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Isolation, Identification, Characterization and Enzymatic Profile of the New Strain of *Pantoea agglomerans*

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A B S T R A C T

Enterobacteriaceae of the genus *Pantoea* are characterized as Gram-negative and have been isolated from a wide variety of environments including soil, water, dust, dairy products, meat, fish, insects, humans and animals. Most of the time they are found associated with a wide variety of plants. The objective of this work was to identify and characterize biochemical, morphological and enzymatic activity, as well as the influence of abiotic factors in a *Pantoea* isolated from industrial laundry effluent in Pernambuco. The results showed that the bacterium presents characteristics similar to those presented by the species of *Pantoea agglomerans*; however, the biochemical and morphological tests were not enough to accurately identify the species. However, the isolated species showed production of enzymes such as cellulase, protease and polyphenoloxidase, demonstrating enzymatic biotechnological potential. In addition to tolerance to high concentrations of salinity, they present as mesophyll, cerscendo in optimal temperature of 30 ° C and good growth in the pH 7.0 and 8.0.

Introduction

The genus *Pantoea* belongs within the family *Enterobacteriaceae* and was proposed by Gavini *et al.*, (1983) for two groups of strains that were, at that time, assigned to the *Erwinia herbicola*–Enterobacter agglomerans complex (Verdonkck, 1987). This complex covered many phena and genomic groups (Brenner at al., 1984), some of which were later designated as new genera (Grimont; Grimont, 2005).

The enterobacterial genus *Pantoea* currently comprises nineteen species of Gram-negative, yellow or beige pigmented, motile rods

(Kageyama at al., 1992). Grimont and Grimont (2005) stated that the genus *Pantoea* can be envisioned to include DNA groups I, II, IV and V as determined by Brenner *et al.*, (1984). It was further observed that the species *P. citrea, P. punctata and P. terrea*, isolated in Japan and described by Kageyama *et al.,* (1992) differed from the ‘‘core’’ *Pantoea* species in several biochemical or nutritional characteristics. Grimont and Grimont determined the phylogenetic position of all currently recognized *Pantoea* species and DNA groups of Brenner *et al.,* (1984) using 16S rRNA- and rpoB-sequence comparisons and found that the ‘‘Japanese’’ species constituted a cluster that joined the *Pantoea* cluster at a lower level. They concluded that more taxonomic work was needed to justify the assignment of these species to the genus *Pantoea* (Braddy *et al.*, 2008; Braddy *et al.,* 2009; Braddy *et al.,* 2010 a,b).

Identification of plant-pathogenic Pantoea species is difficult, due to the high degree of phenotypic similarity between species of this genus and related *Enterobacteriaceae*. *Pantoea* species are typically characterised based on colony morphology, physiological and biochemical tests, and in some cases, fatty acid analysis or quinone composition. This approach has proven to be unreliable though, as identification based solely on phenotypic characteristics has led to the misidentification of many strains belonging to the now obsolete ‘‘*Erwinia herbicola–Enterobacter agglomerans*’’ complex (Brady *et al.,* 2007).

The genus presents short bacilli, Gram negative, 0.5-1.0 μm in diameter and 1.0-3.0 μm in length, are mobile by peritrichal flagella and many samples produce yellow or beige pigment. They are facultative anaerobes, negative indole, Voges-Proskauer and Simmons citrate positive, the reaction of methyl red is variable. They do not decarboxylate lysine, do not produce H_2S and do not hydrolyze urea (Holt *et al.,* 1994; Camatti-Sartori *et al.,* 2008; Cabral, 2010; Roper, 2011; Nadaeasah; Stavrinides, 2014).

Members of this genus *Pantoea* have been isolated from a wide range of environments including soil, water, dust, dairy products, meat, fish, insects, humans and animals (Suen *et al.,* 2010; Prakashi *et al.,* 2015; Büyükcam *et al.*, 2017). However, *Pantoea agglomerans*, is not an obligate infectious agent in humans. However, it could be a cause of opportunistic human infections, mostly by wound infection with plant material, or as a hospital-acquired infection, mostly in immunocompromised individuals (Dutkiewicz *et al.,* 2016).

Most frequently they are found associated with a broad range of plant hosts, as non-pathogenic endophytes or epiphytes, colonizing the leaves, stems and roots. In this context, some *Pantoea* strains can be beneficial to the plant host by contributing to growth promotion through processes such as the production of the plant-growth hormone indole-acetic acid (IAA), phosphate solubilization or nitrogen fixation (Mishra *et al.,* 2011; De Maayer *et al.,* 2012). Some *Pantoea* strains also provide effective protection to plants against various bacterioses as well as fungal diseases and postharvest fruit rots (Smits *et al.*,2011).

