

Isolation Of *Pectobacterium Carotovorum*, Identification With 16s Rrna, Phytase Activity And Characterization Of The Bacteria

Kağan Tolga CİNİSLİ

Ataturk Universitesi Ziraat Fakultesi

Safa Mustafa KILIÇ

Ataturk Universitesi Ziraat Fakultesi

Sevda Uçar

Ataturk Universitesi Ziraat Fakultesi

Emre CANCA

Ataturk Universitesi Ziraat Fakultesi

Neslihan dikbaş (✉ neslidikbas@atauni.edu.tr)

Ataturk Universitesi Ziraat Fakultesi <https://orcid.org/0000-0002-6093-162X>

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Abstract

Background: Phytases can be produced by animals, plants and microorganisms. However, the most promising ones for commercial use and biotechnological applications are those of microbial origin. Phytases are also used in the preparation of myo-inositol phosphates in the food industry, soil remediation and in the paper industry. Biotechnology, along with the increased use of phytase enzymes, is a highly effective technology that is used today and will be used in the future to produce these enzymes and improve their properties.

Results: The aim of this study was to conduct the molecular identification of *Pectobacterium carotovorum* strains isolated from lettuce to produce phytase from a new microbial source and the characterization of the enzyme. The activity and characterization of the phytase obtained from the bacterium was carried out. Isolation of strains was carried out following incubation at 26 ° C for 48 hours using Nutrient agar (Oxoid). The identification was performed using the 16S rRNA method. The phytase produced from *Pectobacterium carotovorum* showed the best activity at pH 8.0. The optimum temperature of the phytase obtained from *Pectobacterium carotovorum* was 60 ° C.

Conclusions: In this study, enzymatic activity of phytase was investigated in *Pectobacterium carotovorum* for the first time. The results showed that it can be used in the industry due to the characteristics of the enzyme produced by *Pectobacterium carotovorum*.

Keywords: *Pectobacterium carotovorum*, 16S rRNA, Phytase, Characterization

1. Background

The term “Biotechnology” can single-handedly explain what biotechnology means. Biology explains its relation to living organisms, while technology means shedding light to existing or emerging problems and developing useful products. Accordingly, biotechnology means using living organisms to shed light to problems and develop more useful products [1]. The application areas of biotechnology are divided into five fields comprising medical, agricultural, food, animal, environmental and industrial biotechnology [2].

In microbial biotechnology, the production of enzymes and organisms that are used in the production of various foods, facilitation of processing and more efficient disposal of industrial wastes has become possible with the manipulation of microorganisms such as bacteria and yeast. The vast number of bacteria and other microorganisms allows various potential biotechnological applications. Microbial biotechnology is a dynamic field that generates a wide range of new applications [3]. The advantages of bacteria including their rapid growth, easy and controlled culture in a small area and simpler structure compared to eukaryotes facilitate their use in the common fields of genetics and biochemistry [4].

Pectobacterium carotovora subsp. *carotovora* is a phytopathogen bacterium that causes soft rot in many economically valuable plant varieties. [5]. *Pectobacterium carotovora* subsp. *carotovora* causes one of the most destructive vegetable diseases worldwide (ECC). *Pectobacterium carotovorum* subsp.

Brasiliense causes serious damage to potatoes and other vegetables due to black spot and soft rot diseases. When the differences between isolated species and subspecies are considered, *Pectobacterium*, which are similar to other species from the Enterobacteriaceae family that cause soft rot in plants, are facultative anaerobic, Gram (-), rod-shaped bacteria that have multiple flagella. In the studies, all isolates of the species (*P. carotovora*. subsp. *carotovora*, *P. carotovora*. subsp. *atroseptica*, *P. carotovora*. subsp. *betavascularum*, *P. carotovora*. sp. *wasabiae*, *P. carotovora*. subsp. *odorifera*) were determined to be catalase positive and oxidase negative and have the ability to carry out glucose fermentation, reduce nitrate to nitrite, produce β -galactosidase and H_2S and use L-arabinose, D-galactose, D-glucose, glycerin, D-mannose, D-ribose and sucrose, do not have the ability to produce urease and have the ability to produce acid from adonitol. Majority of the isolates can use L-rhamnose and D-mannitol but lack the ability to use dextrin. The *Pectobacterium* species in this group cannot be tested using every test used for Enterobacteriaceae and, thus, an ambiguity emerges in their taxonomy. The general methods used in the isolation of phytopathogen bacteria from soil and plant samples can also be used for these bacteria. Enriched or selective media should be used in the isolations from rotten plant tissues and soils [6].

A new standard for the identification of the bacteria was developed in the 1980s. We have come to understand that the comparison of certain regions of the genetic codes of bacteria can help determine the phylogenetic similarity between bacteria [7, 8]. The genes in the bacteria genome used for this purpose include 5S, 16S and 23S rRNA and the regions between these genes. Today, the most commonly used DNA region in the classification of bacteria is the 16S rRNA gene region [9, 10, 11]. 16S rRNA gene sequence region is around 1550 base pairs (bp) long and consists of hypervariable and conserved regions. Using the 500 bp-long region is more cost-friendly and applicable and, thus, the 500 bp-long region is widely used in sequence analyses. 16S rRNA gene sequence analysis steps comprise DNA isolation, PCR, purification, sequence cycle, sequence analysis, evaluation of the electropherogram and data analysis, respectively [12].

