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A NOVEL *BACILLUS PUMILUS* STRAIN FOR ALKALINE XYLANASE PRODUCTION AT AND ABOVE 40°C

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ABSTRACT

The objective of the study was to select a bacterial strain which can produce xylanase at alkaline pH and temperatures above 40°C and to characterize the strain. Among the 45 bacterial strains, isolated from different sources, five strains, which were expected to produce alkaline xylanase, above 40°C, isolated from open media containing xylan as carbon source (GS_7, GS_{15}, GS_{17}, GS_{20} & GS*), were selected for the study. Xylanase production by the strains GS_{17} and GS*, was less affected by pH changes between pH 8.0 and 9.5 than the strains GS_7, GS_{15} and GS_{20}. Strains GS_{17} and GS* produced xylanase at pH 10.0 while the others did not produce. Therefore strains GS_{17} and GS* were selected. Xylanase production, by GS_{17} at 39 hours was 1.2, 1.3, 1.2 and 3.2 times higher at 40, 45, 50 and 55°C than by GS*. At 60°C, GS_{17} produced 4.25 UmL^{-1} (30h) of xylanase activity while GS* did not. As GS_{17} produced xylanase at and above pH 9.0 and 40 to 60°C, it was selected. The xylanase of GS_{17} showed activity in the temperature range from 40 to 95°C showing highest activity at 60°C. Strain GS_{17} is non-branching, gram-positive, sporulating, motile, aerobic, catalase positive, β hemolytic, oxidase positive long rods. Hence, it belongs to the genus *Bacillus*. Slightly oval shaped terminal and subterminal endospores were observed in 24h old cultures. When the culture became old, formation of spores was very much reduced. Further, based on the shape and arrangement of spores, ability to produce acid from glucose, xylose & mannose; growth temperature; inability to hydrolyze starch and tyrosine; inability to produce urease and indole; inability to reduce nitrate; ability to grow in NaCl and ability to grow in different carbohydrate sources, the strain, GS_{17} was identified as *Bacillus pumilus*. Thus, *Bacillus pumilus* which was locally isolated can grow and produce xylanase at and above 40°C and pH 9.0.

Key words: alkaline xylanase, *Bacillus pumilus*, carbon source.

INTRODUCTION

Xylanases are widely used in industries to bleach craft pulp, to increase the brightness in paper industry, to improve the digestibility of animal feed and to clarify the juices in the food industry (Nakamura *et al.,* 1993). When *Trichoderma harzianum* was grown in SSF on sugarcane bagasse, the peak of xylanase activity (287Ug^{-1}DM) was obtained at 7th day (Barbosa *et al*., 2001). Although many bacteria and fungi have been studied for xylanase production, several xylanases commercially available are active at neutral or acidic pH and their optimum temperatures for activity are below 45ºC. Enzymes which are active under alkaline conditions and at higher temperatures have great potential for industrial applications for bleaching process to avoid changes in pH or temperatures. Different strains of *Bacillus,* which are able to produce xylanase under alkaline conditions have been isolated and identified (Duarte, 2000). Among them, the strains that can produce xylanase up to 40ºC were selected and identified using the differences in morphology and biochemical properties.

The objective of the study is to select a bacterial strain which can produce xylanase at alkaline pH and temperature above 40°C from strains isolated from different sources and to characterize the strain by microscopic, biochemical and cultural tests.

MATERIALS AND METHODS

Source of strains

Among the xylanase producing bacterial strains, available at the Biochemistry laboratory, Faculty of Medicine, University of Jaffna, the strains isolated from open xylan agar plate

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medium (GS_7, GS_{15}, GS_{17}, GS_{20} and GS*) were selected (Kanagasingam, *et al.,* 2003).

Chemicals and Media

The chemicals used were from standard sources. The plates and slants containing nutrient agar, 25.0gL^{-1} and xylan, 20.0gL^{-1} were used at pH 8.0 for the storage of the strains. The activation medium contained 20.0 gL^{-1} of xylan and 25.0 gL^{-1} of nutrient broth at different pH values. The fermentation medium contained (gL^{-1}) xylan, 20.0; peptone,20.0; yeast extract, 2.5; $CaCl_2.2H_2O$, 0.005; $MgCl_2.6H_2O$, 0.005; $FeCl_3$, 0.005; K_2HPO_4, 2.5; KH_2PO_4, 1.0; NaCl, 0.1 and $(NH_4)_2SO_4$, 2.0 at the pH required and incubated at different temperatures based on the experimental conditions and at 100 rpm.

