

## Investigation on a Bangladeshi isolate *Bacillus aryabhatai* for promising biotechnological applications

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### Abstract

A new isolate was investigated from soil sample collected from Shahrasti upazilla of Chandpur district of Bangladesh. Based on the physico-chemical studies the strain was identified as gram positive Bacilli. Molecular characterization of the strain was identified as *Bacillus aryabhatai* which is the first report in Bangladesh. The strain can survive in extreme conditions of salt, temperature and pH. This strain was further characterized and screened for the ability to produce useful enzymes. The optimum temperature for growth and production of these enzymes was within the temperature range 35°C to 40°C. The pH was found to be 7 for its growth and production of different enzymes when investigated over 48 h of incubation. The isolate produced various extracellular enzymes such as  $\alpha$ -amylases, cellulases,  $\beta$ -glucosidases, lipases and proteases. The findings of this study provide useful information of the new strain that has potential biotechnological applications.

**Keywords:** *Bacillus aryabhatai*,  $\alpha$ -amylase, protease, lipase, cellulase,  $\beta$ -glucosidase

### INTRODUCTION

Cosmic ancestry is a new theory trying to explain the evolution and the origin of life on earth. It studies the life on earth was seeded from space, and that life's evolution to superior forms depends on genetic foundations that come from space. It is a testable theory for which evidence is increasing. Panspermia hypothesis describes that life on earth is originated from the outer space. The particular version of this theory known as "cometary panspermia", arguing that comets throughout the universe are the incubators and transporters of life, was first anticipated in the 1970's by the Sri Lankan astronomer Chandra Wickramasinghe together with the British astronomer Sir Fred Hoyle (Hoyle & Wickramasinghe, 1977, 1981, 2000; Line 2007, Napier *et al.*, 2007). It is now believed that space contains the ingredients of life, sometimes in chemical form, sometimes in the form of an organism (Arrhenius, 2009; Hoyle & Wickramasinghe, 2000; Wainwright, 2010; Wickramasinghe *et al.*, 2009). This development could be the hints of huge paradigm shift or evidence of panspermia – where – bacteria can survive in harsh environment – away from earth like outer space. The strain *Bacillus aryabhatai* was first reported in the subcontinent by the Indian authors where the sample was collected from high-altitude air samples (altitude 20–41.4 km) (Shivaji *et al.*, 2009). Since then this

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bacterium is used to termed as space organism relating panspermia. Later *B. aryabhatai* was also reported by other authors in this subcontinent where it was collected from the rhizosphere region of *Lemna* sp. in 2012 (Ray *et al.*, 2012).

As a result of random experiment, from a soil sample from Chandpur of Bangladesh, a new bacilli was isolated. From molecular identification and phylogenetic tree analysis it was revealed that the strain was *B. aryabhatai*. We believe that this is the first report in Bangladesh with *B. aryabhatai* that is found to be available in soil – not in space. In this report our study deals with screening of suitable organisms with potential biotechnological applications. Selection of the right organism plays a key role in high yield of desirable biotech products, like enzymes. For production of industrial enzymes, isolation and characterization of new promising strains using cheap carbon and nitrogen sources is a continuous process. This report describes that *B. aryabhatai* is also available in earth soil and it has potential biotechnological applications as we have screened for different industrially important enzyme production by the novel isolate.

## MATERIALS AND METHODS

### Isolation of bacteria from soil sample

**Sample Collection:** The soil samples were collected from Shahrasti upazilla of Chandpur district. Soil, sediment and ground water in the area have been contaminated with arsenic for many years. Soil samples were collected aseptically from the sub-surface (from 0 to 15 cm in depth from ground) in sterile sample bottles and kept in 4°C until further analysis. For isolation of bacteria firstly 2 g soil sample were mixed with 50 ml autoclaved distilled water.

**Strain isolation:** One gram of each soil sample was taken in 100 ml of 0.1 M phosphate buffer solution and mixed well by vortexing for 3 minutes. Each Sample was serially diluted with sterile saline water (0.85%) and plated on Yeast Extract Mannitol (YEM) agar medium {D-Mannitol (Qualikems, India), 10 g; K<sub>2</sub>HPO<sub>4</sub> (Scharlau, Spain), 0.5 g; MgSO<sub>4</sub> · 7H<sub>2</sub>O (MERCK, India), 0.2 g; CaCl<sub>2</sub> (Scharlau, Spain), 0.1 g; yeast extract (TM MEDIA, India), 0.5 g; phosphate buffer solution, up to 1000 ml, pH 7.0. The plates were then incubated at 37°C for 48 hours. Pure cultures of different bacterial strains were obtained by successive isolation of morphologically different colonies through repeated cross streaking (Bergey *et al.* 1984).