Enzymes have an important role in bacteria metabolism and various enzyme systems are needed for metabolic activity. Enzymes are catalyst activators, i.e. has an accelerating effect on a reaction. Reactions can start without enzymes. Enzymes allow a significant amount of freedom for substrates. They show better activity under optimum pH and temperature conditions. The purity of industrial enzymes is usually low and, thus, their cost is lower, whereas scientific, analytic and medical enzymes have high purity and, thus, cost more.

Phytase (myo-inositol hexakisphosphate phosphohydrolase) catalyzes the hydrolysis of myo-inositol hexakisphosphate (phytic acid) to inorganic mono phosphate, reduced myo-inositol phosphates and, in many cases, free myo-inositol. There are basically two phytase enzymes: 3-phytase (E.C. 3.1.3.8) and 6-phytase (E.C. 3.1.3.26). This classification is based on the first phosphate group the enzyme attaches to. 3-phytase is reactive for microorganisms and 6-phytase is reactive for plants [13]. Phytases catalyze the gradual removal of phosphate groups from the myo-inositol ring of phytate. These enzymes can be widely found in nature and more than 2000 soil isolates have been reported. According to their catalytic mechanisms, phytases can be called histidine acid phytases, cysteine phytases and purple acid phytases

[14]. Although phytase was detected in plants, microorganisms and some animal tissues, recent studies have shown that microbial phytase yielded successful results in biotechnological applications [15. 16].

The study investigates the molecular identification of the *P. carotovorum* strain that was isolated from lettuce and procured from the Agricultural Biotechnology Culture Collection, phytase production from a new microbial source and characterization of phytase.

2. Results

In the study, the stock culture of the bacteria strain No. M7 available in the Enzyme and Microbial Biotechnology Science Department was used.

2.1. Morphological Properties of the Microorganism

Certain morphological properties of the isolate were examined. The sizes, endospore formation and Gram properties of the isolates were determined. Table 1 shows the results obtained for the morphological properties of the isolates.

Table 1
Morphological properties of *P. carotovorum*

Identification results	Cell Morphology	Sizes	
		Diameter (µm)	Size(µm)
<i>P. carotovorum</i> subps. <i>carotovorum</i>	Rod	0.5–0.6	2
Endospore	Gram Properties		
	KOH	Gram Staining	
-	-	-	

2.2. Determination of the Properties of the Culture

In the investigation of the culture properties of the bacteria, the results were obtained when mature colonies of each isolate were formed in the agar medium (Nutrient agar). Table 2 shows the culture properties of the isolates in detail. Furthermore, Fig. 1 shows the colony and micron morphologies. The bacteria were isolated using streak plate method. Pure colony structures in the culture were examined and the pure colonies were used in further studies.

Table 2
Properties of the *P. carotovorum* culture

Isolation Results	Colony Shape	Colony Color	Colony consistency
<i>P. carotovorum</i>	Oval	Cream	Dry

2.3. Determination of Some Biochemical Properties

To support the identification with the molecular method, some biochemical tests were performed to obtain a precise identification. Table 3 shows the biochemical test results.

Table 3
Some biochemical test results of *P. carotovorum*

Tests	Citrate test	Indole Test	Phosphatase Test	Catalase Test	KOH Test	Gram Staining Test
<i>P. carotovorum</i> subsp. <i>carotovorum</i>	+	-	-	+	-	-

2.4. Identification of *P. carotovorum* with the Molecular Method

2.4.1. DNA isolation results

The genomic DNA isolation from the *P. carotovorum* isolate was carried out by following the protocol described in Sect. 3.2.5.a with no modification. Figure 2 shows the DNA isolation results.

2.4.2. Gel image of the PCR results

The PCR reactions that were carried out using the genomic DNA obtained from the *P. carotovorum* isolate yielded PCR products of desired sizes of 1400–1500 bp. Figure 3 shows the results. The marked region indicates 1400 bp on the marker.

2.4.3. Sequence analysis results

Gene sequencing was carried out to confirm whether the PCR result was the 16S rRNA gene region. Figure 4 shows the sequences obtained by sequence analysis. The purpose of the tree is to show the relationship between close species and similarities between the samples that were determined to be species and their subspecies.

The tree shown in Fig. 5 was obtained from NCBI and the accession number was given. The examination of Section A revealed three systematic main groups. *Pectobacterium atrosepticum* was the main taxon and identified as the outgroup. There were major similarities between the outgroup and other groups. The second important group was *Pectobacterium carotovorum*, the subject of the article. These serve as two examples of sister taxa. The group showed a high similarity. The other group is currently called *Erwinia* in mass. They had a high similarity among each other. *E. persicinus* and *E. rhamontici* were identified as different species and showed a similarity of 94% and, thus, had a higher similarity even when compared to their subspecies.

2.5. Phytase Activity Results

The activity of isolated and identified *P. carotovorum* was measured at different temperature and pH values and the results are given below.

2.5.1. Optimum pH results for phytase

Measurements were carried out to determine the optimum pH for phytase that was produced and purified from *P. carotovorum*. For optimum pH, 10 different pH values ranging from pH 2.0 to pH 11.0 were investigated. For pH 2.0–6.0, acetate, for pH 7.0–9.0, Tris HCl and for pH 10.0–11.0, carbonate were used as buffers. The activity of the enzyme was measured using the results and “Activity-pH” graphs were drawn. Figure 6 shows the results.

