. The detailed morphological, biochemical and physiological characteristics of the isolate are given in Table 1. On the basis of these characteristics, the isolate was tentatively identified as a species of the genus *Streptomyces* according to Bergey's Manual of Systematic Bacteriology [23].

In xylan–minimal medium, the maximum xylanase yield was 13 IU ml^{-1} after 48 h of incubation at 37°C with shaking (200 rpm). In the same medium, pectinase production was not detected. In pectin-minimal medium, the maximum pectinase yield was 29 IU ml^{-1} after 36 h of incubation under conditions similar to those for xylanase production (data not shown). In xylan–Horikoshi medium, maximum xylanase production was 81 IU ml^{-1} (Figure 1) after 48 h under similar conditions. The protein content and dry biomass were 1.34 mg ml^{-1} and 7.24 mg ml^{-1}, respectively. The pectinase production level in this medium was 12 IU ml^{-1} after 36 h of incubation (Figure 1). Although a number of reports are available regarding the yields of xylanases, it is difficult to compare them, since the enzyme-producing microorganism, production conditions, substrate and assay conditions, and the way of defining the units vary greatly. However, 14.3 IU ml^{-1} of xylanase was reported from *Trichosporon cutaneum* SL409 [22] using 2% (w/v) oat-spelt xylan in the production medium. On the other hand, a xylanase yield of 16.6 IU ml^{-1} was reported from *Streptomyces* T_7 [21] using 2% (w/v) larch-wood xylan as carbon source. In pectin–Horikoshi medium, the maximum pectinase and xylanase yields were 34 IU ml^{-1} and 27 IU ml^{-1}, respectively (Figure 2); the protein content and dry biomass were 1.36 mg ml^{-1} and 6.32 mg ml^{-1}, respectively, after 36 h of incubation. Xylanase was not produced in the pectin–minimal medium, but was produced in pectin–Horikoshi medium (Figure 2), which indicated that peptone and/or yeast extract may have stimulated its production since these ingredients were absent

