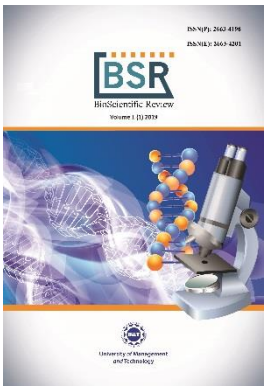


BioScientific Review (BSR)

Volume No.1, Issue No. 3, 2019
ISSN(P): 2663-4198 ISSN(E): 2663-4201
Journal DOI: https://doi.org/10.32350/BSR
Issue DOI: https://doi.org/10.32350/BSR.0103
Homepage: https://ssc.umt.edu.pk/Biosci/Home.aspx

Journal QR Code:

Article: **Extracellular Production of Pectinase from Bacteria Isolated from Rotten Apples from Lahore, Pakistan**

Author(s): Fatima Amanat
Amna Yaqoob
Asif Ali
Muhammad Sajjad

Article DOI: https://doi.org/10.32350/BSR.0103.05

Article QR Code:

Fatima Amnat/M Sajjad

To cite this article: Amanat F, Yaqoob A, Ali A, Sajjad M. Extracellular production of pectinase from bacteria isolated from rotten apples from Lahore, Pakistan. *BioSci Rev.* 2019;1(3):36–44.
Crossref

A publication of the
Department of Life Sciences, School of Science
University of Management and Technology, Lahore, Pakistan

Extracellular Production of Pectinase from Bacteria Isolated from Rotten Apples from Lahore, Pakistan

Fatima Amanat[1], Amna Yaqoob[1], Asif Ali[1], Muhammad Sajjad[1,2*]

[1]Institute of Molecular Biology and Biotechnology, The University of Lahore, Lahore Pakistan
[2]School of Biological Sciences, University of the Punjab, Lahore Pakistan

***Corresponding author:** m_sajjad_khattak@yahoo.com

Abstract

Pectins are intricate blends of polysaccharides which make up around 33% of plant cell wall. Despite of their presence in the greater part of plant body and in other sources, commercial production of pectin is extremely difficult. This is a systematic study that aimed to produce pectinase from bacterial species isolated from rotten apple samples. Zymography and enzyme assay through DNS method were performed to check the pectinolytic activity of bacteria isolated from rotten apple samples. Of all five bacterial species (*Serratia marcescens*, *Klebseilla pneumoniea*, *Pseudomonas aeruginosa* and *Escherichia coli*) maximum enzyme concentration was showed in *P. aeruginosa* and it was 6.2 U/mL. The major achievement of this study was to screen out the most efficient pectinases producing isolate of *S. marcescens* from rotten apples that has never been reported to produce pectinase, previously.

Keywords: Enzyme production, pectinase, rotten apples

1. Introduction

Pectinolytic compounds are of noteworthy significance in the era of biotechnology due to their widely inclusive applications in natural product juice extraction, scouring of cotton, degumming of plant filaments, waste water treatment, vegetable oil extraction, tea and coffee fermentation, blanching of paper, manufacturing of poultry feed, mixed drinks, food industries and in pharmacy as anticancer agents. Pectins are intricate blends of polysaccharides which make up around 33% of the cell wall of the dry substances of plants. They are important polysaccharides due to their increased commercial demand. They are also being used as gelling agents because of their ability to form viscoelastic solutions under optimum conditions [1]. At present, commercial pectins exclusively extracted from apple pomace and citrus peel are used in juice production. In apple pomace and citrus peel, pectin concentration is 10-15% and 20-30%, respectively [2]. Pectinases are also used in food processing. Since the use of non-biodegradable packaging is not ecofriendly; therefore, the use of organic polymers in packaging is preferred. A blend of pectinases and amylases is used to filter fruit juices because it reduces the filtration time up to half [3]. The treatment of fruit pulp with pectinases increases the yield of fruit juice from banana, grapes and apples [4]. Pectinases in combination with different catalysts such as cellulases, arabinases and xylanases are utilized to build the squeezing capacity of different fruits, especially sugar beet pulp, for juice extraction [5]. Apple juice contains a high concentration of unfiltered pulp with a cloudy appearance that is later centrifuged to expel coarse particles. Afterwards,