2.5.2. Optimum temperature results for the phytase obtained from *P. carotovorum*

Measurements were carried out to determine the optimum temperatures for phytase that was produced and purified from *P. carotovorum*. Its activity was calculated using the results and the “Activity-°C” graphs were drawn. The results showed that the optimum temperature for the phytase obtained from *P. carotovorum* was 60 °C. Figure 7 shows the optimum temperature results for the phytase obtained from *P. carotovorum*.

3. Discussion

The individual morphologies of bacteria can only be examined under a microscope due to their small sizes. For this purpose, the pure preparations of the bacteria grown in liquid or agar media are stained with specific dyes. In microscopic examinations, the properties of bacteria such as size, endospore formation and staining (Gram (-) or (+), vb.) are examined [17, 18] *P. carotovorum* is a rod-shaped, non-endospore-forming, Gram (-) bacterium with a diameter of 0.5–0.6 µm and size of 2 µm. The isolates are inoculated into agar media either with spot or streak plate isolation methods and, then, colony formation is waited; then, colony morphology is examined under a microscope to determine their appearance from above, height, edge shape and color [19]. The colony structure of *P. carotovorum* is oval, cream-colored

and dry. Certain biochemical tests were carried out for *P. carotovorum* subps. *carotovorum*. Our results agree with the results obtained in previous studies [6]. The results showed that *P. carotovorum* subps. *carotovorum* tested positive for citrate and catalase and negative for indole and phosphatase. The bacteria were Gram (-) in terms of cell wall structure.

The methods used for identification and characterization can also be classified as conventional and molecular methods [20]. Molecular methods were developed based on the changes stemming from the contents, diversity and ratios of the macromolecules that form microorganisms. Molecular studies use carbohydrates, lipids, proteins and genetic materials (DNA and RNA) as the main materials and identify and characterize microorganisms with the help of one of these materials or their combinations. The sequence analysis of ribosomal operons is the most commonly used method to determine the phylogenetic relationship between organisms [21, 22, 23, 24, 25, 26, 27].

In the study, phytase activity of *P. carotovorum*, which is used in various areas, temperature and pH values that can affect phytase activity and optimum pH and temperature values were investigated. To determine the optimum pH of the phytase purified from *P. carotovorum*, activity measurements were carried out in the pH range of 2.0–11.0. For pH 2.0–6.0, 0.1 M acetate buffer, for pH 7.0–9.0, 0.10 mM Tris/HCl buffer and for pH 10.0–11.0, 10 mM carbonate buffer were used to measure the activity of the enzyme with spectrophotometry. The phytase that was extracellularly produced and purified from *P. carotovorum* had the highest activity at pH 8.0 [28]. Since there were no previous studies on phytase from *P. carotovorum*, the results of the study were compared by using the activities of phytases from other bacteria species as reference. Tran et al. [29] found that the optimum temperature for the *Bacillus* Sp. MD.2 strain was 70 °C. In their study, Erkan [30] determined that the optimum pH for *Bacillus subtilis* was 6.5.

The study is an important contribution to the literature as it is the first study on phytase production from *P. carotovorum*. There were no studies on phytase production from *P. carotovorum* in the literature. With phytase production from *P. Carotovorum*, we are of the opinion that the study will both add to biotechnology in terms of its R&D potential and add diversity to global enzyme industry in terms of production. The phytase activity of *P. carotovorum* differs depending on temperature. In their study, Tran et al. [29] found that the optimum temperature for the *Bacillus* Sp. MD.2 strain was 70 °C. Erkan [30] determined the optimum temperature for *Bacillus subtilis* as 60 °C. Since there were no previous studies on the phytase activity of *P. carotovorum*, we were not able to compare the optimum temperature of 60 °C as found in our study to the literature. The lowest activity was measured at 20 °C. Phylogenetic tree was drawn to determine the position of *Pectobacterium carotovorum* on the tree. Finally, three systematic main groups were generated and our bacteria were highly similar to these groups. In conclusion, in the study, the optimum pH of *P. carotovorum* was determined to be 8.0, while it had the lowest activity at pH 4.0. Optimum temperature was found to be 60 °C, while the lowest activity was measured at 20 °C. The phytase activity of *P. carotovorum* was measured for the first time. The results and the cultural adaptation of the bacteria indicated that, in terms of its production, phytase can be introduced to

technology. Due to the characteristic properties of the enzyme produced by the bacteria, our results support the idea that the enzyme can be used more efficiently and widely in the industrial areas.

4. Material And Method

4.1. Inoculation of bacteria from the stock culture

The stock culture was removed from -80 °C and kept at room temperature until thawing was completed. The culture was inoculated on nutrient agar using the three-phase inoculation method and incubated at 26 °C for 3 days. The experiment was continued when bacteria growth reached a certain level.