Analytical methods

The growth of the organism was measured by measuring the optical densities at 600 nm against the respective fresh fermentation media. Enzyme was diluted with Tris aminomethane buffer (pH-8.5). Enzyme assay was carried out at pH 8.5 and 60°C by preincubating the enzyme extract with 10gL^{-1} xylan in 0.01M Tris aminomethane buffer (pH-8.5) for 5 min. Then 0.5 mL of the enzyme was mixed with 0.5 mL substrate and incubated for 5 min. at 85°C. Reducing sugar was measured by DNS method (Miller, 1959). One unit of xylanase activity is defined as the amount of enzyme that produces 1 μmol of reducing sugar in 1 minute at pH 8.5 and 60°C with 20 gL^{-1} xylan.

Selection of alkaline xylanase producer at high temperatures

Effect of pH on the growth and xylanase production

Strains of GS_7, GS_{15} GS_{17} & GS_{20} and GS* activated in the activation media at different pH levels (7.5, 8.0, 8.5, 9.0 & 10.0) and at 40°C, were inoculated to the fermentation medium at the respective pH values (7.5, 8.0, 8.5, 9.0 & 10.0) and incubated at 40°C and at 100 rpm. The pH was maintained in the test samples and not in the control samples. Growth and the xylanase production were monitored.

Effect of temperature on xylanase production

The bacterial strains GS_{17} and GS* (18 h old; 20%, v/v) activated at optimum pH value and at different temperatures (35, 40, 45, 50, 55 & 60°C) at 100 rpm, were transferred into the fermentation medium at optimum pH value and incubated at the respective temperatures (35, 40, 45, 50, 55 & 60°C) at 100 rpm while maintaining the pH. The experiment was proceeded as said above.

Effect of temperature on xylanase activity

The effect of temperature on the xylanase activity was determined by incubating the appropriately diluted crude enzyme (0.01M Tris buffer at pH 8.6) with 0.5 mL of soluble xylan (2gL^{-1}) at different temperatures, ranging from 40 to 95°C.

Characterization of the Selected Strain

Microscopic studies

The strain GS_{17} was subjected to Gram staining and motality test by hanging drop (Theivendrarajah, 1990 and Barrow and Feltham, 1993).

Biochemical tests

Oxygen requirement test, test for anaerobic growth, Catalase test, Oxidase test, Triple Sugar Iron Agar test and Lactose Fermentation test were done on strain GS_{17} (Theivendrarajah, 1990 & Barrow and Feltham, 1993).

Identification of genus of selected strain

Colony morphology such as form, elevation, margin, opacity, diameter (in mm) after 40 h, colour and surface of the strain GS_{17}, were studied to identify the genus of the selected bacterial strain.

Determination of species of strain GS_{17}

Shape and arrangement of endospore were observed under oil-immersion microscope after Grams staining. Production of acid from different carbohydrates such as glucose, xylose and mannose were tested. Production of urease, hemolysis of blood agar, indole test, nitrate reduction test, decomposition of tyrosine, hydrolysis of starch, citrate utilization test and voges-proskauer (vp) test were done to the selected strain (Theivendrarajah, 1990 & Barrow and Feltham, 1993). Growth of the selected strain was tested at 5, 15, 25, 35, 40, 45, 50, 55 and 60°C at pH 9.0, and 100rpm. Effect of different concentrations of NaCl on the growth of the selected strain was tested at 600nm.

RESULTS AND DISCUSSION

Effect of pH on growth and xylanase production

Five bacterial strains which were isolated from open xylan agar plate as the potential xylanase producers were selected for this study.

When the pH was maintained at 8.0 and 8.5, GS_{15} showed the highest growth, while GS_{17} showed highest growth when the pH was maintained at 9.0, 9.5 and 10.0. Most of the organisms showed better growth in the pH non-maintained media than in pH maintained media (Tables 1 and 2) except GS_7 (at pH 8.0), GS_{15} (at pH 8.0), GS_{17} (at pH 8.5, 9.0 and 9.5) and GS* (at pH 8.5). Strains GS_{17} and GS* were able to grow at pH 10.0. Therefore, among the five strains selected, GS_{17} and GS* were able to grow in alkaline media, up to pH 10.0. Since the strains GS_{17} and GS* were able to grow and produce xylanase under alkaline conditions, they were selected for further studies.

