Characterization of Moderately Thermostable α-Amylase-producing *Bacillus licheniformis* from Decaying Potatoes and Sweet Potatoes

Muhammad Adnan Ashraf,[a,*] Muhammad Imran Arshad,[a] Sajjad-ur-Rahman,[a] and Ahrar Khan [b]

Bacillus licheniformis is an endospore-forming bacterium that is commonly present in soil. The aim of the present study was to isolate and characterize local strains of α-amylase producing *B. licheniformis*. Soil samples were collected from the decaying surfaces of potatoes and sweet potatoes. The samples were identified by Gram staining, spore staining, and motility testing under aerobic conditions. Twenty-three isolates were found to be from the *Bacillus* genus and six of those (26%) were found to be *B. licheniformis*. Two representative samples were run on API 20E and API 50 CH biochemical kits, and 16S rDNA polymerase chain reaction. The isolates were confirmed to be *B. licheniformis* by the API-web program and molecular detection. Partially purified α-amylase was characterized to determine the effect of the incubation period, temperature tolerance, and pH stability. The activity peaked at 740 mU/mL after 42 h of culturing. The relative activity reached a maximum at 55 °C and a pH of 8.0. The decaying surfaces of potatoes and sweet potatoes are promising sources of α-amylase-producing strains of *B. licheniformis* that can tolerate both a high temperature and drastic pH level.

Keywords: Bacillus licheniformis; Microbial amylase; Physico-chemical stability

*Contact information: a: Institute of Microbiology, University of Agriculture, Faisalabad, Pakistan; b: Department of Pathology, Dean, Faculty of Veterinary Science, University of Agriculture Faisalabad, Pakistan; *Corresponding author: a_a@dr.com*

INTRODUCTION

Bacillus sp. are rod-shaped, spore-forming, and Gram-positive bacteria. They are aerobic or facultative anaerobic, and most species are motile in nature. *Bacillus* sp. are commonly present in soil microbiota. These species can withstand harsh conditions, including limited nutrients, because of their endospore forming ability (Tychinskii *et al.* 2007; Soni *et al.* 2016). The spores are very tolerant to heat, cold, radiation, and alkalinity. This ability of the *Bacillus* genus has an increased importance with regards to the enzymes, antibiotics, and metabolites it produces. *Bacillus* sp. ranging from mesophiles to thermophiles can easily adapt to extreme environmental conditions (Schallmey *et al.* 2004; Suganthi *et al.* 2015). *Bacillus* sp. can produce 20 g/L to 25 g/L of extracellular enzymes, which is much higher than that of other microorganisms. The enzyme activity can be improved by optimizing the physical conditions, such as the pH and temperature.

Amylases are important enzymes used in various industries. The global market of amylases has increased each year and is estimated to be 320.1 million USD in 2024. Amylases can be obtained from a number of sources, including plants, animals, and microbes. Microbes are better than other amylase sources because of their vast availability. Microorganisms can produce amylases in bulk economically, and their production process can be easily optimized. Enzymes are excreted extracellularly and can be easily purified

and processed from their microbial origin. *Saccharomyces*, *Aspergillus*, and *Bacillus* sp. have been commonly used to produce enzymes (Sundarram and Murthy 2014). As an extracellular enzyme, α-amylase (EC 3.2.1.1) acts on starch by hydrolyzing α-1,4-glycosidic linkages and degrades it into disaccharides and trisaccharides (Takata *et al.* 2010). The derivatives produced by α-amylases have a variety of applications in the paper, textile, detergent, baking, brewing, and sugar industries (de Souza and de Oliveira Magalhães 2010). In the baking industry, a sufficient quantity of amylase enzyme is required to convert starch into sugars.

Like other *Bacillus* sp., *Bacillus licheniformis* is commonly found in soil. Its optimal growth temperature is 50 °C. It can grow at even higher temperatures, which is helpful for enzymes used in substrate processing at higher temperatures and results in faster reaction rates. *Bacillus licheniformis* produces a variety of extracellular enzymes, including amylases, cellulases, laccases, and proteases (de Boer *et al.* 1994; Kostyleva *et al.* 2016). Because the bacterium can grow at a higher temperature, the enzymes it produces are naturally thermostable. It has the ability to produce α-amylase in large quantities as well (Kubrak *et al.* 2010). This particular characteristic has increased the types of commercial applications of *B. licheniformis* (Singh *et al.* 2001).

