PRODUCTION, OPTIMIZATION, PURIFICATION, AND CHARACTERIZATION OF *STREPTOMYCES* PECTINASE FOR AGRICULTURAL WASTE DEGRADATION: AN IN VITRO AND IN SILICO STUDY

ALSHEHRI, W. A.

Department of Biological Sciences, College of Science, University of Jeddah, Jeddah 21493, Saudi Arabia
(e-mail: waalshehri@uj.edu.sa; phone: +966-55-406-6553)

(Received 19th Sep 2024; accepted 23rd Dec 2024)

Abstract. The agricultural and fruit vegetable sector generate a significant amount of waste, especially in industrialized countries where overproduction and quality standards lead to waste before reaching consumers. Fruit and vegetable waste poses a challenge due to their large quantities and biodegradability. This study identified bacteria capable of producing pectinase enzymes to break down waste-derived pectin and convert it into biofertilizer. Nineteen bacteria were isolated from agricultural soil and fruit dump wastes, with *Streptomyces coelicolor* FMA18 exhibiting the highest pectinase activity. The optimal conditions for pectinase production were determined as 30°C and pH 7, in a medium containing citrus pectin and yeast extract. Molecular docking of pectin and pectinase was carried out with Autodock Vina, followed by a 100 ns Molecular Dynamics simulation (MDS) to confirm the stability of the protein-ligand complex. Analysis of the MDS data indicated that ionic, hydrogen, and hydrophobic bonds were the primary interactions between the ligands and the protein.
Keywords: *pectin hydrolysis, pectinase, streptomyces coelicolor, compost and plant growth, fruit wastes, waste management, sustainable agriculture*

Introduction

Improperly managed waste contributes to pollution and greenhouse gas emissions. Proper waste management is essential to achieving a cleaner environment and transitioning to a zero-waste economy. Pectinase, an enzyme that breaks down pectin in plant cell walls, plays a key role in waste conversion. It has efficient catalytic activity, eco-friendly properties, and low energy consumption. The market size for pectinase was $30 million in 2019 and is expected to reach $35.5 million by 2024 (Prathyusha and Suneetha, 2011; Haile and Ayele, 2022).

Pectinase enzymes break down pectic compounds in plant cell walls, improving juice extraction. They are produced by various organisms, including bacteria, fungi, actinomycetes, and yeast. Different organisms produce different types of pectinases, with alkaline pectinases mainly from bacteria and acid pectinases from fungi. Pectinolytic enzymes are commonly found in spoiled fruits, vegetables, decayed leaves, and decaying organic matter (Xu et al., 2020). Actinomycetes, specifically *Streptomyces* species, play a crucial role in breaking down pectic substances and plant debris using pectinase enzymes. Actinomycetes have stable and pH tolerant pectinase enzymes, giving them an advantage over other species (Bruhlmann, 1995). They can utilize agricultural waste for economical and environmentally friendly production of pectinase enzymes. Actinomycetes species hold potential for the future.

The study aimed to find pectinolytic Streptomyces and evaluate a locally isolated pectinase-producing strain from agricultural soil. It also aimed to determine optimal conditions for enzyme production through submerged fermentation (SmF). Objectives

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

included extracting, purifying, characterizing, and exploring potential applications of pectinases in industrial bioprocessing.

Materials and methods

Chemicals

Absolute ethanol 99.9%, hydrogen chloride 5% (HCl), Sodium hydroxide 3% (NaOH), 3-5-dinitrosalicylic acid were obtained from Sigma company. Pectin, Sodium chloride (NaCl), Potassium iodide, Bovine serum albumin, Coomassie brilliant blue, L-asparagine, L- tyrosine, Ammonium sulphate $(NH_4)^2SO_4$, Sephadex-G150, Gram stain kit, metal ions (Cu^{++}, Zn^{++}, Co^{++}, $Ca^{++}Fe^{++}$, Hg^{++} and Ethylene diamine tetra acetic acid (EDTA) were obtained from HI media company, India.

Isolation of streptomyces

Multiple *Streptomyces* isolates were obtained from agricultural soil and plant waste samples (a grape peel) in Jeddah city. Isolation was done using the serial dilution plate method and incubated at 28°C for 5-7 days. Pure colonies that grew well on the Mineral pectin agar medium were preserved on starch nitrate agar slants. The slants were incubated in a Memmert Incubator at 30°C for 5 days. The isolates were stored at 4°C until further use (Mohandas et al., 2018).

Primary screening of pectin producing streptomyces

A screening was done to find *Streptomyces* that break down pectin. They used a mineral pectin agar medium containing $NaNO_3$, KCL, $MgSO_4$, K_2HPO_4, pectin, yeast extract, and agar. The pH was adjusted to 7.0 (Mohandas et al., 2018). A pure culture was inoculated into the medium and incubated at 28°C for 5 days. After incubation, iodine-potassium iodide solution was added to check for the presence of a clearance zone (Venkata et al., 2013).

Quantitative screening of streptomyces isolates for pectinase production

Streptomyces isolates were screened for pectinase production using pectin broth medium. After incubation, the supernatant was used to evaluate pectinase activity.

Measurement of streptomyces growth

Streptomyces growth was measured in the culture filtrate using a UV spectrophotometer. The optical density at 530 nm was used for detection. Experiments were done in triplicate and averaged (UV-1650PC Spectrophotometric, SHIMADZD).

Enzyme assay

Pectinase activity was measured using the DNS method with 1.0% citrus pectin as a substrate. The assay used 0.5 ml of 1% pectin and 0.5 ml of culture filtrate, incubated at 40°C for 10 min. The resulting mixture was then boiled with 3,5-Dinitrosalicylic acid (DNS) for 5 min at 90°C. One unit of pectinase was defined as the amount of enzyme needed to produce 1 mole of galacturonic acid under standard conditions. The enzyme activity (U/mL) was calculated according to equation:

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

$$\text{Enzyme activity (U/mL)} = (\mu g \text{ galacturonic acid released} \times V) / V \times 194.1 \times t$$

The enzyme broth volume used in the assay is represented by "v", the molecular weight of galacturonic acid is 194.1, and the reaction time is measured in minutes as "t". One unit (U) is defined as 1 μmol of product released per minute. The relative activity of the enzyme is determined by calculating the percentage using the formula (Kadija et al., 2018; Roy et al., 2018):

$$\text{Relative activity} = \text{Activity of the sample} \times 100 / \text{Maximum activity of the sample}$$

Molecular characterization of the selected isolates

Genomic DNA was extracted from bacteria grown in Luria-Bertani medium. The bacterial culture was centrifuged, and the supernatant was collected. DNA extraction was performed using a Genomic DNA Purification kit. Two sets of primers were used: one for species identification and another to find a specific gene. Sanger sequencing was done at BGI in Hong Kong to analyze the 16S rRNA gene. The sequence was compared to the NCBI database using BLAST software. The obtained sequences were aligned in Ugene with the T-Caffee algorithm. A phylogenetic tree was created using iTOL, an interactive tree tool by Letunic and Bork.

Optimization of cultural conditions for pectinase production by streptomyces strain with high pectinolytic activity

The study aimed to optimize pectinase production by investigating the effects of various factors such as different media, yeast extract concentrations, pectin concentrations, temperature, pH, incubation period, and inoculum size. The *Streptomyces* isolate was tested in five different media: Mineral pectin medium (A): (g/l) Pectin 0.30; Sucrose 10.00; $KNO_3$0.60; $MgSO_4$0.25; $KH_2PO_4$1.00; $CaCl_2$0.10; $NaNO_3$2.00; K_2HPO_4 2.00; KCl 0.50; Yeast extract 1.00 at pH 7 and 15-20 agar (Kumar et al., 2012).

The Mineral pectin medium (B) was composed of g/l $NaNO_3$, 1; KCl, 1; $MgSO_4$, 0.50; K_2HPO_4, 1; pectin 10; Yeast extract 0.50 at pH 7 and 15-20 agar (Mohandas et al., 2018).

The Mineral pectin medium (C) was composed of (g/l); Pectin 4, Peptone, 0.5, Beef extract, 0.3; NaCL 0.5 at pH 7 and 15-20 agar (Chandra et al., 2016). The Mineral pectin medium (D) was composed of (g/l); Pectin, 5; $CaCO_3$ 2.0, $MgSO_4.7H_2O$, 1; K_2HPO_4, 1; NH_4SO_4, 2; Na_2SO_4, 2 at pH 7 and 15-20 agar.

Various yeast extract concentrations (0.5-3 g/l) and pectin concentrations (2.5-25 g/l) were then evaluated to determine the optimal conditions. Temperature (20-50°C), pH (5.0-9.0), incubation period (1-10 days), and inoculum size (1-10 ml) were also studied to maximize pectinase production. After selecting the best conditions, the *Streptomyces* isolate was inoculated (2 ml (5.4×10^4 CFU/ml) into the optimal medium and incubated for 7 days at 28°C with agitation at 120 rpm. The growth and pectinase activity were then measured to assess the effectiveness of the chosen parameters.

Purification and molecular weight determination of pectinase enzyme

The purification steps are following (Beulah et al., 2015).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Filtration

It was done using Filter paper and centrifugation is carried out for 15 min at 10,000 rpm.

Ammonium sulphate precipitation

The enzyme was precipitated with 80% ammonium sulfate and incubated overnight at 4°C. After centrifugation for 20 min at 5000 rpm, the resulting pellet was dissolved in T.E. buffer (pH 7.0) (Tripathi et al., 2014).

Dialysis

To remove salt impurities from the protein extract, the tube is suspended in water and incubated overnight (Beulah et al., 2015).

Ion exchange chromatography

The sample is loaded into the column. Tris HCl buffer (pH 7.5) is used to elute proteins. Fractions of 1.5 ml are collected after the void volume, and their absorbance is measured. Fractions with positive pectinase activity are saved and concentrated for further processing (Kashyap et al., 2000).

Gel filtration chromatography

The enzyme was dialyzed and loaded onto a Sephadex G-250 column. A 0.2 M phosphate buffer solution with a pH of 7.5 was used to equilibrate the column and elute the protein. The flow rate was 0.1 ml/min, resulting in the collection of ten fractions. Fractions with pectinase activity were isolated for further analysis (Tapre et al., 2014).

Characterization of the purified enzyme

The purified enzyme was characterized at various temperatures (30–80°C), pH levels (5–11), different substrate and enzyme concentrations. The substrate (pectin) was applied into the reaction mixture at different concentrations (1-6/ml in borate buffer at pH 7.0 and incubation temperature of 45°C for 30 min. The reaction mixture contained 0.5, 1, 1.5, 2, 2.5 and 3 ml, and the mixture was incubated at 45°C and pH 7 for 30 min.

Metal ions and inhibitors

All the ions and chemicals (Ca^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Ni^{2+}, Co^{2+}, Hg^{2+}, Fe^{2+}, EDTA) were added to the reaction mixture at a concentration of 1 mM to detect the activators or inhibitors on the enzyme activity. The pH was adjusted to 8.0, and incubation was done at 45°C for 30 min.

SDS PAGE

The molecular weight of the purified pectinase was determined using vertical gel electrophoresis with a 12% separating gel and a 5% stacking gel, with a low molecular weight protein used as a standard (Laemmli, 1970). Protein bands were visualized after staining with Coomassie Brilliant Blue R-250.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Application of pectinase in bioconversion of fruit waste into fertilizer

Sample collection

Fresh fruit waste from various fruits (banana, orange, sweet lime, pomegranate, guava, pineapple, and apple) was collected in sterile polythene bags from retail fruit outlets in Jeddah. The waste was then transported to the laboratory for immediate indigenous decomposition.

Decomposition studies

A plastic tube (24×25 cm) was used for decomposition studies. 5.0 kg of waste was filled in the tube and mixed gently. Effective Microorganism FMA18 (EM) was sprayed on the tubes after checking for pests and decomposition. The same tube content was used as a control without sprayed with organism. The treated tubes were covered with muslin cloth for aeration and to prevent pests. Every 5 days, microbial growth and decomposition were observed in the tubes. The compost was collected, sieved, and used for further study.

