RESEARCH ARTICLE

Characteristic of Amylase produced by *Bacillus axarquiensis* Isolated from Basi Bhat

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ABSTRACT

Bacillus *axarquiensis* is isolated from Basi Bhat. It had aggregation time (64 min) and produce biosurfactant. It showed protease, lipase, and amylase activity. It passes cell surface hydrophobicity 78.2%; resistance to acidic condition (pH 3 for 90 min) and growing in presence of bile salts (in culture medium containing more than 0.15% bile salt). The thermostable extracellular amylase was isolated and partially purified, the optimum temperature and pH for it was found to be 55^{0}C and 6.5 respectively. The maximum amylase production was seen with maltose as carbon source while among the nitrogen sources, complex nitrogen sources support for maximum amylase production.

Key words: Bacillus, biosurfactant, Basi Bhat, thermostable, amylase.

INTRODUCTION

Bacillus species, such as *Bacillus subtilis, Bacillus licheniformis, Bacillus axarquiensis* and *Bacillus pumilus,* produce biosurfactants (Arima *et al*., 1968; Naruse *et al*., 1990; Yakimov *et al*., 1995), compounds that reduce surface and interfacial tension and thus have excellent detergent, emulsifying, foaming and dispersing properties. They are used extensively in the textile, pharmaceutical and cosmetics industries and also in bioremediation (Banat *et al*., 2000). *Bacillus sp.* are Gram-positive long rods, and classified as Kingdom: *Bacteria*, Phylum: *Firmicutes,* Class: *Bacilli*, Order: *Bacillales,* Family: *Bacillaceae,* Genus: *Bacillus Bacillus axarquiensis* (B-5) is isolated from Basi Bhat, its 16S rRNA gene amplified by PCR using forward primer, Bac 8f (5'AGAGTTTGATCCTGGCTCAG3') and reverse primer, Univ592r (5'ACCGCGGCKGCTGGC3') following standard protocols. The sequences obtained was compared to reference 16S rRNA gene sequences available in the GenBank, and found 78% identical with *Bacillus axarquiensis.* Isolated *Bacillus axarquiensis* has been found to be amylase positive as hydrolyzing starch. The amylases are industrially important like microbial amylase, which has higher yield and thermo stability. They are used also in industries like food, fermentation, textile paper and detergent. The efficiency of microbial amylases has been proved to be better than chemical hydrolysis.

MATERIAL AND METHODS

Isolation of Bacillus: The *Bacillus axarquiensis* was isolated from Basi Bhat on medium; rice powder: 0.5%, peptone: 0.5%, K_2HPO_4: 0.2%, $MgSO_4$: 0.05%, $FeCl_3$: traces, and agar: 2%.

Cell surface hydrophobicity test: It was determined by the method of Rosenberg *et al*. (1980). The strain was harvested after 18h of growth, washed twice and suspended in saline solution to OD of 0.5 at 600 nm. To 3 ml of washed cells, 1 ml of toluene added and mixtures were blended for 90 seconds. The tube was left to stand for 15 min for separation; the OD of the aqueous phase was taken. Hydrophobicity was given by the percentage decrease in the OD of the bacterial suspension due to partitioning of cells into the hydrocarbon layer. Percentage of hydrophobicity = [(OD600 before mixing - OD600 after mixing) / OD600 before mixing] x100 (Handly *et al*).

Amylase Production: The following media were used to study the amylase production. Media I- Starch, 10.0 g; agar, 20 g; dist. water 1 liter and pH adjusted to 7.0.

Media II- Starch, 1.0 g; peptone, 0.5g; $K_2HPO_4.H_2O$, 0.05 g; $FeCl_3$ traces; agar 20g; dist. water 1 liter and pH adjusted to 7.0.

Media III- (composition is same as in media II where peptone replaced by NH_4NO_3).

The colonies identified by starch hydrolysis test using iodine solution.

RESULTS AND DISCUSSION

Effect of carbon and nitrogen sources on production of amylase: For optimization of cultural conditions, media IV was used, whose composition as, starch, 10.0 g; yeast extract, 3.0 g; peptone, 5.0 g; NaCl, 3.0 g; $MgSO_4.7H_2O$, 0.05 g; dist. water 1 liter and pH adjusted to 7.0. For study of effect of carbon source in media IV, starch was replaced by different 1.0% carbon sources as mentioned in Table 3. To study the effect of nitrogen source in media IV, peptone and yeast extract were replaced by different 1.0% nitrogen sources as mentioned in Table 4.

Amylase assay: For amylase assay 0.5 ml of 1 % starch in 0.1M phosphate buffer (pH 6.5) and adding 0.5 ml of enzyme were incubated for 30 min at room temperature i.e. 37^0C. While the reaction was stopped by adding 1.0 ml of dinitrosalicylic acid reagent, heated on boiling water bath 5 min and then to it 10 ml dist. water was added. Absorbance was checked at 540 nm against blank. The blank was the same as above without incubation. One unit of the amylase activity was defined as the amount of enzyme that liberated one µmole of reducing sugar under experimental condition.