Amylases with good hydrolyzing properties can be isolated by an objective approach from bacterial species that can grow on starch-containing kitchen wastes. Potatoes, sweet potatoes, and their peels are used daily in kitchens. Being a natural source of starch, their decaying surfaces have the ability to grow high amounts of α-amylase-producing bacteria (Betiku *et al.* 2013). In this study, natural starch sources, including potatoes and sweet potatoes, were exploited by soil bacilli to get α-amylase-producing isolates of *B. licheniformis*. Bacterial strains with temperature- and salt-tolerant properties were isolated for α-amylase production.

EXPERIMENTAL

Sample Collection

Potatoes and sweet potatoes were cut into slices and buried 1 ft (31 cm) in an agricultural field soil for two weeks. The samples were collected from the decaying surfaces of these slices after two weeks. All of the samples were placed in air-tight polythene bags, labeled accordingly, and stored at room temperature. The study was performed at the General Post-graduate laboratory, BSL-Lab1 and BSL-Lab13, at the Institute of Microbiology, University of Agriculture Faisalabad, Pakistan.

Materials and Chemicals

Nutrient agar (Oxoid, Hampshire, UK), NaCl (Merck, Billerica, USA), biological agar (Bioworld, Dublin, OH, USA), phosphate-buffered saline (MP Biomedicals, St. Ana, CA, USA), soluble starch (UNI-Chem, North Carolina, USA), API 50 CH strips and CH B/E medium (bioMérieux, Marcy-l'Étoile, France), ethyl alcohol (Riedel-de Haen, Seelze, Germany), EDTA (Remel, Lenexa, KS, USA), sodium dodecyl sulphate (Sigma-Aldrich, St. Louis, USA), isoamyl alcohol, isopropanol, and sodium acetate (Fischer Scientific, Nepean, Canada) were used in this study. All of the chemicals were of analytical grade purity and commercially available. The media were adjusted to a pH of 7.0 with 4% NaOH and 4% HCl, and then autoclaved at 121 °C for 20 min. Incubation was done at 37 °C for 24 h unless stated otherwise. All of the other classic culture techniques were used as standard practices for all of the procedures.

Sample Processing and Incubation

One gram of the sample was placed in a test tube and dissolved in distilled water with a total volume of 10 mL. The sample suspensions were labeled accordingly and heated in a water bath at 80 °C for 10 min, as described by Naidu and Devi (2005). Next, 500 µL of the suspension were added to 4.5 mL of the nutrient broth. Sterile conditions were maintained during these procedures. The suspensions were incubated at 37 °C for 4 h and then heated at 80 °C for 3 min. The glass spreader was sterilized by burning it in ethanol. Two hundred microliters of each sample were placed on nutrient agar and the petri plates were labeled accordingly, which were then incubated at 37 °C for 24 h.

Morphological Identification

After incubation for 24 h, the culture characteristics were determined on the basis of the size, shape, color, and texture of the colonies. The desired colonies were streaked on nutrient agar media slants before Gram staining and spore staining. Replicas of each colony were prepared on antibiotic-added media. The Gram staining and spore staining were done according to the modified method described by Claus (1992). The cell morphology was observed under a magnification of 100x with the help of cedar wood oil *via* a light microscope (Nikon, Tokyo, Japan). The motility test was performed *via* the cavity slide method. The samples with a single type of bacilli and that were positive for Gram staining and spore staining were further streaked on antibiotic-added nutrient agar. Quadrant streaking was done to get well-isolated colonies of the samples.

Physiological and Biochemical Identification

The isolates were biochemically characterized by catalase, starch hydrolysis, casein hydrolysis, Voges-Proskauer (VP), and citrate utilization tests. These conventional tests were performed as described in Bergey and Holt (1994). The isolates were further tested by salt tolerance, high-temperature incubation, and growth in anaerobic incubation.

Screening for α-Amylase Enzymes

To determine whether or not the isolates produced α-amylase, they were subjected to the starch hydrolysis test. These bacteria were line inoculated on a starch agar medium with 1% (w/v) soluble starch and incubated at 37 °C for 18 h to 24 h. Later the plates were flooded with 1% iodine in 2% KI. Formation of clear zones around the streaking was regarded as positive for the α-amylase test (Healing 1995). This method determines the dextrinizing activity of α-amylase in terms of the decrease in the iodine color reaction (Saxena *et al.* 2007).