The objective of this study was to isolate from laundry efluent, identification, characterization and enzymatic profile.

Materials and Methods

Sample and Isolation

Bacteria were isolated from industrial laundry effluents. A quantity of 1 ml of water from each of the samples collected was dissolved in

9 ml of sterile distilled water and serial dilutions were made. Each dilution was seeded in Luria Bertani (LB) agar by standard plate spreading method. The plates were incubated at 37 ° C for 3 days and the colonies were transferred from the plates to inclined tubes with the same medium. After plaque growth, bacterial colonies were collected according to their morphological characteristics and purified by striations repeated on plates containing nutrient agar and identified with Gram staining. For the characterization, the biochemical and physiological tests were used for the morphology of the colonies. The strain was routinely cultured in LB medium and maintained at 5 ° C.

Biochemical Tests and Morphology

Bacteria were identified according to macroscopic appearance (colony appearance in solid medium, shape, texture and pigmentation), Gram staining, mobility tests, oxidase, catalase and indole. These tests were done according to Cappuccino and Sherman (1992) and (Grimont;Grimont, 2005). The biochemical Tests were done with various sources of carbon, as monosaccharides (D-glucose, D-fructose, D-xylose, D-mannose, D-mannitol and D-gluconase), disaccharides (lactose, maltose, trehalose and sucrose) (Brown;Dilworth, 1975). Proteins (gelatin) were evaluated according to the standard method (Cappucino; Sherman, 1992).

Abiotic Stress Factors

pH Effect

The pH effect was tested on Nutrient Broth with values of 4, 5, 6, 7, 8, and 10. The medium was inoculated with 100 µl of the culture, grown to a 0.5 standard on the MacFarland scale, And inoculated at 30 ° C / 48h. The experiment was carried out in triplicate. Growth was evaluated using the spectrophotometer at 600nm (Son *et al.*, 2006; Silini-Chérif *et al.*, 2012).

Temperature Effect

The effect of different temperatures (4, 30, 37, 41 and 44 ° C) on bacterial growth was evaluated. The nutrient broth medium was inoculated with 100µl of the culture, grown to a 0.5 standard on the MacFarland scale. The growth time was 48h and the experiment was performed in triplicate. Growth was evaluated using the spectrophotometer at 600nm (Silini-Chérif *et al.*, 2012).

Saline Concentration Effect

The tolerance of the microorganism to an increasing concentration of NaCl (0-100 mM) in Nutrient Broth medium was evaluated. In the medium, 100 µl of the culture was added, grown to a pad of 0.5 on the MacFarland scale. The growth was evaluated at the end of 48h / 30 ° C, using the spectrophotometer at 600nm. The method was performed in triplicate (Son *et al.*, 2006; Silini-Chérif *et al.*, 2012).

Detection of Enzymatic Activity

Detection of amylase

For the detection of aminolytic activity, the methodology described by Hankin and Anagnostakis (1979) was used, using the Nutrient Agar medium containing 0.2% starch, later distributed in Petri dishes. After solidification of the medium, a hole was made in the center of the plate, where a previously prepared bacterial suspension of 100 µL was inoculated with (10^7 / CFU). The plates were incubated at 35 ° C for 96 hours with daily monitoring. The enzyme production was evidenced after washing the plates with a lugol solution, by forming an opaque halo

around the colony. All assays were performed in triplicate.

Detection of Urease

For the detection of urease activity, the method of Hankin and Anagnostakis (1979) was used, using the nutrient agar medium (lower layer), with addition of 5% urea. The top layer was made with phosphate buffer agar plus 5% urea solution and 5% bromothymol blue solution. After solidification of the culture medium, a hole was made in the center of the Petri dish, with a diameter of 0.8 cm, in which 100 µL of the previously prepared bacterial suspension was inoculated. Plates were incubated at 28 and 37 ° C for 96 hours with daily monitoring. After the period of microbial growth, a light yellow halo around the colony indicated the presence of urease. All assays were performed in triplicate.

Detection of Tanase

For the detection of tannic acid activity, a medium having the following composition was used: 3 g of sodium nitrate, 1 g of dibasic potassium phosphate, 0.5 g of magnesium sulfate, 0.5 g of Potassium chloride, 20 g of agar, 4 g of tannic acid, 0.04 g of bromophenol blue and 1000 ml of distilled water (Sharma *et al.*, 2000). Another medium with the same composition was also prepared, however, by removing the bromophenol blue and increasing the tannic acid concentration to 20 g. After sterilization in autoclave (121 ° C, 1 atm, 20 min.), These media were poured into Petri dishes, solidified and inoculated using triplicates and control (uninoculated).