4.2. Determination of morphological properties

The individual structures of bacteria can only be determined using microscopes, since bacteria are too small in size. Thus, the pure bacteria preparations in agar or liquid medium are examined using specific dyes. In the microscopic examinations, properties such as the sizes, endospore formation and staining (Gram (-) or (+), etc.) of bacteria are examined[18, 19]

a. Determination of cell morphology: Samples were prepared from each isolate and simple staining was carried out with crystal violet. The results were examined under a microscope [31, 18].

b. Determination of cell sizes: Cell sizes of the isolates from the preparations that were stained with simple or negative staining were measured by an ocular micrometer using immersion objective [31, 32].

4.3. Determination of the properties of the culture

The pure cultures of bacteria in agar and liquid medium were used in the determination of the properties of bacteria culture.

The isolates were inoculated into agar medium with either spot or streak plate isolation methods to allow colony formation. The colony morphology was examined under a microscope and their appearance from above, edge shape and color were determined [19].

Bacteria can reproduce by forming pellicle at the top and sediment or granules at the bottom. The isolates were inoculated into nutrient broth-containing tubes to determine the properties of the culture in liquid media [17].

4.4. Application of some biochemical tests

4.4.a. Catalase test

The test was used to determine whether catalase was present in the isolates. Catalase is present in facultative and aerobic microorganisms. Catalase converts hydrogen peroxide (H_2O_2) occurring due to respiration into water and oxygen. For the test, a loopful of the 48-h culture was collected and one drop of 5% H_2O_2 was added to observe bubble formation. Bubble formation showed that the test was positive [19].

4.4.b. Citrate test

The test is used to determine the ability of microorganisms to use citrate as the carbon source and ammonium salts as the nitrogen source and identify the genera and species of bacteria. The degradation of citrate (citrate metabolism) by bacteria is carried out by their enzyme systems.

After dilution with physiological water or buffer, the pure cultures to be treated were inoculated into the media and the tubes were incubated at 37 °C for 2–7 days. Inoculation with needle on the Christensen medium was carried out and the needle was also streaked on the slant surface. No sign of growth in the Simmons citrate medium after an appropriate amount of incubation time and a persistently green medium indicate negative reaction, while growth and dark blue color along the inoculation line indicate positive reaction. No color change in Christensen citrate sulfide medium (the light color remains) indicates negative reaction, while observing growth and pink-red color formation along the inoculation line indicate a positive reaction [6].

4.4.c. Indole test

The test is used to examine the indole production of microorganisms by the degradation of tryptophan, an amino acid. After inoculation into a liquid medium or peptone-containing water, the microorganisms were incubated at 37 °C for 1–5 days. A total of 0.5 ml kovacs (or Ehrlich) was added to the cultures and thoroughly stirred. Formation of a red ring at the top of the tubes indicates a positive reaction (indole formation). Presence of a yellowish ring indicates that indole formation did not occur (negative indole test). The color is due to the pyrroles in indole [6].

4.4.d. Phosphatase test

The test is used to determine the presence of phosphatase especially the phosphatase synthesized by staphylococcus (coagulase positive). Microorganism cultures were inoculated into phenolphthalein phosphate agar and incubated at 37 °C for 1–2 days. At the end of this period, the colonies were exposed to ammonia vapor (1 ml NH₄OH is put on the lid of the petri dish and culture is closed upside down). If a liquid medium is used, one drop of sodium hydroxide is dropped on the tubes. Red coloration in the colonies or tubes indicates a positive reaction. No change in color is observed if the reaction is negative [6].

4.4.e. Determination of Gram properties

The test was used to determine the differences between the cell walls of the isolates. Two different methods can be used for this purpose.

In the first method, 1–2 drops of 3% KOH solution is dropped on the microscope slide. Then, the bacteria collected with a loop is mixed with the KOH solution for 5–10 seconds and the loop is lifted up. If the microorganism is Gram (-), the peptidoglycan layer in the cell wall has fewer layers and does not contain teichoic acid and thus, is easily disintegrated by KOH and cytoplasm and cell content are released. This leads to viscous stretching. While in Gram (+) bacteria, KOH-bacteria mixture is an aqueous liquid and no stretching is observed when the loop is lifted up [19].

A drop of pure water is dropped on the microscope slide; then, a loopful of the bacteria that were grown on the medium for 24 hours is thoroughly streaked and fixed by fire. The preparation is first treated with crystal violet for 1 minute and rinsed with pure water for 30 seconds; then, it is treated with Lugol for 1 minute and rinsed with 96% ethyl alcohol. The preparation is re-rinsed with pure water and finally stained

with safranin. The preparation is washed and dried and, then, examined under a microscope. Gram (+) bacteria are purple colored, Gram (-) bacteria are pink colored [33].

4.5. Molecular Identification of the Microorganism

4.5.a. DNA isolation from bacteria isolates

The isolation of the isolate DNAs was carried out using a DNA isolation kit (Qiagen) by following the instructions of the manufacturer.

The steps include;

- 1- A loopful of the bacteria grown in nutrient agars was collected into sterile Eppendorf tubes.
- 2- To the tubes, 200 µl Lysozyme + TrisHCL solution was added.
- 3- The mixture was thoroughly vortexed to obtain a homogenous dissolution.
- 4- The vortexed mixtures were incubated at 37 °C for 45 minutes. During this period, vortex was repeated every 15 minutes.
- 5- At the end of incubation, the samples were centrifuged at 12000 rpm and 24 °C for 5 minutes.
- 6- The liquid part of the centrifuged samples was poured and the pellets attached to the side walls were left.
- 7- The samples were placed on the sections of the robot and isolation was commenced.