**Identification of bacterial isolates:** After single colony isolation, the morphological, biochemical and molecular characteristics of the isolated bacterial strains were evaluated. Color, gram stain and cell morphology were investigated under microscope (Optika B-350) (1000X magnification). Biochemical properties of the selected bacterium were tested according to Bergey's Manual of Systemic Bacteriology (Bergey, *et al.*, 1984). The following biochemical tests were performed: Methyl Red (MR) test, Voges-Proskaur (VP) test, catalase test, glucose, fructose, sucrose, maltose, mannitol fermentation test, indole, citrate, gelatin and nitrate reduction test (Cappucino, *et al.*, 2001). Molecular characterization was done by 16S rRNA sequence analysis. Genomic DNA was extracted

by automated DNA extractor (Model: Maxwell 16, Origin: Promega, USA) and qualified by Nanodrop Spectrophotometer (Model: ND2000, Origin: Thermo Scientific, USA). The 16S rDNA from the extracted DNA was amplified by PCR using 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-ACGGCTACCTTGTTACGACTT-3') primers. The reaction mixture composed of 12.5 µl Hot Start Master Mix (Origin: Promega, USA), 1 µl extracted genomic DNA, 1 µl 37F primer (concentration 10 pMol), 1 µl 1492R primer (concentration 10 pMol), 9.5 µl nuclease free water. PCR was done by Gene Atlas (Model: G2, Origin: Astec, Japan). PCR profile was as follows: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation (95°C, 30 seconds), annealing (48°C, 30 seconds), extension (72°C, 1 minute) and final extension at 72°C for 5 minutes. To confirm amplification gel electrophoresis was done using agarose, 100 bp DNA ladder, ethidium bromide, TAE buffer, all from Promega, USA. Gel documentation was done by Alpha Imager (Model: Mini, Origin: Protein Sample, USA). Sequencing was performed by ABI 3700 Genetic Analyzer, 1st Base Laboratory SdnBhd, Malaysia. The 16S rDNA gene sequence was BLAST searched against GenBank database (<http://www.ncbi.nlm.nih.gov/>) and phylogenetic analysis was performed using Mega6-tree explorer after multiple alignment by BioEdit software.

**Growth at different temperatures:** In order to study the effect of temperature on bacterial growth, cultivation temperature of the selected isolate was adjusted to 30°C, 35°C, 37°C, 40°C and 47°C. After 16 hour of incubation the absorbance was measured at 660 nm.

**Growth at different pH levels:** In order to determine the appropriate pH on bacterial growth, the pH of the medium was adjusted to different pH level (pH 4 to 10). After 16 hour of incubation the absorbance was measured at 660 nm.

**Growth at different salinities:** To determine the appropriate salt concentration for the growth of *B. aryabhatai*, LB medium with different concentration of salt (1% to 7%) having pH 7 were prepared. After 16 hour of incubation the absorbance was measured at 660 nm.

**Amylolytic activity assay:** Bacterial colonies first grown on 1% starch plate {beef extract 3 g/L, starch 10 g/L, agar 12 g/L pH 7.5±0.2 (at 25°C)}. Amylase activity can be detected by flooding the plates of 48 hours old culture on starch agar with iodine complex. Amylase positive organisms show clear zone around colony while development of blue to purple zone indicates starch is not hydrolyzed. Size of the clear zone is directly proportional to the starch hydrolyzing activity of the strain (Islam *et al.*, 2017).

**Proteolytic activity assay:** Skim milk agar plates (SMA) are used for providing nutrition and for the detection of proteolytic activity of microorganisms. SMA is composed of skim milk powder (28 g/L), casein enzymichydrolysate (5 g/L), Yeast extract (2.5 g/L), dextrose (1 g/L), agar 15 (g /L) with final pH 7. The plates are inoculated with a population of microorganisms to test for the ability to digest casein hydrolysis (Rahman *et al.*, 2018).

**Cellulytic activity assay:** For primary screening of cellulytic activity, purified strains were inoculated in 5 ml of number 3 medium. After 24 h incubation at 37°C in water bath (VS-1205SW1, Korea), 5 µl of culture was transferred in CMC agar plates (CMC 1%, peptone 3%, KH<sub>2</sub>PO<sub>4</sub> 0.1%, MgSO<sub>4</sub> 0.01% and agar 2% with pH 6.8). CMC plates were incubated at 37°C for 24 to 48 hour. After incubation formation the plates were flooded with gram's iodine solution and washed with 70% alcohol.

