Relationship of Polygalacturonase Enzyme Activity between Brown Rot Isolates and their Virulence on Potato Cultivars

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ABSTRACT

***Ralstonia solanacearum*, the bacterium that causes bacterial wilt in many plant species, produces a number of extracellular plant cell wall-degrading enzymes that are thought to be virulence factors. Endopolygalacturonase (endo-PG), PehA, and two exo-PGs, pehB and pehC, are among them. *R. solanacearum* was isolated and identified from sick potato tubers, yielding five isolates (BR1, BR2, BR3, BR4, and BR5). The pathogenicity of the isolates was determined, results revealed that the isolates differed in their virulence. *R. solanacearum* isolates (BR3, BR2, and BR5) had different levels of virulence on the potato stems of the examined cultivar (strong, moderate, and weak) respectively, so these isolates were chosen to assess potato cultivars for susceptibility to *R. solanacearum* isolates. *R. solanacearum* susceptibility differed amongst potato varieties. Spunta cultivar was the most sensitive, whereas Nicola cultivar was the most resistant. Mondial was a fairly sensitive cultivar. The activity of the PG enzyme was measured in the five isolates. *BR3*, *BR2* and *BR5* isolates were high, moderate and low activity, respectively. The optimum PG activity was observed at pH 5 and 5.5 and PG activity reached the highest level at 2 and 2.5 from incubation time, the maximum activity of PG was observed at 30 ºC and 40 ºC of incubation temperature, the optimum PG activity was observed with NaCl followed by $CaCl_2$, The optimum NaCl concentrations for PG activity were 0.1 and 0.15 M**

Key words: pathogenicity, PG, Optimum, sensitive, incubation, cultivar.

INTRODUCTION

The potato (*Solanum tuberosum* L.), which one of the main food crops, is one of the world's most important vital vegetable crops. It's also one of the most popular solanaceous plants in the world (Birch *et al.,* 2012 and Liu *et al.,* 2016 Potato is a reliable crop for nutrition-security that holds a lot of promise in the fight against malnutrition in poor nations (Devaux *et al.,* 2020). In Egypt, Potato is one of the most economically important vegetable crops for local consumption, processing and export, with a production of approximately 5.08 million tons, produced from approximately 417.05 feddans Egypt is the 15th worldwide and is Africa's largest producers of potato (FAO STAT, 2019). The most dangerous bacteria to potato production in Africa, Asia, South and Central America is *Ralstonia solanacearum* Smith caused bacterial wilt disease in potato plants (Charkowski *et al.,* 2020). The *R. solanacearum* may live under a variety of environments, including soil, weeds, plant waste, rhizospheres, and alternate hosts. It spreads through diseased plant debris and irrigation water, making pathogen eradication more difficult (Hayward, 1991, Laferriere *et al.,* 1999, and Liu et al., 2016). Generally, bacteria are soil-borne pathogens that attack plants by penetrating xylem vessels via roots (Garcia *et al.,* 2019). symptoms such as yellowing the leaves, coloration of the vascular bundles, necrosis, and Complete wilt on infected plants, then followed by physiological disorders in diseased plants, such as increasing the respiratory rate and reducing transpiration and photosynthesis ,all of this is due to the multiplication of bacteria in large numbers inside the vascular bundles, which leads to blockage and impedes the process of transporting water and nutrients (Karim and Hossain, 2018). The bacteria that causes potato wilt is divided into five races based on their host range, and five biovars based on their ability to oxidise three hexose sugar alcohols and three disaccharides (Hayward, 1964). R. solanacearum race (3) and biovar (2) are responsible for potato crop attack. (Prior and Fegan, 2005). The disease severity and incidence were increased with R3bv2 as well as significant damage was found (Chávez *et al.,* 2012).

The disease severity and incidence vary between potato cultivars, according to the presence of genetic variations between them. Studies have pointed that Mondial cultivar is one of the tolerant cultivars to wilt disease, unlike Bellini and Spunta cultivars that are sensitive (Karim and Hossain, 2018). There are many factors were found to contribute to the virulence of *R. solanacearum*. The important factor in *R. solanacearum* virulence and a lot of pathogenic bacteria is the Type III Secretion System (T3SS) (Büttner and He, 2009; Coburn *et al.,* 2007). It delivers effector proteins inside the plant cells and hijacking the cellular machinery for bacterial benefit (Peeters *et al.,* 2013). Another key virulence factors are the extracellular polysaccharide I

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(EPS I). EPS I directly cause wilting by physically blocking the vascular system and thereby alters water movement (Denny and Baek, 1991). *R. solanacearum* may also be protected from the plants' antimicrobial defenses by hiding bacterial surface characteristics that are recognized by hosts (Araud-Razou *et al.*, 1998; Saile *et al.*, 1997).