4.5.b. Amplification of the 16S rRNA region with PCR

The region synthesizing 16S rRNA, which has an important role in bacterial systematics, was selected and amplified using universal primers (27F forward 5'- AGA GTT TGA TCC TGG CTC AG -3', 1492R backward 5'- GGT TAC CTT GTT ACG ACT T -3') under laboratory conditions.

The preparation of the PCR reaction: 30 µl reaction; Using 3 µl 10 x PCR buffer, 0.6 µl dNTPs, 0.3 µl 50 µM primer, 0.3 µl 50 µM primer, 1.2 µl DMSO, 1.8 µl MgCl₂, 0.3 µl 5 unit/µl Taq DNA polymerase and 21.5 µl sterile pure water, 29-µl reaction mixture was prepared. Lastly, 1 µl template DNA was added to the mixture and the final volume was brought to 30 µl [19].

PCR program: The PCR program was set to denaturation at 95 °C for 120 seconds followed by 40 cycles of denaturation at 94 °C for 60 seconds, annealing at 53 °C for 60 seconds and extending at 72 °C for 120 seconds followed by extending at 72 °C for 5 minutes [19].

Electrophoresis of 16S rRNA PCR products: 50 ml 1XTAE buffer was added to 0.6 gr agarose. The mixture was boiled in a microwave oven until obtaining a clear appearance. The agarose gel was cooled to 50 °C or to a temperature that won't burn hands and 0.6 µl gel red chemical was added to the agarose gel and the gel was poured into the electrophoresis gel bath with installed combs. After waiting to make sure the gel was frozen, the combs were carefully removed from the gel. 1XTAE buffer was placed in the electrophoresis tank so that the gel surface was completely covered. To the first well in the gel, 8 µl of the DNA marker was loaded and the sample was loaded to the other well.

Electrophoresis gel setup: The sample was run at 90 Volt for 60 minutes. After running, the gel was scanned using a UV light system (Gel Doc 2000 BIO RAD) and analyzed using a computer [19].

4.5.c. Collection of DNA sequence analysis and evaluation of the results

Sequence analysis results were analyzed with BioEdit and converted into the Fasta format. Primer regions were identified and top and bottom parts were removed. Then, the region that was read from both sides in the middle was controlled and aligned. Moreover, using the MEGA7 program of the NCBI

databank (with the bacteria sequences obtained with the accession number) the sequence results were compared in accordance with the Neighbor Joining cluster analysis and the phylogenetic tree was drawn.

4.6. Measurement of enzymatic activity

In the phytase activity measurements, activity measurement was performed for all supernatants obtained in each stage. The ranges in which phytase was active were determined. In the activity measurements, 250 µL substrate was added to 100 µL enzyme solution and the mixture was vortexed. Then, the mixture was placed in the water bath at 50 °C for 10 minutes to allow reaction. To stop the reaction, 500 µL 10% TCA was added. After a 15-minute waiting period at room temperature, 500 µL coloring solution was added to the tubes. After incubation for 15 minutes, the tubes were centrifuged at 3200 rpm for 10 minutes. Following centrifugation, the absorbance values of the samples at 700 nm were measured using spectrophotometry and a blind sample.

4.6.a. Determination of the optimum pH for the enzyme

For the determination of the optimum pH value at which the enzyme was active, 2 mM sodium phytate-containing substrate solutions at different pH values (pH 2.0–11.0) were prepared using the carbonate (pH 10.0–11.0), Tris (pH 7.0–9.0), sodium citrate (pH 4.0–6.0) and acetate (pH 2.0–3.0) buffer solutions. For the determination of activity, 100 µL enzyme and 250 µL substrate were vortexed in a 2-ml tube. Then, the mixture was left in a water bath at 50 °C for 10 minutes to allow reaction. To stop the reaction, 500 µL 10% TCA was added. After the addition of 500 µL coloring solution to the tubes collected from the water bath and waiting for 15 minutes, the samples were centrifuged at 4000 rpm for 5 minutes. After centrifugation, the absorbance values of the samples at 700 nm were measured using spectrophotometry and a blind sample.

4.6.b. Determination of the optimum temperature for the enzyme

For the determination of the temperature at which the enzyme showed optimum activity, 10 °C, 20 °C, 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C and 90 °C were selected. A water bath was used for temperature measurements. For the activity measurements, 100 µL of the enzyme and 250 µL of the substrate were mixed and the standard activity measurement method was followed.

Declarations

Authors' contributions

ND and SMK conceived and planned the experiments. SMK, KTC, SU, and EC carried out the experiments. ND, SMK, KTC, SU and EC contributed to sample preparation. ND, SMK, KTC, SU and EC contributed to the interpretation of the results. ND took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

