

## Study the Susceptibility of Plant Isolated Bacteria against Some Antibiotics

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**Abstract:** 15 isolate of *Pantoea dispersa* were isolated from soil samples included two isolates with highest producing isolates for cell wall degrading enzymes of cellulase. The two isolates were subjected to susceptibility test against chloramphenicol 30mg, imipenem 10mg, nalidixic acid 30 mg, Amoxicillin 25mg, Oxacillin 5mg and Methicillin 10mg. the results indicated there were no resistance to chloramphenicol, imipenem, nalidixic acid, oxacillin and methicillin, while one of isolates was resist to amoxicillin.

**Keywords:** *Pantoea dispersa*, Antibiotics, Cellulase, Methicillin, Oxacillin.

### Introduction

The *Pantoea* genus contained twenty species, the colonies of this bacteria are pigmented with yellow color, lactose fermented, motile, mucoid in form and appear quorum sensing (Walterson. & Stavrinides, 2015). Also it is Gram-negative, non capsulated, do not forming of spore, straight rod in shape and it is isolated from plant surfaces, human feces and from the environmental sources (Nobuhiro et al., 2019).) A genus of *Pantoea* is uncommon to be pathogen in a clinical cases, but *P. agglomerans* is the distinguished species which infected the humans, previously designate *Enterobacter agglomerans* (Schmid et al., 2003)).

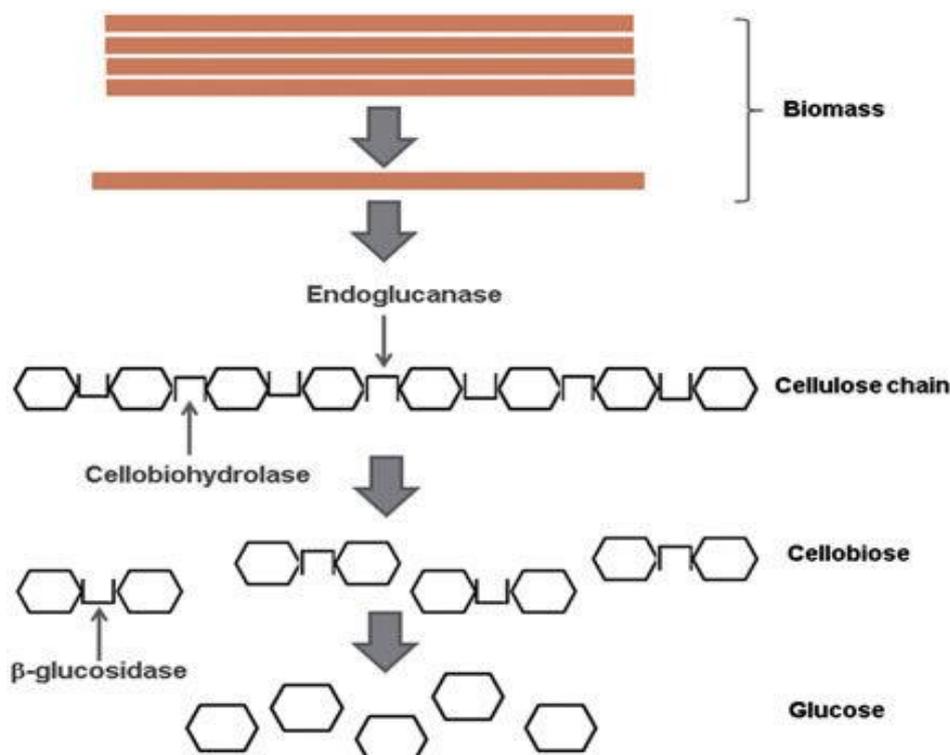


Figure 1: Cellulose hydrolysis by three type of enzymes. (Eva& Anas, 2013)

*Pantoea dispersa* species is recorded to cause infections, like respiratory, neonatal sepsis, and bloodstream infections, also to infections in immune system of patients and in addition to acute cholangitis (Layla & Mayyada, 2016). The development of resistance in bacteria against antibacterial substances has detect a failure therapy for infection. Killing these bacteria is a major challenge for scientists. In addition to some information on the pathogenicity of this bacterium in humans, there is little or no data on resistance in this bacterium in Iraq. Therefore, the study was important to detect environmental bacteria and their susceptibility against some types of antibiotic (Atlas & Brown, 1995).

Cellulose are important plant cell wall degrading enzyme, which consider the most numerous and renewable source of power. Cellulose is enzyme that produced by bacteria which works as virulence agent to break down the components of cell wall (Liu et al., 2005), the full enzymatic hydrolysis for cellulose needs three kinds of enzymes named cellobiohydrolase, carboxymethylcellulase and glucosidase (figure 1) (Yi et al.,1999). Numerous of bacteria, yeast and fungi can produce cellulose enzyme, but *Pantoea* have most important pathogen for plant around the world, because it cause some of plant diseases like leaf blight and bulb rot in onion (Sangharak et al., 2012).