**Lipolytic activity assay:** Tributyrin agar (TBA) is a differential agar medium (peptone, 5 g; beef extract, 3 g; tributyrin, 10 ml and agar-agar, 20 g per litre ) that tests the ability of a strain to produce an exoenzyme, called lipase and hydrolyzes tributyrin oil. At first the strain was inoculated in TBA plates and grown at 37°C for 24 to 48 hour. Lipase allows the microorganisms that produce it to break down lipids into smaller fragments and forms a clear halo surrounding the areas where the lipase-producing organism has grown.

**Secondary Screening for extracellular enzymes:** For secondary screening, the isolate was cultured on freshly prepared different mediums according to enzymes to be screened. After 24 hours of culture, submerged fermentation conical flasks were taken off from the incubator and shaking water bath, homogenize fermented culture using homogenizer (IKA®T10, Germany) for 15 seconds. Then the homogenized cultures were centrifuged at 10000 × g for 20 minutes using bench top centrifuge machine (Eppendorf 5418, Germany) and supernatants were collected. These supernatants were used as crude enzymes for measuring enzyme assays.

**Amylase assay:** A colorimetric method was used to measure the α-amylase activity with 3,5-dinitrosalicylic acid (DNS) reagent. After 16 h culture in number 3 medium (polypeptone 30 g, starch 10g, KH<sub>2</sub>PO<sub>4</sub> 1 g and MgSO<sub>4</sub> 0.5 g per litre) the culture was centrifuged and supernatant was used for assay. In this method, starch is converted into glucose by α-amylase. Glucose released from starch was measured by the reduction of 3,5-dinitrosalicylic acid. DNS binds with glucose and changes color. This intensity change in color was measured using a spectrophotometer as the absorbance at 575 nm wavelength. Wave length was set to 575 nm because it is the region where orange-red color absorbs. 1 mg per ml of enzyme carries 30 unit of activity. Sodium acetate was used to soluble the control enzyme. 500 µl starch (1%) is mixed with 500 µl of enzyme in a test tube and used as control. 1 ml of only starch is taken in another test tube. Both tubes were incubated for 30 min at 37°C on water bath shaker. 500 µl of each solution was taken and mixed with 500 µl of DNS in two tubes. Tubes were kept for 10 min at 90°C in water bath. Immediately 250 µl of 40% Rochelle's salt was added in both tubes and cooled down to room temperature. Absorbance was measured at 575 nm.

**Protease assay:** To determine the activity of protease, azocasein protease assay was done. 200 µl of overnight selected bacterial culture aliquot was transferred to 100 mL conical flask containing 20 ml No. 3 medium [Peptone- 3% (Uni-chem, china), D-Glucose- 1% (Merck, Germany), KH<sub>2</sub>PO<sub>4</sub>- 0.1% (Scharlau, Spain), MgSO<sub>4</sub>.7H<sub>2</sub>O- 0.05% (Merck, India), pH- 6.8]. Then the conical flasks was kept in shaking water bath (Model: VS-1205SW1, Origin: Vision Scientific Co., Ltd, Korea) at 37°C, 120 spm for submerged

fermentation. After 24 hours, the conical flask was taken off from the shaking water bath, homogenized the culture using homogenizer (IKA®T10 basic, Germany) for 15 seconds. Then the homogenized culture was centrifuged at 10000xg for 20 minutes using bench top centrifuge machine (Eppendorf 5418, Germany) and supernatant were collected. This supernatant was used as crude enzymes for measuring protease activity.

To measure protease activity of the crude enzymes, 100 µl of 1% azocasein [0.1 g azocasein (Sigma, Germany) dissolved in 10 ml phosphate buffer solution] was added to 200 µl of crude enzyme, mixed well by vortexing and incubated at 37°C for 30 minutes. Then 700 µl trichloroacetic acid (TCA) (BDH, London) was added and kept on ice for 20 minutes. After centrifugation at 15000 x g for 5 minutes, 600 µl supernatant was added to 600 µl 0.5 M sodium hydroxide (Merck, Germany) and measured optical density (OD) at 440 nm using spectrophotometer (Mecasys Optizen Pop UV/Vis Spectrophotometer, Korea). Blanks were prepared by following the same procedure except crude enzymes were added after adding TCA. From the OD<sub>440</sub>, enzyme activity (units/ml) was measured. One unit is defined as change in absorbance at 440 nm of 0.01/hours (Kohlmann *et al.*, 1991).