All the organisms produced xylanase in parallel with their growth. When the enzyme production decreased, all the selected organisms stopped growing. This indicated that the organism was unable to survive beyond that level due to the non-production of xylanase. The enzyme production was maximum, when the cell population entered into stationary phase, suggesting the enzyme production is not growth associated. Xylanase production by strains GS_{17} and GS* was less affected than strains GS_7, GS_{15} and GS_{20} when the pH of the medium was changed from 8.0 to 9.5. Xylanase production by strains GS_{17} and GS* was better than by strains GS_7, GS_{15} and GS_{20} when the pH of the medium was maintained at 8.0, 8.5, 9.0 and 9.5 during fermentation. Xylanase was produced by strains GS_{17} and GS* when the pH of the medium was maintained at 10.0 but strains GS_7, GS_{15} and GS_{20} did not produce xylanase. Therefore, strains GS_{17} and GS* were selected as the best alkaline xylanase producers from the selected strains.

Effect of temperature on xylanase production

In order to select the bacterial strains which are capable of producing xylanase at high temperatures, the effect of different temperatures on xylanase production, was studied at pH 9.0 and the results are summarized in Table 3.

Strains GS_{17} and GS* started to produce xylanase at 14 h at all the tested temperatures. At the commencement of the xylanase production, GS_{17} produced higher activity (8.85UmL^{-1}) at 40°C than GS*. GS* produced higher activity at 35°C (6.25UmL^{-1}). Both the strains GS_{17} and GS* produced xylanase activity at 14h of fermentation (1.21 and 0.16 UmL^{-1} respectively) at 55°C. At 60°C, strain GS_{17} produced 0.22UmL^{-1} of xylanase activity while the strain GS* did not produce the enzyme. Both the strains GS_{17}, and GS* produced highest xylanase activity [(24.52UmL^{-1} (42h) and 19.26 UmL^{-1} (48h)] at 40°C. With the increase in temperature above 40°C, the enzyme production also decreased. Both strains produced xylanase activity up to 55°C. Though both strains GS_{17} and GS* produced xylanase at higher temperatures, xylanse production by strain GS_{17} at 39 hours and at pH 9 was 1.21, 1.22, 1.31, 1.17 and 3.20 times higher at 35, 40, 45, 50 and 55°C respectively, than that produced by strain GS*. The strain GS* did not produce xylanase at 60°C and at pH 9.0 while the strain GS_{17} produced 2.12 UmL^{-1} (39h) of xylanase activity at 60°C. As strain GS_{17} gave a higher xylanase activity than strain GS*, at alkaline pH and higher temperatures, GS_{17} was selected for further studies.

Effect of temperature on xylanase activity

The supernatant xylanolytic activities were assayed at different temperatures ranging from 40°C - 95°C at pH of 9.0 with 20gL^{-1} xylan. When the temperature was varied from 40 to 95°C, the activity of xylanase increased upto 60°C (25.65 UmL^{-1}). Xylanase from the isolate exhibited a temperature profile with a sharp peak of maximal activity at 60°C and activity between 40 - 95° C. The optimum temperature for xylanase enzyme activity was 60°C at pH 9.0. When the enzyme was kept at room temperature, 84% (21.45UmL^{-1}) of the original activity was observed for more than 5 min. The optimum temperature for activity depends on the type of organism.

Characterization of the selected bacterial strain

Microscopic studies

Microscopic study was carried out to identify the genus of the strain. The strain GS_{17} was stained as blue-violet rods with spores indicating that it is a gram positive rod. Strain GS_{17} moved rapidly across the microscopic field with twisting and this indicated the true motility. The hanging drop method used here is a type of wet mount slide preparation that permits the observation of living, unstained cells in a fluid medium. Gram-positive motile non-branching spore forming rods belong to Family Bacillaceae (Ananthanarayan *et al*., 1997; Prescott, 1996). Thus, the strain GS_{17} may belong to the Family Bacillaceae.