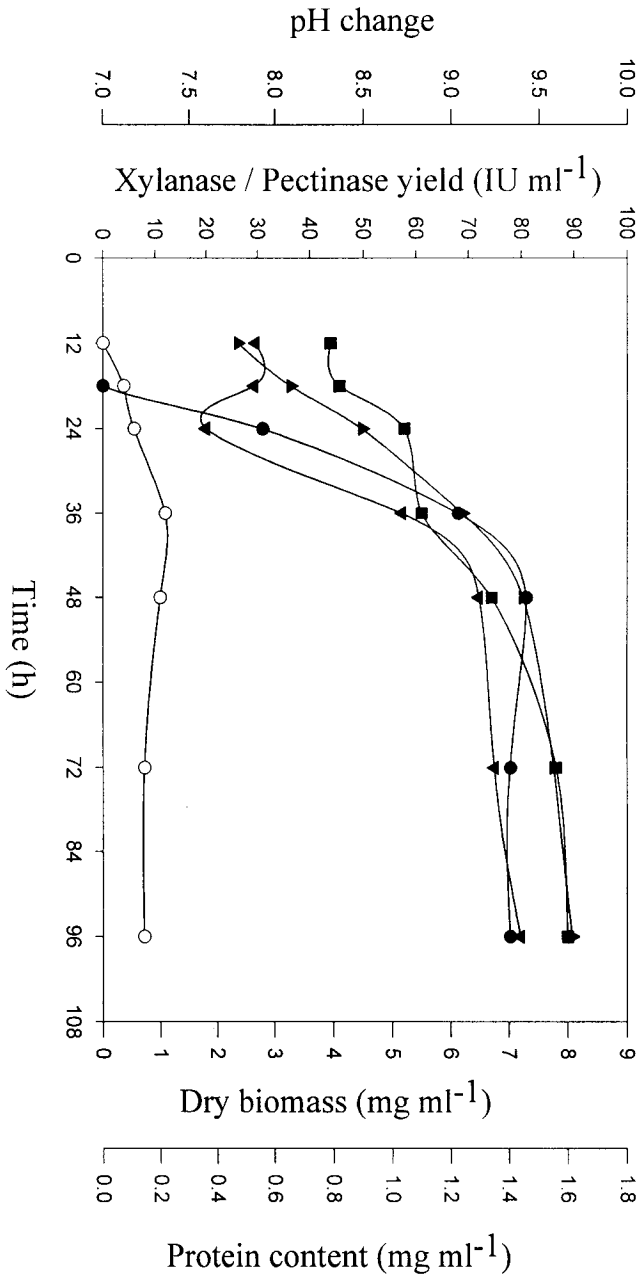

Figure 1 Growth parameters for xylanase and pectinase production from *Streptomyces* sp. QG-11-3 in xylan–Horikoshi medium at 37°C (pH 8.0) with shaking (200 rpm): xylanase yield (●), pectinase yield (○), protein content (■), dry biomass (▲) and pH change (▼).

in the pectin–minimal medium. Production of xylanase and pectinase is not only stimulated by their own substrates but also by the undefined nutrients such as peptone and yeast extract, which contain a number of amino acids, as previously reported for xylanase, α-amylase and β-galactosidase [18,19].

Xylanase and pectinase production was either negligible or very low in Horikoshi medium supplemented with glucose, galactose, xylose, arabinose, lactose, sucrose, starch, neem cake or crab shell chitin (Table 2). Similar results have been reported [3,13,20] where glucose, lactose, galactose, starch and xylose have been shown to suppress xylanase production. The more promising carbon sources for xylanase production were wheat bran, rice bran, birch-wood xylan and corn cobs (Table 2). Some of the hemicellulosic carbon sources that have been reported to enhance xylanase production are wheat bran [3,21], xylan [14,22], rice straw [21] and corn cobs [28]. The favorable carbon sources for pectinase production were wheat bran, pectin and cotton seed cake (Table 2). A better production of xylanase and pectinase using wheat bran, rice bran, xylan and pectin served as evidence that *Streptomyces* sp. QG-11-3 prefers polysaccharides for production of xylanase and pectinase rather than simpler sugars. Inhibition of