**Cellulase assay:** By using a method described by Miller (1959) with some modifications (Wood & Bhat 1988), the carboxy methyl cellulase activity was assayed. In this assay, 500 µl crude enzyme was mixed with 500 µl of substrate (1% CMC) in a test tube. In the negative control, 500 µl substrate was mixed with 500 µl citrate buffer and in positive control 500 µl standard cellulase enzyme was mixed with 500 µl of substrate. The tubes were incubated at 60°C for 30 min in incubator. The reaction was stopped by the addition of 2 ml of DNS reagent and subsequently incubating the tubes at 90°C for 15 min. After incubation 1ml of 40% Rochelle salt was added and kept in ice to stabilize the color for 2 min. The absorbance was measured at 575 nm.

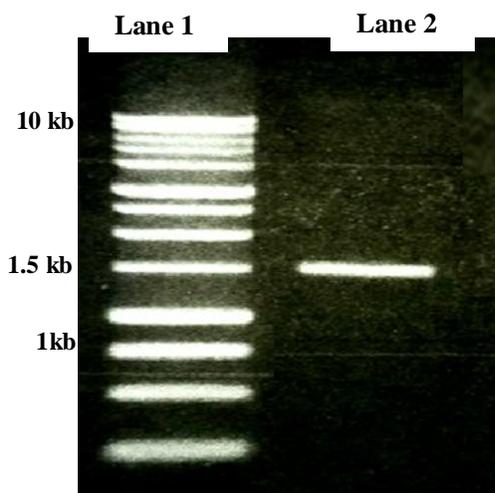
**β-glucosidase activity:** To assay β-glucosidase activity p-nitrophenyl-β-D glucopyranoside (pNPG) was used as substrate. 375 µl crude enzyme was mixed with 50 µl of pNPG and 75 µl of sodium acetate buffer (2 M, pH 5.0) and incubated at 50°C for 10 min in hot water bath. To terminate the reaction 1750 µl of 1 M Na<sub>2</sub>CO<sub>3</sub> was added. The developed yellow color was read at 410 nm against negative control using a spectrophotometer (Neesa *et al.*, 2017).

**Lipase assay:** With modified methods of Lee *et al.* (1999), the lipase activity was determined spectrophotometrically. To prepare the reaction solution 100 mM potassium phosphate buffer (pH 7.0), ethanol and 50 µM para- nitrohenyl palmitate (*p*-NPP) at a concentration of 95:4:1 was mixed. To carry enzymatic reaction the test tubes were inoculated at 60°C for 15 min and the reaction stopped by storing the solution at -20°C for 8 min in order to cool down. The release of *p*NP (para-nitrophenol) at 400 nm was measured, and one unit of enzyme was defined as the amount of enzyme needed for the hydrolysis of 1 µM *p*-NPP per minute at 60 °C, pH 7.0 (Koc *et al.*, 2015).

## RESULTS AND DISCUSSION

**DNA quantification:** DNA quantification was done to determine the amount of DNA in the sample. From novel bacterial isolates genomic DNA was extracted and quantified by Nanodrop Spectrophotometer and PCR was done by Takara PCR thermal cycler. The concentration of DNA (ng/ $\mu$ l) was 2.7 and the 260/280 ratio was 2.42 which means that DNA sample was not pure and RNA contamination was present. The 260/230 ratio was 0.63 which indicated possible other contaminants absorbing light 230 nm or less.

**Agarose gel analysis of genomic DNA:** Isolated DNA of the bacteria obtained from soil sample was loaded, PCR product in Lane 2 and 1kb DNA Ladder was loaded on Lane 1. To visualize the band size agarose gel analysis was performed. It was observed that the PCR product of genomic DNA of isolate fall within the size range approximately 1500 bp when compared to 1kb DNA Ladder (Marker DNA) (Figure 1).



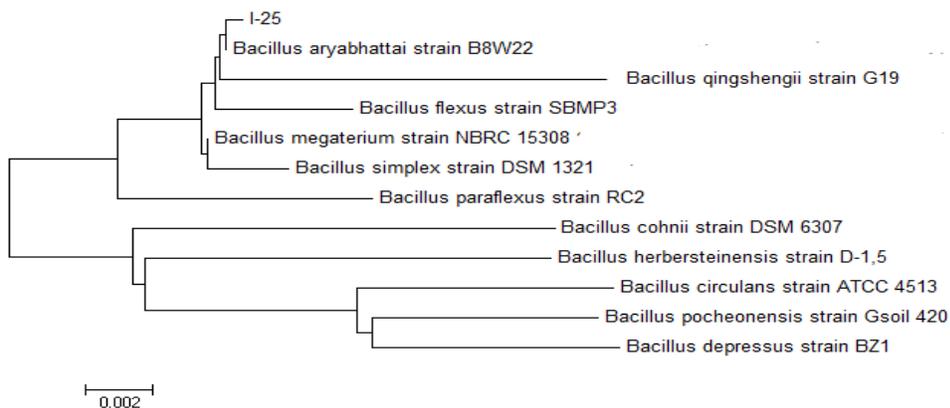
**Fig. 1. Agarose gel analysis of genomic DNA and PCR product of genomic DNA of arsenic metabolizing bacteria with Marker DNA (1kb DNA Ladder)**