Table 1. The effect of pH on the growth of bacterial strains GS_7, GS_{15}, GS_{17}, GS_{20} and GS*, at 40°C and 100rpm in fermentation medium.

pH	Highest growth (600nm)									
	GS_7		GS_{15}		GS_{17}		GS_{20}		GS*	
	T	C	T	C	T	C	T	C	T	C
8.0	1.94 (60)	1.89 (54)	2.42 (66)	2.36 (66)	2.35 (54)	2.43 (66)	1.71(60)	1.74 (66)	2.36 (66)	2.41(60)
8.5	1.80 (42)	1.84 (60)	2.34 (60)	2.45 (60)	2.31(60)	2.14 (60)	1.61(54)	1.69 (60)	2.22 (60)	2.12 (60)
9.0	1.67 (54)	1.82 (54)	1.84 (60)	2.42 (66)	2.37 (60)	2.09 (60)	1.41(66)	1.62 (60)	2.11 (66)	2.09 (66)
9.5	1.31(54)	1.48 (60)	1.26 (60)	1.52 (66)	1.58 (54)	1.41(66)	1.16 (60)	1.45 (66)	1.46 (60)	1.68 (66)
10.0	-	-	-	-	1.29 (66)	1.21(42)	-	-	1.22 (60)	1.35 (66)

T - pH was maintained at the respective pH values C - pH was not maintained
Time at which highest optical density was obtained is given in parenthesis.

Table 2. The effect of pH on xylanase production by different bacterial strains (GS_7, GS_{15}, GS_{17}, GS_{20} and GS*) at 40°C and 100rpm in fermentation medium.

pH	Highest xylanase activity (UmL^{-1})									
	GS_7		GS_{15}		GS_{17}		GS_{20}		GS*	
	T	C	T	C	T	C	T	C	T	C
8.0	21.28 (66)	21.23 (66)	15.84 (42)	16.38 (42)	35.68 (54)	33.29 (54)	32.75 (60)	33.24 (60)	39.53 (54)	36.59 (54)
8.5	18.35 (60)	21.24 (60)	16.42 (42)	17.88 (42)	38.65 (54)	32.44 (54)	30.64 (66)	33.24 (66)	31.54 (54)	28.32 (60)
9.0	21.6 (60)	22.96 (60)	22.40 (42)	23.97 (42)	26.40 (54)	19.46 (54)	22.40 (54)	24.96 (54)	23.75 (60)	20.56 (60)
9.5	9.54 (54)	13.68 (60)	7.88 (42)	11.32 (42)	18.22 (54)	14.95 (54)	8.69 (54)	15.64 (54)	14.26 (60)	13.02 (66)
10.0	-	-	-	-	8.48 (54)	6.88 (54)	-	-	8.16 (60)	7.36(66)

T - pH was maintained at the initial level C - pH was not maintained
Time at which the highest activity was obtained is given in parenthesis.

Table 3. The effect of temperature on the xylanase production by the strains GS_{17} and GS* grown at different temperatures at 100rpm in fermentation medium.
(The pH of the medium was maintained at 9.0, by the manual addition of 0.1N NaOH.)

Temperature (^oC)	Activity at 14th h (UmL^{-1})		Highest activity (UmL^{-1})	
	GS_{17}	GS*	GS_{17}	GS*
35	7.35	6.25	22.48 (34)	18.42 (48)
40	8.85	5.96	24.52 (42)	19.26 (48)
45	7.54	4.16	18.84 (39)	15.94 (42)
50	4.56	2.24	12.42 (34)	11.69 (48)
55	1.21	0.16	7.85 (39)	4.48 (54)
60	0.22	-	2.12 (39)	-

Biochemical tests

Biochemical tests were carried out to confirm the genus of the strain and to identify the species. The strain GS_{17} had shown good growth under aerobic condition but did not grow under anaerobic condition (in anaerobic jar). This indicated that the strain GS_{17} is a strict aerobe. The strain GS_{17} produced O_2 from H_2O_2. This showed that the strain GS_{17} is a catalase producer. If the bacterium oxidizes tetramethyl-p-phenylenediamine dihydrochloride, it will turn purple, indicating that the organism can produce cytochrome oxidase. No change in colour indicates that there is no production due to lack of cytochrome oxidase. The strain GS_{17} did not bring about a colour change. Therefore, it does not produce cytochrome oxidase. Strain GS_{17} ferment lactose, sucrose and glucose. The strain inoculated slants showed pink red butt and yellow slope. These results indicated that strain GS_{17} ferments glucose. When Mac Conkey agar medium was inoculated with strain Gs_{17} it did not change colour into red. This indicated that strain Gs_{17} does not ferment lactose.