The *R. solanacearum* also produces a number of Cell-Wall-Degrading Enzymes (CWDEs), including three PGs (PehA, PehB, and PehC) (Huang and Allen, 1997; Schell *et al.*, 1988), an endoglucanase (Egl) (Roberts *et al.*, 1988), a pectin methylesterase (Pme) (Tans-Kersten *et al.*, 1998), and a cellobiohydrolase (CbhA) (Liu *et al.*, 2005). Plant invasion by *R. solanacearum* appears to be facilitated by a trio of PGs that break down plant cell walls. The three PGs hydrolytically split the pectic polymer, but their reaction products are distinct. An endo-PG (EC 3.2.1.82), PehA (also known as PglA), cleaves the pectic polymer internally at random, releasing large oligomers, predominantly galacturonic acid (galUA) trimers (Allen *et al.*, 1991; Roberts *et al.*, 1988). Many investigators studied the effects of pH, temperature, incubation time, metals ions and Na concentration on the PG activity (Mathew *et al.*, 2008; Maisuria *et al.*, 2010; Anam and Latif., 2012; Martins *et al.*, 2013; Rahman *et al.*, 2019; Almulaiky *et al.*, 2020; Berber and Çetinkaya, 2020). They assayed the enzyme activity at different pH, different temperatures, different incubation times, different metals ions and different NaCl concentrations under standard assay conditions.

Therefore, the aim of this work was to isolate, purify and identify the bacterial potato brown rot in Egypt based on morphological, biochemical and cultural approaches. Evaluation of potato cultivars susceptibility against *R. solanacearum*. Determination PG enzyme activity in *R. solanacearum* isolates and study the effect of pH, temperature, incubation time, metals ions and Na concentration on PG activity.

MATERIAL AND METHODS

1. Sampling

Potato tubers exhibiting classic brown rot symptoms were collected from farms, marketplaces, and cold storage facilities in the El-Behera Governorate were kept separate in polyethylene bags and transported to the laboratory.

2. Isolation and purification of the causal organism.

Discolored vascular tissues and oozes of infected potato tubers were mixed with sterile water, suspension of bacteria was streaked on plats Contains tetrazolium agar medium (TZC) (Abo-El-Dahab and El Goorani, 1969). 5 gm of Peptone, 3 gm of beef extract, 20 ml of glycerol, 15 g of agar, one liter of distilled water and 0.05% of TZC (pH 7.0). At 28 C° plates were incubated for 48 hrs. For differentiating fluidal colonies of virulent isolates, the TZC medium was used (Kelman, 1954). Both avirulent and virulent strains of *Ralstonia solanacearum* were continuously cultured at room temperature on glycerol nutrient agar (GNA) medium

3. Biochemical and physiological bacterial identification experiments

The isolated bacteria's morphological, physiological, and biochemical features were investigated using the standard assays specified by (Fahy and Persley (1983), Adhikari (1993), Popoola *et al.* (2015), and Kumar *et al.* (2017). Cell morphology, motility, gram staining, sporulation, growth at 40°C, anaerobic growth, kovac's oxidase, gelatin liquefaction, starch hydrolysis, arginine hydrolysis, and acid production from Arabinose, xylose, lactose, Mannose, maltose, Adonitol, Raffinose, Sorbitol, Dextrin, and Sucrose were evaluated. He et al., 1983 described how carbohydrates were sterilized. Tests were carried out at 30°C for 2, 4, and 6 days, and acid production was monitored. Five brown rot isolates, (*BR1*: brown rot isolate1, *BR2*: brown rot isolate2, *BR3*: brown rot isolate3, *BR4*: brown rot isolate4 and *BR5*: brown rot isolate5) of *Ralstonia solanacearum* bacterial were identified.