Evaluation of nutritional parameters of compost

The nutrient parameters of the compost obtained from waste decomposition were evaluated, including Nitrogen (N), Phosphorus (P), Potassium (K), organic carbon (TOC), Ca, Mg, and C: N ratio. Nutrient analysis was conducted on dried and finely dispersed compost samples.

Phytotoxicity evaluation

The study employed a pot culture experimental design to evaluate the phytotoxicity of various compost concentrations on Vigna mungo (black gram) plants.

Plant species: Vigna mungo (monocot); growth period: 90 days; growth medium: sandy soil, cultivated with Vigna mungo.

Four compost concentrations were tested: 10%, 20%, 30%, and 40%. A control group was also maintained for comparison. Only five growth parameters were assessed: root length, shoot length, no. of leaves, and moisture and dry weights. Measurements were taken at three distinct growth stages to evaluate the plant's response to different compost concentrations over time. The data collected allowed for a comprehensive analysis of the compost's effects on Vigna mungo's growth and development throughout the 90 days experimental period.

Statistical analysis

Statistical analyses were performed using the statistical Package for Social Science (SPSS for windows, version 16). The variability degree of the result is expressed as mean ± standard deviation (Mean ± SD). The significance of the difference between samples was determined using t-test. The difference was regarded significant when $P < 0.05$ and non-significant when $P > 0.05$.

In silico study

Structure prediction and validation

The 3D structure of *S. coelicolor* strain enzyme pectinase was predicted by AlphaFold (Jumper, et al., 2021). The preliminary structural evaluation of the predicted

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

protein models was conducted using ERRAT (Dym, et al., 2012) and PROCHECK (Laskowski et al., 1993) tools from the UCLA-DOE LAB – SAVES v6. For the ERRAT score, a good model typically exhibits an average overall quality factor of approximately 91%. Further quality assessment of the predicted models was performed using PROCHECK tool which generated the Ramachandran plots for both models. This comprehensive evaluation helped ensure the reliability and quality of the predicted protein structures.

Molecular docking

The predicted structure of the pectinase enzyme was prepared for the molecular by adding the polar hydrogen atoms in MGL tools the Kollman charges were computed. The protein was then converted to pdbqt format for docking. After protein preparation, the ligand (pectin) was prepared by optimizing its geometry using MMFF94 forcefield (Halgren et al., 1996). The prepared ligand was then docked to the protein and the binding affinities were calculated using Vina tool (Trott and Olson, 2010).

Molecular dynamics (MD) simulation

Desmond was used to perform MD simulations of selected compounds for 100 ns (Bowers et al., 2006). We performed Molecular Dynamics simulations to evaluate the stability of the protein and ligand complexes. Molecular Dynamics simulation was used to evaluate the stability of complexes after several stages, including preprocessing, optimization, and reduction. Minimization was done using the OPLS_2005 force field (Shivakumar et al., 2012). The compounds were solvated in a periodic box with a 10 Å size containing the TIP3P water molecules (Price and Brooks, 2014). Neutralization of the systems was done by adding counter ions and 0.15 M NaCl salt as needed to mimic physiological conditions. A pressure of 1 atm and a temperature of 300 K were set using the NPT ensemble. The systems went through a relaxing period before the simulation started. Trajectories were recorded and saved at 40 ps intervals during the simulation, allowing for a later study of the outcomes.

Results

Selection of pectinase producing actinomycetes

Nineteen isolates from Jeddah, Saudi Arabia were filamentous bacteria (*Streptomyces*) found on Mineral pectin agar medium B (*Table 1*). The different colored and shaped *Streptomyces* colonies displayed impressive growth and efficient degradation of pectin, their carbon source. The level of growth varied from high to moderate to poor. The most active isolate was chosen for further study.

Primary screening of pectinolytic bacteria and actinobacteria

Pectin agar medium was used to screen for pectinolytic *Streptomyces*. Nineteen isolates showed activity, forming clear zones on the medium indicating pectinase activity. Six isolates (FMA3, FMA5, FMA7, FMA10, FMA15, FMA18) exhibited the highest pectinolytic activity. These isolates were selected for further screening.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Pectinase production in liquid medium from Streptomyces

Pectinase was produced by *Streptomyces* after 5 days of growth at 30°C in Mineral pectin broth medium. Four out of nineteen *Streptomyces* isolates (FMA5, FMA9, FMA11, and FMA18) showed high pectinase production. Growth was measured using a spectrophotometer at A 540 nm.

Selective of most active streptomyces isolate for pectinase production

Streptomyces isolate FMA18 pectinase production was tested on Mineral Pectin Agar Medium (B) (*Fig. A1a, b*) The isolates were cultured on mineral pectin agar medium and starch nitrate agar medium for five days. The isolates capable of producing the enzyme and breaking down the pectin showed high growth and the formation of a clear zone around the colony (*Fig. A1C*).

Table 1. *Pectinase production on agar medium (mm) and in Mineral pectin broth medium (U/ml) by the different Streptomyces isolates*

Bacterial isolates	Growth on pectin agar medium	Enzyme activity	
		Diameter of clear zone (mm)	U/ml
FMA 1	+ + + +	28.00 ± 0.05	0.663 ± 0.03
FMA 2	+ + + +	21.33 ± 0.08	0.634 ± 0.03
FMA 3	+ + + +	33.00 ± 0.09	0.840 ± 0.01
FMA 4	+ + + +	29.67 ± 0.01	0.882 ± 0.04
FMA 5	+ + + +	35.00 ± 0.05	0.961 ± 0.05
FMA 6	+ + + +	22.00 ± 0.01	0.862 ± 0.05
FMA 7	+ + + +	36.33 ± 0.03	0.733 ± 0.06
FMA 8	+ + + +	24.33 ± 0.02	0.839 ± 0.04
FMA 9	+ + + +	27.33 ± 0.03	0.916 ± 0.03
FMA 10	+ + + +	39.67 ± 0.04	0.644 ± 0.02
FMA 11	+ + + +	23.00 ± 0.01	0.991 ± 0.04
FMA 12	+ + + +	26.00 ± 0.07	0.692 ± 0.03
FMA 13	+ + + +	19.67 ± 0.008	0.787 ± 0.05
FMA 14	+ + + +	28.33 ± 0.01	0.654 ± 0.06
FMA 15	+ +	35.33 ± 0.02	0.839 ± 0.05
FMA 16	+ + + +	26.33 ± 0.08	0.869 ± 0.03
FMA 17	+ +	13.33 ± 0.1	0.676 ± 0.04
FMA18	**+ + + +**	**41.33 ± 0.01**	**1.001 ± 0.03**
FMA 19	+ + + +	18.33 ± 0.02	0.663 ± 0.06

+: low growth. ++: moderate growth; +++: high growth; ++++: very high growth

Identification of the selected bacterium to the genus level

The gene sequences of isolate FMA18 were found to be comparable to other *Streptomyces* species in the database, confirming their classification as *Streptomyces.* The FMA18 isolate was found to have a 100% similarity to *Streptomyces coelicolor* when used as a query. As a result, it was proposed to be named *Str. coelicolor strain WA* (GenBank Accession Number PP837735.1). A neighbor-joining phylogenetic tree

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

was then created to determine its relationship with other *Streptomyces* strains by comparing partial 16S rRNA gene sequences (*Fig. 1*).

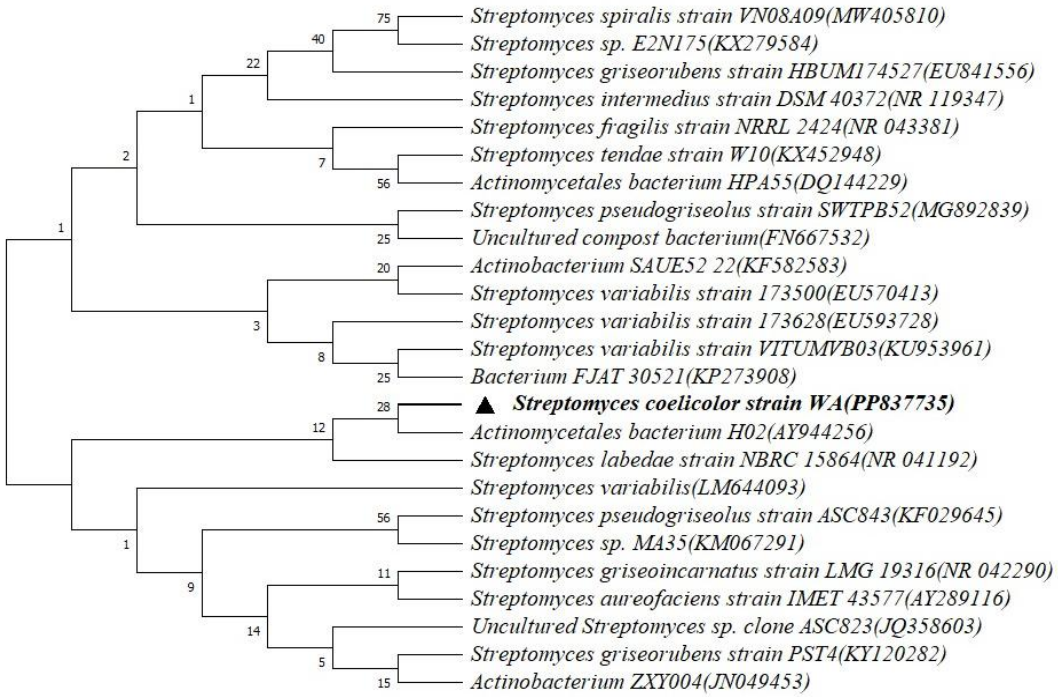


Figure 1. *Phylogenetic tree of Streptomyces coelicolor strain WA with accession number PP837735 and its closest relatives based on 16S rRNA sequence. The tree was created using the Neighbor-Joining method using the MEGA software version 11*

Factors affecting growth and pectinase production

Effect of different media on growth and pectinase production

Five different media were tested to study the impact of media on growth and pectinase production. *Str. coelicolor strain WA* was grown in Mineral pectin broth media with varying compositions and pectin as a carbon source. Results revealed that medium B supported the highest growth and maximum pectinase activity (1.051 U/ml). This medium was selected for further optimization of enzyme production under different conditions (*Table 2*).