through enzymatic treatment filtered clear or amber colored juice is formed [6]. Pectinases which can depolymerize exceptionally esterified pectin is the proteins utilized in the processing of apple juice. The mixture of cellulase or pectinase accounts for the production of 100% pure juice. Starch is also a potential supporter of cloudiness and unripe apple may contain 15% of starch. The addition of amylase optimizes pH and consequently, pectinase gives a filtered yield of apple juice [7]. Pectinases are depolymerizing pectins that bring down the cationic request of pectin solution during paper making [8]. Microbial alkaline pectinases have a variety of other commercial applications such as textile processing, waste water treatment, and mineralization of the environmental traces of pectin [9]. All these commercial benefits of pectinases necessitate the development of methods to increase its high quality yield. This is a systematic study and a part of it has already been completed with the isolation and screening of bacterial isolates from rotten apples. The current study intends to optimize the parameters utilized for the maximum production of pectinase from the bacteria isolated from rotten apples.

2. Material and Methods

This study was sequenced after the isolation and screening of bacterial isolates obtained from rotten apple samples (method described by Amanat et al. (2020)).

The production of crude enzyme was carried out by preparing the inoculum of 5mL LB broth culture at shaking condition for 24 hours at 37°C. At that point, the production medium was set up in all five flasks marked as A, B, C, D, and E, respectively. After that 5mL LB broth culture was inoculated and incubated in shaking condition for 24 hrs. at 37°C. Afterwards, the media was set at 6500rpm at 4°C for 20 minutes to get cell free supernatant. Since the enzyme produced was extracellular, so the supernatant was collected as crude enzyme and stored at 4°C till further processing. The extracellular action of the crude enzyme was checked through enzyme assay utilizing DNS technique and enzyme plate assay.

2.1. Enzyme Assay by Dinitro-salicylic Acid (DNS) Method

The activity of enzyme was estimated by calculating the amount of reducing sugars released in extracellular environment using dDinitrosalicylic Acid (DNS) reagent assay [10].

2.2. Preparation of the Standard Graph

In order to check the activity of the crude enzyme, a standard graph of glucose was plotted. For standard graph, two stock solutions were prepared and labelled as stock solution SS (A) and SS (B). SS (A) was prepared by adding 10mg glucose in 10ml water (mg/ml). SS (B) was prepared by adding 2ml of SS (A) in 18ml dH_2O. From SS (B), further dilutions were prepared for 1ml solution. All dilutions were placed in the boiling water bath for 15 minutes and then cooled at room temperature. Dilution 1 was considered as blank for all sample readings. The absorbance of each sample was noted at 540nm and a standard graph was plotted.

2.3. Pectinase Assay

After plotting the standard curve graph, pectinase assay of samples containing the crude enzyme was performed. For this purpose, solution was prepared in five different test tubes by adding 0.5ml of each supernatant sample and 0.5ml of 1% pectin. This solution was incubated at 37°C for 2-3 hours. Then, reaction mixture was prepared by adding 100µl of solution

A, 900µl dH_2O, and 3mL of DNS reagent in every test tube. Test tubes were placed in the boiling water bath for 15 minutes and then the reading at 540nm was noted.

2.4. Pectinase Plate Assay for Pectinolytic Activity of Isolates

Pectinase plate assay is also called well plate assay. In this assay, wells are created in the petri plate containing MS medium and then filled with supernatant samples. Using this method, we were able to quantify the amount of reducing sugars according to the size of the clear zone around the wells or holes to identify the enzyme activity.

2.5. SDS-PAGE

SDS-PAGE was performed following the protocol described by Smith (1984) [11].

2.6. Zymography

Gel obtained after SDS-PAGE was washed with phosphate buffer containing 10% isopropanol for 1 hour. After 1 hour, the gel was again washed with phosphate buffer of pH 6 for 30-40 minutes. Then, it was placed in a plate containing phosphate buffer, 1% pectin, and 1.5% agar for 4-5 hours in incubation at 37°C. The researchers took out the gel and stored it at 4°C. The agar media plate was then stained with iodine for 30 minutes and the bands were visualized.

3. Results

3.1. Enzyme Assay

Pectinase assay was performed to measure the pectinolytic activity of bacterial isolates. In order to depict the concentrations of pectinase activity, a glucose standard curve was plotted.

Different dilutions with known concentration of glucose were prepared along with DNS reagent and then the absorbance of these dilutions was measured in spectrophotometer. From the observed readings of absorbance, a standard curve or graph was plotted shown in Figure 1.