Salt Tolerance in an Anaerobic Environment

Nutrient agars were prepared with three concentrations of NaCl (4.5%, 6.5%, and 8.5%) and isolated colonies were streaked on the prepared media. The cultures were placed in anaerobic jars, and a candle was lit to consume the oxygen present. The anaerobic jars were incubated at 37 °C for 24 h. The presence of growth and the colony morphology were observed after 24 h.

Temperature Tolerance in an Anaerobic Environment

Two sets of nutrient agar plates were prepared for each isolate. The isolated colonies were streaked on the nutrient agar and placed in anaerobic jars. Each isolate was incubated at 37 °C, 55 °C, and 60 °C in an anaerobic environment. The colony morphology was observed after 24 h.

Characterization *via* API Kits

Further identification of the presumptive *B. licheniformis* isolates was done *via* the analytical profile index (API) using API 50 CH, API 20E strips, and CH B/E medium, (bioMérieux). This system is a miniaturized identification method that employs substrates for fifty biochemical tests. The sterile saline solution was inoculated and mixed thoroughly with the presumptive *B. licheniformis* isolates. Ampoules with 10 mL and 5 mL of API CHB medium and API 20E, respectively, were inoculated with prepared saline suspensions. Substrates present in the tubules and cupules of the API strips were rehydrated and filled with bacterial cultures. The cupules of the API strips were covered with a mineral oil in the prescribed tubules according to the instructions given by the manufacturer. The strips were incubated at 37 °C for 48 h. Positive and negative tests were read after 48 h.

Molecular Analysis by PCR

Overnight bacterial growth was obtained in nutrient broth, and the genomic DNA was extracted *via* the conventional phenol-chloroform method described by Green and Sambrook (2012). The extracted DNA samples were then processed further as templates for targeting different genes of polymerase chain reaction (PCR). Primers (Table 1) were reconstituted in a T buffer. A master mix of the reaction mixture (25 µL for each sample) was prepared. Amplification was performed in a thermal cycler (Bio-Rad, Hercules, CA, USA) for 30 cycles with denaturation at 94 °C for 3 min, annealing at 56 °C for 1 min, and extension at 72 °C for 2 min using bacterial universal primers.

Table 1. Forward and Reverse Primers

Primer	Sequence
27F	5'- AGAGTTTGATCCTGGCTCAG-3'
1492R	5'-TACGGTTACCTTGTTACGACTT-3'

Electrophoresis of the PCR Products

The amplified product was electrophoresed on 1.5% agarose gel. Next, 7 µL of the PCR product were mixed with 3 µL of the tracking dye and loaded in the well properly. Then, 5 µL of a 100 bp of DNA ladder (Invitrogen, Carlsbad, CA, USA) were also loaded into a well to detect the approximate size of the molecule run on the gel during electrophoresis. The voltage was kept at 120 V for 10 min, which was followed by 100 V for 50 min. The gel was visualized by an ultraviolet illumination system and the image was captured with a gel documentation system (Wealtec, Sparks, NV, USA).

Production Medium and DNS Assay

The bacterial isolates were cultured in a broth culture (1000 mL, pH = 8) containing 10 g of starch, 10 g of peptone, 10 g of yeast extract, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of K_2HPO_4, and 0.01 g of $CaCl_2$. The broth culture was centrifuged at 10000 rpm for 10 min at 4 °C. Cell-free supernatant was used as a crude enzyme for the enzyme assay. The enzyme activity was determined by the amount of maltose released. Then, 500 µL of 1% (w/v) soluble starch solution in 20 mM sodium phosphate buffer and 500 µL of enzyme extract were incubated at 45 °C for 10 min and the reaction was stopped by 2 mL of 96 mM 3,5 dinitrosalicylic acid (DNS). The tubes containing these reactions were put in a 100 °C water bath for 10 min. The optical density was measured at 540 nm after the tubes were cooled down (Bernfeld 1955). One unit of enzyme activity was defined as the amount of enzyme that released 1 µmol of the maltose at 45 °C in 1 min in the phosphate buffer.

Characterization of the α-Amylase

The isolate with a better α-amylase activity was chosen for further characterization. Isolate 4 was cultured for 6 h, 12 h, 18 h, 24 h, 30 h, 42 h, 48 h, and 60 h at 45 °C to determine its effect on the α-amylase activity. The partially purified enzyme extract was incubated at different temperatures (35 °C to 85 °C) for 10 min to assess the thermal stability. The activity of the crude enzyme was measured at different pH levels (4 to 10) to determine the effect of the pH on the stability. The enzyme activity was assayed by the above-mentioned method.

