The UV light effects on pectinase production of *Pantoea* sp. bacteria

Mohmmed Lefta Atala*
Department of Biology, College of sciences, University of Baghdad, Iraq

**Article History:**
Received on: 12 Jan 2020
Revised on: 19 Mar 2020
Accepted on: 23 Mar 2020

**Keywords:**
Pantoea sp, UV light, Pectinase, Mutagenesis, Physical effects

**ABSTRACT**
The highest pectinase producing isolate A3 out of forty isolates of *Pantoea* sp. bacteria was subjected to physical effects by using UV light to change in their ability for producing of pectinase, the results appeared increasing their activity and production in some of the mutants more than wild type bacteria, the increase was ranged between 14 to 20% in specific activity between bacterial mutants compared with wild type bacteria. Also, the two highest enzyme-producing isolates were tested for their susceptibility against a group of antibiotics (Rifampin 50mcg, Imipenem 10mcg, Vancomycin 30mcg, Amoxicillin 25mcg, Chloramphenicol 30mcg, Trimethoprim 5mcg, Nitrofurantoin 100mcg, Nalidixic acid 30mcg), results appeared different susceptibilities ranged between resist for amoxicillin, and Nalidixic acid, and sensitive for other antibiotics. The susceptibilities of studied bacteria to some antibiotics were different between resist and sensitive, which considered an indicator of dangerous to human life. The study was aimed to increase the ability of these isolate in producing this enzyme which plays an essential role in different industries in food, cosmetics manufacturing and scientific research, besides, their ability in resisting some of the common antibiotics to confirm the transferring of genetic materials between bacteria that isolated from soil samples, which is considered crucial in the microbial genetics. The study appeared there were apparent effects on the genes that responsible for enzyme production either for increase or decrease their activity and creation of an enzyme, which belong to using physical effects to improvement these feature in bacteria.

**INTRODUCTION**
Enzymes are the essential biomolecules for life. The microorganisms produce many enzymes, which have been commercially role, today the enzyme technology depends on bacteria and actinomycetes (Li *et al.*, 2012). The biotechnological methods represent study to screen and report enzymes producing microbes (Vuppusuneetha, 2011). The bacterial pectinases represent 25% of the enzyme sales (Jayani *et al.*, 2005). Potentially the bacteria are so susceptible to gene manipulation experiments that provide hope for strain improvement. The production of pectinase enzyme from bacteria uses in many of industrial processes, like juice clarification, juice extraction, production of pectin free starch, refinement of plant fibres, degumming of plant fibres, wastewater treatment (Murad and Azzaz, 2011; Atala *et al.*, 2015). To change and improve the ability of bacteria in producing enzymes, there were some methods by using chemicals and physical ways. The physical methods involved UV light. Which has high geno-
toxic effects to produce genetic damage, induce mutations, and maybe causes the development of tumours? The primary source of UV is the sunlight, which represents one of the common causes of skin cancers (Setlow, 1974; Brash et al., 1991). UV light effects on DNA particle when it causes DNA lesions, give dimers of any one of adjacent pyrimidine bases causing T-T, C-T, and C-C dimers, but T-T dimers are the most common cyclobutane pyrimidine dimers (Wang, 2001). UV radiation may be a hazard to the human and animals; also, it represents environmental stress for other organisms like bacteria. Such environmental stress that caused some induces different evolutionary changes on bacteria that would have otherwise not been selected. This area thus provides fact physiological, ecological and genetic investigation because mutations play a crucial role in biological processes such as evolution, carcinogenesis and generation of somatic genetic diversity (Livneh et al., 1993).

MATERIALS AND METHODS

Collection and preparation of samples
To isolate pectinase producing bacteria, soil samples were collected from different places that were cultivated with wheat crops located in Al-Jaderia, Baghdad university. One gram of each soil sample was suspended in 9ml of sterilized DW and mixed vigorously. After that, 0.1 ml of supernatant was spread on nutrient agar plates and incubated at 30°C for 24 hrs. After incubation, the growing colonies were selected and screened to determine their ability to produce a pectinase enzyme.

Identification
The pectinase producing isolates were identified depending on their cultural and morphological features of size, shape, edge, gram staining, colour and biochemical tests of VITEK 2 kit tests depending on (Atlas et al., 1995).