4. Pathogenicity test:-

The virulence of *R. solanacearum* isolates were tested on Spunta potato cultivar which is susceptible to R. solanacearum (Rs) (Khairy *et al.*, 2021) was obtained from the International Potato Center (CIP). The tubers were external sterilized with (1% NaOCl for five minutes, then washed with sterile water), plastic pots 15 cm in diameter contains on sterile peat moss were used for planting potato tubers (one tuber per pot).

When plants reached about 15-20 cm in tall, plants were inoculated by injecting 0.25 ml of bacterial suspension (109 cfu /ml) into the stems at a height of 5 cm from the soil level by using forcing a sterilized needle (Prior and Steva, 1990). Plants were inoculated with three isolates, place in a green house at 25 + 2ºC. Four replicates, five pot with each replicate were used and plants control were injected with sterile distilled water. The severity of the disease was measured 19 days post inoculation (dpi), the percentage of disease severity (DS %) was calculated according to the formulae given by Bereika *et al*. (2020) as follows:

$$DS\% = \{\Sigma (ni \times vi) / N \times S\} \times 100$$

Where, Σ = Summation; *ni* represents the number of plants for each disease rating, *vi* represents the disease rating, *V* represents the highest disease rating (5), and *N* represents the total number of plants observed. The scale of (He *et al*., 1983) was used to determine disease severity as follow:-1 = no symptoms, 2 = one leaf

wilted, 3 = two to three leaves wilted, 4 = four or more leaves wilted, and 5 = plant was dead.

5. Sensitivity of different potato cultivars to *R. solanacearum*

Potato plants of the three potato cultivars (Spunta, Mondial and Nicola) were used to study the sensitivity of these potato cultivars to *R. solanacearum* isolates, BR3 strong isolate, BR2 intermediate isolate and BR5 weak isolate (according to pathogenicity test) were used. Potato plants were inoculated, the severity of the disease was measured and the percentage of disease severity (DS %) was calculated as mentioned in the pathogenicity experiments. Control plants, from each cultivar, were prepared, four replicates of each treatment were conducted.

6. Determination of polygalacturonase (PG) activity produced by *R.solanacearum* isolates.

6. 1. Polygalacturonase(PG) enzyme preparation.

The nutrient broth (NB) medium supplemented with 0.2% polygalacturonic acid (PGA) as the sole carbon source for 48 hrs. Bacteria were incubated at 30ºC ±2. The centrifugation at 12000 rpm for 20 min at 4 Cº was used to separate the bacterial cells from cultures (Universal 32 R centrifuge, Hettich-Zentrifugen, Germany). The supernatant was saturated with ammonium sulfate crystals to about 60–80 percent saturation , and the mixture was agitated overnight at 4°C. Centrifugation was used to collect the precipitates, which were then redissolved in small amounts of 0.05 M citrate-phosphate buffer pH 5.5 and dialyzed against the same buffer (Nasuno and Starr, 1966)

6. 2. Polygalacturonase (*PG*) assays.

The thiobarbituric acid (TBA) method was used to determine polygalacturonase (PG) activity with minor modifications (Lei et al., 1985a and Lei et al., 1985b). The crude enzyme was added to tubes containing 2.5 ml 0.5 percent polygalacturonic acid in 0.05 M sodium acetate buffer, pH 5.5, and 0.1 M NaCl to determine PG (Nasuno and Starr, 1966). The reaction mixture was incubated for 2 hours at 30°C before being halted by heating the tubes for 10 minutes at 100°C. The reaction mixture was centrifuged at 12000 rpm for 10 minutes after cooling at ambient temperature, and 2 ml of the supernatant was combined with an equal volume of TBA reagent (Ayers *et al.,* 1966) and heated at 100 Cº for 30 minutes. The rise in absorbance (optical density) at 515 nm was used to determine the activity (OD_{515}). Zero time reaction mixture containing active enzyme was used as control. The amount of galacturonic acid released per mL per minute was calculated from standard curve of galacturonic acid. One Unit of PGase activity was defined as the amount of enzyme required to release one micromole of galacturonic acid per mL per minute under standard assay conditions

6. 3. Effect of pH, temperature, incubation time, metals ions and Na concentration on PG activity.