RESULTS AND DISCUSSION

Morphological Identification of the *Bacillus* Isolates

Well-isolated colonies were obtained after culturing the heat-treated soil suspensions on nutrient agar. The size, shape, margin, and elevation of the colonies were observed under a microscope at 4x magnification. The colonies were yellow in color and variable in size, shape, and margin. The shape varied from round to irregular, the margin varied from entire and smooth to undulate, and the elevation varied from flat to convex. Two streaking replicas were prepared for each of the colonies. All of the isolates were found to be Gram positive rods and spore positive. All of the isolates showed typical Brownian movement in the cavity slide method.

Table 2. Results for the Staining and Classical Biochemical Tests of the Positive Isolates of *B. licheniformis*

Test	Isolates from Soiled Potato		Isolates from Sweet Potato
	Results of Isolate 1 and 2	Results of Isolate 3	Results of Isolate 4, 5 and 6
Gram Staining	+	+	+
Spore Staining	+	+	+
Catalase Test	+	+	+
Starch Hydrolysis Test	+	+	+
VP Test	+	+	+
Citrate Utilization Test	+	+	+
Casein Hydrolysis Test	+	+	+
Anaerobic Growth	+	+	+
4.5% NaCl	+	+	+
6.5% NaCl	+	+	+
8.5% NaCl	+	-	+
Incubation at 37 °C	+	+	+
Incubation at 55 °C	+	+	+
Incubation at 60 °C	+	-	+
Results	*B. licheniformis*		*B. licheniformis*

Classical Biochemical Identification of the *Bacillus* Isolates

Manual biochemical tests were performed, which included catalase, VP, and starch hydrolysis tests. The bacilli were tested for their resilience in a high-salt medium (4.5%, 6.5%, and 8.5%), high-temperature incubation (55 °C and 60 °C), and anaerobic environment. The strains that could survive a 6.5% NaCl concentration and 55 °C incubation temperature in an anaerobic environment were concluded to be *B. licheniformis*.

Six isolates were identified to be *B. licheniformis via* the biochemical tests. Both

the soiled potatoes and sweet potatoes provided three isolates. All of the isolates, except for one from the potato, were resilient to the salt and temperature tolerance testing conditions. These results are given in Table 2.

Starch Hydrolysis Test of the *Bacillus* Isolates

Starch hydrolysis is of paramount importance in the identification of the *Bacillus* sp. It is based on the production of amylase enzymes, which has a high commercial value. The zone of starch hydrolysis was determined by the qualitative method of line inoculation on a 1% nutrient-starch medium. All of the six isolates tested positive during the starch hydrolysis test. Starch hydrolysis of *B. licheniformis* isolates 1, 2, 3, 4, and 5 recovered from soiled potatoes and sweet potatoes is shown in Fig. 1.

Fig. 1. Starch hydrolysis test of confirmed *Bacillus* licheniformis isolates on the nutrient agar supplemented with 1% soluble starch; Halo zone around the bacterial growth indicates starch hydrolysis; Parts **a** and **b** show starch hydrolysis for isolates 1 and 2, Part **c** and **d** show starch hydrolysis for isolates 4 and 5, Part **e** shows starch hydrolysis for isolate 3.

Identification of *Bacillus licheniformis* by the API 50 CH Kit

Commercial biochemical API 50 CH and API 20E test kits along with the respective media were used to identify the isolates. All tests were performed according to the instructions given by the manufacturers. Fourty-nine biochemical tests were done for the fermentation of carbohydrates with the help of API 50CH kit. Amongst the 49 carbohydrates, positive fermentation of starch and maltose were relatively more important for the identification of *B. licheniformis*. Twelve tests were performed from API 20E kit which were based on the production of specific enzymes. Eight tests were skipped from the API 20E as the biochemical tests were already present in API 50CH kit. Fermentation results of API 50CH are summarized in Table 3, while the results of the API 20E are summarized in Table 4. The resulting biochemical profiles from both of the kits were interpreted using the API-web software (bioMérieux). The similarity index of the isolates was found to be 99% for taxa *B. licheniformis*.