Table 2. *Effect of different media on growth and pectinase production by Str. coelicolor strain WA*

Mineral pectin broth media	Pectinase activity U/ml
A	0.854 ± 0.194
B	1.051 ± 0.113*
C	1.005 ± 0.061
D	1.016 ± 0.139*
E	1.014 ± 0.075

*Significant results at $p < 0.05$

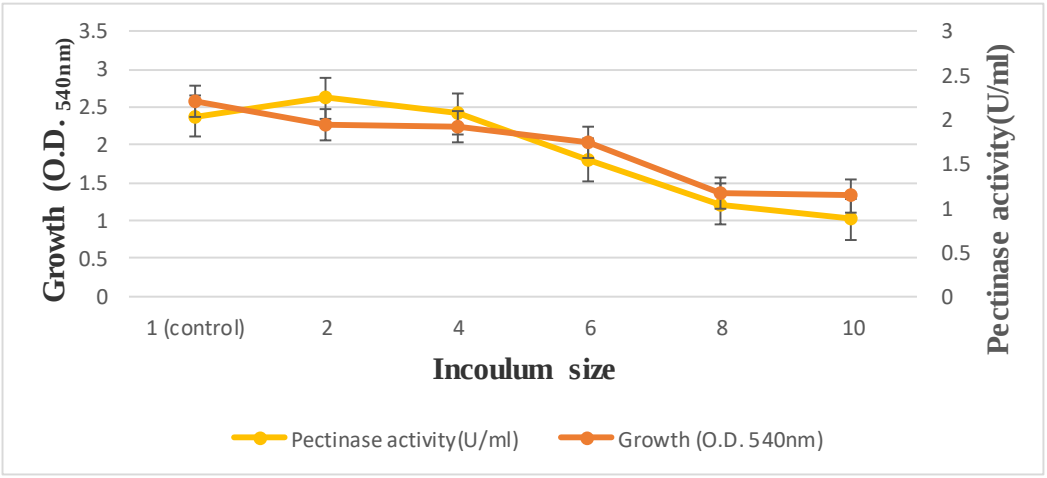
http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Effect of different inoculum sizes on growth and pectinase production

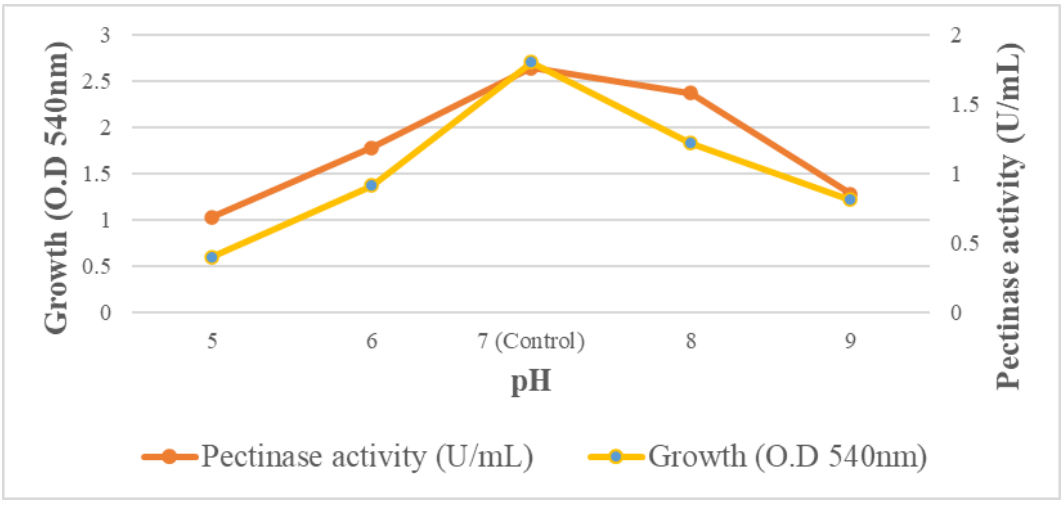
The impact of inoculum size on the growth and pectinase production of *Str. coelicolor strain WA* was examined. The highest pectinase activity recorded was 2.636 U/ml with an inoculum size of 2 × 107 CFU/ml (*Fig. 2A*).

Effect of different pH values on growth and pectinase production

The impact of pH values on growth and pectinase production with *Str. coelicolor strain WA* was investigated. The strain was cultivated in a medium with pH values from 5 to 10. The best growth and highest pectinase activity (2.643 U/ml) were seen at pH 7. (*Fig. 2B*).



A



B

Figure 2. *(A and B) Effect of different inoculum size and different concentration of pH value on growth and pectinase production by Str. coelicolor strain WA*

Effect different concentrations of pectin on growth and pectinase production

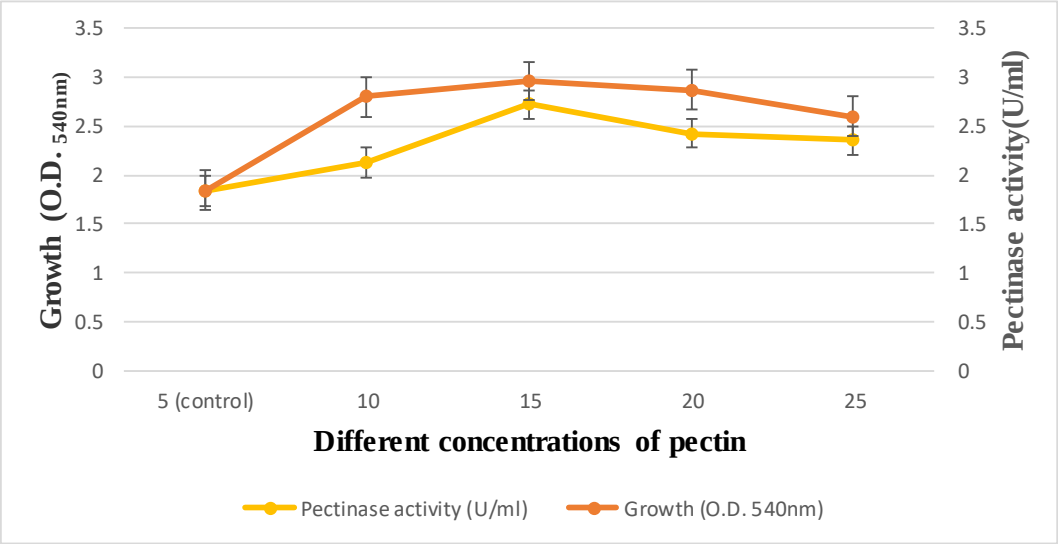
After determining optimal pH values, varying concentrations of pectin (5, 10, 15, 20, and 25 g/l) were added to the media. The flasks were incubated for 5 days at 30ºC with

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

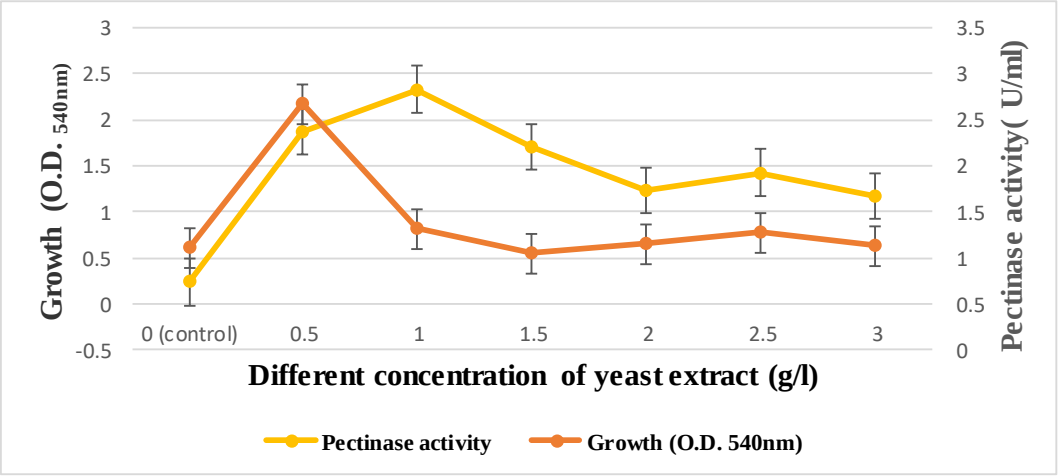
a rotation speed of 120 rpm for *Str. coelicolor*. Enzyme activity and growth were measured at 540 nm using a UV spectrophotometer after the incubation period. The highest pectinase activity (2.727 U/ml) was observed with a concentration of 15 g/l of pectin for *Str. coelicolor strain WA*. Therefore, 15 g/l of pectin was chosen for optimizing enzyme production under different cultural and nutritional conditions. (*Fig. 3A*).

Effect of different concentrations of yeast extract on growth and pectinase production

After determining optimal pectin concentrations, the impact of yeast extract concentration on growth and pectinase production was observed. *Str. coelicolor strain WA* showed the highest pectinase production (2.760 U/ml) with 1 g/l yeast extract. Therefore, yeast extract was included in the medium to optimize enzyme production under different cultural and nutritional conditions (*Fig. 3B*).



A



B

Figure 3. *(A and B) Effect of different concentrations of pectin and yeast extract on growth and pectinase production by Str. coelicolor strain WA*

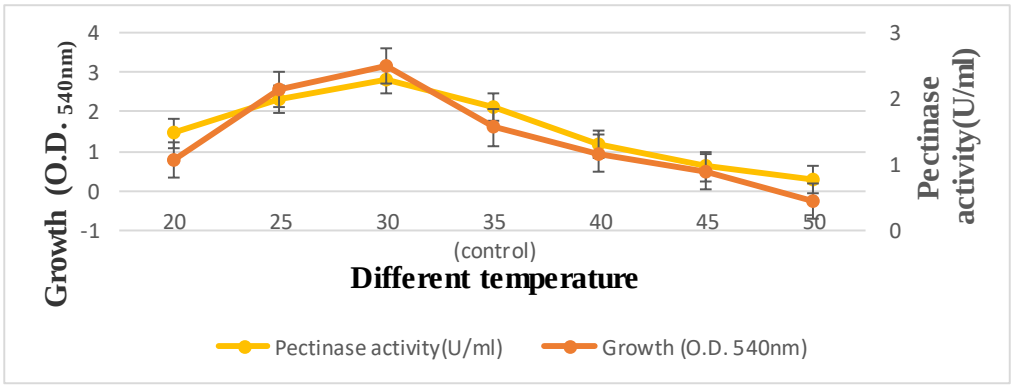
http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Effect of different incubation temperature on growth and pectinase production

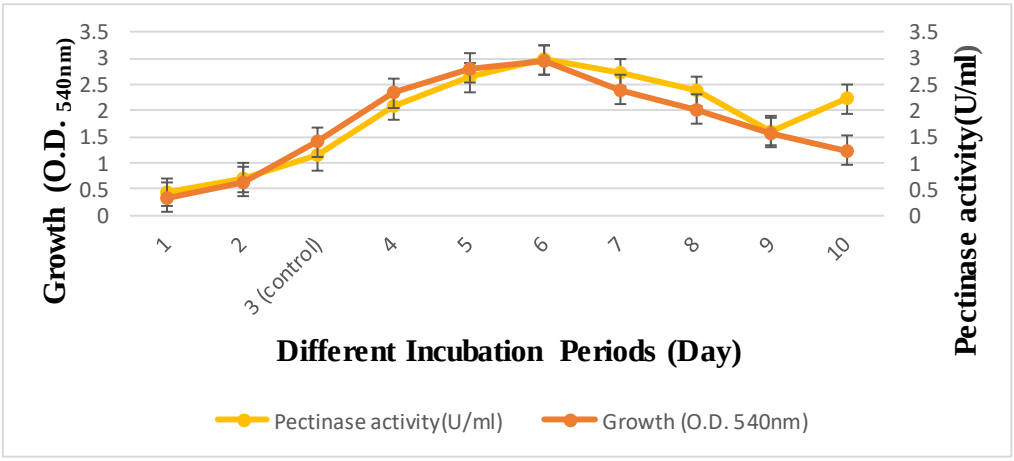
The effect of incubation temperature on the growth and enzyme production of *Str. coelicolor strain WA* was studied after determining the best yeast extract concentrations. Pectinase activity was measured in Mineral pectin broth medium at temperatures ranging from 25°C to 50°C after 5 days. The highest pectinase activity of 2.824 U/ml was observed at 30°C, which was also the optimal temperature for growth. The lowest pectinase activity of 0.273 U/ml was recorded at 50°C (*Fig. 4A*).

Effect of incubation periods on growth and pectinase production

The optimal incubation time for *Str. coelicolor strain WA* was determined to be 6 days, based on measurements of growth and pectinase production. This time period yielded the highest growth and maximum pectinase activity of 2.987 U/ml. Therefore, 6 days was chosen as the ideal duration for optimizing enzyme production in different cultural and nutritional conditions for *Str. coelicolor strain WA* (*Fig. 4B*).



A



B

Figure 4. *(A and B) Effect of different incubation temperature and incubation periods on growth and pectinase production by Str. coelicolor strain WA*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Purification of pectinase enzyme

Str. coelicolor strain WA was cultivated in pectin broth medium B at pH 7 for 6 days. The pectinase was purified using column chromatography, and the active fractions with pectinase activities were collected. Fractions 30-40 with high absorption at 280 nm and pectinase activities were lyophilized and stored. The lyophilized fractions were used to characterize the enzyme and determine its molecular weight. Finally, the enzyme was obtained, lyophilized, and examined (*Fig. A2*).