**16S rRNA gene sequences analysis:** 16S rRNA gene was used for the identification of the bacterial isolate. This gene is highly conserved and encodes a component of the small subunit in the ribosome. Sequences were compared to other sequences from the genetic nucleotide library by using BLAST. The 16S rRNA gene sequences size was modified for the sample by eliminating undefined nucleotides at the beginning of the sequences because the peaks on those sections were not well defined. The nucleotide sequences coding for the 16S rRNA gene after BLAST query revealed that this gene is 99.93 % homologous to *B. aryabhatai* (Table 1).

**Table 1. BLAST analysis results for *B. aryabhatai* strain**

Bacterial strains	Total Score	Identity (Nucleotide nos)	Identity (%)	Accession Number
<i>Bacillus aryabhatai</i> strain B8W22	2621	1421/1422	99.93%	NR 115953.1
<i>Bacillus megaterium</i> strain NBRC 15308	2610	1419/1422	99.78%	NR 112636.1
<i>Bacillus flexus</i> strain SBMP3	2531	1406/1423	98.81%	NR 118382.1
<i>Bacillus qingshengii</i> strain G19	2484	1398/1424	98.17%	NR 133978.1
<i>Bacillus simplex</i> strain DSM 1321	2444	1362/1382	98.56%	NR 115603.1
<i>Bacillus paraflexus</i> strain RC2	2364	1358/1393	97.48%	NR 135732.1
<i>Bacillus pocheonensis</i> strain Gsoil 420	2326	1373/1427	96.21%	NR 041377.1
<i>Bacillus bataviensis</i> strain NBRC 102449	2326	1370/1427	96.01%	NR 109068.1
<i>Bacillus cohnii</i> strain DSM 6307	2307	1367/1425	95.93%	NR 026138.1
<i>Bacillus depressus</i> strain DZ1	2303	1368/1426	95.93%	NR 146034.1
<i>Bacillus circulans</i> strain ATCC 4513	2303	1368/1426	95.93%	NR 104566.1
<i>Bacillus herbersteinensis</i> strain D-1,5	2300	1369/1430	95.73%	NR 042286.1

**Phylogenetic analysis:** The 16S rRNA sequences of the novel strain subjected to nucleotide BLAST query. Based on the sequence alignment, a phylogeny tree was found. Evolutionary analysis was conducted in MEGA6. The evolutionary history was inferred using the Neighbor-Joining method. The key, 0.002 indicates nucleotides per site that gives a measure of the scale of the genetic distance between each species (Table 2). Because of the fact that genetic distance is proportional approximately to time it can be said that the novel strain and *B. aryabhatai* are closely related and comes from a common ancestral species than other members of the node.



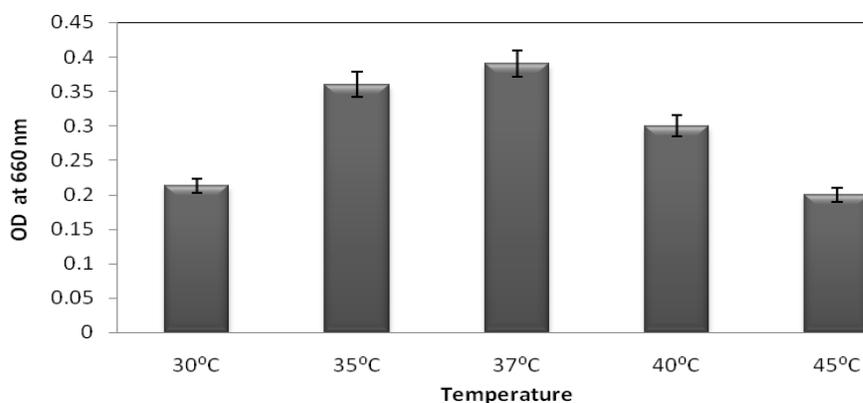
**Fig. 2. Phylogenetic tree based on 16S rRNA gene sequences. The relationship between the novel strain with other related bacteria showed using phylogenetic tree based on 16S rRNA gene sequences**