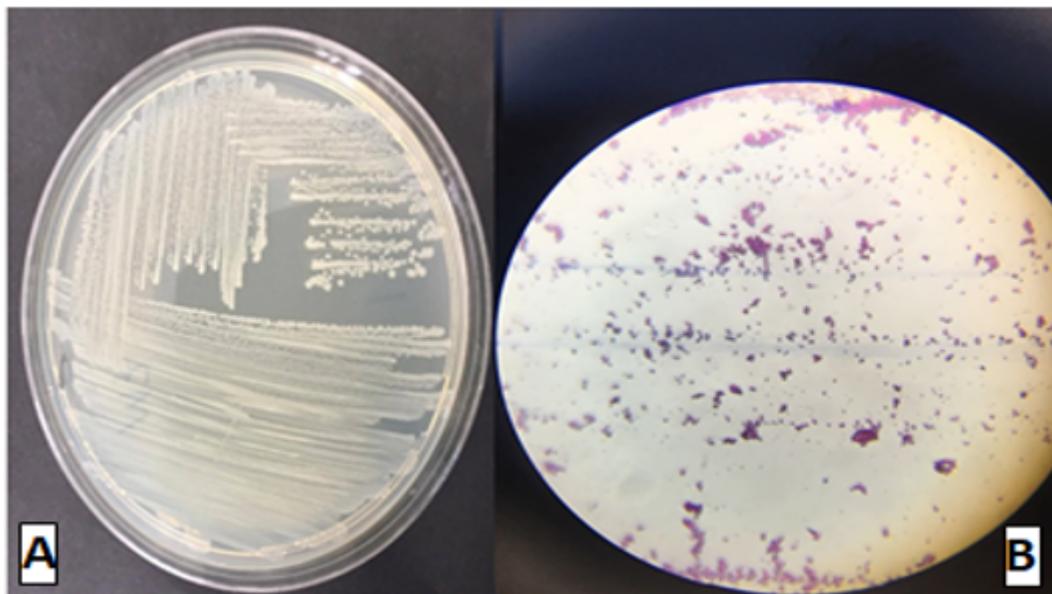


Figure 2

The colony and cell morphology of *P. carotovorum* *A. Petri image B. cell morphology (10*40)