PH, temperature, incubation time, metals ions and Na concentration were used to study there effect on PG activity were determined by assaying the enzyme activity at different pH values ranging from 4.5 to 7.5, different temperatures, ranging from 20 to 70 ºC and different incubation times from 0.5- 4 hrs., different metals ions (NaCl, $CaCl_2$, $MgCl_2$, $CoCl_2$ and KCl) and different NaCl concentrations ranging from 0 to 0.3 M, were performed using 0.05M sodium acetate buffer under standard assay conditions (Almulaiky *et al* 2020).

Statistical Analysis

Enzyme assays and pathogenicity test were carried out as repeated measures over time with 4 replicates per treatment. Data were statistically analyzed as repeated measures over time using the MIXED procedure of the statistical analysis software (SAS) version 9.4 Cary, NC, SAS Institute Inc. (SAS, 2014). Least significant means were compared using Dunnett's post-hoc Test ($P < 0.05$).

RESULTS

1. Biochemical and physiological bacterial Identification experiments

Five brown rot isolates, (*BR1*: brown rot isolate1, *BR2*: brown rot isolate2, *BR3*: brown rot isolate3, *BR4*: brown rot isolate4 and *BR5*: brown rot isolate5) of *Ralstonia solanacearum* bacterial were isolated from infected potato tubers showing wilt symptoms collected from fields and cold stored houses in El-Behera governorate. All isolates have rod shape, non sporic, motile and gram negative. The isolates showed a positive reaction for catalase activity, gelatin liquefaction, kovac's oxidase and acid production from Arabinose, lactose, Mannose, Raffinose, Sorbitol and Sucrose as well as grow at 40 ^{0}C. However, the isolates showed negative reaction for hydrolysis of starch, anaerobic growth, arginine hydrolysis and unable to produce acid from Maltose, Adonitol and Dextrin (Table1).

2. Pathogenicity tests:

Pathogenicity tests of the five isolates of *R. solanacearum* were performed on plants of Spunta cultivar, indicated that there were significant different between isolates in pathogenicity. The tested isolates were differed in their virulence (Table 2 and Fig. 1). The isolates *BR3* and *BR4* were highly virulent on Spunta cultivar and no significant different was found between they. On the contrary to, the isolates *BR1* and

BR5 were weakly virulent, however, the isolate *BR2* was moderately virulent.

Table 1. Morphological traits, physiological and biochemical activities of *R. solanacearum* isolates obtained from infected potato tubers.

Characteristics		*BR 1*	*BR 2*	*BR3*	*BR4*	*BR 5*
Cell shape (Rods, single)		+	+	+	+	+
Sporulation		-	-	-	-	-
Motility		+	+	+	+	+
Gram reaction		-	-	-	-	-
Catalase activity		+	+	+	+	+
Gelatin liquefaction		+	+	+	+	+
Hydrolysis of starch		-	-	-	-	-
Anaerobic growth		-	-	-	-	-
Kovac's oxidase		+	+	+	+	+
Growth at 40 º c		+	+	+	+	+
Arginine hydrolysis		-	-	-	-	-
Production of acid from						
	Arabinose	+	+	+	+	+
	Lactose	+	+	+	+	+
	Mannose	+	+	+	+	+
	Maltose	-	-	-	-	-
	Adonitol	-	-	-	-	-
	Raffinose	+	+	+	+	+
	Sorbitol	+	+	+	+	+
	Dextrin	-	-	-	-	-
	Sucrose	+	+	+	+	+

Table 2. Pathological reaction of Spunta potato cultivar.to five isolates of *R. solanacearum*

Isolates	Disease severity (%)* (Mean ± SD)
brown rot isolate1(*BR1*)	24 ± 0.056^{c}
brown rot isolate2 (*BR2*)	42 ± 0.156^{b}
brown rot isolate3 (*BR3*)	73 ± 0.090^{a}
brown rot isolate4 (*BR4*)	70 ± 0.065^{a}
brown rot isolate5 (*BR5*)	20 ± 0.166^{c}

* Data were average of four replicates.

$LSD_{0.05}$ for BR isolates = 5.017

Fig. 1. Pathogenecity tests of five brown rot isolates,*(BR1: brown rot isolate1, BR2: brown rot isolate2, BR3: brown rot isolate3, BR4: brown rot isolate4 and BR5: brown rot isolate5)* on Spunta potato cultivar and Cont.=control, 19 days after inoculation.