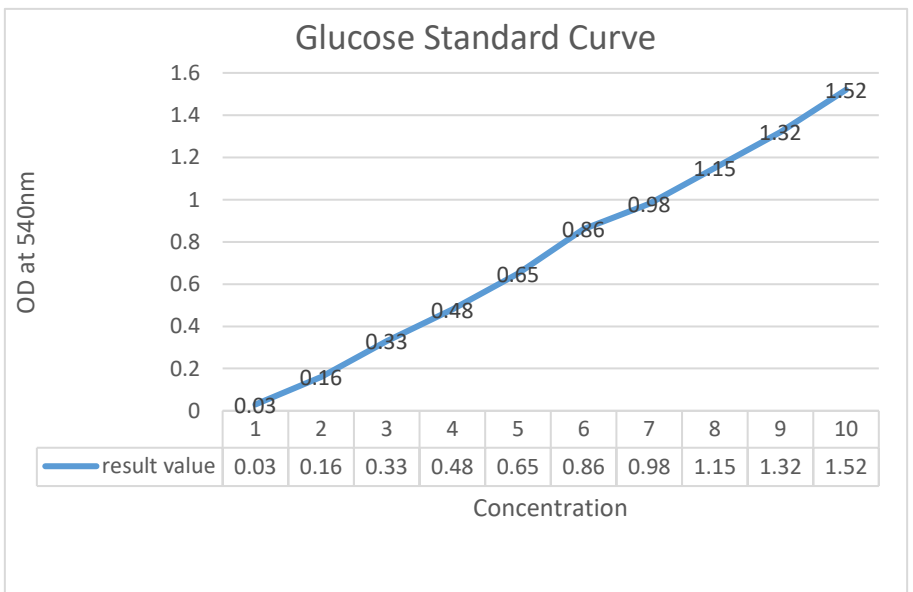
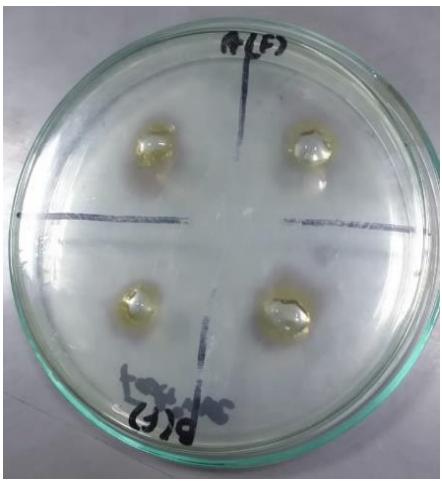


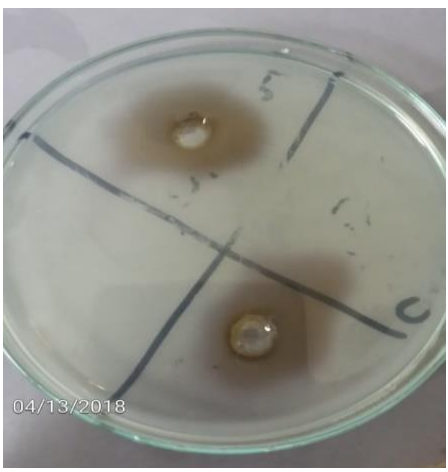
Figure 1. Glucose standard curve

3.2. The Determination of Pectinase Activity by Well Plate Method

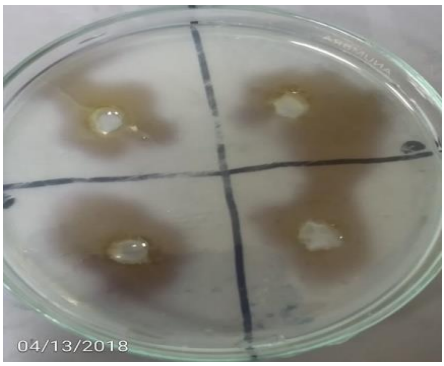
Wells of same sizes were created on plates containing modified MS medium of 1% pectin. They were filled with enzyme supernatant and incubated for 24 hours. After incubation and iodine staining, there was no formation of clear zones around the wells which showed that the reducing sugar was not able to hydrolyze their substrates and it indicated the negative results of pectinase for the well plate method.