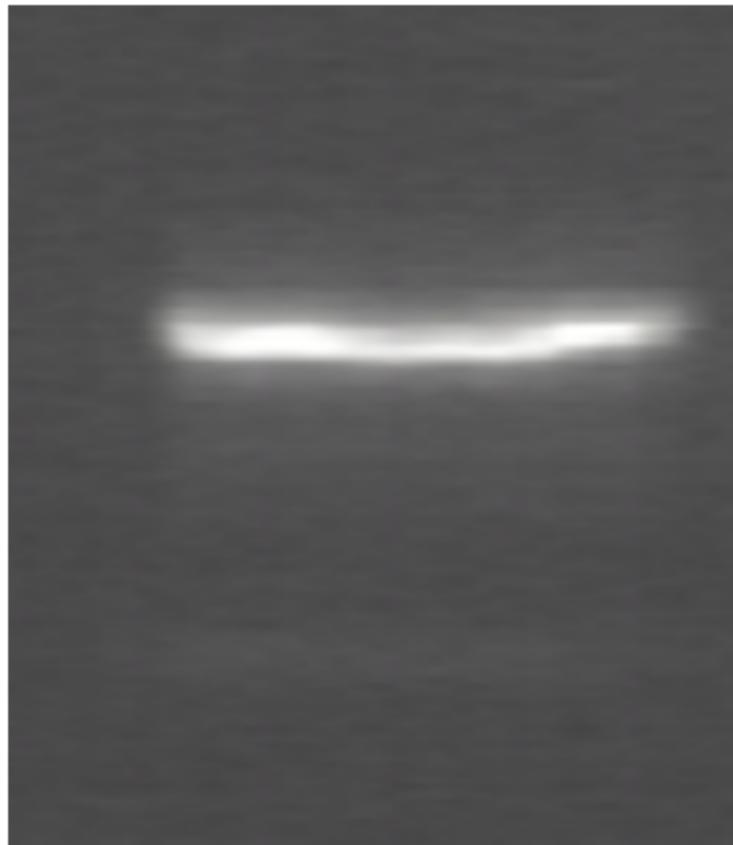


Figure 3

Gel image after DNA isolation

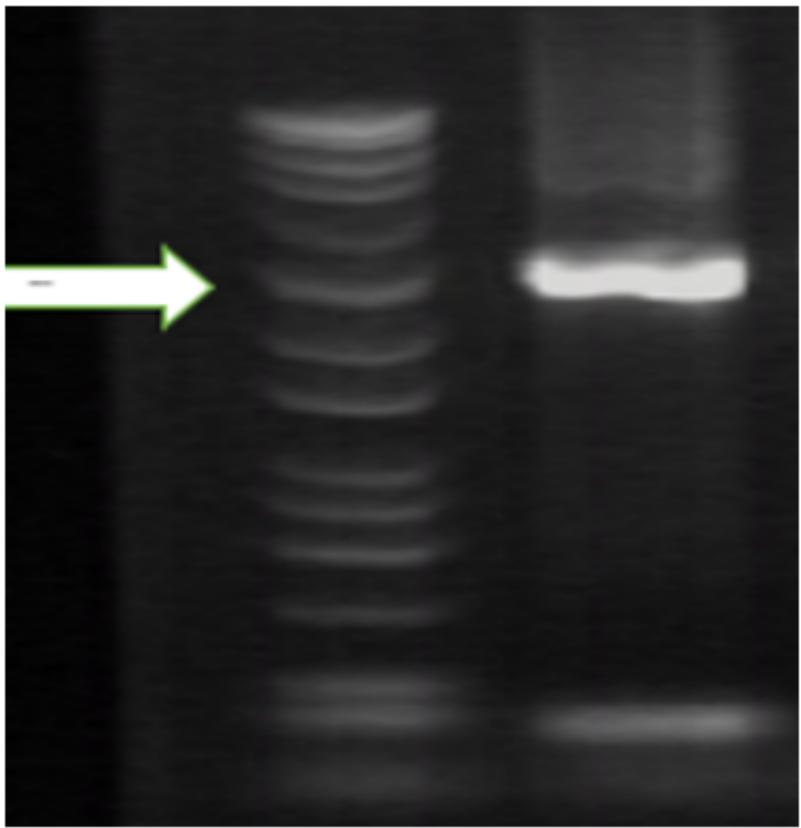


Figure 6

Gel image of the PCR results *Marked region indicates 1400 – 1500 bp.

> *Pectobacterium carotovorum*

GCTTGCTTTGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGAAACTGCCTGATG
GAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACCTCGCAAGAGCAAAGAG
GGGGACCTTCGGGCCTCTGCCATCAGATGTGCCAGATGGGATTAGCTAGTAGGTGAGG
TAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGA
ACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGAATATTGCACAATGGGC
GCAAGCCTGATGCCATGCCCGTGTGAAGAAGGCCTCGGGTTGAAAGCACTT
CAGCGAGGAGGAAGGCGTAAGGTTAATAACCTTATCGATTGACGTTACTCGCAGAAGA
AGCACCGGCTAACTCCGTGCCAGCAGCCGGTAATACGGAGGGTGCAAGCGTTAATCG
GAATGACTGGCGTAAAGCGCACGCAGCGGTCTGTTAAGTTGGATGTGAAATCCCCGG
GCTTAACCTGGAACTGCATTCAAAACTGACAGGCTAGAGTCTTAGAGGGGGTAGA
ATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGC
CCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGAGCAAACAGGATTAGAT
ACCTGGTAGTCCACGCTGAAACGATGTCGATTGGAGGGTTGCCCCCTGAGGCGTGGC
TTCCGGAGCTAACCGTTAAATCGACCGCTGGGAGTACGGCCGCAAGGTTAAA
AATGAATTGACGGGGGCCGCACAAGCGGTGGAGCATGTGGTTAATTGATGCAACGC
GAAGAACCTTACCTACTCTGACATCCAGAGAATTAGCTAGAGATAGCTGAGTGCCTCG
GGAACCTGAGACAGGTGCTGCATGGCTGTCGTAGCTCGTGTGAAATGTTGGTTA
AGTCCCGCAACGAGCGAACCCCTATCCTTGTGCCAGCGATTGGTCGGGAACTCAA
GGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGATGACGTCAAGTCATCATGGCC
TACGAGTAGGGCTACACACGTGCTACAATGGCGTATAACAAAGAGAAGCGACCTCG
AGCAAGCGGACCTCATAAAGTACGTCGTAGTCCGGATTGGAGTCTGCAACTGACTCC
GAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTT
TGTACACACCGCCCCGTACACCATGGGAGTGGGTTGCAAAA

Figure 8

Sequence analysis results of the 16S rRNA gene region from the *P. carotovorum* isolate