After solidification of the culture medium, a hole was made in the center of the 0.8 cm diameter Petri dish, where 100 µL of the bacterial suspension (10^7 / CFU) was inoculated. The plates were incubated at 35 °C for 96 hours, with daily monitoring (dark). The degrading activity was evaluated by the appearance of clear zones around the bacterial growth (Hankin; Anagnostakis, 1975).

Detection of Polyphenoloxidase

The colonies were aseptically removed and transferred to the center of the Petri dish containing modified nutrient agar medium (5g meat extract, 10g peptone, 5g sodium chloride, 750ml distilled water, pH 6.0, Tannic acid 5 g to 125 ml water and gallic acid 5 g to 125 ml distilled water) and incubated for 24 h / 35 °C. The enzymatic activity was observed by the brown halo formation around the colony on the rest of the plaque (Harkin; Obst, 1973).

Detection of Lipase

For the detection of lipolytic activity a medium was used with the following composition: 10 g peptone, 5 g sodium chloride, 0.1 g calcium chloride bihydrate, 20 g agar, 20 ml tween 20 And tween 80 and 1000 mL of distilled water. The tween was autoclaved separately in flowing steam and added to the medium before dispensing into Petri dishes. After sterilization in autoclave (121 °C, 1 atm, 20 min.), These media were poured into Petri dishes, solidified and inoculated using triplicates and control (without tween). Methodology described by Hankin and Anagnostakis (1979).

Results and Discussion

Isolation and Characterization of Bacteria

The isolated bacteria were cultured in nutrient agar (AN) for 24h at 30 °C. The colonies obtained had the following macroscopic characteristics: circular, smooth colonies, regular and flat borders, 1 mm in diameter and yellow pigment. Microscopic

examination revealed to be a Gram-negative bacillus with rounded ends. They were presented alone or in pairs. It is mobile, catalase positive, facultative anaerobic, non-fermenting glucose (Table 1).

The results obtained corroborate those found by Silini-Cherif and collaborators (2012) in the identification of a strain of *Pantoea agglomerans* IMA2 isolated from wheat rhizosphere. Fujikawa and Akimoto (2011) also show similar results for Pantoea agglomerans. The biochemical characteristics presented by the bacterium isolated from industrial laundry residue are also similar to the strains of *P. ananatis* and *P. stewartii* (Delétoile *et al.,* 2009).

Gavini *et al.,* (1989) and Mergaert (1993) describe the genus *Pantoea* as bacilli of 0.5-1.3 × 1.0-3.0µm. Non-encapsulated and non-spore forming. Most of the strains are mobile by means of perimeter, Gram-negative flagella and colonies when grown on nutrient agar are smooth, translucent with covex or heterogeneous margins in whole consistency and adhering to agar.

The colonies are yellow, beige or non-pigmented, facultative anaerobes. The optimum temperature of growth is around 28 and 30 °C. Oxidase negative.

Glucose dehydrogenase and gluconate dehydrogenase are produced and are active without an added cofactor. Lysine and ornithine are not decarboxylated, urease negative, does not degrade pectin, H_2S is not produced from thiosulfate. Most of the strains are Voges-Proskauer-positive and indol-negative. The acid is produced from the fermentation of L -arabinose, D -ribose, D -xylose, D-galactose, D-fructose, Lramnose, D-mannitol, N-acetylglucosamine, maltose and trehalose. The sources of carbon used at 28 °C (Biotype-100) are D-glucoside, D-fructose, D-galactose, trehalose, D-mannose, cellobiose, 1-O-methyl β-D-glucopyranoside, L-arabinose, Glycerol, and L -serine. The sources of unused carbon at 28 °C (Biotype-100) are L-sorbose, palatinose, melezitose, maltitol, turanose, tricarballylate, 4-hydroxybenzoate, gentisate, methyl 3-hydroxybenzoate, methyl benzoate, 3-phenylproprionate, M -cammarate, histamine, caprate, caprylate, glutarate, 5-aminovalerate, ethanolamine, tryptamine, itaconate, 3-hydroxybutyrate, propionate and L-tyrosine. Reference strains were isolated from plants, seeds, fruits, soils and water, and from humans (urine, blood, wounds, internal organs) and other animals. Strains of various species are phytopathogenic in a wide range of facilities and agricultural machinery. The G + C content of the DNA varies from 52.7-60.6 mol% (Deletoile *et al.,* 2009; Duron *et al.,* 2016).