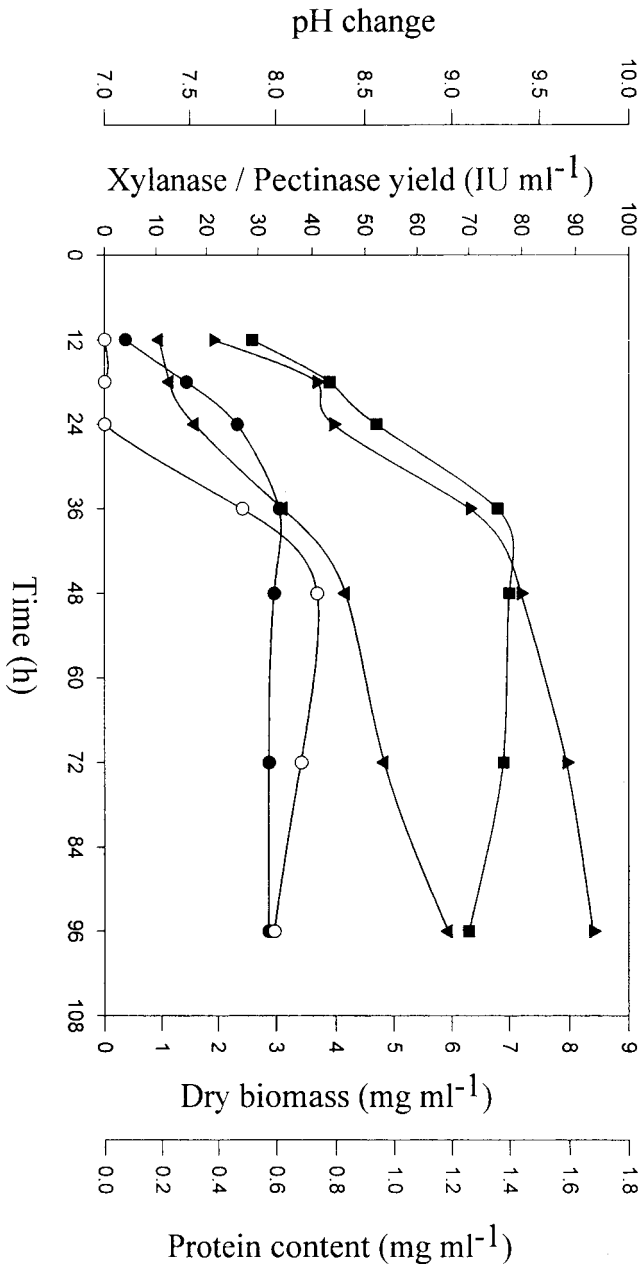

Figure 2 Growth parameters for xylanase and pectinase production from *Streptomyces* sp. QG-11-3 in pectin–Horikoshi medium at 37°C (pH 8.0) with shaking (200 rpm): xylanase yield (●), pectinase yield (○), protein content (■), dry biomass (▲) and pH change (▼).

Table 2 Production of xylanase and pectinase by *Streptomyces* sp. QG-11-3 on various carbon sources at 37°C with shaking (200 rpm)

Carbon sources	Concentration (% w/v)	Enzyme activity (IU ml^{-1})	
		Xylanase	Pectinase
Monosaccharides			
Glucose	1	N.D.	N.D.
Galactose	1	N.D.	N.D.
Arabinose	1	3 (48)	N.D.
Xylose	1	5 (48)	N.D.
Disaccharides			
Lactose	1	N.D.	N.D.
Sucrose	1	N.D.	8 (36)
Polysaccharides			
Carboxymethyl cellulose	0.5	24 (48)	6 (36)
Starch	1	N.D.	4 (36)
Pectin	0.5	41 (48)	34 (36)
Crab shell chitin	0.5	4 (48)	3 (36)
Birch wood xylan	0.5	81 (48)	12 (36)
Agro and industrial residues			
Rice bran	1	82 (72)	20 (48)
Wheat bran	1	96 (48)	46 (36)
Cotton seed cake	1	58 (48)	34 (48)
Corn cobs	1	78 (48)	29 (36)
Sugarcane bagasse	1	20 (48)	24 (48)
Neem cake	1	9 (48)	4 (48)
Oil cake	1	52 (48)	4 (48)
Poplar wood	1	26 (48)	24 (48)
Sugarcane molasses	1	18 (72)	12 (36)

N.D.: Not Detected.
Figures in parentheses represent optimum time (h) of incubation.

enzyme production in the presence of simpler sugars may be due to catabolite repression.

The xylanase and pectinase from *Streptomyces* sp. QG-11-3 were one-step purified with 60% ammonium sulphate saturation. The specific activities and percentage recovery of xylanase and pectinase were 43 U mg^{-1} and 116 U mg^{-1}, and 83% and 80%, respectively, with a purification fold of 2.8 and 1.9, respectively. The partially purified enzymes were used for further characterization.