16S rDNA Detection of *Bacillus licheniformis*

The DNA extraction was confirmed by measuring the density *via* the nanodrop method. The corresponding band (~1.5 kb), after amplification and 1% agarose gel electrophoresis, confirmed the amplification of the 16S rDNA fragments, which is shown in Fig. 2. The 16S rDNA gene was sequenced to confirm the identification. For this purpose, sequences of the full-length 16S rDNA gene from *B. licheniformis* were BLAST searched in the GenBank sequence database to determine the homology with the already sequenced and deposited genes.

Table 3. Biochemical Profile of the API 50 CH Kit of the Two Representative *B. licheniformis* Isolates

	Biochemical tests	Result
1.	Glycerol	+
2.	Erythritol	-
3.	D-Arabinose	-
4.	L-Arabinose	+
5.	Ribose	+
6.	D- Xylose	+
7.	L-Xylose	+
8.	Adonithol	-
9.	Methyl xyloside	-
10.	Galactose	-
11.	D-Glucose	+
12.	D-Fructose	+
13.	D-mannose	+
14.	Sorbose	-
15.	Rhamnose	-
16.	Dulcitol	-
17.	Inositol	-
18.	Mannitol	+
19.	Sorbitol	-
20.	Methyl-D-mannoside	+
21.	Methyl-D-glucoside	+
22.	N-acetyl-glucosamine	+
23.	Amygdalin	+
24.	Arbutin	+
25.	Esculine	+
26.	Salicin	+
27.	Cellobiose	+
28.	Maltose	+
29.	Lactose	-
30.	Melibiose	-
31.	Sucrose	+
32.	Trehalose	+
33.	Inulin	+
34.	Melizitose	-
35.	D-raffinose	+
36.	Starch	+
37.	Glycogen	+
38.	Xylitol	-
39.	Gentibiose	+
40.	Turanose	+
41.	Lyxose	-
42.	Tagatose	+
43.	D-fucose	-
44.	L-fucose	-
45.	D-Arabitol	+
46.	L-Arabitol	-
47.	Gluconate	-
48.	2, Keto-gluconate	-
49.	5, keto-gluconate	-

Table 4. Biochemical Profile of the API 20E Kit of the Two Representative *B. licheniformis* Isolates

	Biochemical tests	Result
1.	ONPG	+
2.	ADH	+
3.	LDC	-
4.	ODC	-
5.	Citrate	+
6.	Hydrogen sulfide	-
7.	Urease	-
8.	TDA	-
9.	Indole	-
10.	Voges-Proskauer	+
11.	Gelatin	+
12.	Nitrate	+

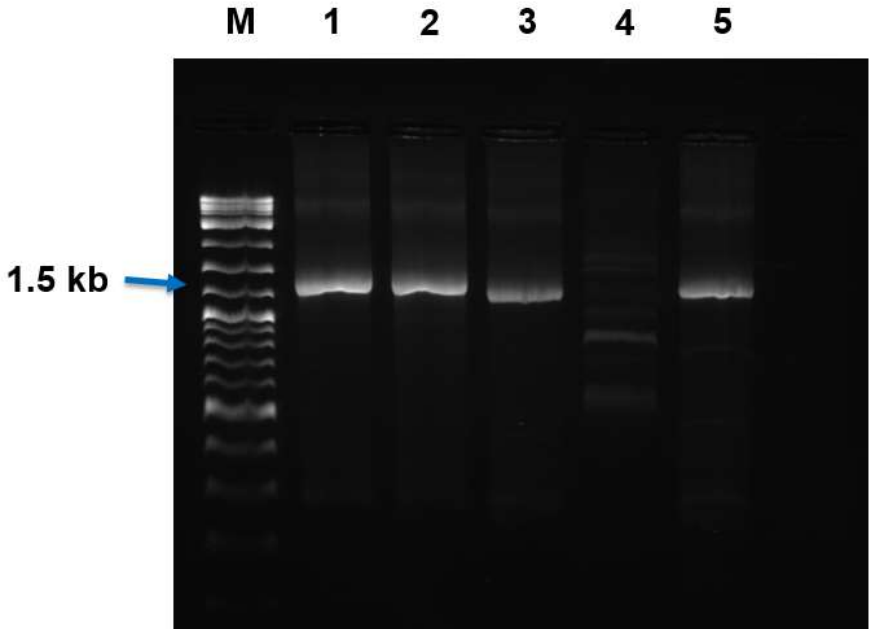

Fig. 2. PCR based detection of *B. licheniformis*: Lane M represents 1 kb marker. Lanes 1, 2, 3 and 5 showed the positive bands for 1.5 kb 16S rDNA gene.