Molecular weight of pectinase

Str. coelicolor strain WA was cultivated under optimal conditions to produce pectinase. After precipitation with 80% ammonium sulfate and purification through column chromatography, the enzyme was characterized and its molecular weight determined to be 43 kDa using gel electrophoresis (*Fig. A3*).

The pectinase from *Str. coelicolor strain WA* was purified using several steps, including precipitation with ammonium sulphate, dialysis, and purification through DEAE-Sephacel and Sephadex G200 column. Pectinase activity and protein levels were measured at each stage. The enzyme was purified 2.86-fold after the Sephadex G100 step. Electrophoresis on 10% SDS-PAGE showed a single band, indicating complete purification of the enzyme. The size of the purified enzyme was determined to be 43 kDa using standard protein markers.

Using gel filtration and SDS-PAGE, respectively, the native and subunit molecular weights of the enzyme were ascertained. The pectinase's molecular weight, as determined by SDS-page analysis, was 43 kDa, which is quite similar to what earlier researchers on pectinases from *Streptomyces* spp. found.

Enzyme properties

After purifying the enzyme, its properties were studied. This included analyzing the effects of temperature, pH, enzyme and substrate concentrations, and heavy metal concentrations on enzyme activity.

Effect of temperature

The mixture was incubated at different temperatures (30-60°C) for 30 min to study the temperature's impact. Actinomycete enzyme activity increased as the temperature rose from 30 to 40°C. The optimal temperature for pectinase activity in *Str. coelicolor strain WA* was found to be 40°C, while the lowest activities were observed at 100°C (*Fig. A4*).

Effect of different pH

The impact of pH on enzyme activity was investigated using a reaction mixture containing 0.3 ml of enzyme preparation and substrate at pH levels ranging from 5 to 9. The optimal pH for the pectinase enzyme from *Str. coelicolor strain WA* was found to be 7, while the lowest activities were observed at pH 5 and pH 9 (*Table 3*).

Effect of different enzyme concentrations on pectinase activity

The impact of different enzyme concentrations on pectinase activity was studied at 40°C. The reaction mixture was incubated with varying enzyme concentrations (0.25,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

0.5, 1, 1.5, and 2 U/ml). Results showed that as the enzyme concentration increased from 0.25 to 2 U/ml, the enzyme activity gradually decreased. Statistical analysis confirmed a significant effect of enzyme concentration on activity, with the highest activity observed at a concentration of 0.25 U/ml (relative activity %). The lowest activities were recorded at the 2 ml concentration (*Table 4*).

Table 3. *Effect of different pH on the crude Pectinase obtained from the Str. coelicolor strain WA*

Different pH value	Pectinase activity U/ml	Relative activity (%)
5	0.744 ± 0.005	**43.00**
6	1.086 ± 0.007	**62.77**
7 (control)	1.730 ± 0.005	**100**
8	1.373 ± 0.003	**79.39***
9	0.548 ± 0.004	**31.67**

*Significant results at $p < 0.05$

Table 4. *Effect of different enzyme and Substrate concentration on the crude pectinase obtained from the Str. coelicolor strain WA*

Enzyme and substrate concentration	Enzyme concentration		Substrate concentration	
	Pectinase activity (U/ml)	Relative activity (%)	Pectinase activity (U/ml)	Relative activity (%)
0.25	1.841 ± 0.007	100*	0.952	**80.78**
0.5	1.571 ± 0.007	85.33*	1.207	**100***
1	0.910 ± 0.008	49.42	1.156	**95.77***
1.5	0.785 ± 0.016	42.64	0.775	**64.21**
2	0.649 ± 0.004	35.25	0.517	**42.83**

*Significant results at $p < 0.05$

Effect of different substrate concentrations

The impact of substrate concentrations on pectinase activity was studied. Different substrate concentrations (0.25, 0.5, 1, 1.5, and 2 ml) were tested at 40°C. The pectinase activity of *Str. coelicolor strain WA* decreased as substrate concentration increased from 0.025 to 2 ml. Statistical analysis showed a significant effect of substrate concentration on enzyme activity, especially at a concentration of 0.5 ml. Results are in *Table 4* (100% relative activity).

Effect of metal ions on pectinase activity

The impact of heavy metal ions on enzyme activity was investigated by preparing a reaction mixture. Various heavy metal ions (Ni^{++}, Zn^{++}, Cu^{++}, Ca^{++}, Fe^{++}, Hg^{++}, Mn^{++}, and $EDTA^{++}$) at a concentration of 1 mM were added to the mixture. The activity of the heavy metals was measured and the results showed that Mn^{++} and Ca^{++} enhanced the enzyme activity of *Str. coelicolor* FMA18, while Hg^{++} and EDTA had the lowest activities (*Table 5*).

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Application of pectinase

Bioconversion of fruit waste into fertilizer using Str. coelicolor strain WA

The fruit waste decomposed when inoculated with *Str. coelicolor strain WA*. Within five days, the waste degraded as *Str. coelicolor strain WA* grew. This rapid degradation caused significant changes in color, texture, and volume of the compost. After 90 days, the fruit waste completely disintegrated, with a ¾ reduction in volume. In the experiment, 210 organic carbons were detected, compared to 23 in the control (*Fig. 5*). The composted fruit waste had a C:N ratio of 9.13 (*Table A1*).

Table 5. *Effect of different heavy metal ions on the crude pectinase obtained from the Str. coelicolor strain WA*

Metal ions (1 mM)	Pectinase activity (U/ml)	Relative activity (%)
Ni^{++}	1.351±0.018	60.66
Cu^{++}	1.003 ± 0.044	45.03
Zn^{++}	0.978 ± 0.063	43.91
Mn^{++}	1.944 ± 0.035	87.29*
$EDTA^{++}$	0.444 ± 0.033	19.93
Fe^{++}	1.254 ± 0.034	56.30
Hg^{++}	0.324 ± 0.033	14.54
Ca^{++}	2.227 ± 0.036	100*

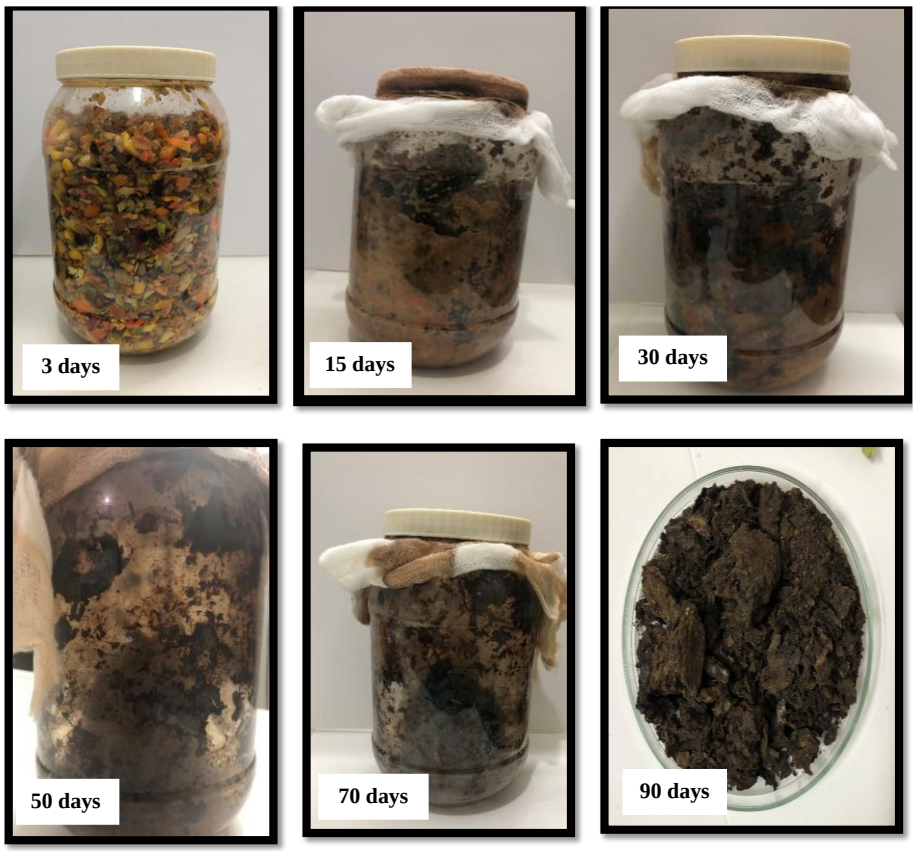


Figure 5. *Bioconversion of fruit wastes into fertilizer using Str. coelicolor strain WA*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Compost phytotoxicity test

The obtained results were presented in *Table A2.*

Effect of compost on plant growth parameters of Vigna mungo

After 80 days, the effectiveness of compost on plant growth was evaluated. Vigna mungo plants treated with experimental compost showed better growth compared to control plants. The combination of vegetable waste from *Str. coelicolor strain WA* increased shoot and root length in seedlings. The root length of control plants was 4.3 cm, while it reached 11.133 cm in plants treated with 40% compost made from *Str. coelicolor strain WA* ($p < 0.0001$). Shoot length increased from 14.033 cm to 14.333 cm in plants treated with 10% compost after sprouting ($p < 0.001$). The total plant weight increased by approximately 15% with the compost made from *Str. coelicolor strain WA* fruit waste. Overall, the compost enriched with *Str. coelicolor strain WA* significantly promoted plant growth, as supported by statistical significance (*Table A3*).

In silico study

Molecular protein docking binding results with pectinase

Structure validation

The quality of 3D pectinase protein predicted by AlphaFold was validated by calculating the ERRAT quality factor and by observing the Ramachandran plots (*Fig. 6a*). A good model usually shows an average overall quality factor of about 91% for the ERRAT score. The ERRAT quality factor of the predicted structure was 92.16% (*Fig. 6b*). Similarly, the Ramachandran plot of the protein showed that all residues were in the favored region while no residue was observed in disallowed region (*Fig. 6c*).

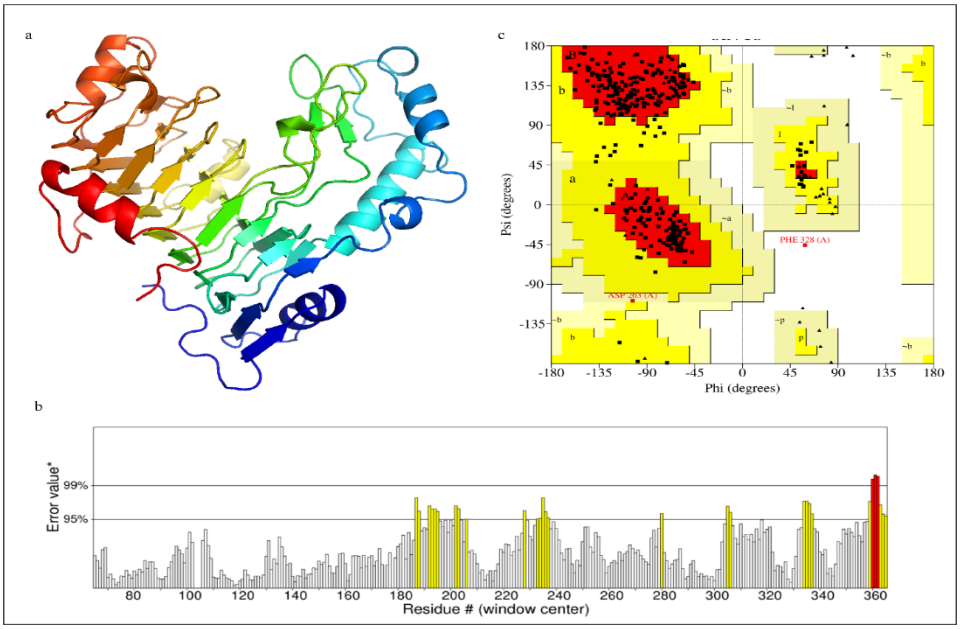


Figure 6. *(a) The predicted model of the pectinase enzyme. (b) The ERRAT quality factor of the predicted protein. (c) The Ramachandran plot of protein. The yellow region shows the allowed region while the white region shows the disallowed regions*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Molecular docking

The molecular docking pectin and pectinase was performed by using Autodock Vina. There was a total of 10 poses generated and the binding pose with highest binding affinity was selected for further analysis. The binding affinity of the selected pose was -5.6 kcal/mol. The binding pose of the selected compound was analyzed and it was observed that pectin occupied the space in the binding pocket of the protein (*Fig. 7a*). Further, the molecular interactions were analyzed by using Discovery studio. Upon analysis, it was observed that pectin made six hydrogen bonds with Ser249, Glu284, Asp248, Asp243, Ser341, and Asn318 (*Fig. 7b*).