Identification of the genus of selected strain

The colonies of the strain GS_{17} have a circular form with an entire margin, white in colour, moist and shiny with a convex elevated surface. After 40 h of growth, the colony diameter was 1.5 to 2.0 mm. The strain produced opaque single colonies. Based on studies so far carried out, it can be confirmed that the strain GS_{17} belongs to the genus *Bacillus*. *Bacillus* is distinguished from the other endospore-forming bacteria on the basis of being a strict or facultative aerobe, rod-shaped, and (usually) catalase-positive. According morphological, cultural and biochemical tests carried out so far, GS_{17} belongs to genus *Bacillus*. Its species has been identified, in the following experiments.

Determination of species of strain GS_{17}

To determine the species of a bacterial strain, it is important to know its endospore formation, acid production from sugars, urea hydrolysis, blood hemolysis, indole production, nitrate reduction, tyrosine utilization, starch hydrolysis, citrate utilization, glucose fermentation, growth temperature and growth in NaCl.

When serum-water glucose medium, serum-water xylose medium and serum-water mannose medium were inoculated with strain GS_{17} and incubated at 37^oC for 24h, the colour changed to red. This indicated that strain GS_{17} can produce acid from glucose, xylose and mannose.

If the strain is a urease-producer, the enzyme will hydrolyze urea by releasing ammonia and carbon dioxide. Ammonia released will change the phenol red indicator to red pink colour due to the change in pH of the medium to alkaline conditions. Medium inoculated with Strain GS_{17} did not show any colour change. This indicated that strain GS_{17} is not a urease producer.

When blood agar plate was inoculated with strain GS_{17}, around the bacterial colony a transparent clear zone was observed. This indicated that GS_{17} has broken down the haem in hemoglobin present in blood. Thus, strain GS_{17} is a β-hemolytic organism.

When tryptophan is metabolised by an organism using the enzyme tryptophanase, indole is produced and the presence of indole can be detected using Kovac's reagent. Kovac's reagent is yellow in colour. It contains 4(p)-dimethylaminobenzaldehyde, which reacts with indole and produces a red colour on the surface of the reagent. Light red or pink colour at the border of Kovac's indole reagent and tryptophan

solution (tryptone water) indicates the formation of indole (Theivendrarajah, 1990). When tryptone water medium was inoculated with strain GS_{17} and mixed with Kovac's indole reagent, no colour change was observed. This indicated that strain GS_{17} does not utilise tryptophan and produce indole.

Reduction of nitrate to nitrite by an organism can be tested by incubating one loopful of inoculum in a broth containing nitrate and after 4 h adding sulphanilic acid reagent to the broth. If nitrite is present the acid reagent is diazotized and forms a pink-red compound with alpha napthalamine. The strain GS_{17} did not show any colour change, indicating that it does not have the ability to produce nitrate reductase.

If the organism decomposes tyrosine crystals there should be a clear zone under and around the bacterial growth. When the tyrosine-agar plates were inoculated with strain GS_{17}, no clear zone was observed around and under the colonies. This indicated that the strain GS_{17} did not utilize tyrosine.

If the organism produces starch hydrolysing enzymes it can hydrolyse starch into monosaccharides. To check whether the organism has utilized starch, after the growth of the organism I_2 has to be added. If there is blue colour formation, it indicates the production of starch hydrolyzing enzyme (Theivendrarajah, 1990). When starch-agar medium was inoculated with strain GS_{17} and I_2/KI was added after 48 h of incubation, a blue colour was formed. This indicated that the strain GS_{17} did not produce starch-hydrolyzing enzymes.

Bacteria that utilize citrate can extract nitrogen from the ammonium phosphate incorporated in the medium, resulting in the production of ammonia, which combines with water to form NH_4OH. These reactions in combination produce an alkaline pH, resulting a colour change in the indicator from green to blue. If the organism does not utilize citrate, the medium remains green in colour. When citrate phosphate broth was inoculated with strain GS_{17}, the medium remained green. This indicated that the strain GS_{17} did not utilize citrate as the carbon source.

If acetoin is produced by the organism, in the presence of sodium hydroxide acetoine is oxidised to diacetyl. The diacetyl will give a pink compound with creatine. GS_{17} was cultured in glucose-phosphate-peptone water and incubated at 37°C for 48 h. When creatine and sodium hydroxide were added the culture and exposed to air, a pink colour was obtained. This indicated that GS_{17} had produced acetoin by the fermentation of glucose.