## **Materials and Methods**

### **Soil Samples Collection**

To isolation of bacteria that producing plant cell wall degrading enzymes, soil samples were collected from corn crop located Baghdad university's fields.

### **Preparation of Samples**

A quantity of 1gm of soil samples were suspended in 9ml of D.W. and mixed vigorously, then 0.1ml of supernatant were spreading on nutrient agar dishes and incubated at 30C° for 24 hrs.

### **Identification of Bacteria**

The isolates are identified according to Atlas *et al.* (1995).

### **Cellulase Enzyme Production**

The cellulase enzyme production was determined depending on degrading crystalline cellulase in cellulose agar plates after incubation at 30C° for 24hrs, then the plates were flooded with congo red solution and left for 15min., then the plates were destained with 1M NaCl solution. The appearance halo zone around colonies due to cellulase production indicates positive results (Oxoid, 1982).

### **Susceptibility Test for Test Bacteria**

The isolates were streaked on nutrient agar, Then a discs of antibiotics of amoxicillin, chloramphenicol, imipenem, nalidixic acid, Oxacillin 5mg and Methicillin 10mg were placed on agar. Then plates were incubated at 37°C for 24hrs. After that a diameters of inhibition zone were recorded and compared with standard value of scientific references (Selvakumar et al., 2008).

## **Results and Discussion**

### **Isolation and Identification of Bacteria**

15 isolate of bacteria were isolated from soil samples after incubation period reached to 24 hrs. The isolates were identified depending on the cultural and biochemical properties. The results appeared that these isolates belong to *Pantoea dispersa* which appeared negative results for tests of Gram stain, Oxidase, Methyl red,

Indole, Sorbitol, Urease. While they appeared positive results to Catalase, Motility, VP, Gelatinase, Citrate utilization, Fermentation / Oxidation of Mannitol, Lactose, Rhamnose, Maltose, Sucrose, Salicin, also for Growth at 30 °C or 41°C. In addition to results of morphological tests that showed these isolates were as straight rods in shape, motile, non-haemolytic, non-capsulated, non-sporeforming. Also the colonies were smooth, mucoid, yellow pale pigmented, irregularly round, rough. The previous results were accordance to these reported by Selvakumar *et al.* (2008) that mentioned these isolates belong to *Pantoea dispersa* species. The isolation of these bacteria from the soil is very important, when this species it can produce essential materials in industry, in addition to their role in the pathogenesity for human and plant.

### **Cellulase Production of Pantoea Dispersa Bacteria**

To determine the producer isolates for cellulose, the isolate were screened using CMC agar media which involved CMC as sole source of carbon, the results indicate that the 15 isolates were produced to cellulase enzyme with halo zone ranged between 10 into 15 mm, the results was similar to these reported by Atala *et al.* (2015), and Nurachman (2010) that mentioned these bacteria are able to produce cellulase enzyme which can utilize the cellulose as substrate. Also the results were accordance to these mentioned by Nurachman *et al.*, (2010) if the isolates were grow on cellulose with variable degree according to the diameters of clear halo around the colonies of bacteria,

### **Sensitivity Test for Antibiotics**

The results of susceptibility test appeared that these isolates were not resist to each used antibiotics involved methicillin and oxacillin, but only one of them was resist to amoxicillin antibiotic, the results were accordance to these reported by Mardaneh & Mohammad (2013) who mentioned that 62-100% of *Pantoea dispersa* isolates sensitive to each of imipenem, chloramphenicol and nalidixic acid and there were 25% of isolates resist to amoxicillin. Also nearly, there were similar results between different isolate that isolated from different sources. Also the results were similar to these mentioned by Richter *et al.* (2013) which appeared this species was susceptible to all antimicrobial agents tested in the study like imipenem, ampicillin, cefazolin, gentamicin, LVFX, and trimethoprim-sulfamethoxazole. In other study dissimilar result was appeared that this species was resist to imipenem antibiotic (Hagiya & Otsuka, 2014). It is clear from the studies it must performe further researches to determine sensitivity and pathogenesity of this bacteria, when there were a few knowledge on the pathogenesity of this bacteria in human and the development their resistant to antibiotics.

### **Conclusion**

The study concluded there were similar results for sensitivity test between isolates that isolated from different sources of isolation for antibiotics. But there were unexpected results of antibiotic resistance in soil isolated bacteria. Also the production of cellulose enzyme it was different between the isolate that isolated from the same source.

### **Recommendations**

It is recommended to perform further studies on the resist of bacteria that isolated from the soil against another antibiotics, in order to determine the fact sensitivity and pathogenesity of this bacteria, because there were a little knowledge on the pathogenesity of this bacteria in human and the development their resistant to antibiotics.