Biochemical Characterization

The results of several biochemical tests were listed in Table 1. The *Pantoea*sp. Degraded some carbon sources such as D-mannitol, D-mannose, D-glucose, D-gluconase, D-fructose and sucrose. And also the gelatin protein. He presented H_2S production and was positive for the Voges-Proskauer test, methyl red and lactose. According to Delétoile *et al.,* (2009) and Mergaert *et al.,* (1993) these are characteristics of the strains of *Pantoea agglomerans.*

Responses to Abiotic Stress

Pantoea sp. Demonstrated great growth ability over a broad pH range, ranging from pH 4.0 to pH 8.0. There was inhibition of growth for alkaline pHs (pH 9.0 and 10.0). The bacteria showed optimum growth at pH 7.0 (Figure 1). The results were similar to those reported by *Pantoea agglomerans* CPA-2 and *Pantoea agglomerans* IMA2 in works

presented by Costa *et al.*, (2002), Son *et al.*, (2006) and Silini-Chérif *et al.*, (2012), respectively. Other studies have reported that the highest development of Pantoea occurs in culture medium with pH values ranging from 6.0 to 7.0 (Costa *et al.*, 2002).

Table.1 Biochemical tests and acid production to bacterialidentification

BiochemicalTests	**Bacterial strain**	**Acid production**	**Bacterial strain**
Motility	+	D-glucose	+
Yellow Pigment	+	D-fructose	+
Catalase	+	D-xylose	_
Citrate	_	D-mannose	+
Lactose	+	D-mannitol	+
Methyl red	+	D-gluconase	+
Glucose	_	Lactose	_
Indol production	_	Maltose	_
Production H2S	+	Trehalose	+
Reaction Voges-Proskauer	+	Sucrose	+

+: Positive Test; -: Negative Test.

Fig.1 Effect of different pHin nutrient broth medium on the growth of*Pantoea* sp.at48hof incubation

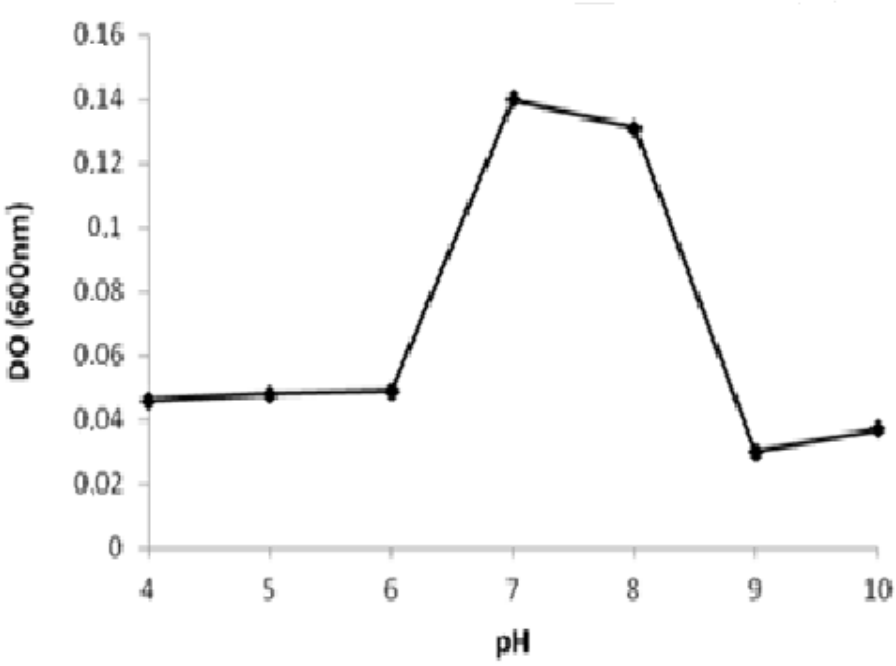


Table.2 Enzyme production by bacterial strain isolated from laundry effluent

Enzyme Activity	Bacterial strain
Urease	_
Protease	_
Amylase	_
Tanase	+
Polyphenoloxidase	+
Cellulase	+

The turbidity measurement, for growth at different temperatures, showed a significant result at 30 ° C, indicating that the tested *Pantoea lineage* is mesophilic (Figure 2). Similar results were found by Camatti-Sartori *et al.,* (2008) when evaluating the influence of temperature on the growth of *Pantoea agglomerans* and obtained the best results at temperatures of 30 ° C. The microorganism showed good viability of growth at high temperatures of 40 and 44 ° C (Figure 2), which could explain the presence of these organisms in arid regions (Silini-Chérif *et al.*, 2012).

However, Camatti-Sartori *et al.,* (2008) presented discordant results regarding the temperature of 40 ° C, because there was a significant decrease in the development of the microorganism. Son *et al.,* (2006) worked with insoluble phosphate solubilization by *Pantoea agglomerans* and obtained good microorganism growth results in a temperature range between 5 and 45 ° C. With best results at 25-35 ° C. Temperature is one of the most important factors governing the physiology and growth of microorganisms, as reported by Rahman *et al.,* (2006).