**Table 2.** Physicochemical characteristics of *B.aryabhatai*

Tests	<i>B.aryabhatai</i>
Gram staining	+Ve
Shape	Rod Shaped
Methyl Red (MR) Test	+Ve
Voges-Proskaur (VP) Test	+Ve
Catalase Test	+Ve
Urease Test	-Ve
Oxidase Test	-Ve
Citrate Test	-Ve
Gelatin Test	+Ve
Nitrate Reduction Test	+Ve

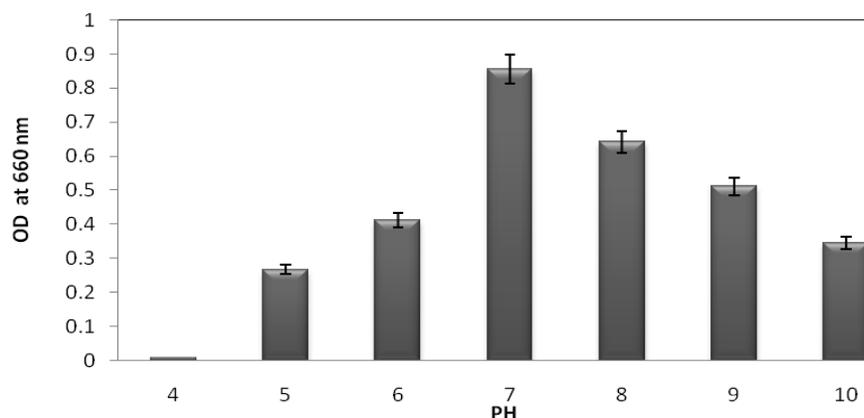
Note: +ve = positive result    -ve = negative result

**Growth at different temperature and pH:** The isolate grew at various temperatures ranging from 30°C to 45°C. From this observation it was found that this strain was similarly grown better at 37°C in comparison to other incubation temperatures (Figure 3). For optimum pH determination, the cultures were incubated overnight following inoculation at 37°C. It was observed that the can frequently grow above the neutral pH as it was conducted up to pH10 (Figure 4). The optimum pH for growth was found to be at neutral and gradually decreased. The strain was unable to grow at pH 4 as the growth profile indicates the likeliness of the strain in neutral to alkaline condition.

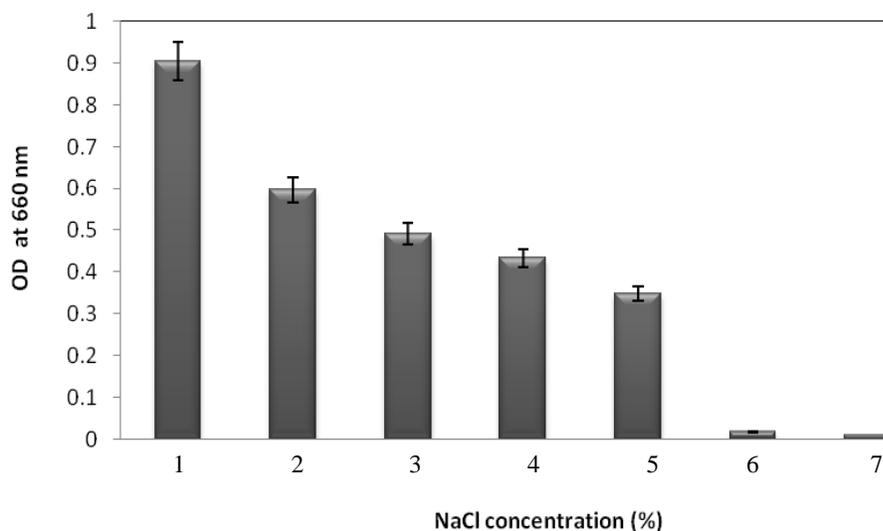


**Fig. 3.** Growth of *B. aryabhatai* at different temperature. Number of independent experiments (n ≥3)

**Growth at different salinities:** The new strain was able to grow in a range of salty condition. When it was cultured at different salinity (NaCl) levels, the isolate grew well within salinity range of 1%-5% (Figure 5). Further increase of salt concentration sharply reduced the growth of the cell when culture was investigated after overnight incubation at 37°C. As the new *B. aryabhatai* showed growth in different ranges of temperature, salt concentration and alkaline conditions. These characteristics indicates *B. aryabhatai* in category of haloalkaliphiles.