3. Susceptibility of potato cultivars to *R. solanacearum*.

The isolates of *R. solanacearum* (BR3, BR2 and BR5) differed in their virulence on potato stem of the tested cultivars (high, moderate and weak, respectively) were chosen dependent on symptoms disease on potato plants from Fig (1). These isolates were performed on Spunta, Mondial and Nicola potato cultivars. (Table 3 and Fig 2) showed that there are significant between potato cultivars in susceptibility degrees to *R. solanacearum* isolates. Nicola cultivar was the most resistant, while Spunta cultivar was the most susceptible to the infection with isolates of *R. solanacearum*. Mondial cultivar proved to be moderately susceptible to the infection with the test isolates.

Table 3. Susceptibility of potato cultivars to *R. solanacearum*.

isolates	Disease severity (DS%)		
	Spunta cv	Mondail cv	Nicola cv
BR2	42±0.146	35±056	24±112
BR3	73±0.130	50±152	32±046
BR5	20±0.142	120±134	12±156
Mean	45±0.129[a]	33.3±0.112[b]	22.6±0.104[c]

* Data were average of four replicates.

$LSD_{0.05}$ for cultivars = 8.237

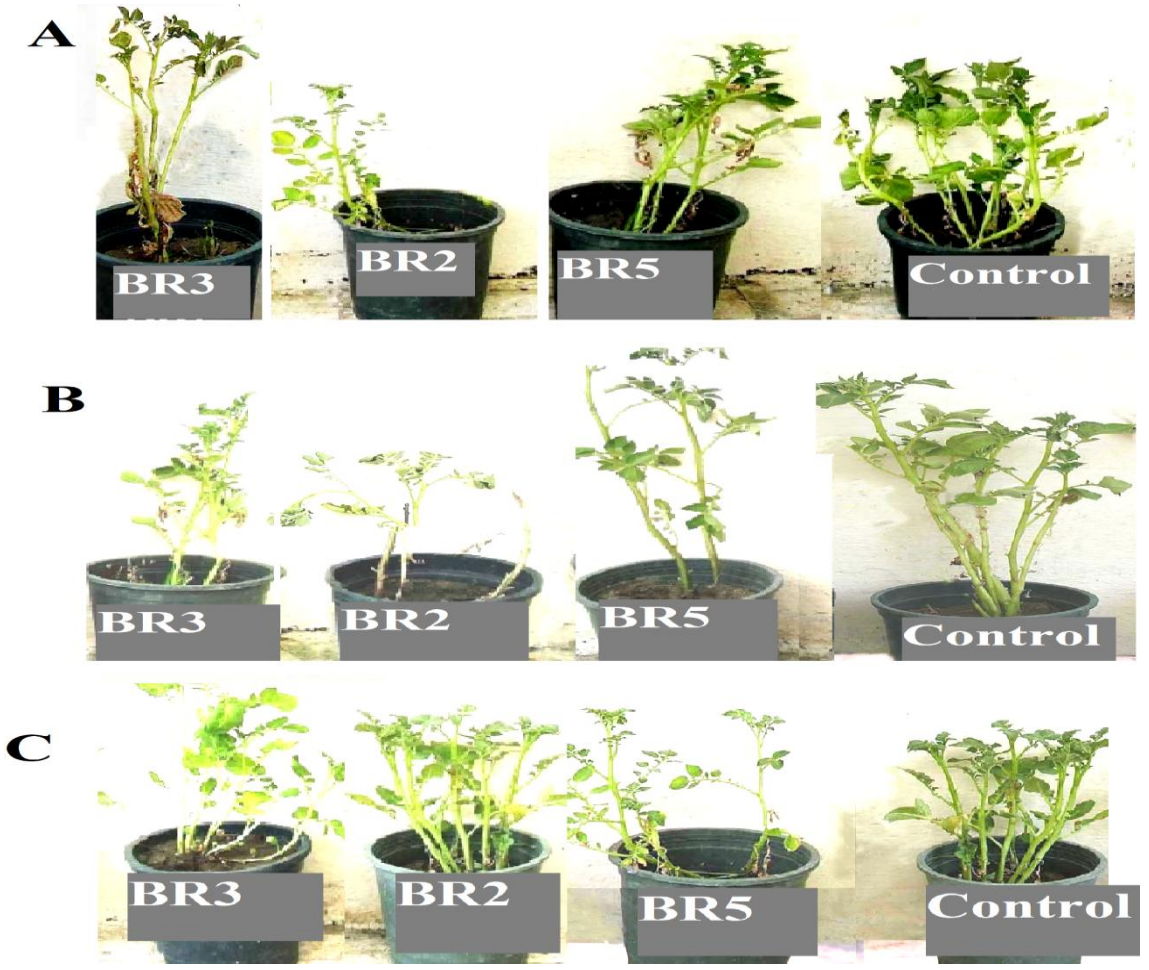

Fig. 2. Artificially inoculated aerial stems of Spunta (A), Mondial (B) and Nicola(C) with *R. solanacearum* isolates (*BR3: brown rot isolate3, BR2: brown rot isolate 2and BR5: brown rot isolate5)* and control, 19 days after inoculation.

4. Determination of polygalacturonase (PG) enzyme activity in *Ralstonia solanacearum* isolates