Characterization of xylanase

The optimum pH for xylanase activity was 8.6 at 60°C and the enzyme retained more than 75% of its activity over a broad pH range of 5.0 to 9.2 (Figure 3a). Xylanase was stable in the pH range of 5.4 to 9.4 at 50°C for 1 h, retaining more than 85% of its activity (Figure 3b). The pH optima of xylanases from actinomycetes, yeasts and fungi generally fall in an acidic to neutral range; e.g. the optimum pH for xylanase from *S. viridosporus* T7A [25] has been reported to be 7.0 to 8.0, and the pH optima and pH stability of xylanase from *T. cutaneum* SL409 [22] were 6.5, and 6.0 to 8.0, respectively. On the other hand, the optimum pH for xylanases from *Aspergillus fischeri* Fxnl [29], *A. oryzae* NRRL 1808 [10] and *Gliocladium viride* CBS 658.70 [10] are 6.0, and their pH stabilities were between 5.5 to 9.5, 5.0 to 6.0, and 5.0, respectively, retaining about 80% of their activity. Puchart et al. [28] reported xylanases from 12 different strains of the thermophilic fungus *Thermomyces lanuginosus* having pH optima between 6.5 and 7.0. The xylanases from *Bacillus* sp. strain K-1 [30] and *Bacillus* sp. strain AR-009 [15] had pH optima of 5.5 and 9.0, respectively, and were stable between 5.0 to 9.0 and 8.0 to 9.0, respectively. Xylanases active in the alkaline range are important for the processing of kraft pulps [35]. Xylanase from *Streptomyces* sp. QG-11-3 exhibited a temperature optimum at 60°C (Figure 4) at pH 8.6 and was completely stable for more than 24 h at 50°C. The half-lives of xylanase at 65, 70, 75