Strain GS_{17} was grown at varying temperatures, ranging from 5 to 60°C in activation medium. The temperature range for growth of strain GS_{17} was 25 to 55°C. GS_{17} grows at 55°C but no growth at 5 and 15°C (Figure1). Strain GS_{17} grew well at temperatures between 25-55°C. Strain GS_{17} did not grow at or below 15°C. At 60°C less growth (OD_{600nm} 0.14) was observed.

When different concentrations of NaCl were added to the fermentation medium, the growth (OD at 600nm) of the strain GS_{17} was increased from 0 to 1.678 at 42 h in the media with NaCl concentration up to 10gL^{-1} and beyond that concentration growth of the strain decreased. In the presence of 70gL^{-1} NaCl, very low growth (OD $_{600}$nm 0.021) was observed (Figure 2).

Final confirmation of species of identified strain Bacillus GS_{17}

Characteristics of the selected strain GS_{17} was compared with 13 species of *Bacillus*. If the character of strain GS_{17} is similar to the known species its score would be 1. If the character is not similar and variable, it will not get any score. Total score was counted, divided by total characteristics and it was multiplied by 100 and presented as a percentage.

Based on these morphological findings and biochemical studies, the strain GS_{17} got the highest score of 86% showing similarities with *Bacillus pumilus*, followed by *Bacillus subtilis* (81%, Table 4). The strain GS_{17} showed clear characteristics of *Bacillus pumilus* than the other *Bacillus* species. As the strain GS_{17} got the highest score, it was identified as *Bacillus pumilus*. Summary of the identification studies of the strain GS_{17} is present in Table 4.

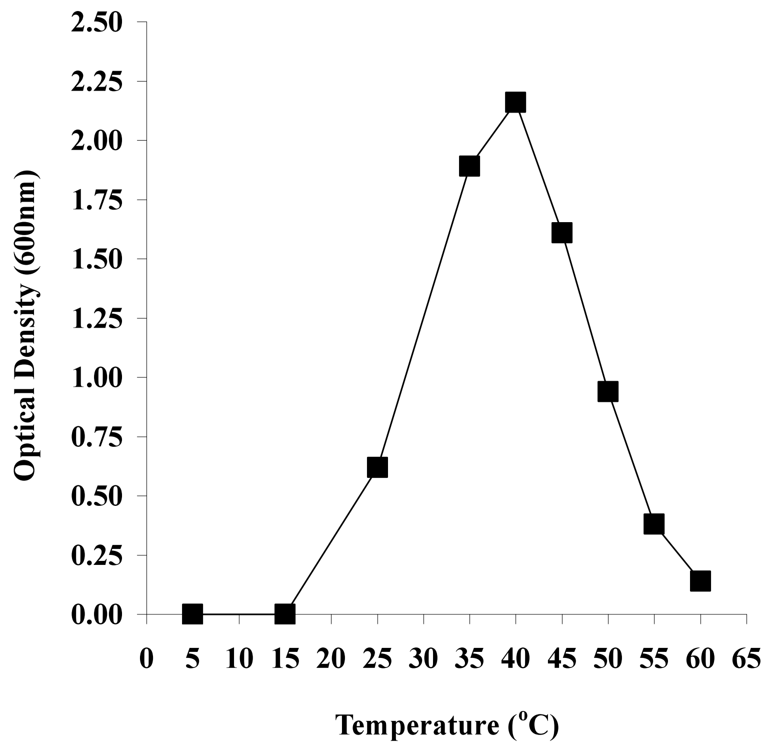

Figure 1. Growth of strain GS_{17} at 20 h in the activation medium at pH 9.0 and at different temperatures and at 100rpm.