Polygalacturonase (PG) enzyme activity of all isolates and the result indicated there are significant between isolates in obtained polygalacturonase (PG) enzyme activity. The maximum enzyme activity was detected with enzyme produced from isolate (BR3) followed by isolate (BR4), while minimum enzyme activity was observed in case of enzyme produced by isolate (BR5) followed by isolate (BR1), while, (BR2) was moderate activity. The isolates namely *BR3*, *BR2* and *BR5* were chosen from Pathogenicity and PG activity test, these isolates were showed variation in Pathogenicity (Fig 3). Strong, intermediate and weak isolate, respectively were used for study the relationship between production and activity of polygalacturonase enzyme and pathogenesis.

4. 1. Effect of some external factors on the activity of PG:

The effect of some environmental conditions such as, pH, incubation temperature, incubation time, metal ions and concentrations of Na^+ (Fig 3) on the activity of PG enzyme *in vitro* using the isolates *BR3*, *BR 2* and *BR 5* to optimize conditions related to highest enzyme activity.

4. 1. 1. Effect of pH on the activity of PG

Polygalacturonase (PG) enzyme activity has wide range of pH ranged from 3.5-7.0. The optimum polygalacturonase (PG) activity was observed at pH 5, 5.5 and 6.0 in isolates *BR5*, *BR3* and *BR2*, respectively, however a further increased from pH 6.5 to 7.5 decreased the polygalacturonase activity rapidly, while the pH before 5 decrease the PG activity (Fig 4).

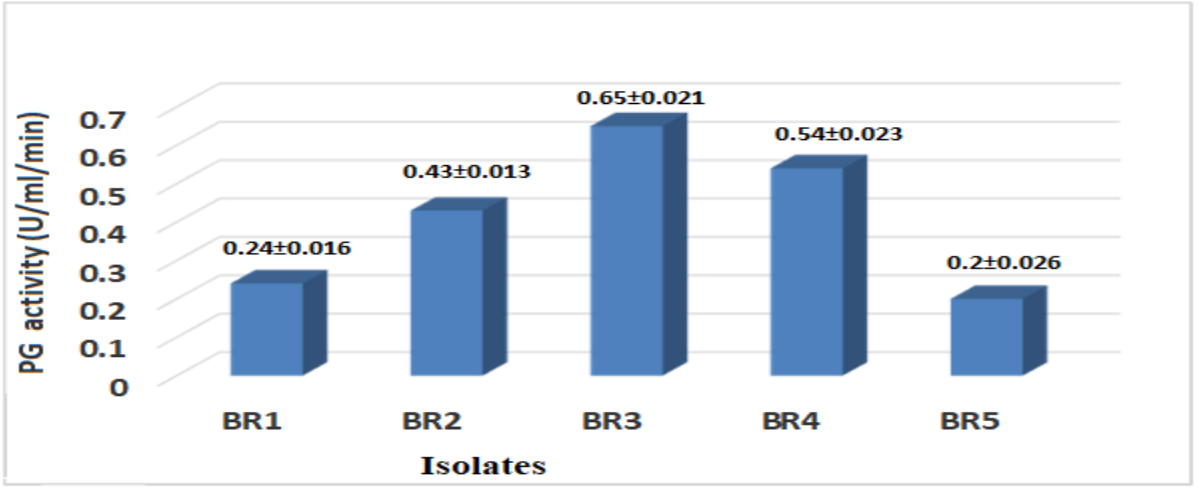

Fig.3. The Polygalcturonase activity (PG) in five isolates of Ralstonia solanacearum,*(BR1: brown rot isolate1, BR2: brown rot isolate2, BR3: brown rot isolate3, BR4: brown rot isolate4 and BR5: brown rot isolate5)*.