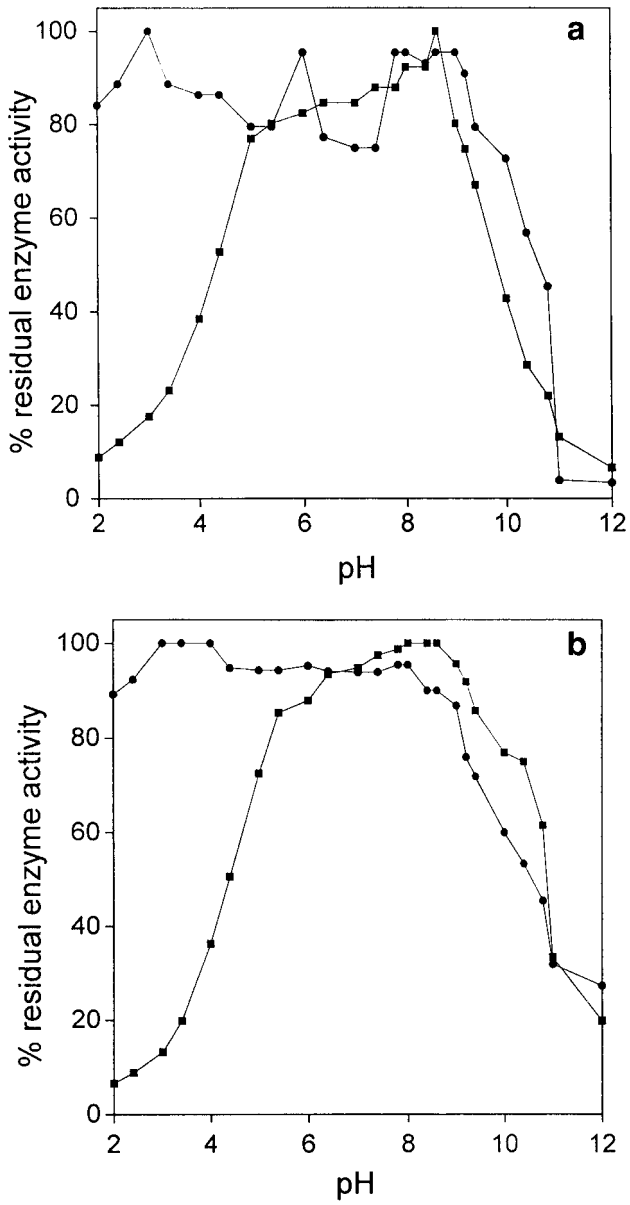

Figure 3 Effect of assay pH (a) and pH stability (b) on xylanase (■) and pectinase (●) from *Streptomyces* sp. QG-11-3. 100% xylanase and pectinase activity was equivalent to 90 IU ml^{-1} and 44 IU ml^{-1}, respectively. The enzymes were assayed in the pH range of 2.0–12.0 using buffers of different pH range (citrate:phosphate buffer 2.0–7.8, sodium:phosphate buffer 5.0–8.0, Tris:HCl buffer 7.4–9.0, borate:NaOH buffer 7.8–9.2, carbonate:bicarbonate buffer 9.2–10.8 and glycine:NaOH buffer 8.6–12.0). pH stability was determined by incubating the enzyme with an equal amount of buffer for 1 h at 50°C and thereafter, the residual activity was determined under standard assay conditions.

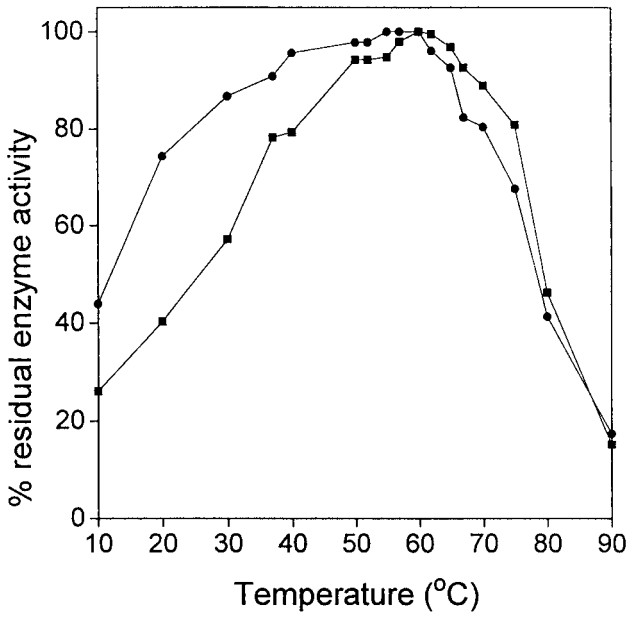

Figure 4 Effect of assay temperature on activity of xylanase (■) and pectinase (●) from *Streptomyces* sp. QG-11-3. 100% xylanase and pectinase activity was equivalent to 90 IU ml^{-1} and 44 IU ml^{-1}, respectively. The optimum assay temperatures for xylanase and pectinase were determined by incubating the assay mixture at pH 8.6 for xylanase and pH 3.0 for pectinase at different temperatures between 10°C and 90°C.

and 80°C were 120, 90, 75 and 9 min, respectively (Figure 5a). A similar temperature optimum of 60°C at pH 6.0 was reported for xylanases from *A. fischeri* Fxnl [29] and *A. oryzae* NRRL 1808 [10] and at pH 5.5 from *Bacillus* sp. strain K-1 [30]. A temperature optimum of 50°C at pH 6.5 was reported for xylanase from *T. cutaneum* SL409 [22]. A higher temperature optimum of 70°C was reported for xylanases of *Bacillus* sp. NG-27 [17], *S. viridosporus* T7A [25], and 75°C at pH 6.0 to 9.0 for the xylanase of *Bacillus* sp. SPS-0 [3]. Significant thermostability has been reported for xylanases from *Streptomyces* T_7 (50°C, pH 5.0, 6 days) [21], *Bacillus* sp. NCIM 59 (50°C, pH 7.0, 4 days) [11] and *Bacillus* sp. NG-27 (60°C for 4 h) [17].