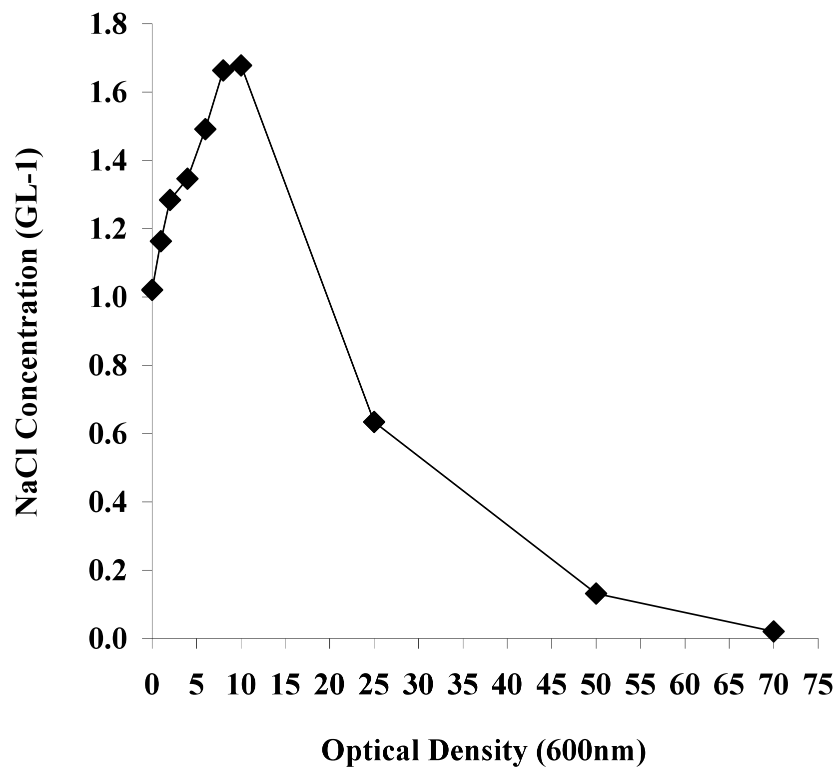

Figure 2. Growth of strain GS_{17} at 24 h in fermentation medium containing different concentrations of NaCl at pH 9.0 and 40°C while shaking at 100 rpm.

Table 4. Cultural and biochemical characteristics of different species of *Bacillus* (Fisher, 1975; Barrow and Feltham, 1993).

	Gram stain ing	Motility	Growth in 7% NaCl	Anaerobic growth	Acid from mannose	Acid from xylose	Utilization of citrate	Urease activity	Production of indole	VP test	Nitrate reduction	Starch hydrolysis	Production of oxidase	Production of catalase	Chains of cells	Hydrolysis of tyrosine	Hemolysis	Swelling of cells	Growth at 45°C	Growth at 50°C	Growth at 55°C	Total scoring	% of suitability
subtilis	+	+	+	-	+	+	+	-	-	+	+	+	-	+	d	-	d	-	+	+	+	17	81
pumilus	+	+	+	-	+	+	+	-	-	+	-	-	-	+	+	-	d	-	+	+	-	18	86
licheniformis	+	+	+	+	+	+	+	d	-	d	+	+	-	+	d	-	d	-	+	+	+	14	67
cereus	+	+	+	+	-	-	+	d	-	+	+	+	d	+	+	+	β	-	+	+	-	11	52
anthracis	+	-	+	+	-	-	-	-	-	+	+	+	d	+	+	d	-	-	-	+	-	8	38
thuringiensis	+	+	+	+	d	-	+	-	-	+	+	+	d	+	+	d	d	-	+	+	-	11	52
megaterium	+	+	+	-	d	+	+	d	-	-	+	+	d	+	+	d	d	-	+	-	-	10	48
firmus	d	+	+	-	d	-	-	-	-	-	+	+	-	d	d	d	d	d	+	d	-	7	33
laterosporus	+	+	-	+	+	-	-	-	-	+	+	-	-	+	-	-	d	+	+	d	-	12	57
fastidiosus	+	+	d	+	d	-	d	+	d	d	-	d	d	+	d	d	d	-	+	-	-	6	29
popillae	d	d	-	+	d	-	-	d	-	d	-	-	d	-	d	-	d	d	+	-	-	5	24
lentimorbus	d	-	-	+	d	-	-	d	-	d	-	-	d	-	d	-	d	d	+	-	-	5	24
amyloliquifaciens	+	+	+	+	d	-	d	-	-	+	+	+	-	d	d	d	d	-	+	+	-	10	48
Selected *Bacillus*	+	+	+	-	+	+	+	-	-	+	-	-	-	+	-	-	β	-	+	+	+	21	

+ Positive results for the test - Negative results for the test d Variable

CONCLUSION

Selected bacterial strain GS_{17} can produce xylanase at pH 9.0 and above and temperatures above 40°C. Based on the microscopic, biochemical and culture studies, the strain GS_{17} was identified as *Bacillus pumilus*.

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