Thermostability and Alkali Tolerance of the α-Amylase

Crude enzymes were extracted from the six isolates, which were identified to be *B. licheniformis*, and screened by starch hydrolysis. The DNS assay of all of the isolates was performed in triplicate. The enzyme from Isolate 4 (Enzyme 4) had the highest activity compared with the others, which was 740 mU/mL. When the enzyme was extracted at different intervals after bacterial culturing, it played an important role in the enzyme activity. The enzyme activity was considerably higher after 42 h of incubation. The activity decreased to 635 mU/mL at 48 h, and further decreased to 561 mU/mL at 60 h of culturing (Fig. 3).

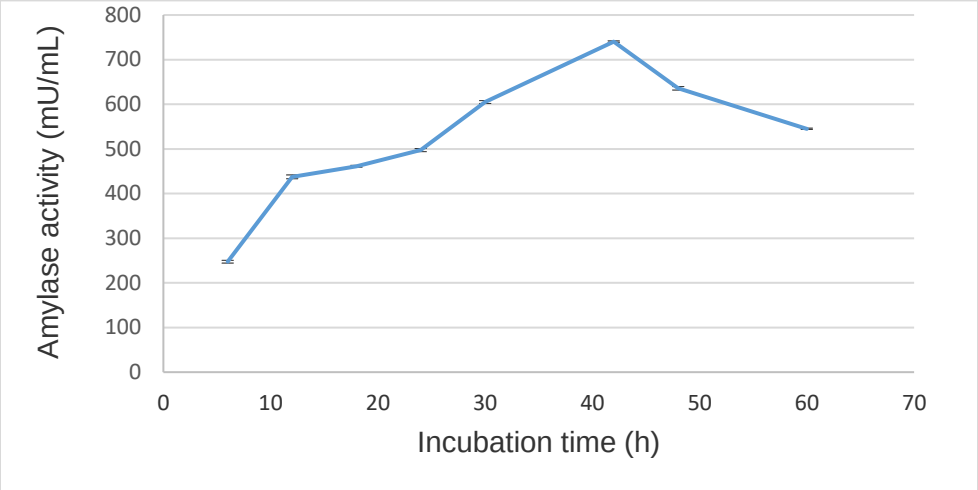

Fig. 3. Amylase activity for the different incubation times; the maximum activity was observed at 42 h of incubation.

The thermal stability of the α-amylase was assayed while incubating at various temperatures (30 °C to 85 °C) for 10 min. The residual activity of the α-amylase was measured at given temperatures. The α-amylase activity increased gradually until 55 °C until 740 mU/mL, and then it decreased with a further increase in the temperature. The α-amylase retained 77% of its activity (576 mU/mL) while undergoing incubation at 85 °C (Fig. 4).

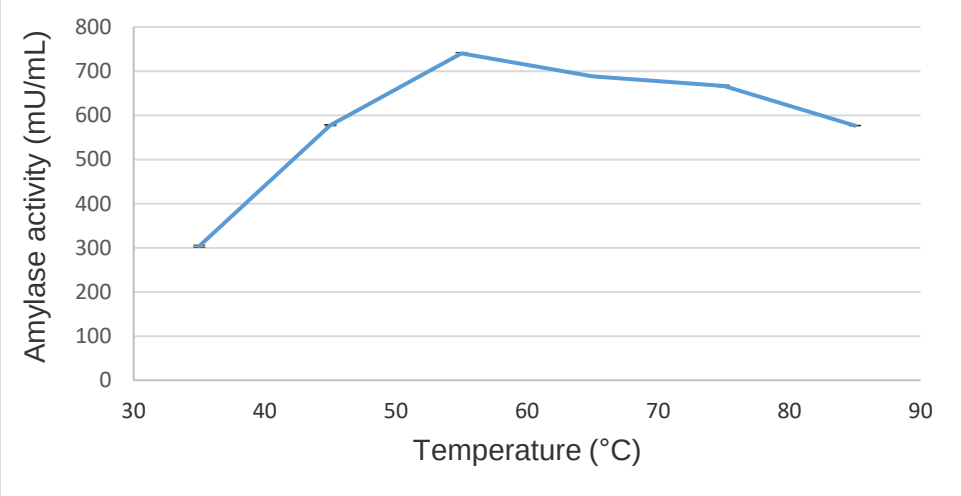

Fig. 4. Residual activity of the amylase after incubation at different temperatures; the maximum activity was observed at 55 °C.