Pectinase production
Pectinase was produced by using medium involved of pectin 10, MgSO4.7H2O 0.5, KH2PO4 1 and NaNO3 1. The pH was adjusted to 6, then 50 ml of the medium was sterilized and cooled at 37°C and inoculated with 0.5 ml of the bacterial suspension. After that, the cultures were incubated for 48 hr. At 30°C and 140 rpm. The supernatant was extracted by cooling centrifugation (6000 g 10 min. and 4°C) and used for further analysis (Thangaratham and Manimegalai, 2014).

Pectinase assay
Pectinase enzyme was estimated through determining their activity in the filtrate of production medium which contained pectin broth, the action of the enzyme was estimated by mixing one millilitre of 1% pectin in 0.1 M of sodium acetate buffer pH 5 for 30 min at 37°C. The reducing sugar was determined to depend on Miller method (Biz et al., 2014).

Protein assay
To determine of protein in the production medium, it was added 0.1 ml of a crude enzyme to 0.4 ml of Tris-HCl after that 2.5 ml of coomassie brilliant blue. Then the absorbance was read. After that, the protein was estimated by using a standard curve for BSA (Miller, 1959).

Mutagenesis of bacteria
Mutagenesis of bacteria was performed by subjecting culture of bacterial suspension to UV light in a dark place. 1 ml of overnight bacterial culture was cultivated in 100 ml of nutrient broth for 6 hrs. At 37°C, then 10 ml of the suspension was transferred into sterilized plates and placed under UV ray at 11 cm of the UV source with total time was 15 sec, with time intervals of 1 sec. For all time interval, 100 µl was taken. Serially diluted and spread on nutrient medium agar which incubated in the dark at 37°C for 24 hrs. To estimate the survivals and detection of mutants at the killing percentage of more than 90%. The suspected mutated bacteria were screened to determine their ability to produce pectinase (Xu et al., 2011).

Susceptibility for antibiotics
The Susceptibility test was performed by using discs of antibiotics named Rifampin (RA) 50mcg, Imipenem (IPM) 10mcg, Vancomycin (VA) 30mcg, Amoxicillin (AX) 25mcg, Chloramphenicol (C) 30mcg, Trimethoprim (TMP) 5mcg, Nitrofurantoin (F) 100mcg, Nalidixic acid (NA) 30mcg that were placed on an agar plate which cultivated previously with bacteria, the dish is left to incubate at 37°C for 24 hrs. Then the results were recorded through measure the diameters of inhibition zones around the antibiotic disc compared to a database of zone standards to determine if the bacteria was susceptible, moderately susceptible or resistant to these antibiotics.

RESULTS AND DISCUSSION

Collection and preparation of samples
To get bacteria producing enzymes that degrade plant tissues, soil samples were collected from fields of wheat located in Baghdad university. The result appeared isolation forty bacterial isolates after that
the isolates subjected to pectin medium to detect their ability for producing pectinase. The highest producing isolates for pectinase were subjected to further studies (Brown and Kothari, 1975).

**Identification**

The selected isolate with high producing of pectinase was identified depending on the morphological features and VITEK 2 kit tests. The results were in accordance with those reported by (Deletoile et al., 2009) when the results showed the isolate was as straight rods in shape, non-haemolytic, motile, non-capsulated, non-spore-forming. The colonies appeared smooth, yellow pale pigmented, mucoid, irregularly round, rough and wrinkled that are difficult to remove with a platinum wire. In addition to it was able to grow at 30°C and 41°C and unable to grow at 4°C and 44°C. The results of identification was completed by using VITEK 2 that appeared to the isolate belong to *Pantoea* sp., the results seemed to be similar features to these reported in (Atlas et al., 1995).

**Pectinase production**

The production of pectinase was screened by using a qualitative method through determining enzyme-specific activity infiltrate of culture for isolates after culturing in pectin broth at 30 °C for 48 hrs. The results indicate that these isolates produced pectinase in various abilities that ranged between 0.70-1.30 U/mg for five isolates out of forty isolates that represent high producers for pectinase depending on their specific activity. The results were similar to those mentioned by (Soares et al., 1999) who reported that *Pantoea* spp. were produce pectinase enzyme which degrading plant cell wall and there were various values of activity within each one of isolates (Jabeen et al., 2015).