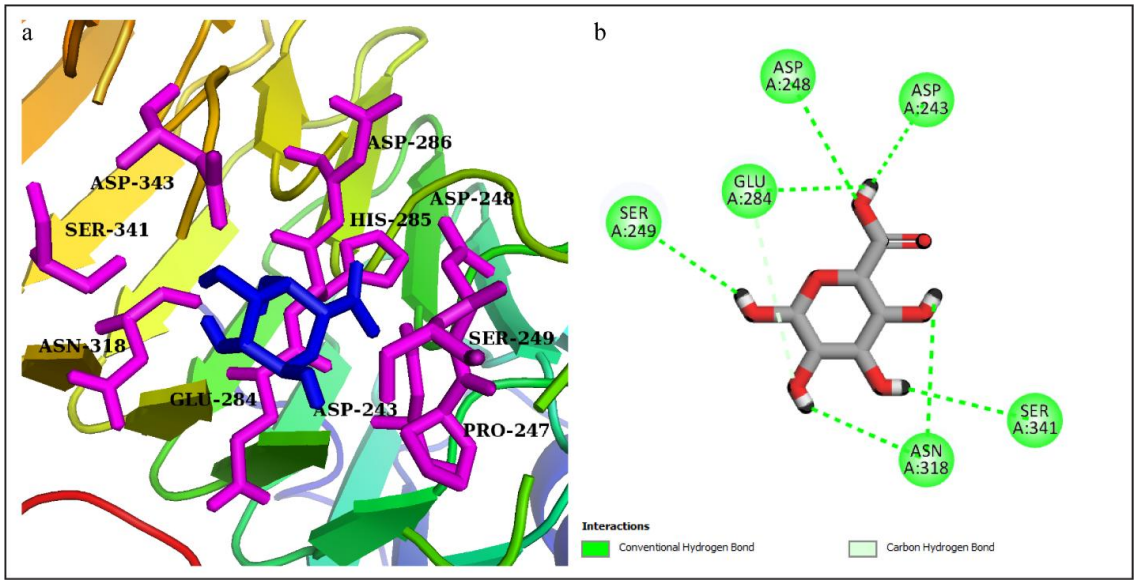


Figure 7. *(a) The plausible binding mode of the pectin inside the pectinase binding pocket. (b) The molecular interactions of the pectin with pectinase enzyme*

Molecular dynamics (MD) simulation

Root-mean-square deviation (RMSD)

To confirm the stability of the protein-ligand complex, Molecular Dynamics (MD) simulation of 100 ns was employed. The RMSD of the carbon alpha (C) atoms was calculated in order to look into the complex' deviation and general structural changes during the simulation (Sargsyan et al., 2017). The RMSD values of the pectinase protein remained in the range of 2 Å till 50 ns and showed deviations of up to 3.2 Å in second half of the simulation. However, the RMSD dropped to 2.4 Å towards the end of simulation. While the RMSD of pectin remained less than 0.5 Å throughout the simulation (*Fig. 8*).

Root mean square fluctuations (RMSF)

RMSF values have been calculated in order to identify the fluctuation of the protein while they are bound to the pectin (Martínez, 2015). RMSF values give detailed information on the residue's mobility and flexibility. Based on the RMSF values, most of the residues did not show the fluctuations while some residues ranging from 30 to 40, 60 to 90, 145 to 160, 180 to 200, and 230 to 250 shows fluctuations i.e., RMSF values

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

greater than 2 Å. While all other residues remained under 2 Å indicating that the protein did not undergo confirmational changes during simulation (*Fig. 9*).

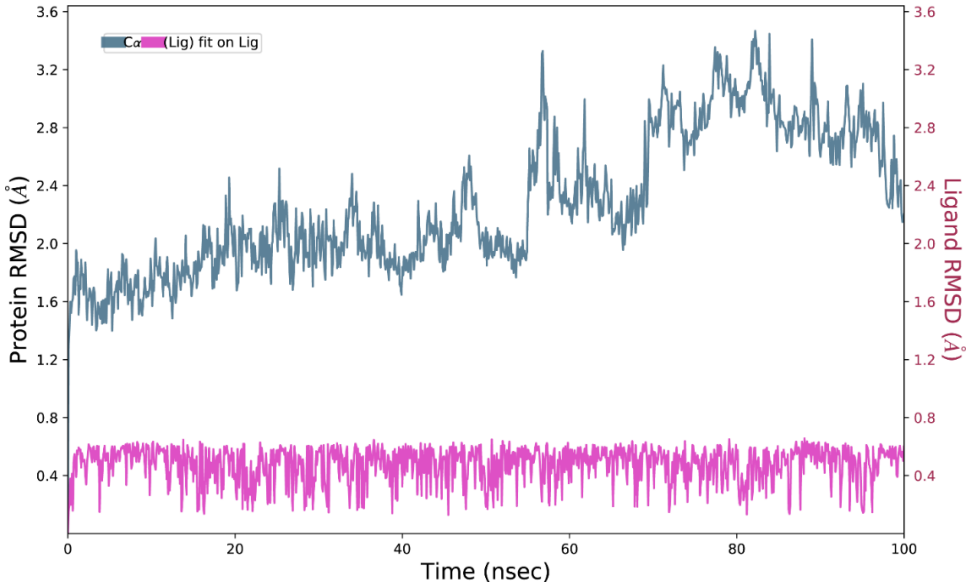


Figure 8. *The RMSD of pectinase-pectin complex calculated during 100 ns simulation. Blue color shows the RMSD of pectinase while magenta shows the RMSD of pectin*

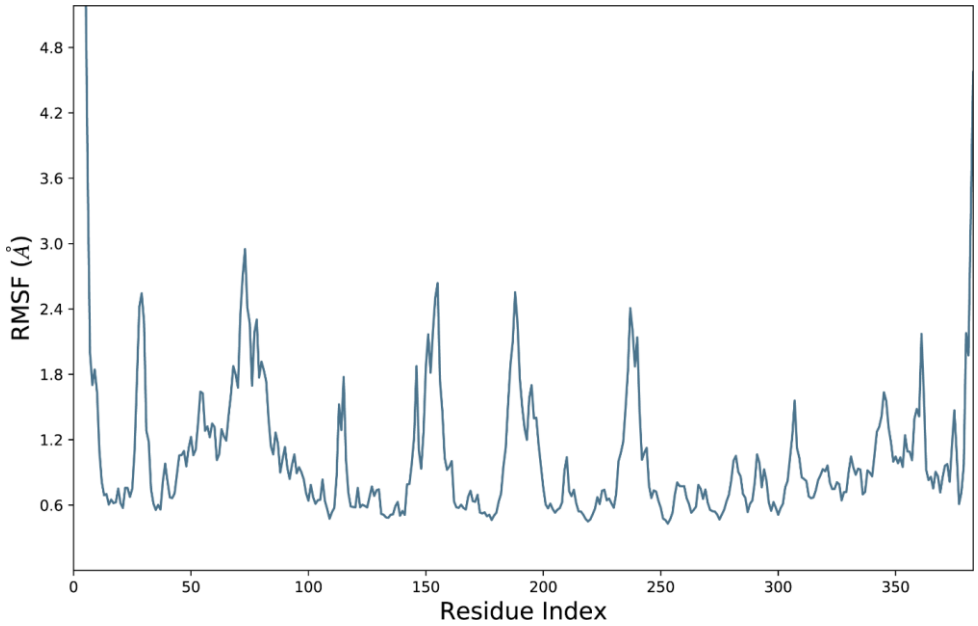


Figure 9. *The residual fluctuations of the pectinase receptor upon binding of the pectin*

Protein-ligand contacts

The MD Simulation analysis showed that ionic, hydrogen, and hydrophobic bonds were the most important types of interactions between the ligands and the protein. The functional properties of the protein-ligand complex are stabilized and regulated by these interactions. Residues that form hydrogen bonds with Pectin were Arg158, Lys161,

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Gln185, Lys187, Asp237, Asp248, Glu284, Asn318, Asp343, Lys376, and Arg444. Arg158, Lys161, Asp248, and Glu284 were also involved in ionic interactions with pectin (*Fig. 10*). These hydrogen bonding interactions, which were displayed during the MD simulations, not only highlighted the specific residues that were crucial for stabilizing the protein-ligand complex, but they also provided insight into the crucial interactions that maintain the complexes' general stability and binding affinity.

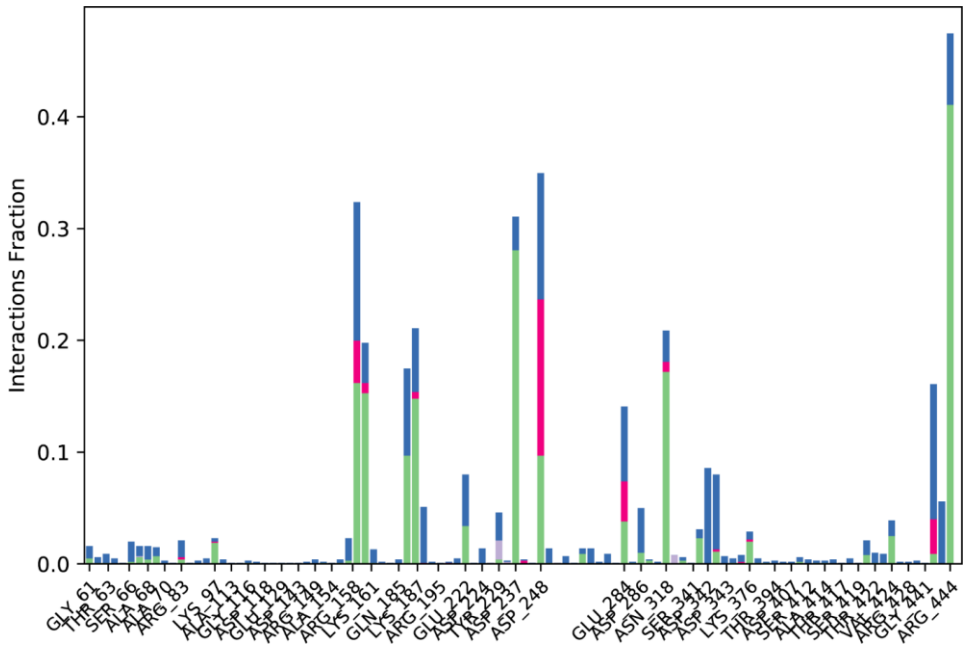


Figure 10. *The interaction of protein-ligand during MDS. The residues that interact are shown as tall, stacked bars. Hydrogen bonding is represented by green bars, hydrophobic interactions by grey bars, and water bridges by blue bars*

Discussion

Population growth and industrialization have caused a surge in pollution, raising global concerns due to its harmful effects on humans, animals, and plants. Microbial biodiversity is crucial for various reasons, including its aesthetic appeal and utility in biotechnology, specifically in the production of enzymes for feed and fuel. The agricultural and fruit processing industries generate a significant amount of waste, leading to pollution and waste management challenges. Untreated waste can contribute to greenhouse gas emissions, water, and soil pollution. Millions of tons of fruit and vegetable waste (FVW) and agricultural waste (AW) are produced annually, rich in organic polysaccharide components. Converting FVW and AW into value-added products is essential. The polysaccharides in FVW and AW can serve as nutrients for microbes, enabling the production of industrially important enzymes like amylase and pectinase through microbial bioprocessing of organic waste (Panda et al., 2016; Sagar et al., 2018; Anand et al., 2020).