The stability of the enzyme was measured in acidic to alkaline pH conditions (4 to 10) by determining the residual activity in relation to the maximum activity, which was observed at a pH of 8. It was shown that the α-amylase remained quite stable at a pH of 9, as it retained 88% of its activity (651 mU/mL). The activity decreased by both increasing and decreasing the pH from 8. The activity decreased by to 540 mU/mL with an increase in the pH to 10. A drastic decrease in the activity was observed at pH values of 4 and 5 (Fig. 5).

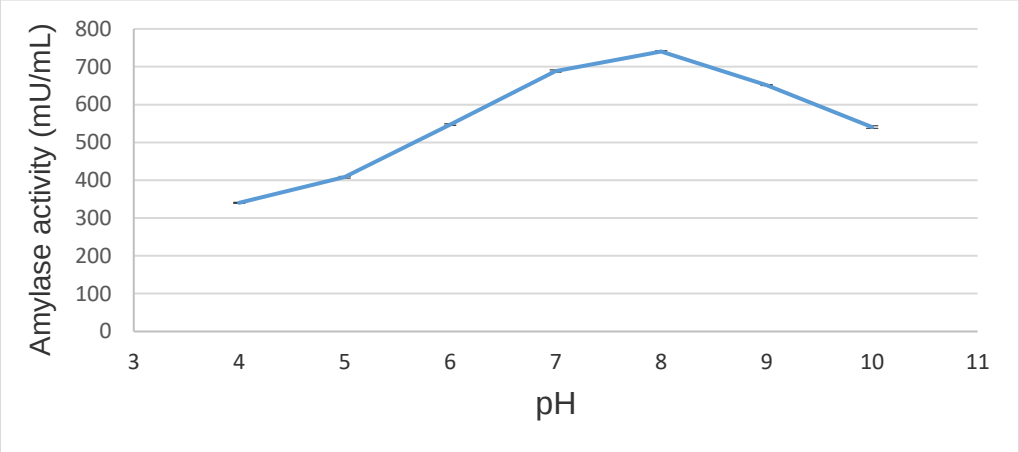

Fig. 5. Residual activity of the amylase at different pH values; the maximum activity was observed at a pH of 8.

Based on the optimal activity and relative activity at different temperatures and pH values, the α-amylase was found to be thermostable, as well as alkali tolerant.

Discussion

The current study revealed the importance of *B. licheniformis* strains for α-amylase production from potato and sweet potato peel waste. For this purpose, samples were collected from the soiled surfaces of sliced potatoes and sweet potatoes. The isolates from the decaying surfaces of the potatoes and sweet potatoes harbored high α-amylase-producing *Bacillus* sp. (Elmarzugi *et al.* 2013). Initially, the heat shock method was used to kill vegetative growth of any kind of bacterial species. The remaining spores were germinated in a nutrient broth to grow vegetative colonies later. The colony morphology was similar to those reported in Suganthi *et al.* (2013). As the procedure was performed in aerobic conditions, the characteristics of rod-shaped, spore-producing, Gram-positive, and motile bacteria confirmed that the isolates were part of the *Bacillus* genus (Wei *et al.* 2015).

After confirmation of the *Bacillus* genus, the starch hydrolysis test was performed, which differentiates the major species of bacilli. It was found that clear and bigger halo zones around the colonies formed when the medium was supplemented with soluble starch. In contrast, little to none of these halo zones were found for the same species when the medium was supplemented with insoluble starch. The isolates from the sweet potatoes showed a relatively bigger zone of starch hydrolysis than those from the potatoes. Exclusive biochemical tests were chosen according to Bergey's Manual to differentiate and identify *B. licheniformis* (Bergey and Holt 1994). Further positive tests for catalase, citrate, and VP narrowed down the possibilities to *B. licheniformis*. Similar results have been reported by Khusro and Sankari (2014) and Olanbiwoninu and Fasiku (2015).