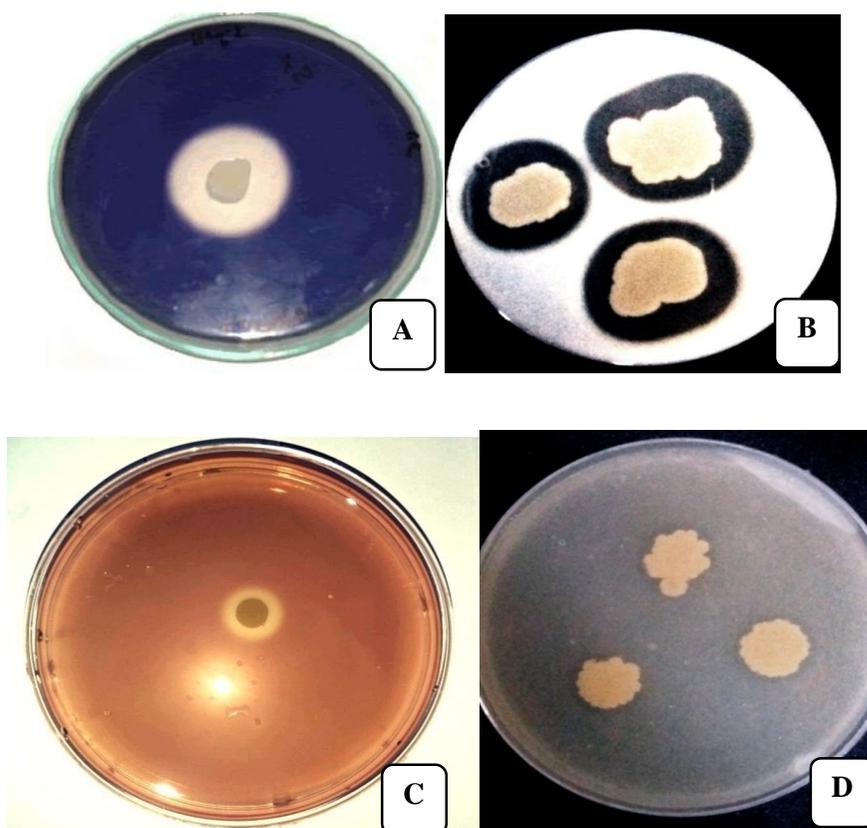
**Fig. 4. Growth of bacteria *B. aryabhatai* at different p<sup>H</sup> level. Number of independent experiments (n≥3)**



**Fig. 5. Growth of *B. aryabhatai* at different NaCl concentration. Number of independent experiments (n≥3)**

**Enzymatic activity screening:** Bacterial colonies were grown on 1% starch agar plate. After 24 hour of incubation the plates were flooded with Gram’s iodine (Gram’s iodine-250 mg iodine crystals added to 2.5 gm potassium iodide solution) to produce a deep blue colored starch -iodine complex. In the zone of degradation no blue color formed, which is the basis of the detection and screening of an amylolytic strain (Figure 6 A). In the assay for α-amylase, glucose released from starch was measured by the reduction of 3, 5-dinitrosalicylic acid (Miller, 1959). This intensity change in color is measured using a

spectrophotometer as the absorbance at 575 nm wavelength. When compared the standard  $\alpha$ -amylase enzyme it was observed that the crude sample is resulted with 0.298 units of absorbance compared to 0.471 (Figure 7 A). As a first screening data, this information was quite impressive.