(AB)



(C)



(DE)

Figure 2. Negative results of well plate method

Pectinase activity of *Serratia marcescens, Klebsiella pneumoniea, Escherichia coli, and Pseudomonas aeruginosa* was calculated through DNS (Dinitrosalicyclic Acid) method. The pectinase activity of *S. marcescens, K. pneumoniea, P. aeruginosa,* and *E. coli* was 3.8 U/mL, 5.8 U/mL, 6.2 U/mL, 2.8 U/mL and 4.2 U/mL, respectively.

3.3. SDS-PAGE

Since the enzymes are protein in nature, so extracellular pectinase was observed by polyacrylamide gel electrophoresis. The gel was loaded with samples of enzymes from different bacterial isolates. After running, staining and de-staining a gel, clear bands of different molecular weight from different samples were separated and visualized. The molecular weight of sample was 65kDa (as shown in Figure 3).

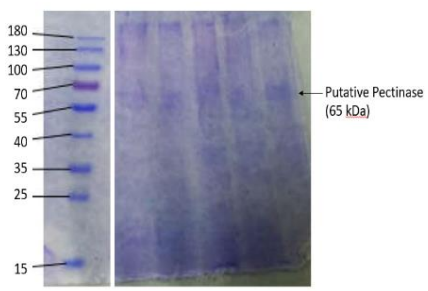


Figure 3. SDS-PAGE results

3.4. Zymography

A duplicate and unstained gel from SDS-PAGE was used for zymography. After iodine staining and 1M NaCl de-staining, bands were observed on plate.

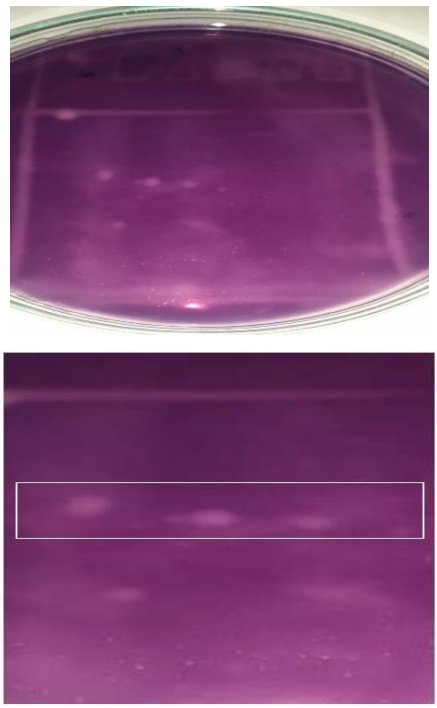


Figure 4. Zymography bands of different strains (*Serratia marcescens, Klebseilla pneumoniea, Pseudomonas aeruginosa and Escherichia coli)* on plate. zymography on starch plate: boxed region corresponds to 65kDa

4. Discussion

Pectin is commonly present in organic plant materials and in leafy foods in a vast amount. It is an intricate polysaccharide containing irregular complexes of different molecular weight ranging between 25-360 kDa. They contain galacturonic acid to a greater or lesser extent based on their types. A group of enzymes that break pectin into simpler sugars are called pectinases. They diminish the plant tissues' inflexibility and intracellular glue properties [12]. Pectinase is the common term used for pectic enzymes such as pectin methylesterase, polygalacturonase, and pectin lyase. Pectic enzymes are mostly used in fruit processing industries and they alone account for one fourth of the production of world's food enzymes [13]. Microorganisms such as yeast, fungi and bacteria also secrete pectic enzymes [14, 15]. Enzymes produced by organisms *Rhizopus*, *Penicillium*, and *Aspergillus niger* are generally seen as safe (GRAS) and can easily be recovered easily [16]. Different fruit and vegetable preparing wastes have been utilized also for pectinase production [17, 18].

Pectinolytic action was detected by the visualization of a clear zone around the wells on the plate using the potassium iodide flooding method [19]. However, in our study no clear zones around wells were observed which means that no reducing sugar was being hydrolyzed by the enzyme. Jayani et al. (2011) and Rashmi et al. (2008) also performed well plate assay and they obtained colorless hydrolytic zones of 22mm which became visible around the wells after flooding them with potassium iodide solution [20, 21]. Since we obtained no results through the well plate assay, so the strains were further screened for enzyme activity through zymography. For this purpose, SDS gel was carried out and placed in a plate containing the medium of phosphate buffer, 0.1% pectin, and 1.5% agar and it was incubated for 4 hours. Clear bands were observed on the plate after its staining and de-staining with iodine solution and 1M NaCl solution, respectively. Hadj-Taiebet al. (2011) characterized pectic enzymes by staining the gel with Cetyl Trimethyl Ammonium Bromide (CTAB), also known as cetramide [22]. Giacobe et al. (2014) also

observed pectinolytic activity by flooding the gel with 0.05% ruthenium red stain for 10 minutes [23].