Fig.2 Evaluationof temperatures(°C) of incubation on the growth of *Pantoea* spin the nutrient broth medium at 48h

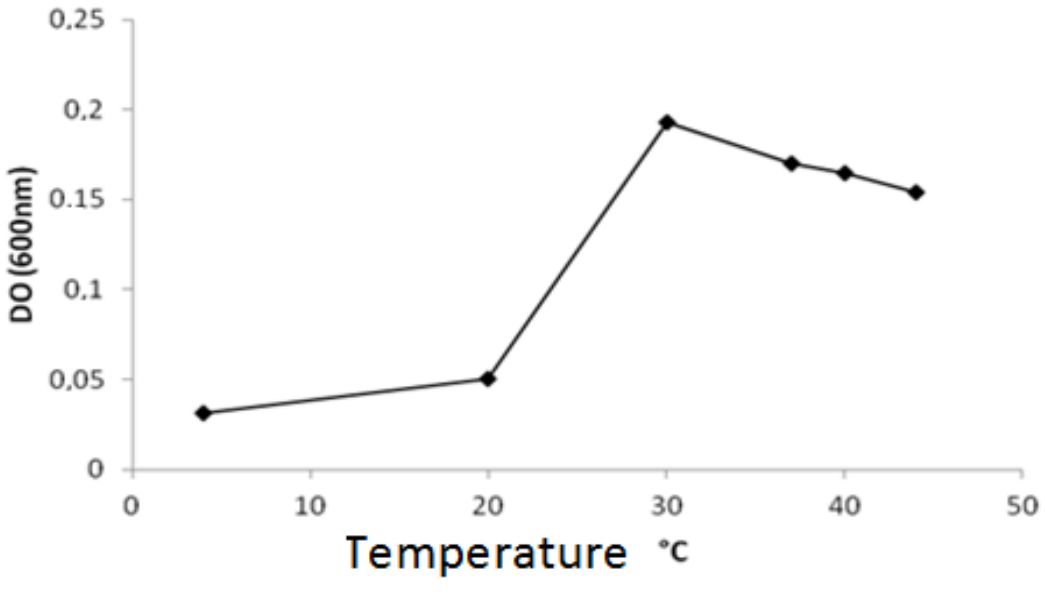
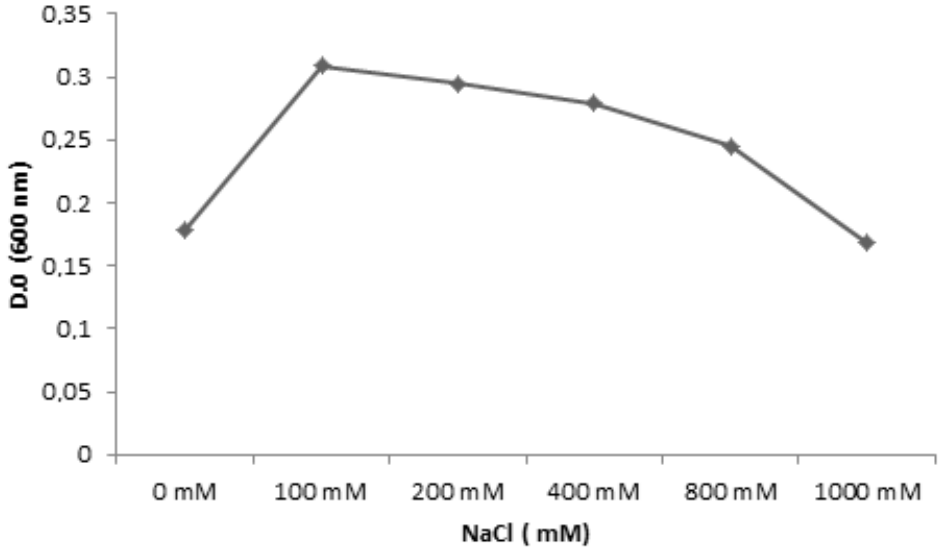


Fig.3 Effect of different concentrations of NaCl (mM)in nutrient broth medium on the growth of *Pantoea* sp.at 48h of incubation



Growth of the nutrient broth strain with variations in salt concentration from 0 to 1000 mM showed a high tolerance capacity at high salt concentrations. The high point of growth was with 100 mM. However, it presented excellent results at concentrations of 400, 800 and 1000 mM NaCl (Figure 3). According to Borneman *et al.,* (1996), soil salinity plays an important role in the microbial selection process. Several studies indicate that bacteria isolated from saline environments are better able to survive inhibition concentrations compared to non-saline isolates (Sharma, 2006). Similar results were found by Silini-Chérif *et al.,* (2012) when working with a strain of *Pantoea agglomerans.*