The biochemical tests performed up to this stage could not differentiate exclusively between *B. licheniformis* and *B. subtilis*. As *B. licheniformis* and *B. subtilis* are very closely related bacteria, it is difficult to differentiate between both species using the colony morphology, size and shape of the bacteria, and conventional biochemical tests (Huang *et al.* 2016). For this purpose, the special characteristics of *B. licheniformis* were exploited. As *B. licheniformis* is a facultative anaerobe (Li *et al.* 2014), thermophilic, and salt tolerant, the isolates were cultured on a nutrient agar with 6% to 6.5% NaCl at 55 °C in anaerobic jars. The isolates had the ability to grow at 55 °C, and so the isolates met the criteria of being thermophilic organisms (Brock 2012).

For further biochemical tests, well-isolated colonies were run on the API 20E and API 50 CH strips. Basic tests of the API 20E kit are specific to differentiating species of Enterobacteriaceae. However, they can also be used for the identification of *Bacillus* sp. (Lin and Madida 2015). These kits were combined because the API 50 CH kit provided fermentation results for carbohydrates and the API 20E kit provided results for basic substrate utilization. The results were similar to those of Laribi-Habchi *et al.* (2015).

The pH of the growth medium and DNS reaction is crucial for the growth of bacteria and its ability to synthesize α-amylase enzymes. The ability of the isolates to continue producing α-amylase with a good activity until 42 h of incubation indicated its ability to withstand a low pH caused by metabolites. This result contrasts with the study by Asgher *et al.* (2007), where the activity peaked at 48 h for *B. subtilis* α-amylase. In both cases, the bacteria were in the stationary phase of growth, which is necessary for enzyme production (Wandserley *et al.* 2004). The activity decreased considerably at 60 h of culturing. This might have been because of fewer nutrients, a fast metabolism, and toxic metabolites. In contrast, the α-amylase from *Penicillium chrysogenum* did not produce a considerable activity until four days because of the slow growth and metabolism of fungus (Ertan *et al.* 2006). The slow metabolism of fungus was also supported in another study in which amyloglucosidase from *Aspergillus niger* produced its optimum activity after 72 hours of growth (Santana *et al*. 2012). The α-amylase in this study yielded a better activity at an alkaline pH of 8 compared with that reported by Ibrahim *et al.* (2013), who obtained a maximum activity at a pH of 5. The α-amylase of this study had a higher alkaline performance (at a pH of 8.0) than that of Rashid *et al.* (2009), who obtained a maximum activity at a pH of 6. The activity decreased at higher or lower pH values. The activity was reduced by 12% at a pH of 9 and 23% at a pH of 10. As 88% of the activity was retained at a pH of 9, the enzyme was found to be alkali tolerant.

Another important factor for enzyme production and activity is the temperature it endures (Vidyalakshmi *et al.* 2009). Enzyme production becomes slow at low temperatures because bacterial growth slows. As the temperature increases bacterial growth and enzyme production increases, but only to a certain extent. Thermophilic bacteria can grow optimally at 55 °C. In this study, the maximum activity was observed at 55 °C. The activity was reduced by 7% at 65 °C and 10% at 75 °C. The reduced activity was likely because of the hydrolysis of the peptide chain, change in the conformation of the protein, or aggregation (Wanderley *et al.* 2004). In contrast with thermophilic organisms, *Arthrobacter* sp. had a peak activity at 40 °C (Hampel *et al.* 1994). In different studies, α-amylase is either thermostable or alkali stable. As it was both thermostable and alkali tolerant and originated from the decaying waste of potatoes, which is effortless and can be performed optimally at a pH of 8.0, the α-amylase in this study was unique compared with the α-amylase from the metagenomic study by Vidya *et al.* (2011). Another study characterized α-amylase from *B. pseudofirmus* and found it to have a high relative activity at a pH of 8.5, but it could only produce an optimum activity at 40 °C (Lu *et al.* 2014).

CONCLUSIONS

1. The local strains of *B. licheniformis* were characterized and could tolerate the higher temperature and high salt concentration of the biochemical and molecular method.

2. This α-amylase was unique in being able to withstand industrial processes because it was thermostable and alkali tolerant at the same time.
3. More α-amylase producing strains of *B. licheniformis* could be isolated from the decaying surfaces of potatoes and sweet potatoes. The α-amylase from *B. licheniformis* is a good prospect in various industries as it can be easily genetically modified to produce the desired properties.
4. These strains are future candidates for α-amylase production and can be optimized for industrial conditions.

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