Table1: Attributes of *B. axarquiensis*

Parameter	
Amylase activity	+
Protease activity	+
Lipase activity	+
Aggregation time (min)	64
Cell surface hydrophobicity	78.2%

Table2: B. *axarquiensis*-characteristics

Parameters	Characteristics
Morphology Colony on Agar	Straight rods, Gram +ve, Sporulating
Growth temp.	Opt growth at 32^0C range 15-45^0C
Gelatinase	Positive
Casein hydrolysis	Positive
Amylase	Positive
Catalase	Positive
Indole test	Negative
Urease	Negative
Nitrate reduction	Positive
Methyl red test	Positive
Citrate utilization	Positive

Table 3: Effect of carbon sources on amylase production

Carbon source	Activity (µmole/min/ml)
Arabinose	0.0523
Dextrin	0.1543
Fructose	0.4110
Galactose	0.2803
Glucose	0.1311
Glycerol	0.0215
Lactose	0.0101
Maltose	0.5120
Mannitol	0.1245
myo-Inositol	0.1542
Raffinose	0.3512
Ribose	0.2843
Sod. Acetate	0.0084
Sod. Citrate	0.1431
Starch	0.0661
Sucrose	0.2865
Xylose	0.2664

Partial purification of amylase:

From Basi Bhat total 32 *Bacillus* were screened based on aggregation time, antibacterial effects, enzymatic

activity, cell surface hydrophobicity, co-aggregation, tolerance to bile salts and acidic condition and finally selected *Bacillus* B-5 for study because of its peculiar characteristics in comparison to the other isolated strains from the Basi Bhat. The results showed that isolated *Bacillus axarquiensis* had amylase, lipase, and protease activity. This isolate was selected for partial purification of amylase. The inoculums was prepared from slant culture by transferring a loop-full of cells in inoculums media 50ml in 250ml fermentation flask and incubating at room temp in a rotary shaker at 120 rpm for 48 h. The fermentation medium was inoculated with 0.1% inoculums (medium 100ml in 250ml flask) and incubated for 72 hrs. On 48 hrs of fermentation, broth was centrifuged at 6000 rpm for 15 min at 4^0C. The partial purification of enzyme was carried out by ammonium sulphate precipitation (40%). Bradford method was used to estimate enzyme protein using bovine serum albumin as standard (Kotiranta *et al*., 2000).

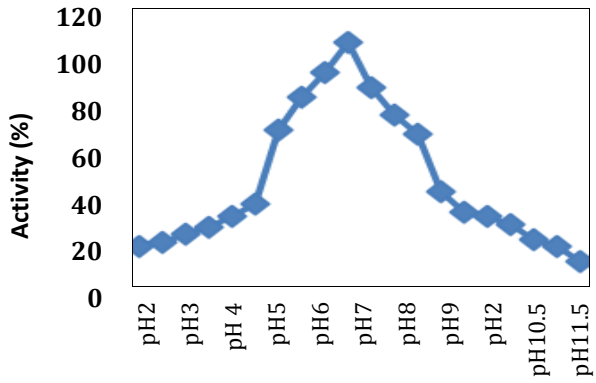

Fig. 5: Effect of pH on Amylase activity.

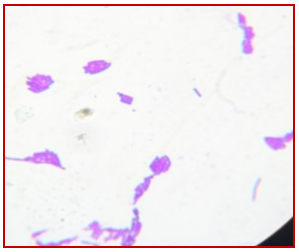

Fig.1: *B. axarquiensis* (autoaggregation)

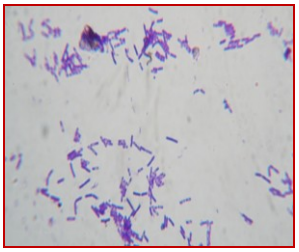

Fig. 2: *B. axarquiensis* (endospore)

Fig.3: Protease activity (+ve)

Fig. 4: Amylase activity (+ve)

Effect of pH and temperature on amylase: Effect of pH was from pH 2.0 to 12.0 (using HCl/KCl buffer for pH 2; glycine/HCl buffer for pH 2.5 to 3.5; acetate buffer for pH 4 to 5.5 phosphate buffer for 6 to 7.5; tris/HCl buffer for pH 8 to 9; glycine/ NaOH buffer for 11 to 12). Effect of temp was examined from 5^0 to 80^0C.

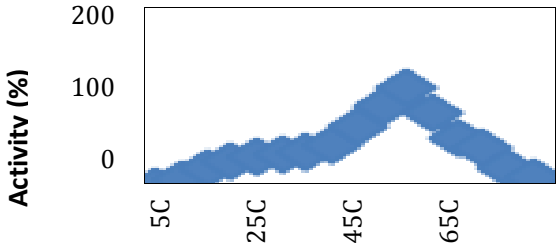

Table 4: Effect of various nitrogen sources on Amylase production

Nitrogen sources	Amylase Activity(µ mole/ min/ml)	Protein (µg/ml)	Specific activity (U/mg)
*$(NH_4)_2SO_4$	0	9	0
*$(NH_4)_2NO_3$	0	3	0
*NH_4Cl	0	8	0
*$(NH_4)H_2PO_4$	0	2	0
*CH_3COONH_4	0	3	0
*L-Glutamic acid	0	2	0
*KNO_3	0.06	0.001	60
*Urea	0.05	0.003	16.66
#Peptone	0.33	0.11	3
#Yeast extract	0.30	0.13	2.72
#Tryptone	0.25	0.35	0.72
#Soybean meal	0.25	0.46	0.543
#Beef extract	0.29	0.38	1.03
#Gelatine	0.09	0.22	0.4

**Simple Nitrogen Source; #Complex Nitrogen Source*

The morphological and physiological characteristics of the *Bacillus axarquiensis* are shown in Table 2. The optimum pH and temperature for its amylase activity was found to be 6.5 and 55^0C (Fig. 5 & 6), respectively. The maltose is found to induce amylase activity to 0.512 U, followed by fructose, ribose, raffinose, sucrose and xylose, but starch, and arabinose have very low inducing effect. In addition to soluble starch the lactose, glucose and dextrin were also found suitable for amylase production.

This selected *Bacillus axarquiensis* gives higher yield of amylase with complex nitrogen sources than with simple nitrogen sources as given in Table 4. Further enzyme purification is required for more characterization.

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