Enzymatic Profile of *Pantoea agglomerans*

Microbial enzymes have an essential role in the processes related to soil quality, since it is through them that the soil microorganisms degrade complex organic molecules in simple and that can be assimilated. In addition to allowing microorganisms to have access to the energy and nutrients present in complex substrates, extracellular enzymes are responsible for the decomposition and mineralization of nutrients in the soil, also making them available to plants and promoting the cycling of nutrients in the soil (Makol; Ndakidemi, 2008). The bacterium of the genus *Pantoea*sp was submitted to enzyme activity detection tests for amylase, urease, protease, tannase, cellulase and polyphenoloxidase. *Pantoeaagglomerans* isolated from laundry efluent showed positive results for enzymes tanase, polyphenoloxidase and cellulase (Table 3). Tannase is an enzyme that hydrolyzes esters and side bonds of hydrolysable tannins producing glucose and gallic acid (Banerjje *et al.,* 2001). It is mainly used for the production of gallic acid, instant teas, wine color stabilization, leather treatment process, food detanification, antioxidant production and effluent treatment in the leather industry (Battestin *et al.*, 2004;).

Madhukar *et al.,* (1996) and Souza and Magalhãs (2010 detected the enzymatic activity of amylase and cellulase by *Pantoea agglomerans* isolated from pea leaves (*Lathyrus maritimus* L). Cellulases are used in many biotechnological applications. In the

textile industry, these enzymes are used to give better finishing to the fabrics, making them smoother, smoother and with better trim. Cellulases are also used in the beverage industry for the production of fruit juices and in winemaking processes. Also, they play an important role in animal nutrition, detergent manufacturing, and the paper pulp industry, making the paper whiter and smoother. However, interest in these enzymes has increased greatly due to their use in the ethanol production process (Castro; Pereira, 2010).

Phenol oxidase-catalyzed reactions are related to changes in cell wall properties (increased impermeability and hydrostatic resistance), intracellular interactions and removal / detoxification of certain secondary metabolites (Mayer; Harel, 1979; Mardaneh; Dallal, 2013; Dushyanthie to al., 2014).

The biochemical and morphological tests showed that the microorganism isolated is of the *Pantoea* genus and presents characteristics similar to those presented by *Pantoea agglomerans*. The Pantoea strain studied showed tolerance to high salt concentrations, optimum growth in mesophilic temeprature codons, 30, 37 ° C, and alkaline pH 7 and 8 indicating an enzymatic biotechnological potential for cellulase, tannase and polyphenoloxidase.

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References

Banerjje, D., Mondal, K.C., Pati, B.R. 2001.Production and characterization of extracellular and intracellular tannase from newly isolated *Aspergillus aculeatus* DBF9. J. Basic Microbiol., 41, 313-318.

Battestin, V., Matsuda, L. K., Macedo, G. A. 2004. Fontes e Aplicações de Taninos e Tanases em alimentos. Alimentos e Nutrição Araraquara, 15, 63-72.

Brady, C. L., Venter, S. N., Cleenwerk, I., Vandemeulebroecke, K., De Vos, P.; Coutinho, T. A. 2010.Transfer of *Pantoea citrea*, *Pantoea punctata* and *Pantoeaterrea* to the genus *Tatumella* emend. As *Tatumella citrea* comb.nov., *Tatumellapunctata* comb.nov. and *Tatumella terrea* comb. nov. and description of *Tatumella morbirosei* sp. nov. Int. J. Syst. Evol. Microbiol, 60, 484–494 b.

Brady, C. L.; Cleenwerck, I.; Venter, S. N.; Vancanneyt, M.; Swings, J.; Coutinho, T. A. 2008. Phylogeny and identification of *Pantoea*species associated with plants, humans and the natural environmentbased on multilocus sequence analysis (MLSA). Syst. Appl. Microbiol. 31, 447–460.

Brady, C. L.; Venter, S. N.; Cleenwerck, I.; Engelbeen, K.; Vancanneyt, M.,;Swings, J.; Coutinho, T. A. 2009. *Pantoea vagans sp. nov., Pantoea eucalypti sp. nov., Pantoea deleyi sp.* nov. and*Pantoea anthophila* sp. nov. Int J Syst Evol Microbiol 59, 2339–2345.

Brady, C.,Venter, S.; Cleenwerck, I.; Vancanneyt, M.; Swings, J.; Coutinho, T. 2007. A FAFLP system for the improved identification of plant-pathoganic and plant-associated species of the genus *Pantoea*, Syst. Appl. Microbiol., 30, 413-417.

Brady, C.L.; Cleenwerck, I.; Venter,S.V.;

Engelbeen,K.; De Vos, P.; Coutinho, T.A. 2010.Emended description of the genus *Pantoea*, description of four species from human clinical samples, *Pantoea* septica sp. nov., *Pantoea eucrina* sp. nov., *Pantoeabrenneri* sp. nov. and*Pantoea conspicua* sp. nov., and transfer of *Pectobacterium cypripedii* (Hori 1911) Brenner *et al.*, 1973 emend. Hauben *et al.*, 1998 to the genus as *Pantoea cypripedii* comb. Nov. Inter. J. Syst.Evol.Microbiol., 60, 2430–2440a.