Fruits and vegetables contain pectin, which helps pectinolytic isolates grow (Anuradha et al., 2010). Fruits and vegetables are great sources of pectinolytic isolates, including exopolygalacturase produced by various fungi and yeasts. (Birgisson et al., 2003), These microbes grow slowly. The study aims to find fast-growing *Streptomyces* strains that can produce lots of polygalacturonase to meet the nation's needs. It is

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

crucial to discover beneficial microorganisms using innovative methods to meet industry demands and boost national demand.

In this study, we isolated 19 *Streptomyces* from different sources, including soil and decomposing fruits and vegetables. Isolating and screening Streptomyces strains is important for efficient and cost-effective pectinase production. The sequential dilution approach was used to recover various actinomycetes strains. (Adeleke et al., 2012). Spot inoculation was used to screen for pectinase-producing *Streptomyces*. Pectin hydrolysis screening was done on agar plates with pectin. Addition of potassium iodide solution showed a hydrolysis zone, indicating effective degradation of pectin by bacteria-produced pectinase, preventing iodine complex formation. Takcı and Turkmen (2016) A direct relationship was found between hydrolysis area size and pectinase activity, with larger areas indicating higher activity. *Streptomyces* isolates in our study showed hydrolysis zones ranging from 18.33 mm to 41.33 mm.

After selection, pectinolytic isolates from *Streptomyces* were further screened using the well diffusion method. Four strains were chosen for their ability to depolymerize pectin and form a colorless hydrolytic zone. The maximum zone size observed after flooding the plate with potassium iodide solution was reported to be 41.33 mm by Janani et al. (2011) and Rashmi et al. (2008). The producers lacked pectinolytic activity and distinct lysis zones. *Streptomyces sp.* was the main isolate based on its morphological characteristics.

A grape peel isolate of *Streptomyces* species produces a high amount of polygalacturonase. The strain with the highest pectinase activity is identified as *Str. coelicolor strain WA.*

Different kinds of pectinolytic enzymes have been produced by a variety of microorganisms (Jayani et al., 2010). 25% of all food and industrial enzyme sales worldwide are made up of microbial pectinases, and this market is growing daily (Murad and Azzaz, 2011). A significant factor in the breakdown of plant residues is actinomycete.

Actinomycetes are efficient decomposers of plant material and produce extracellular enzymes such as cellulase, xylanase, and pectinase. Due to spore dispersal, *Streptomyces* species are distributed globally among antimicrobial populations (Babu et al., 2008). *Streptomyces* species are the source of approximately 80% of the metabolites produced by actinomycetes (Watve et al., 2001).

Selecting the appropriate fermentation media is crucial for achieving high enzyme yields as it delivers the nutrients and energy required for microbial growth. This study examined five different fermentation media for pectinase synthesis. The B medium exhibited the highest pectinase productivity for *Str. coelicolor strain WA* (1.051 U/ml). This could be attributed to the components of the B medium promoting optimal growth of *Streptomyces.* The presence of pectin in the medium provided sufficient nutrients to support microbial growth, leading to the maximum enzyme production (Balkan and Ertan, 2007).

The optimal inoculum size for producing pectinase in *Str. coelicolor strain WA* is 2 × 107 (U/ml), resulting in a maximal enzyme activity of 2.636 U/ml. larger inoculum sizes hinder growth and enzyme production due to excessive spore collection and competition for nutrients. On the other hand, low inoculum densities may not be enough to initiate pectinase production and promote growth (Mrudula and Anitharaj, 2011; Jacob and Prema, 2008).

The pH level is a crucial factor for pectinase synthesis. Ramesh and Lonsane (1990) found that pH greatly affects the production of extracellular enzymes by microbes. They

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

tested pH values ranging from 3 to 9 to determine the ideal pH for pectinase production by *Str. coelicolor strain WA*. The highest enzyme yield was achieved at pH 7.0.

The explanation could be that the bacteria may survive under ordinary conditions, and any changes to the media's pH have a significant impact on the enzyme's activity (Turner, 2010). pH values higher or lower than ideal cause the enzyme to become inactive and cease production (Amadioha, 1993). Similar results were shown by Ramanujam and Subramani (2008), who found that a pH of 7 was ideal for *A. niger* to produce pectinase. Adeleke et al. (2012), however, indicated that the ideal pH range for pectinase synthesis was 5-5.5.

After incubating for 5 days, *Str. coelicolor strain WA* showed growth on liquid and solid media with pectin as the only carbon source. It also secreted pectolytic enzymes, as evidenced by the presence of pectinolysis zones around colonies. The strain reached maximum enzyme activity in the liquid culture medium after 6 days at 30°C. Ladjama et al. (2007) found similar results, suggesting that 5 or 6 days of incubation at 28°C is optimal for *Streptomyces* to produce high levels of pectinase in liquid culture media.

The incubation temperature affects the growth and enzyme synthesis of *Streptomyces*. In this study, enzyme synthesis was tested at temperatures from 20°C to 50°C. *Str. coelicolor strain WA* produced the most enzymes at 30°C (2.824 U/ml). At 50°C, minimal pectinase was generated, possibly due to enzyme denaturation at higher temperatures (Thangaratham and Manimegalai, 2014). Lower temperatures result in slower bacterial growth and reduced enzyme production by actinomycetes (Bailey and Pessa, 1990).

The pH and temperature of the culture medium impact enzyme production. Different organisms can produce pectinase under varying pH and temperature conditions. A pH range of 6.0-10 was studied to understand its effect on pectinase synthesis (Stanbury et al., 1997).

The incubation period is a crucial factor in the fermentation process. It is influenced by the type of microbe, the chemical composition of the medium, and other physiological factors. In this study, the incubation period was varied from one day to ten days to observe its impact on pectinase production by *Str. coelicolor strain WA*. Pectinase estimation was done every 24 h. The findings revealed that the highest pectinase production (2.987 U/ml) by *Str. coelicolor strain WA* occurred after 6 days.

As the incubation period lengthens, enzyme synthesis decreases due to depleted nutrition and accumulated metabolites in the medium (Palaniyappan et al., 2009). Our findings contradict those of Sethi et al. (2015), a study proposed that a fermentation period of 96 h is ideal for pectinase synthesis. A shorter period would increase the strain's industrial importance and advantages. Thus, our findings hold greater significance compared to previous studies.

Mn^{++} and Ca^{++}ions led to an increase in the pectinase activity. Ni²z ions moderately, whereas EDTA ions strongly inhibited its activity. Ca^{++} ions reportedly play a vital role in pectinase confirmation maintenance (Alana et al., 1990). The discrepancy in the pectinase metal ion preference indicated that the enzyme might have differential flexibility at the active site (Oumer et al., 2017). The stability of enzymes in the presence of agents promotes their use in various industries. Previous studies have shown that pectinases remain stable even in the presence of surfactants (Beg et al., 2000).

The pectinase from *Str. coelicolor strain WA* was purified using several steps, including precipitation with ammonium sulphate, dialysis, and purification through DEAE-Sephacel and Sephadex G200 column. Pectinase activity and protein levels were measured at each stage. The enzyme was purified 2.86-fold after the Sephadex G100 step. Electrophoresis on 10% SDS-PAGE showed a single band, indicating complete

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

purification of the enzyme. The size of the purified enzyme was determined to be 43 kDa using standard protein markers.

Using gel filtration and SDS-PAGE, respectively, the native and subunit molecular weights of the enzyme were ascertained. The pectinase's molecular weight, as determined by SDS-page analysis, was 43 kDa, which is quite similar to what earlier researchers on pectinases from *Streptomyces* spp. found. Pectinase originating from microorganisms is typically found in the 30-70 kDa size range (Klug-Santner et al., 2006; Tari et al., 2008; Banu et al., 2010). Nevertheless, studies have reported pectinases from microorganisms with molecular weights beyond 70 kDa (Kashyap et al., 2000; Arotupin et al., 2012). The enzyme's molecular weight of 43 kDa, as determined by the subunit molecular weight, has been proposed to be that of a monomer compared to its natural molecular weight. It has been reported that most microbial pectinases have a monomeric structure (Polizeli et al., 1991; Schols et al., 2009).

At 50°C, the maximal activity of the isolated pectinase from *Str. coelicolor strain WA* was observed. Pectinase from *Bacillus subtilis* (Al-Ajlani et al., 2012) and pectinase from other sources (Silva et al., 2002; Phutela et al., 2005; Yadav et al., 2009) have both been shown to have a comparable optimal temperature for action. Most bacteria's optimal temperature range for pectinase is between 45 and 60°C (Silva et al., 2002; Phutela et al., 2005; Yadav et al., 2009; Al-Ajlani et al., 2012).

One of the main factors in waste biodegradation is microbial populations. The amount of organic matter available and the physicochemical conditions during composting affect the microbial load and variety. This study explored the bacterial population, enzymatic potential, and the impact of abiotic factors on their community during fruit waste composting. Composting fruit waste with *Str. coelicolor strain WA* resulted in brown color and volume reduction after two weeks. The compost had a carbon-to-nitrogen ratio of 9.13, showing the effectiveness of *Str. coelicolor strain WA* in breaking down fruit waste.

Various research groups have already used fruit and vegetable waste to compost Karnchanawong and Nissaikla (2014). Organic waste was used to create compost with microbial consortia, and the quality of composting was enhanced by using mature compost as a starting material. Mature compost contains various microbial consortia, including *Streptomyces*. In a specific study, the Actinomycetes consortium alone was used to compost domestic agro-residues, while mature compost was utilized to treat pulp-paper mill effluent. (Limaye et al., 2017).

Conclusion

Pectinase enzymes are a cost-effective and environmentally friendly alternative to chemicals in many industries. In this study, extracellular pectinase was produced using *Str. coelicolor strain WA* isolated from agricultural soil in Jeddah, Saudi Arabia. The strain showed maximum pectinase activity after 6 days of growth at pH 7.0 and 30°C. After purification, the pectinase was characterized and its molecular weight determined. Utilizing pectinolytic *Str. coelicolor strain WA* for fruit waste degradation and biofertilizer production is a sustainable approach for environmental remediation and promoting plant growth. Overall, pectinase enzymes offer a sustainable and versatile solution for various industrial and agricultural processes.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Availability of data and material. The nucleotide sequence of the chromosome and plasmids of *Streptomyces coelicolor strain WA* have been deposited in GenBank under accession number PP837735.1. All raw data and supporting materials related to this paper can be obtained from the corresponding author.

REFERENCES

[1] Adeleke, A. J., Odunfa, S. A., Olanbiwonninu, A., Owoseni, M. C. (2012): Production of cellulase and pectinase from orange peels by fungi. – Nat and Sci. 10(5): 107-12. http://www.sciencepub.net/nature.

[2] Al-Ajlani, M. M., Ahmad, Z., Hasnain, S. (2012): Pectinase activity of wild-type Bacillus subtilis isolated from Pakistan. – Biotechnol Res. 1(2): 7-12.

[3] Alana, A., Alkorta, I., Dominguez, J. B., Llama, M. J., Serra, J. L. (1990): Pectin lyase activity in a *Penicillium italicum* strain. – Appl Environ Microbiol. 56: 3755-3759. https://doi.org/10.1128/aem.56.12.3755-3759.1990.

[4] Amadioha, A. C. (1993): Production of cellulolytic enzymes by *Rhizopus oryzae* in culture and Rhizopus-infected tissues of potato tubers. – Mycologia 85: 574-578. https://doi.org/10.1080/00275514.1993.12026310.