Sohail and Latif (2016) reported that the molecular weight of crude pectinase obtained from strains *K. pneumoniea* and *Pseudomonas spp* [24] was found to be approximately 29kDa when compared with standard molecular weight marker, whereas the molecular weight of pectinase produced in our study was 65kDa.

Pectinase activity of *S. marcescens*, *K. pneumoniea*, *E. coli*, and *P. aeruginosa* was calculated through DNS (Dinitrosalicyclic acid) method. The activity of *S. marcescens*, *K. pneumoniea*, *P. aeruginosa*, and *E. coli* was 3.8 U/mL, 5.8 U/mL, 6.2 U/mL, 2.8 U/mL and 4.2 U/mL, respectively. *P. aeruginosa* obtained from oranges showed maximum activity which was 4.4 U/µL. The production of polygalacturonase from *Enterobacter aerogenes* (18.54 U/mL) was much smaller as compared to its production from *Bacillus* and *Pseudomonas* which was 4.4 U/µL each [25]. Patil and Dayanand (2006) reported that the pectinase produced by *Pseudomonas fluorescens* led to the soft rot of potato tubes and also reported the production of pectinase by *Pseudomonas cepacia* in diseased onions [13]. *P. aeruginosa*, isolated from tannery waste polluted soil, has the ability to produce pectinases, phosphatases, cellulases and proteases [26]. However, in our investigation, *P. aeruginosa* showed the highest pectinolytic activity as compared to the other four strains. In other works, *P.aeruginosa* that was isolated from oranges showed the highest pectinolytic activity. In our study, *P. aeruginosa* was isolated from rotten apples and it also showed the maximum activity for pectinase production. So, according to our information, it is the first report about the isolation of pectinolytic bacteria such as *S. marcescens*, *K. pneumoniea* and *P. aeruginosa* from rotten apples.

5. Conclusion

Pectinase is a group of chemicals used to catalyze the pectic substance through depolymerization response. Pectinase has been used in different industries where the elimination of pectin is essential such as in fruit juice processing, extraction of vegetable oil, and tea and coffee processing. In this study, different pectinolytic microbial strains were isolated from rotten apples on the MS medium and were identified as *Serratia marcescens*, *Klebseilla pneumoniea*, *Pseudomonas aeruginosa* and *Escherichia coli* after 16S rRNA sequencing. The strains were then further analyzed for their pectinolytic activity by SDS-PAGE zymography. All four strains showed pectinase activity. Pectinase assay was also performed to quantify the pectinase activity of isolates. The maximum activity was shown by the strain *P. aeruginosa* which was 6.2 U/mL.

References

[1] Thakur BR, Singh RK, Handa AV. Chemistry and uses of pectin: a review. *Crit Rev Food Sci Nutr*. 1997; 37(1): 47–73.

[2] May CD. Industrial pectins: sources, production and applications. *Carbohydr Polym*. 1990;12(1): 79–99.

[3] Blanco P, Sieiro C, Villa TG. Production of pectic enzymes in yeasts. *FEMS Microbiol Lett*. 1999;175(1):1–9.

[4] Kaur G, Kumar S, Satyanarayana T. Production, characterization and application of a thermostable polygalacturonase of a thermophilic mould Sporotrichum thermophile

Apinis. *Bioresour Technol.* 2004; 94(3): 239–243.

[5] Gailing MF, Guibert A, Combes D. Fractional factorial designs applied to enzymatic sugar beet pulps pressing improvement. *Bioprocess Biosyst Eng*. 2000; 22(1): 69–74.

[6] Kilara A. Enzymes and their uses in the processed apple industry: a review. *Process Biochem*. 1982; 17: 35–41.

[7] Alkorta I, Garbisu C, Llama MJ, Serra JL. Industrial applications of pectic enzymes: a review. *Process Biochem*. 1998;33(1): 21–28.