Brenner, D.J., *et al.*,1984. Attempts to classify *Herbicola* group-*Enterobacter agglomerans* strains by deoxyribonucleic acid hybridization and phenotypic tests, International Journal of Systematic Bacteriology, Ames, 34, 45–55.

Brown, C.M, Dilworth. 1975. Ammonia assimilation by *Rhizobium* cultures and bacterioids. J. Gen. Microbiol., 86 39-48.

Büyükcama, A.;Tuncer,Ö.; Gür, D.; Sancak, B.; Ceyhana, M. ; Cengiz, A.B.; Kara, A. 2017.Clinical and microbiological characteristics of *Pantoea agglomerans*infection in children.J. Infect Public Health, 783, 1-6.

Cabral,J.P.S. 2010. Water Microbiology. Bacterial Pathogens and Water.Int J Environ Res Public Health. 7(10): 3657–3703.

Camatti-sartori, V.,Valdebenito-Sanhueza, R.M.,Ribeiro, R.T.S. 2008. Desenvolvimento de *Pantoea agglomerans* em diversas temperaturas, pH e concentrações de carboxi-metil-celulose e o seu impacto no controle de *Rosellinia necatrix*, Summa phytopathologica,, 34, 1, 22-28.

Cappuccino, J.C., Sherman, N, 1992. Microbiology: a laboratory manuel. 3rd Edn, Benjamin/cummings Pub. Co., New York, USA, pp: 125-179.

Castro, A. M., Pereira JR, N. 2010. Produção, propriedades e aplicação de celulases na

Costa, E. *et al.*, 2002. Water activity, temperature and pH effects on growth of the biocontrol agent *Pantoea agglomerans* CPA-2. Canadian Journal of Microbiology, 48, 1082-1088.

De Maayer, P., Chan, W.Y., Blom, J., Venter, S.N., Duffy, B., Smits, T.H.M., Coutinho, T.A. The large universal *Pantoea* plasmid LPP-1 plays amajor role in biological and ecological diversification. BMC Genomics, 625,1471-2164.

Delétoile, A. *et al.*,2009. Phylogeny and Identification of *Pantoea* species and Typing of *Pantoea agglomerans* strains by multilocus gene sequencing.Journal of Clinical Microbiology, 47,300-310.

Duron, O.; Noël, V. 2016. A wide diversity of Pantoea lineages are engaged in mutualistic symbiosis and cospeciation processes with stinkbugs. Envir.Microbiol. Reports, 8,5, 715–727.

Dutkiewicz, J.; Mackiewicz, B.; Kinga Lemieszek, M.; Golec, M.;, Milanowski, J. 2016.Pantoea agglomerans: a mysterious bacterium of evil and good. Part III. Deleterious effects: infections of humans, animals and plants.Ann. Agric. Environ. Med.,23,2, 197-205.

Fujikawa, H., Akimoto, R. 2011. New blue pigment produced by *Pantoea agglomerans* and its production characteristics at various temperatures. Applied and Environmental Microbiology, 77, 172-178.

Gavini, F. *et al.*,1983.Taxonomic study of strains belonging or related to the genus *Erwinia*, *herbicola* group, and to the species *Enterobacter agglomerans*. Systematic and Applied Microbiology, 4, 218–235.

Gavini, F., *et al.*,. 1989. Transfer of *Enterobacter agglomerans* (Beijerinck

1888) Ewing and Fife 1972 to Pantoea gen. nov. as*Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov. Inter. J. Syst. Bacteriol., 39, 3, 337-345,

Grimont, P.A.D.; Grimont, F. Genus: *Pantoea*, in: D.J.Brenner, N.R. Krieg, J.T. Staley (Eds.), Bergey's Manual of Systematic Bacteriology, vol. 2, The Proteobacteria, Part B, The Gammaproteobacteria, second ed., Springer, New York, 2005, pp. 713–720.

Gupta, R; Mohapatra, H; Goswami, V.K.; Chauhan, B. 2003. Microbial α-Amylases: Biotechnological Perspective. Process Biochem.,38, 199-1616.

Hankin, L.; Anagnostakis, S. L. 1979.The use of solid media for detection of enzymes production by fungi. Mycologia, 67, 597-607.

Harkin, J.M., Obst, J.R. 1973.Syringaldazine an affective reagent for detecting laccase and peroxidase in fungi. Experientia, Basel, 29, 381-387.

hidrólise de resíduos agroindustriais. Química Nova, 33,181-188.