**Mutagenesis of bacterial isolate**

To improve the capability of *Pantoea* sp. isolate for pectinase production, the isolate named A3 was subjected to UV light as physical mutagens which cause random mutations that may give a positive effect on pectinase production, after that selecting the over producer mutants depending on pectinase specific activity in culture filtrate of these mutants. The results of UV light was achieved after bacterial culture of *Pantoea* was subjected to this light at a 254 nm wavelength for durations of 1, 2, 3, 4, 5, 10 and 15 seconds. Then 100 µl of aliquots from each treatment were taken and spread on a nutrient agar medium. The results appeared Figure 1, that LD90 was achieved after 3 seconds of irradiation under UV light, the most of isolate cultivability was lost after 5 seconds of radiation. The survivals of irradiated bacterial cells were screened to determine their ability for pectinase producing as showed in Table 1 only six out of one hundred mutants exhibited an increase in enzyme production compared with wild type.

![Figure 1: Survivals of *Pantoea* sp. Isolate after UV irradiation effects](image)

**Table 1: Pectinase specific activity for mutated and wildtype bacteria**

| Isolate name | Specific activity U/mg |
|--------------|-----------------------|
| Wild type    | 1.30                  |
| Mutant 1     | 2.19                  |
| Mutant 2     | 3.01                  |
| Mutant 3     | 2.86                  |
| Mutant 4     | 2.67                  |
| Mutant 5     | 2.86                  |
| Mutant 6     | 2.08                  |

On the other hand, all mutants showed lower enzyme-producing. The results appeared similarity with (Siddalingeshwara et al., 2010) who reported that UV light at 254 nm is mild mutagens, which may cause induction of mutations. Also, the agreement of the results with the report of (Kanokphornsangkharak, 2012) who mentioned that mutagenic effects lead to an increase or decrease of the enzyme production or activity.

**Susceptibility for antibiotics**

To study the susceptibility of highest producing isolates against some antibiotics, the results appeared to resist to AX for isolate A1 and resist to NA for isolate A3 as mentioned in Table 2. Others antibiotics were effective on these isolates with different degrees of inhibition zones as they reported in the table.

It was clear that isolate A1 has a high resistance to amoxicillin, and this result similar to these mentioned by (Abdalhussen and &darweesh, 2016) who indicated that *Pantoea* spp. Has highly resistant...
Mohmed Lefta Atala, Int. J. Res. Pharm. Sci., 2020, 11(3), 4578-4582

Table 2: Susceptibility test for highest producing enzyme isolates

| Antibiotic | Isolate A1(cm) | Isolate A3 (cm) |
|------------|----------------|-----------------|
| RA         | S (4 cm)       | S (2.5 cm)      |
| IPM        | S (3.5 cm)     | S (3.5 cm)      |
| VA         | S (2.5 cm)     | S (1.5 cm)      |
| AX         | S (0.9 cm)     | S (1.8 cm)      |
| C          | S (3.2 cm)     | R               |
| TMP        | S (4 cm)       | R               |
| F          | S (2.1 cm)     | R               |
| NA         | S (2 cm)       | R               |

to amoxicillin which considered most commonly used.

Also, the results were similar to these reported by (Walterson and Stavrinides, 2015) who mentioned that other antibiotics are given various activity against this genus of bacteria. Results appeared the two isolates have sensitive against imipenem which was in accordance with details indicated that these antibiotics are more active on this genus and the susceptible reached to (95%).

Pantoea sp. causes many of human diseases like nosocomial infections, Intensive Care, burn, haemodialysis and oncology department this lead to sepsis as a complicated, multifactorial syndrome that can evolve into circumstances of various seriousness, portray as acute sepsis or septic shock. All these diseases may need drugs of antibiotics which may lead to MDR in bacteria in future. All these effects considered dangerous for human health.

CONCLUSIONS

The study appeared there were apparent effects on the genes that responsible for enzyme production either for increase or decrease their activity and production of an enzyme, which belong to using physical effects to improvement these feature in a protein. The susceptibilities of studied bacteria to some antibiotics were different between resist and sensitive, which considered an indicator of dangerous to human life.

Funding Support
None.

Conflict of interest
None.

REFERENCES

Abdalhussen, L. S., & Darweesh, M. 2016. Prevalence and antibiotic susceptibility patterns of Pantoea spp. Isolated form clinical and environmental sources in Iraq. International Journal of Chemical Studies, 9(8):430–437.

Atala, M., Ali, L., Kadhim, M. 2015. Optimization of Pectinase Production from Pseudomonas sp. Isolated from Iraqi Soil Iraqi Journal of Science, 56(3C):2595–2600.