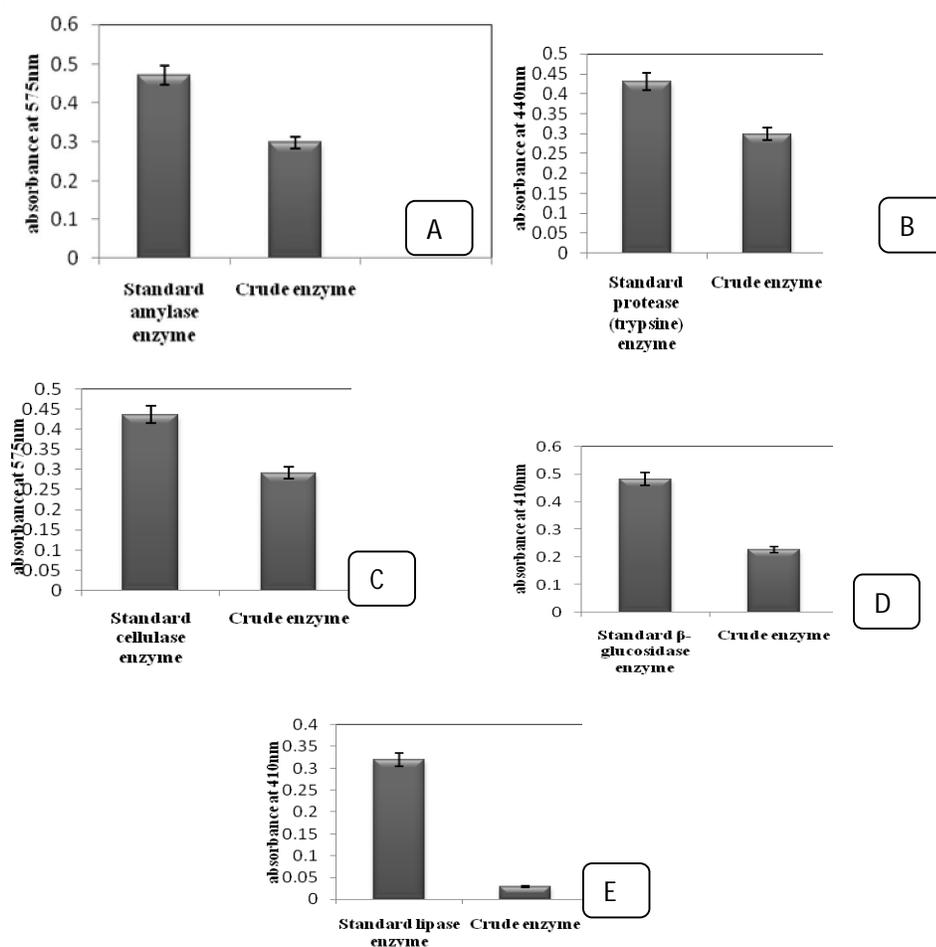
**Fig. 6. Primary Screening of enzymes (A. Amylase on starch plate, B. Protease on SMA plate, C. Cellulase on CMC plate and D. Lipase on TBA plate)**

In regular screening tests for proteolytic bacteria in agar plates hydrolyzed casein and formed soluble nitrogenous compounds indicates clear zone surrounding the colonies. In our investigation, a clear zone of casein hydrolysis indicates the strong proteolytic activity of the isolate (Figure 6 B). Azo-casein assay for proteolytic enzyme activity resulted with about eighty percent activity with that of the standard enzyme used (Figure 7 B). The production of protease by this strain show significant promise in biotechnological applications.

The new isolate was tested on CMC agar plate for cellulase activity and formed clear zones after application of Gram's iodine solution indicated cellulose digestion by the production of cellulase enzyme in the corresponding bacterial strain. CMCCase (using 1%

CMC as a substrate) assay resulted with absorbance of 0.292 while the positive control resulted with 0.437. This result is interesting as well because this will facilitate us to use this organism for increasing demand cellulolytic enzyme production. The  $\beta$ -glucosidase productivity of the strain was further investigated, and noticeably the strain resulted with the absorbance of *p*-nitrophenol of 0.227 compared to the control enzyme that resulted with 0.483 units of absorbance (Figure 7 D).

In the TBA plate assay, lipase/esterase producing microorganisms produced a zone of clearance (hydrolysis) when their appropriate dilutions were spreaded on the TBA plates (Figure 6 D). The novel strain resulted with slight activity as it was seen the agar plate that was also verified with the spectrophotometric assay (Figure 7 E).



**Fig. 7.** Comparison of the activity of the standard enzymes and crude enzymes produced by *B.aryabhatai* (A.  $\alpha$ -Amylase, B. Protease, C. Cellulase, D.  $\beta$ -glucosidase and E. Lipase) (n $\geq$ 3)

*Bacillus* is well known for its production ability of different industrially important enzymes like amylase (Souza & Magalhaes 2010, Das *et al.*, 2011); proteases (Gupta *et al.*, 2002); cellulases (Li & Yu, 2012); lipase (Horikoshi, 1999) etc. But in these cases different strains were reported for their production of respective enzymes. In our study this new strain of *B. aryabhatai* was primarily screened for four most industrially useful enzymes and showed excellent result with amylase, protease and cellulase but poor in lipase.

The bacterium isolated in this study was identified as *B. aryabhatai* from its phylogenetic analysis of 16S rRNA sequence. This novel strain was primarily screened for four useful enzymes. It showed excellent production results for amylase, protease and cellulase but poor for lipase. The isolate was grown in different ranges of temperature, pH and salt concentrations. The optimum growth was observed at 37°C, at pH 7 and in 1% salt concentration. The ability of *B. aryabhatai* to survive and proliferate in harsh conditions is an important factor in their success as inoculants for industrial production of biotechnological products. Since this is a new strain in Bangladesh, more advance research for a deeper understanding about this bacterial strain is needed to improve their production for biotechnological applications.

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