### **Acknowledgements or Notes**

Finally, the study was not very easy to isolate these bacteria, but rather it took a long time to isolate them.

### **References**

- Atala, M. L., Hameed, M. J., & Kadhim, M. I. (2015). Production, purification and characterization of cellulose from local isolate of *Pantoea* spp. *Iraqi Journal of Science*, 56(2B): 1324-1330.
- Atlas, M., Parks, C. & Brown, A. (Eds) (1995). *Laboratory manual of experimental microbiology*. Mosby-Year-Book, Inc., USA.
- Eva, M. G. D. P. & Anas, S. (2013). The cellulolytic system of *Thermobifida fusca*. REVIEW ARTICLE. *Informa health care*. pp1-12. Retrieved 2020 from internet database. [https://www.researchgate.net/publication/236088767\\_The\\_cellulolytic\\_system\\_of\\_Thermobifida\\_fusca/figures?lo=1&utm\\_source=google&utm\\_medium=organic](https://www.researchgate.net/publication/236088767_The_cellulolytic_system_of_Thermobifida_fusca/figures?lo=1&utm_source=google&utm_medium=organic).
- Hagiya, H. & Otsuka, F. (2014). *Pantoea dispersa* bacteremia caused by central line-associated bloodstream infection. *Braz J Infect Dis*, 18, 696–7..
- Layla, S. A., & Mayyada, D. F. (2016). Prevalence and antibiotic susceptibility patterns of *Pantoea* spp. isolated from clinical and environmental sources in Iraq. *International Journal of Chem Tech Research*, 9(8): 430-437.
- Liu, H., Zhang, S., Schell, M.A. & Denny, T.P. (2005). Pyramiding unmarked deletions in *Ralstonia solanacearum* shows that secreted proteins in addition to plant cell-wall-degrading enzymes contribute to virulence. *Mol. Plant-Microbe Interact.*, 18, 1296-1305.
- Mardaneh, J. & Dallal, M. M. S. (2013). Isolation, identification and antimicrobial susceptibility of *Pantoea (Enterobacter) agglomerans* isolated from consumed powdered infant formula milk (PIF) in NICU ward: First report from Iran. *Iran J Microbiol*, 5(3): 263–267.
- Mehar, V., Yadav, D., Sanghvi, J., Gupta, N., & Singh, K. (2013). *Pantoea dispersa*: a n unusual cause of neonatal sepsis. *Braz J Infect Dis*, 17, 726–8.
- Nobuhiro, A., Yusuke, K., Atsuko, Y., Daisuke, S., Hiroki, W., Hideo, K., Arufumi, S., Mao H., Hiroyuki S., Yuka Y. & Hiroshige M. (2019). *Pantoea dispersa* bacteremia in an immunocompetent patient: a case report and review of the literature. *Journal of Medical Case Reports*, 13(33), 1-5.
- Nurachman, Z., Kurniasih, S. D., Puspitawati, F., Hadi, S., Radjasa, O. K. & Natalia, D. (2010). Cloning of the endoglucanase gene from a *Bacillus amyloliquefaciens* PSM 3.1 in *E. coli* revealed catalytic triad residues ThrHis-Glu. *Americ. J. of Biochem. and Biotech.*, 6(4), 268-274.
- Oxoid. (Eds) (1982). *The oxoid manual of culture media, ingredients and other laboratory services*. 5<sup>th</sup>.ed. Oxoid limited. Wade road. Basingstoke. Pp. 214-215.
- Richter, S.S., Sercia, L., & Branda, J.A, (2013). Identification of *Enterobacteriaceae* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the VITEK MS system. *Eur J Clin Microbiol Infect Dis*, 32, 1571–8.
- Sangkharak, K., Vangsirikul, P. & Jantachai, S. (2012). Strain improvement and optimization for enhanced production of cellulase in *Cellulomonas* sp. TSU-03. *Afric. J. of Microbial. Res.*, 6(5): 1079-1084.
- Schmid, H., Schubert, S., Weber, C., & Bogner, J.R.(2003). Isolation of a *Pantoea dispersa*-like strain from a 71-year-old woman with acute myeloid leukemia and multiple myeloma. *Infection*, 31, 66–7.
- Selvakumar, G., Kundu, S., Joshi, P., Nazim, S. A. D., Mishra, P. K. & Gupta, H. S.(2008). Characterization of a cold-tolerant plant growth- promoting bacteria *Pantoea dispersa* 1A isolated from a sub-alpine soil in the north western Indian Himalayas. *Journal Microbial. Biotechnol*, 24, 955-960.
- 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.
- Yi, J. C., Sandra, A. B. J. & Shu, T. C. (1999). Production and distribution of endoglucanase, cellobiohydrolase, and  $\beta$ -glucosidase components of the cellulolytic system of *Volvariella volvacea*, the edible straw mushroom. *Appl. Environ. Microbiol*, 65, 553-559.

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