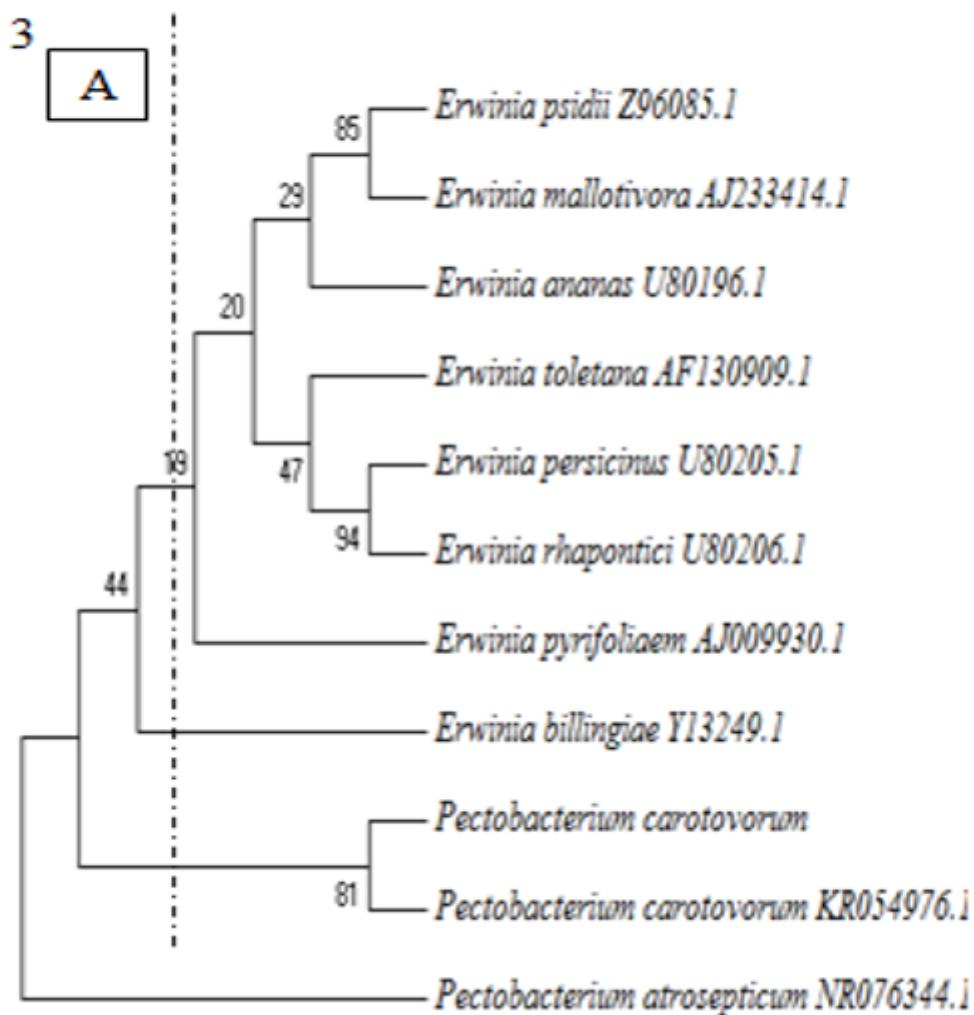


Figure 10

Determination of the location of *P. carotovorum* on the phylogenetic tree

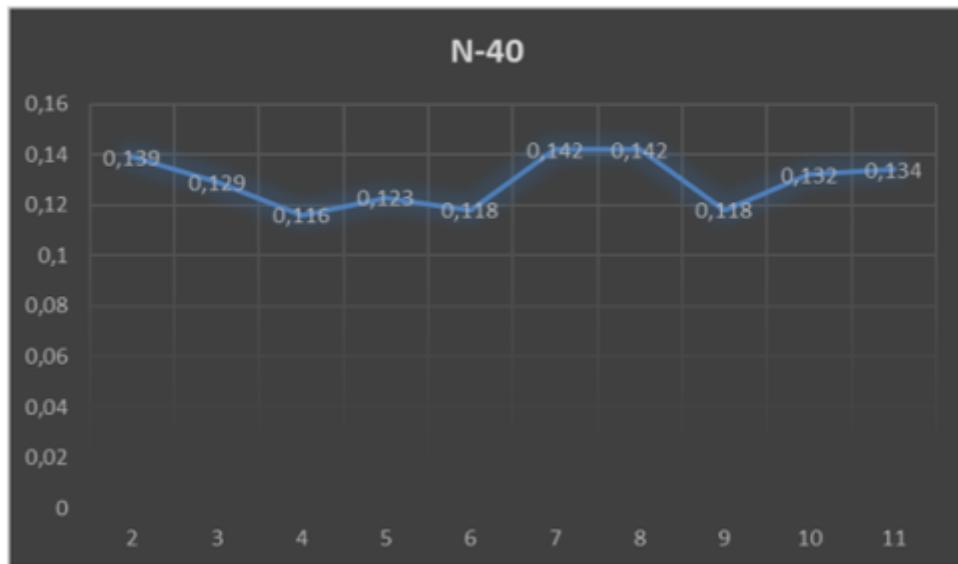


Figure 11

Effects of pH on the enzymatic activity of the extracellular phytase obtained from *P. carotovorum*

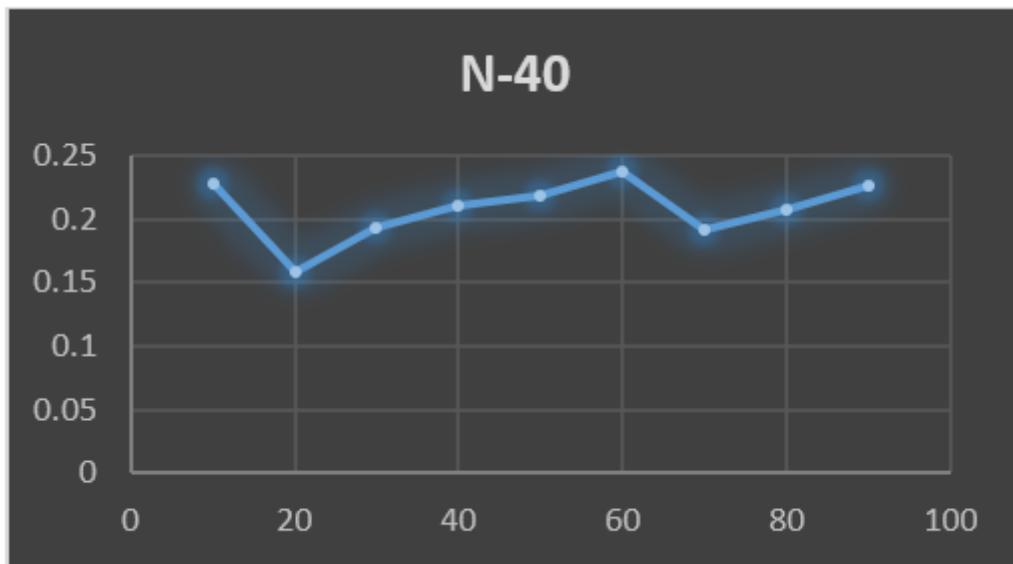


Figure 13

Effects of temperature on the enzymatic activity of the phytase that was obtained extracellularly and purified from *P. carotovorum*