[5] Anand, G., Yadav, S., Gupta, R., Yadav, D. (2020): Pectinases: From Microbes to Industries. – In: Chowdhary, P. et al. (eds.) Microorganisms for Sustainable Environment and Health. Elsevier, Amsterdam, pp. 287-313. https://doi.org/10.1016/b978-0-12-819001-2.00014-0.

[6] Anuradha, K., Padma, P. N., Venkateshwar, S., Reddy, G. (2010): Fungal isolates from natural pectic substrates for polygalacturonase and multienzyme production. – Ind J Microbiol. 50: 339-344. https://doi.org/10.1007/s12088-010-0054-5.

[7] Arotupin, D. J., Akinyosoye, F. A., Onifade, A. K. (2012): Purification, characterization and application of polygalacturonase from *Aspergillus niger* CSTRF. – Malaysian J of Microbiol. 8(3): 175-183.

[8] Babu, A. S., Stach, J. E. M., Goodfellow, M. (2008): Genetic and phenotypic evidence for *Streptomyces griseus* ecovars isolated from a beach and dune sand system. – Antonie Leeuwenhoek 94: 63-74. https://doi.org/10.1007/s10482-008-9246-y.

[9] Bailey, M. J., Pessa, E. (1990): Strain and process for production of polygalacturonase. – Enz and Microb Technol. 12(4): 266-271. https://doi.org/10.1016/0141-0229(90)90098-B.

[10] Balkan, B., Ertan, F. (2007): Production of α-amylase from *Penicillium chrysogenum* under solid-state fermentation by using some agricultural by-products. – Food Technol and Biotechnol. 45(4): 439-432. https://hrcak.srce.hr/23996.

[11] Banu, A. R., Devi, M. K., Gnanaprabhal, G. R., Pradeep, B. V., Palaniswamy, M. (2010): Production and characterization of pectinase enzyme from *Penicillium chrysogenum*. – Ind J of Sci and Technol. 3(4): 377-81.

[12] Beg, Q. K., Bhushan, B., Kapoor, M., Hoondal, G. S. (2000): Production and characterization of thermostable xylanase and pectinase from a *Streptomyces* sp. QG-11-3. – J of Indust Microbiol and Biotechnol 24: 396-402. https://doi.org/10.1038/sj.jim.7000010.

[13] Beulah, D., Sunitha, E. M., Srilakshmi, T. (2015): Production, purification and assay of pectinase enzyme from *Aspergillus niger*. – Helix 1: 673-U45.

[14] Birgisson, H., Delgado, O., García Arroyo, L., Hatti-Kaul, R., Mattiasson, B. (2003): Cold-adapted yeasts as producers of cold-active polygalacturonases. – Extremophiles 7: 185-93. https://doi.org/10.1007/s00792-002-0310-7.

[15] Bowers, K. J., Chow, E., Xu, H., Dror, R. O., Eastwood, M. P., Gregersen, B. A., Klepeis, J. L., Kolossvary, I., Moraes, M. A., Sacerdoti, F. D., Salmon, J. K. (2006):

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

Scalable algorithms for molecular dynamics simulations on commodity clusters. – Proceedings of the 2006 ACM/IEEE Conference on Supercomputing Nov 11.

[16] Bruhlmann, F. (1995): Purification and characterization of an extracellular pectate lyase from an *Amylocota* sp. – Appl Environ Microbiol. 61: 3580-3589. https://doi.org/10.1128/aem.61.10.3580-3585.1995.

[17] Chandra Reddy, M. P., Saritha, K. V. (2016): Effects of the culture media optimization on pectinase production by *Enterobacter* sp. PSTB-1. – 3 Biotech. 6(2): 207.

[18] Dym, O., Eisenberg, D., Yeates, T. O. (2012): Examples: detection of errors in structures. – International Tables for Crystallography Vol. F. ch. 21.3, pp. 679-682

[19] Halgren, T. A. (1996): Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. – J. Comput. Chem. 17(5-6): 490-519. https://doi.org/10.1002/(SICI)1096-987X(199604)17: 5/6 < 490::AID-JCC1 > 3.0.CO;2-P.

[20] Jacob, N., Prema, P. (2008): Novel process for the simultaneous extraction and degumming of banana fibers under solid-state cultivation. – Braz J Microbiol. 39: 115-21. https://doi.org/10.1590/S1517-83822008000100025.

[21] Janani, L., Kumar, G., Rao, B. (2011): Screening of pectinase producing microorganisms from agricultural waste dump soil. – Asian J Biochem Pharm Res. 2(1): 329-37.

[22] Javed, R., Nawaz, A., Munir, M., Hanif, M., Mukhtar, H., Haq, I. U., Abdullah, R. (2018): Extraction, purification and industrial applications of pectinase: a review. – J Biotechnol Biores. 1(1).

[23] Jayani, R. S., Shukla, S. K., Gupta, R. (2010): Screening of bacterial strains for polygalacturonase activity: its production by *Bacillus sphaericus* (MTCC 7542). – Enz Res. 5. https://doi: 10.4061/2010/306785.306785.

[24] Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A. (2021): Highly accurate protein structure prediction with AlphaFold. – Nature 596(7873): 583-9. https://doi.org/10.1038/s41586-021-03819-2.

[25] Kadija, Tul Kubra, S. A., Manam Walait, H. S. (2018): Potential applications of pectinases in food, agricultural and environmental sectors. – Journal of Pharmaceutical, Chemical and Biological Sciences 6(2): 23-34.

[26] Karnchanawong, S., Nissaikla, S. (2014): Effects of microbial inoculation on composting of household organic waste using passive aeration bin. – Int J Recycl Org Waste Agricult 3: 113-119. https://doi.org/10.1007/s40093-014-0072-0.

[27] Kashyap, D. R., Chandra, S., Kaul, A., Tewari, R. (2000): Production, purification and characterization of pectinase from a Bacillus sp. DT7. – World J Microbiol and Biotechnol. 16: 277-82. https://doi.org/10.1023/A: 1008902107929.

[28] Klug-Santner, B. G., Schnitzhofer, W., Vršanská, M., Weber, J., Agrawal, P. B., Nierstrasz, V. A., Guebitz, G. M. (2006): Purification and characterization of a new bioscouring pectate lyase from *Bacillus pumilus* BK2. – J Biotechnol. 121(3): 390-401. https://doi.org/10.1016/j.jbiotec.2005.07.019.

[29] Kumar, P., Suneetha, V. (2015): Pectinases from actinomycetes: a thorough study. – Int J Chem Tech Res. 8(7): 345-50.

[30] Ladjama, A., Taibi, Z., Meddour, A. (2007): Production of pectinolytic enzymes using *Streptomyces* strains isolated from palm grove soil in Biskra area (Algeria). – Afr Crop Sci Conf Proc. 8: 1155-58.

[31] Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. – Nature 227(5259): 680-685. https://doi.org/10.1038/227680a0.

[32] Laskowski, R. A., MacArthur, M. W., Moss, D. S., Thornton, J. M. (1993): PROCHECK: a program to check the stereochemical quality of protein structures. – Journal of Applied Crystallography. 26(2): 283-91. https://doi.org/10.1107/S0021889892009944.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

[33] Limaye, L., Patil, R., Ranadive, P., Kamath, G. (2017): Application of potent actinomycete strains for bio-degradation of domestic agro-waste by composting and treatment of pulp-paper mill effluent. – Adv Microbiol. 7(1): 94-108 https://doi.org/10.4236/aim.2017.71008.
[34] Martínez, L. (2015): Automatic identification of mobile and rigid substructures in molecular dynamics simulations and fractional structural fluctuation analysis. – PloS ONE 10(3): e0119264. https://doi.org/10.1371/journal.pone.0119264.
[35] Mohandas, A., Raveendran, S., Parameswaran, B., Abraham, A., Athira, R. S., Mathew, A. K., Pandey, A. (2018): Production of pectinase from *Bacillus sonorensis* MPTD1. – Food Technol Biotechnol. 56(1): 110. https:// doi: 10.17113/ftb.56.01.18.5477.
[36] Mrudula, S., Anitharaj, R. (2011): Pectinase production in solid state fermentation by *Aspergillus niger* using orange peel as substrate. – Global J Biotechnol Biochem 6(2): 64-71.
[37] Murad, H. A., Azzaz, H. H. (2011): Microbial pectinases and ruminant nutrition. – Research Journal of Microbiology 6(3): 246-269. DOI: 10.3923/jm.2011.246.269.
[38] Oumer, O. J., Abate, D. (2017): Characterization of pectinase from *Bacillus subtilis* strain Btk 27 and its potential application in removal of mucilage from coffee beans. – Enz Res. 2017(1): 7686904.
[39] Palaniyappan, M., Vijayagopal, V., Viswanathan, R., Viruthagiri, T. (2009): Screening of natural substrates and optimization of operating variables on the production of pectinase by submerged fermentation using *Aspergillus niger* MTCC 281. – Afr J Biotechnol. 8(4): 682-686.
[40] Panda, S. K., Mishra, S. S., Kayitesi, E., Ray, R. C. (2016): Microbial-processing of fruit and vegetable wastes for production of vital enzymes and organic acids: biotechnology and scopes. – Environ Res. 146: 161-72. https: doi: 10.1016/j.envres.2015.12.035. Epub 2016 Jan 4. PMID: 26761593.
[41] Phutela, U., Dhuna, V., Sandhu, S., Chadha, B. S. (2005): Pectinase and polygalacturonase production by a thermophilic *Aspergillus fumigatus* isolated from decomposting orange peels. – Braz J Microbiol. 36: 63-69. https://doi.org/10.1590/S1517-83822005000100013.
[42] Polizeli, M. D., Jorge, J. A., Terenzi, H. F. (1991): Pectinase production by Neurospora crassa: purification and biochemical characterization of extracellular polygalacturonase activity. – Microbiol. 137(8): 1815-23. https://doi.org/10.1099/00221287-137-8-1815 .
[43] Prathyusha, K., Suneetha, V. (2011): Bacterial pectinases and their potent biotechnological application in fruit processing/juice production industry: a review. – J Phytol. May 17;3(6): 16-19.
[44] Price, D. J., Brooks III, C. L. (2004): A modified TIP3P water potential for simulation with Ewald summation. – The Journal of Chemical Physics 121(20): 10096-10103. https://doi.org/10.1063/1.1808117.
[45] Ramesh, M. V., Lonsane, B. K. (1990): Characteristics and Novel Features of Thermostable α-Amylase Produced by *Bacillus licheniformis* M27 under Solid State Fermentation. – Starch-Stärke 42(6): 233-8. https://doi.org/10.1002/star.19900420608.
[46] Rashmi, R., Murthy, K. R., Sneha, G., Shabana, S., Syama, A., Radhika, V. (2008): Partial purification and biochemical characterization of extracellular pectinase from *Aspergillus niger* isolated from groundnut seeds. – J. Appl. Biosci. 9(1): 378-84.
[47] Roy, K., Dey, S., Uddin, M. K., Barua, R., Hossain, M. T. (2018): Extracellular pectinase from a novel bacterium *Chryseobacterium indologenes* strain SD and its application in fruit juice clarification. – Enzyme Research 2018(1): 3859752. https://doi.org/10.1155/2018/3859752.
[48] Sagar, N. A., Pareek, S., Sharma, S., Yahia, E. M., Lobo, M. G. (2018): Fruit and vegetable waste: bioactive compounds, their extraction, and possible utilization. – Compr. Rev. Food Sci. Food Saf. 17: 512-531. https://doi: 10.1111/1541-4337.12330.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