Atlas, M., Parks, C., Brown, A. 1995. Laboratory Manual of Experimental Microbiology. Mosby-Year Book, Inc., USA.

Biz, A., Farias, F. C., Motter, F. A., de Paula, D. H., Richard, P., Krieger, N., Mitchell, D. A. 2014. Pectinase Activity Determination: An Early Deceleration in the Release of Reducing Sugars Throws a Spanner in the Works! PLoS ONE, 9(10):e109529–e109529.

Brash, D. E., Rudolph, J. A., Simon, J. A., Lin, A., McKenna, G. J., Baden, H. P., Halperin, A. J., Ponten, J. 1991. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. Proceedings of the National Academy of Sciences, 88(22):10124–10128.

Brown, D. F., Kothari, D. 1975. Comparison of antibiotic discs from different sources. Journal of Clinical Pathology, 28(10):779–783.

Deletoile, A., Decere, D., Courant, S., Passet, V., Audo, J., Grimont, P., Arlet, G., Brisae, S. 2009. Phylogeny and Identification of Pantoea Species and Typing of Pantoea agglomerans Strains by Multilocus Gene Sequencing. Journal of Clinical Microbiology, 47(2):300–310.

Jabeen, A., Hanif, Hussain, Q., Munawar, M., Farooq, A., Bano, N. 2015. Screening, isolation and identification of pectinase producing bacterial strains from rotting fruits and determination of their pectinolytic activity. Science Letters, 3(2):42–45.

Jayani, R. S., Saxena, S., Gupta, R. 2005. Microbial pectinolytic enzymes: A review. Process Biochemistry, 40(9):2931–2944.

Kanokphornsangkharkar, 2012. Strain improvement and optimization for enhanced production of cellulase in Cellulomonas sp. TSU-03. African Journal of Microbiology Research, 6(5):1079–1084.

Li, S., Yang, X., Yang, S., Zhu, M., Wang, X. 2012. Technology Prospecting On Enzymes: Application, Marketing And Engineering. Computational and Structural Biotechnology Journal, 2(3).

Livneh, Z., Cohen-Fix, O., Skaliter, R., Elizur, T. 1993. Replication of Damaged DNA and the Molecular Mechanism of Ultraviolet Light Mutagenesis. Critical Reviews in Biochemistry and Molecular Biology, 28(6):465–513.
Miller, G. L. 1959. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. *Analytical Chemistry*, 31(3):426–428.

Murad, H. A., Azzaz, H. H. 2011. Microbial Pectinases and Ruminant Nutrition. *Research Journal of Microbiology*, 6(3):246–269.

Setlow, R. B. 1974. The Wavelengths in Sunlight Effective in Producing Skin Cancer: A Theoretical Analysis. *Proceedings of the National Academy of Sciences*, 71(9):3363–3366.

Siddalingeshwara, K. G., Devi, N., Mohanty, S., Lodh, S., Sumanta, K. P., Karthic, J., Sumanth, B. K. 2010. Mutational studies on L-glutaminase - A tumour inhibitor from microbial origin. *International Journal of Pharmaceutical Sciences Research and Review*, 4(2):176–179.

Soares, M. M., da Silva, R., Gomes, E. 1999. Screening of bacterial strains for pectinolytic activity: characterization of the polygalacturonase produced by Bacillus sp. *Revista de Microbiologia*, 30(4):299–303.

Thangaratham, T., Manimegalai, G. 2014. Optimization and Production of Pectinase Using Agro Waste by Solid State and Submerged Fermentation. *Int.J.Curr.Microbiol.App.Sci*, 3(9):357–365.

Vuppusuneetha, M. M. 2011. An Overview Of Some Reported Soil Enzyme Producing Microorganisms. *Indian Journal of Fundamental and Applied Life Sciences*, 1(4):180–186.

Walterson, A. M., Stavrinides, J. 2015. Pantoea: insights into a highly versatile and diverse genus within the Enterobacteriaceae. *FEMS Microbiology Reviews*, 39(6):968–984.

Wang, K. L. A. A. 2001. UV mutagenesis in Escherichia coli K-12: Cell survival and mutation frequency of the chromosomal genes lacZ, rpoB, ompF, and ampA. *Journal of Experimental Microbiology and Immunology*, 1:32–46.

Xu, H., Jia, S., Liu, J. 2011. Development of a mutant strain of Bacillus subtilis showing enhanced production of acetoin. *African Journal of Biotechnology*, 10(5):779–788.