Xylanase from *Streptomyces* sp. QG-11-3 was marginally stimulated by 1 mM Ca^{2+} (Table 3). On the other hand, Cd^{2+}, Co^{2+}, Cr^{3+}, iodoacetic acid and iodoacetamide inhibited the xylanase up to 35% at 1 mM, whereas Hg^{2+} inhibited the enzyme completely (Table 3). Similar reports are available where Hg^{2+} inhibited the xylanases completely [14,15,22,25]. Inhibition of xylanase activity by Zn^{2+}, SDS, Co^{2+} [22] and the stimulation of enzyme activity in the presence of Mn^{2+}, Fe^{2+} and β-mercaptoethanol [9] has been reported earlier. In an unusual report, xylanase activity was stimulated by $FeCl_2$, $CaSO_4$, $FeSO_4$ and $MgSO_4$ by 161%, 91%, 189% and 74%, respectively [30].

Characterization of pectinase

The pectinase from *Streptomyces* sp. QG-11-3 was optimally active at pH 3.0 at 60°C (Figure 3a). Besides having its optimum pH in an acidic range, the pectinase was highly stable in the broad pH range of 2.0 to 9.0, retaining more than 85% of its activity (Figure 3b); hence it can be used in acidic as well as alkaline environments. Most pectinases known to date are active in the acidic pH range. Recently, pectinases with optimum pH in the acidic range have been reported in yeasts [5]. An important application of acidic pectic enzymes is their role in fruit juice extraction [1,5,27]. Beside their uses in the acidic range, the alkaline pectic enzymes have been used in the degumming of jute, hemp, bast and ramie fibers [6–8,31]. Three thermostable extracellular pectinases having pH optima of 5.4, 7.0 and 10.4 have been reported in *Bacillus* GK-8 [12]. Other pectinases with pH optima in the alkaline range have been reported from *Bacillus* sp. NT-2, NT-6, NT-33 and NT-82 [8] and *Amycolata* sp. [7].

The optimum temperature for pectinase production from *Streptomyces* sp. QG-11-3 was 60°C (Figure 4) and it retained