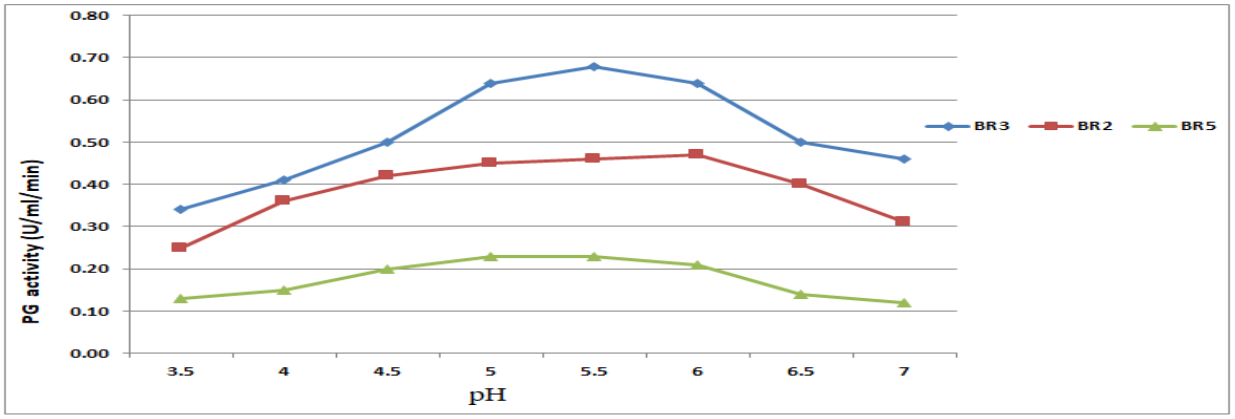

Fig.4. Effect of pH on the PG activity of *BR2*, *BR3* and *BR5* isolates

4.1.2. Effect of incubation time on the activity of PG.

A continuous increase in polygalacturonase enzyme activity was observed with increasing incubation time, but the enzyme activity reached its highest value after an incubation time at 2.5 and 2 hrs in isolates BR3, BR5and BR2, respectively, then the activity was declined (Fig. 5).

4.1.3. Effect of incubation temperature on the activity of PG.

The highest activity of the PG enzyme was at30 to 40 ºC, 30 and 35 ºC in isolates *BR3*, *BR2*and *BR5*, respectively. It was also observed that the lowest activity of the PG enzyme was at 50 ºC in all tested isolates (Fig 6).