Coelho, R.S.,Amarante Junior, O.P., Melo, I.S., Parma, M.M, Vieira, E.M.Identificação de bactérias degradadoras de fluidos de corte por análise dos ácidos graxos. Revista ACTA Tecnológica, 6, n. 2, jul-dez. 2011.

Holt, J.G. *et al.*, Bergey's Manual of Determinative Bacteriology. 9ed. Baltimore: Williams & Wilkins, p. 787, 1994..

Kageyama, B. *et al.*, 1992. *Pantoea punctata* sp. nov., *Pantoea citrea* sp. nov., and *Pantoea terrea* sp. nov. isolated from fruit and soil samples, Inter. J. Syst. Bacteriology,, 42, 203–210.

Madhukar, B. K., Arya K. B., Lisa D. N., John, A. G. 1996. *Pantoea agglomerans* is the etiological agent for black spot necrosis on beach peas. Can. J. Microbiol., 42, 1252-1257.

Makol, J. H. J. R., Ndakidemi, P. A. 2008. Selected soil enzymes: examples of their potential roles in the ecosystem. African J. Biotechnol., 3, 181-191.

Mardaneh, J. and 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, 263–267.

Mergaert, J., Verdonck, L., Kersters, K. 1993.Transfer of *Erwinia ananas* (synonym, *Erwinia uredovora*) and *Erwinia stewartiito* the genus *Pantoea* emend. as*Pantoeaananas* (Serrano 1928) comb. nov. and*Pantoea stewartii* (Smith 1898) comb. nov., respectively, and description of *Pantoea stewartii* subsp. indologenes subsp. nov., Inter. J. Syst. Bacteriol., 43,162–173.

Mishra, A.; Chauhan, P.S.; Chaudry, V.; Tripathi, M.; Nautiyal, C.S. 2011.Rhizosphere competent *Pantoea agglomerans* enhances maize (*Zea mays*) and chickpea (*Cicer arietinum L.*) growth, without altering the rhizosphere functional diversity. Antonie Van Leeuwenhoek, 100, 405– 413.

Murray, P. R.; Rosenthal, K. S.; Pfaller, M. A. Microbiologia Médica, 5th ed., Elsevier editora, Rio de Janeiro, 2006.

Nadarasah,G.; Stavrinides, J. 2014. Quantitative evaluation of the host-colonizing capabilities of the enteric bacterium *Pantoea* using plant and insect hosts.Microbiol., 160, 602–615.

Prakash,O.; Nimonkar, Y.; Vaishampayan,A.;Mishra, M.; Kumbhare, S.; Josef, N.; Shouche, Y.S.;. 2015. *Pantoea intestinalis* sp. nov., isolated from the human gut. Inter.

J. Syst. Evol. Microbiol., 65, 3352–3358.

Rahman, M. *et al.*,2006.Effect of some environmental factors on the growth of *Azospirillum* species isolated from saline soils of Satkhira district, Bangladesh. Bangladesh J. Microbiol., 23,145-148.

Roper, C.M. 2011.P*antoeastewartii subsp. stewartii*: lessons learned from a xylem-dwelling pathogen of sweet corn. Molecular Plant Pathol.,12,628–637.

Sharma, S.; Bhat, T. K.; Dawra, R. K. 2000.A spectrophotometric method for assay of tannase using rhodanine. Analytical Biochem., 279, 85-89.

Silini-Chérif, H., Silini, A., Ghoul, M.; Yadav, S. 2012.Isolation and characterization of plant growth promoting traits of rhizobacteria: *Pantoea agglomerans* Ima2. Pakistan J. Biol. Sci., 15, 267-276.

Smits, T.H.M., Rezzonico, F., Kamber, T., Blom, J., Goesmann, A., Ishimaru, C.A.,Frey, J.E., Stockwell V.O., Duffy, B. 2011.Metabolic versatility and antibacterial metabolite biosynthesis are distinguishing genomic features of the fire blight antagonist *Pantoea vagans* C9-1. PLoS One, 6, 22-247.

Son, H.J., Park, G.T., Cha, M.S., Heo, M.S. 2006. Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant *Pantoea agglomerans* R-42 isolated from soybean rhizosphere. Biores. Technol., 97, 204–210.

Souza, P.M., Magalhães, P.O. 2010. Application of microbial α-amylase in industry – a review, Braz. J. Microbiol., 41, 850-861.

Suen, G. *et al.*,2010. An insect herbivore microbiome with high plant biomass-degrading capacity. PLoS Genet, 6, 1001-1029.

Verdonck, L. *et al.*,1987. *Genus Erwinia*: Numerical analysis of phenotypic features, Inter. J. Syst. Bacteriol., 37 4–18.

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