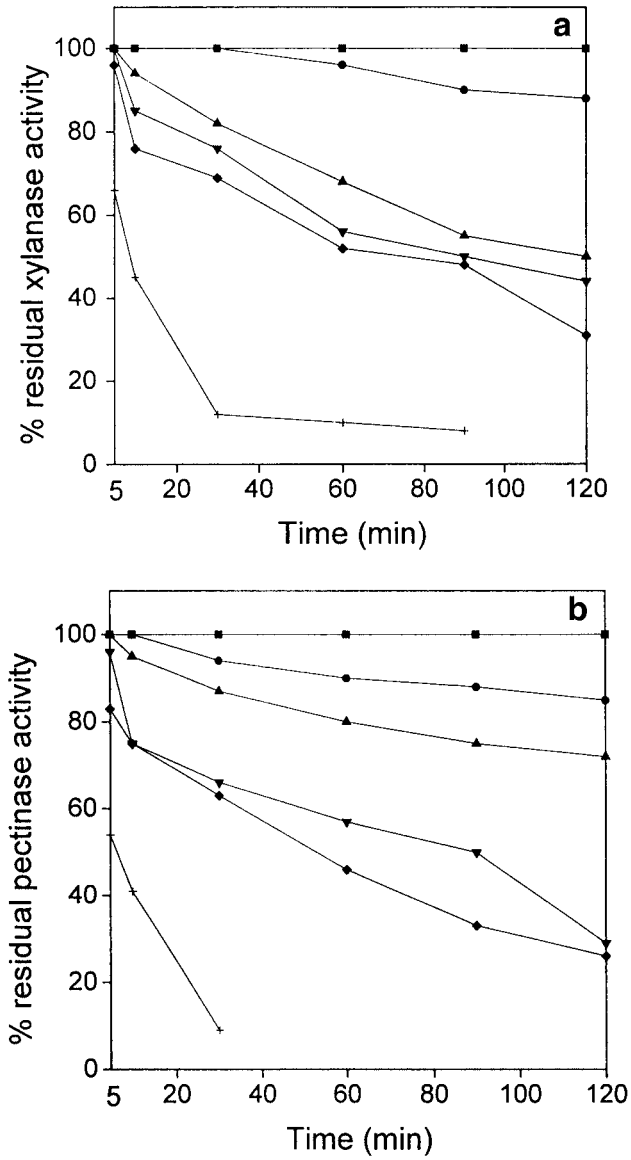

Figure 5 Thermostability profiles of xylanase (a) and pectinase (b) from *Streptomyces* sp. QG-11-3. Thermostability was determined at temperatures of 55°C (■), 60°C (●), 65°C (▲), 70°C (▼), 75°C (✦) and 80°C (+) as a function of time. 100% xylanase and pectinase activity was equivalent to 90 IU ml^{-1} and 44 IU ml^{-1}, respectively.

Table 3 Effect of metal ions (1 mM) and chemical compounds on the xylanase and pectinase activities of *Streptomyces* sp. QG-11-3

Metal ion/Chemical compound (1 mM)	Percentage residual activity	
	Xylanase	Pectinase
Control	100	100
Ag^{+}	83	76
Ba^{2+}	84	82
Ca^{2+}	107	105
Cd^{2+}	72	65
Co^{2+}	77	66
Cr^{3+}	72	79
Cu^{2+}	80	78
Fe^{2+}	84	86
Fe^{3+}	85	90
Hg^{2+}	N.D.	N.D.
Li^{+}	80	76
Ni^{2+}	87	81
NH_4^{+}	98	98
Mn^{2+}	83	83
Pb^{2+}	80	81
Zn^{2+}	88	89
EDTA	80	78
Iodoacetic acid	65	37
Iodoacetamide	70	41
Triton X-100 (1% v/v)	100	100
SDS (0.1% w/v)	84	79
H_2O_2 (0.1% v/v)	84	86
Bleaching powder (0.1% w/v)	80	70

N.D.: Not detected.
100% xylanase and pectinase activity was equivalent to 90 IU ml^{-1} and 44 IU ml^{-1}, respectively. The effect of metal ions (1 mM) and organic compounds on enzyme activity was determined by incubating the enzyme with varying concentrations of different compounds at room temperature for 1 h, thereafter, the residual activities were determined under standard assay conditions.

more than 80% activity in the range of 30°C to 70°C. A similar temperature optimum of 60°C was reported for pectinases from *Bacillus* sp. GK-8 [12]. Pectinases from *Bacillus* sp. NT-2, NT-6, NT-33 and NT-82 were optimally active at 70°C [8]. *Streptomyces* sp. QG-11-3 pectinase was 100% stable at 50°C for more than 24 h. The half-lives of pectinase at 70, 75 and 80 were 90, 53 and 7 min, respectively (Figure 5b). Ca^{2+} ions slightly stimulated the pectinase from *Streptomyces* sp. QG-11-3, whereas Cd^{2+}, Co^{2+}, Li^{+}, iodoacetic acid and iodoacetamide inhibited activity up to 63% at 1 mM (Table 3). Hg^{2+} inhibited the pectinase completely. In our previous study, the three pectinases (PI, PII and PIII) from *Bacillus* sp. GK-8 [12] were stimulated by Mg^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Mn^{2+} and were drastically inhibited by Cr^{3+} and Ni^{2+}.

Conclusions

A thermostable, alkaliphilic and cellulase-free xylanase (Table 1) and a thermostable and acidophilic pectinase are produced by *Streptomyces* sp. QG-11-3. It is rare to obtain two biotechnologically/commercially important enzymes from a single microorganism and in comparatively high amounts. Both enzymes exhibited a broad range of thermostabilities, pH stabilities and metal ions tolerances. On the basis of these properties, the xylanase and pectinase from *Streptomyces* sp. QG-11-3 qualify them for use in biobleaching of kraft pulps, clarification of fruit juices and degumming of plant bast fibers. Further work on the scale-up production and application of these enzymes, and their economic/commercial feasibility is currently underway.

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