[49] Sargsyan, K., Grauffel, C., Lim, C. (2017): How molecular size impacts RMSD applications in molecular dynamics simulations. – Journal of Chemical Theory and Computation 13(4): 1518-24. https://doi.org/10.1021/acs.jctc.7b00028.
[50] Schols, H. A., Visser, R. G., Voragen, A. G. (eds.) (2009): Pectins and Pectinases. – Wageningen Academic Publishers, Wageningen, pp 101-135.
[51] Sethi, B. K., Panda, B., Sahoo, S. L. (2015): Biosynthesis of industrially relevant extracellular pectinase from *Aspergillus terreus* NCFT 4269.10 using orange peels as substrate. – Asian J Biotechol 7(1): 13-22. https://doi.org/10.3923/ajbkr.2015.13.22.
[52] Shivakumar, D., Harder, E., Damm, W., Friesner, R. A., Sherman, W. (2012): Improving the prediction of absolute solvation free energies using the next generation OPLS force field. – Journal of Chemical Theory and Computation 8(8): 2553-8. https://doi.org/10.1021/ct300203w.
[53] Silva, D., Martins, E. D., Silva, R. D., Gomes, E. (2002): Pectinase production by *Penicillium viridicatum* RFC3 by solid state fermentation using agricultural wastes and agro-industrial by-products. – Brazi J Microbiol. 33: 318-24. https://doi.org/10.1590/S1517-83822002000400008.
[54] Stanbury, P. F., Whitaker, A. and Hall, S. J. (1997): Principles of Fermentation Technology. 2nd Ed. – Aditya Books (P) Ltd, New Delhi, pp. 93-122.
[55] Tapre, A. R., Jain, R. K. (2014): Pectinases: enzymes for fruit processing industry. – Int Food Res J. 21(2): 447.
[56] Tari, C., Dogan, N., Gogus, N. (2008): Biochemical and thermal characterization of crude exo-polygalacturonase produced by *Aspergillus sojae*. – Food Chem. 111(4): 824-829. https://doi.org/10.1016/j.foodchem.2008.04.056
[57] Thangaratham, T., Manimegalai, G. (2014): Optimization and production of pectinase using agro waste by solid state and submerged fermentation. – Int J Curr Microbiol Appl Sci. 3(9): 357-65.
[58] Tripathi, G. D., Zoya, J., Singh, A. K. (2014): Pectinase production and purification from *Bacillus subtilis* isolated from soil. – Advances in Applied Science Research 5(1): 103-105.
[59] Trott, O., Olson, A. J. (2010): Auto Dock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. – J Comput Chem. 31(2): 455-61. https://doi.org/10.1002/jcc.21334.
[60] Turner, B. L. (2010): Variation in pH optima of hydrolytic enzyme activities in tropical rain forest soils. – Appl and Environ Microbiol. 76(19): 6485-6493.
[61] Venkata, E., Naga, Rajuand, Divakar, D. (2013): Production of pectinase by using *Bacillus circulans* isolated from dump yards of vegetable wastes. – International Journal of Pharmaceutical Science and Research 4(7): 2615-2622. DOI: 10.13040/IJPSR.0975-8232.4(7).2615-22.
[62] Watve, M. G., Tickoo, R., Jog, M. M., Bhole, B. D. (2001): How many antibiotics are produced by the genus Streptomyces? – Arch Microbiol. 176: 386-390. https://doi.org/10.1007/s002030100345.
[63] Xu, Z., Cen, Y. K., Zou, S. P., Xue, Y. P., Zheng, Y. G. (2020): Recent advances in the improvement of enzyme thermostability by structure modification. – Crit Rev Biotechnol. 40(1): 83-98. https://doi.org/10.1080/07388551.2019.1682963
[64] Yadav, S., Yadav, P. K., Yadav, D., Yadav, K. D. (2009): Purification and characterization of pectin lyase produced by *Aspergillus terricola* and its application in retting of natural fibers. – Appl Biochem and Biotechnol. 159: 270-283. https://doi.org/10.1007/s12010-008-8471-1.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

APPENDIX

Table A1. *Quantification of major elements in compost using Str. coelicolor strain WA*

Elements	Units (mg/g)
Physical properties	
Color	Black
Moisture	18.33
Chemical properties	
pH	7.67
TOC	210
N	23
P	36.8
K	377
Mg	31.4
Ca	132.5
C:N ratio	9.13

Table A2. *Compost phytotoxicity test obtained from Str. coelicolor strain WA*

Elements	Units (mg/g)
Physical properties	
Color	Black
Moisture	18.33
Chemical properties	
pH	7.67
TOC	210

Table A3. *Effect of compost on growth parameters of Vigna mungo after treatment by Str. coelicolor strain WA*

Compost concentration	Plant growth parameters				
	Moisture weight (%)	Dry weight (gm)	Root length (cm)	Shoot length (cm)	No. of leaves
Control	2.4	0.523	4.3	14.033	6.333
10%	4.1	0.596	6.6	14.333	12
20%	4.366	0.683	7.133	15.066	12
30%	6.766	1	7.666	15.6	13.333
40%	15.366*	1.24*	11.133*	18.533*	21.666*

*Significant results at $p < 0.05$

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

(A) (B) (C)

Figure A1. *(A, B and C). Growth of Str. coelicolor strain WA on: A. Pectin agar medium, B. Starch casein nitrate, C: Zone of pectin hydrolysis on pectin agar medium after 5 days' incubation*

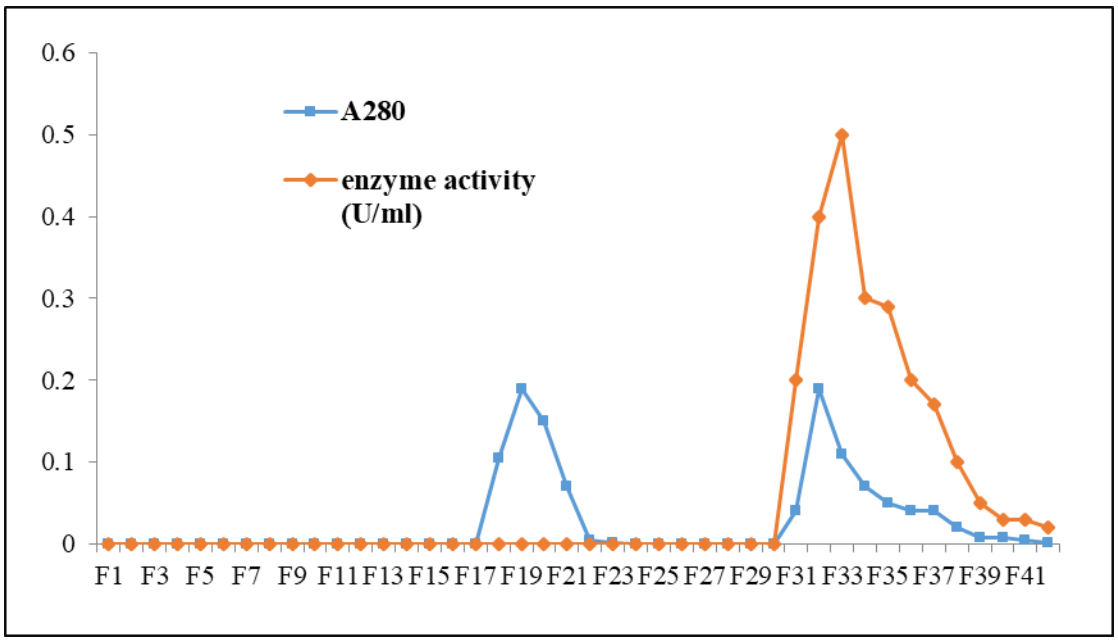


Figure A2. *Elution profile of pectinase after sephadex G-100 chromatography*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

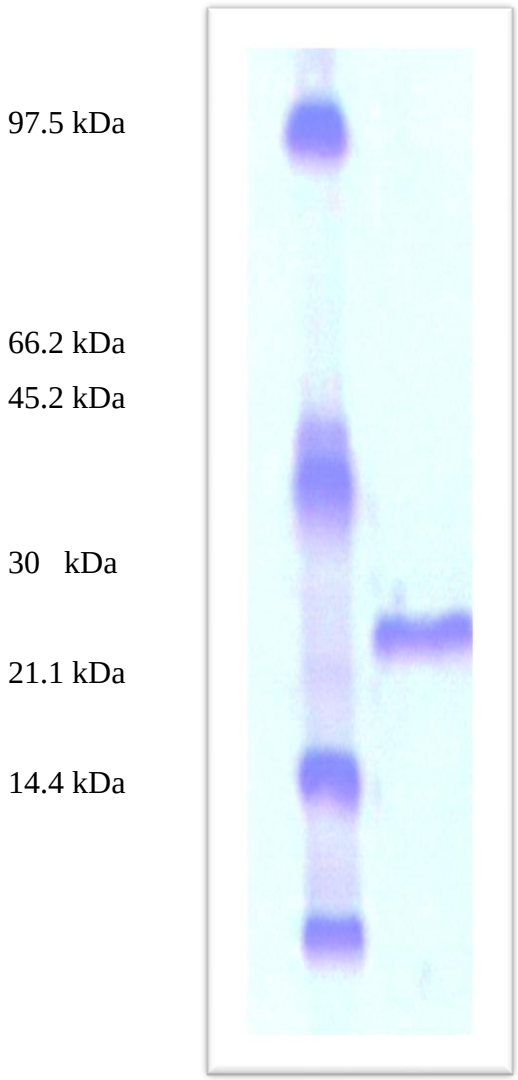


Figure A3. *Molecular weight of pecinase isolated from Str. coelicolor strain WA. by SDS-PAGE, M: protein standards employed were phosphorylase (97kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa)*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483

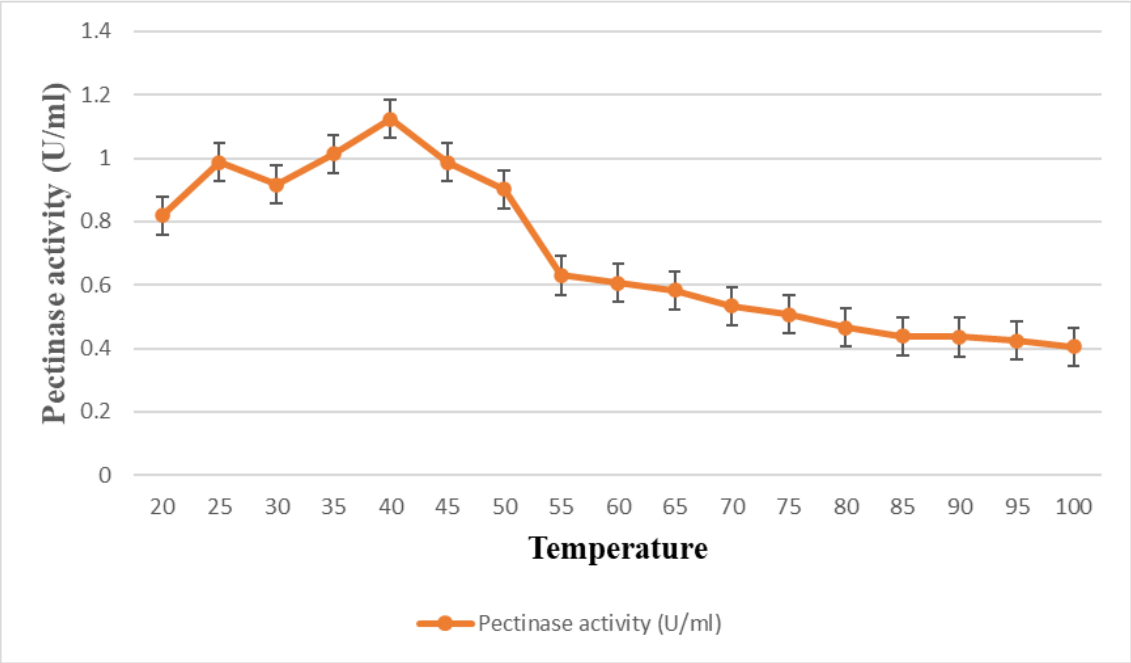


Figure A4. *Effect of different incubation temperature on the crude pectinase activity obtained from the Str. coelicolor strain WA*

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_24552483