[8] Ricard M, Reid ID. Purified pectinase lowers cationic demand in peroxide-bleached mechanical pulp. *Enzyme Microbial Technol*. 2004; 34(5): 499–504.

[9] Hoondal GS, Tiwari RP, Tewari R, Dahiya N, Beg QK. Microbial alkaline pectinases and their industrial applications: a review. *Appl Microbiol Biotechnol*. 2002; 59(4-5): 409–418.

[10] Miller G. Modified DNS method for reducing sugars. *Anal Chem.* 1959; 31(3): 426–428.

[11] Smith B. *SDS polyacrylamide gel electrophoresis of proteins'* in *Proteins*. New York: Springer; 1984.

[12] Pires TdCR, Finardi F-F. Extraction and assay of pectic enzymes from Peruvian carrot (Arracacia xanthorriza Bancroft.). *Food Chem*. 2005; 89(1): 85–92.

[13] Patil SR, Dayanand A. Production of pectinase from deseeded sunflower head by Aspergillus niger in submerged and solid-state conditions. *Bioresour Technol*. 2006; 97(16): 2054–2058.

[14] Rombouts F, Pilnik W. Pectinases and other cell-wall degrading enzymes of industrial importance. *Symbiosis*. 1986; 2: 79–90.

[15] Rombouts, FM, Voragen AGJ, van Leeuwen MF, Geraeds CCJM, Schols HA, Pilnik W. The arabinanases of Aspergillus niger—purification and characterisation of two α-l-arabinofuranosidases and an endo-1, 5-α-l-arabinanase. *Carbohydr Polym*. 1988; 9(1): 25–47.

[16] Mrudula S, Anitharaj R. Aspergillus niger Using Orange Peel as Substrate. *Global J Biotechnol Biochem*. 2011; 6(2): 64–71.

[17] Bari MR, Alizadeh M, Farbeh F. Optimizing endopectinase production from date pomace by Aspergillus niger PC5 using response surface methodology. *Food Bioprod Process*. 2010; 88(1): 67–72.

[18] Kumar YS, Kumar PV, Reddy OVS. Pectinase production from mango peel using Aspergillus foetidus and its application in processing of mango juice. *Food Biotechnol*. 2012; 26(2): 107–123.

[19] Fernandes-Salomäo TM, Amorim ACR, Chaves-Alves VM, Coelho JLC, Silva DO, Araujo EF. Isolation of pectinase hyperproducing mutants of Penicillium expansum. *Rev Microbiol*. 1996; 27(1): 15–18.

[20] Rashmi R, Muthy KRS, Sneha G, Shabana S, Syama A, Radhika VS. Partial purification and biochemical characterization of extracellular pectinase from Aspergillus niger isolated from groundnut seeds. *J Appl Biosci*. 2008; 9(1): 378–384.

[21] Jayani RS, Shukla SK, Gupta R. Screening of bacterial strains for

polygalacturonase activity: Its production by Bacillus sphaericus (MTCC 7542). *Enzyme Res*. 2010; 2010: 1–5.

[22] Hadj-Taieb N, Tounsi H, Chabchoub A, Abid N, Gargouri A, Birolo L, Vinciguerra R,. Studies on the zymogram method for the detection of pectinolytic activities using CTAB. *Appl Biochem Biotechnol*. 2011; 165(7-8): 1652–1660.

[23] Giacobbe S, Pepe Olimpia, Ventorino V, Faraco V. Identification and characterisation of a pectinolytic enzyme from Paenibacillus xylanolyticus. *BioResour*. 2014;9(3):4873–4887.

[24] Sohail M, Latif Z. Phylogenetic analysis of polygalacturonase-producing Bacillus and Pseudomonas isolated from plant waste material. *Jundishapur J Microbiol*. 2016; 9: e28594.

[25] Zeni J, Cence K, Grando CE, Tiggermann L, Colet R, Lerin L, Cansian RL, Toniazzo G, de Oliveiraet D, Valduga E. Screening of pectinase-producing microorganisms with polygalacturonase activity. *Appl Biochem Biotechnol*. 2011;163(3): 383–392.

[26] Obi SK, Umezurike GM. Pectic enzyme activities of bacteria associated with rotted onions (Allium cepa). *Appl Environ Microbiol.* 1981;42(4): 585–589.