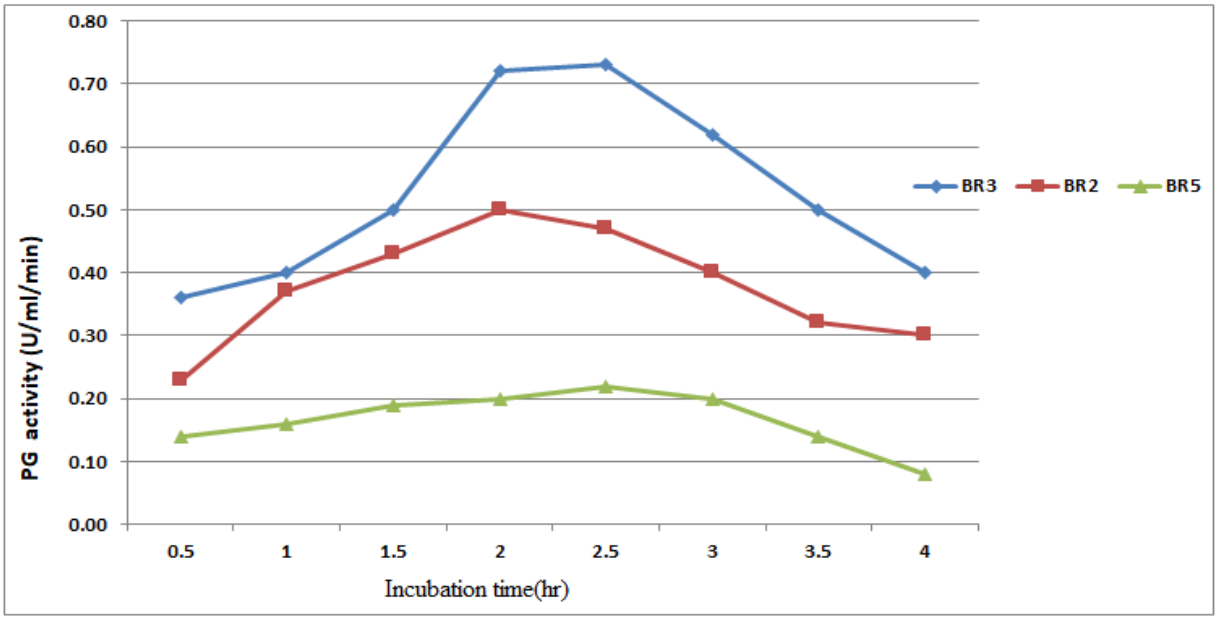

Fig.5. Effect of incubation time on the PG activity of *BR2*, *BR3* and *BR5* isolates

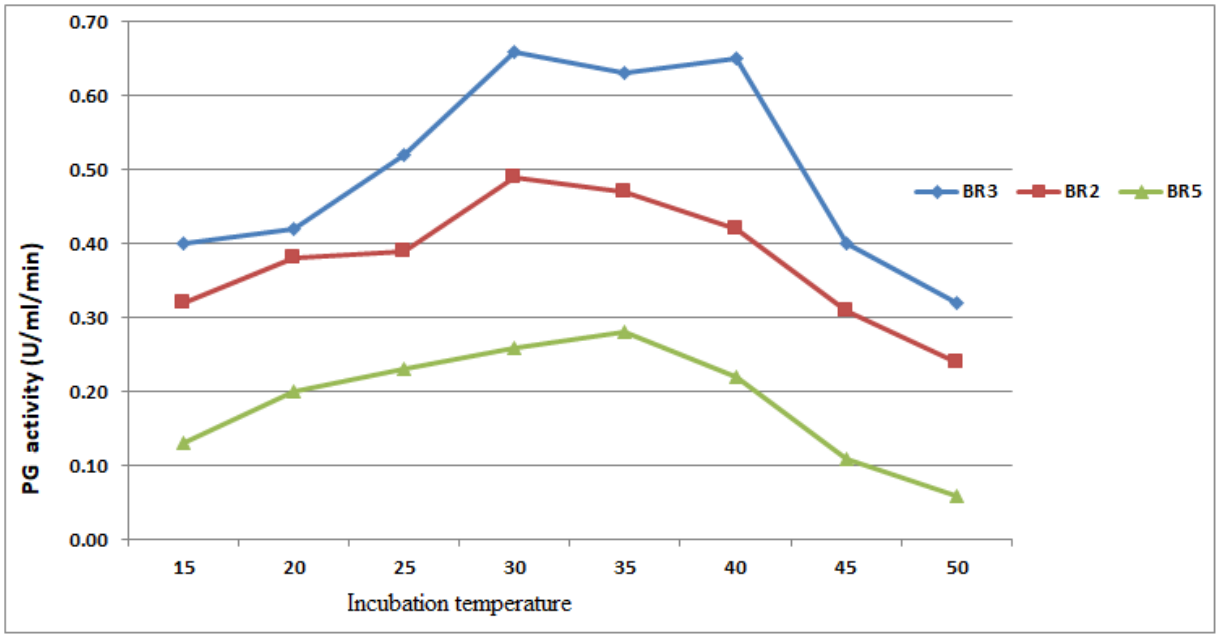

Fig.6. Effect of incubation temperature on the PG activity of *BR2*, *BR3* and *BR5* isolates

4.1.4. Effect of metal ions on the activity of PG

Clarified effect of various metal ions on the activity of PG enzyme. Data indicated that the optimum PG activity was observed with NaCl followed by CaCl2 in BR3 isolate the opposite of what is in isolate BR5 and there was no obvious difference was observed in PG activity when using NaCl and CaCl2 in BR2 isolate, while the inhibition of PG activity was observed with co^{2+} and K^{+} ions (Fig 7).

4.1.5. Effect of Na^{+} concentrations on PG activity:

The addition of sodium chloride increases the PG activity. The optimum NaCl concentration for PG activity at about 0.1 and 0.15 M. A higher concentration of NaCl (0.25 M) or lower concentration (0.05 M) decreased the PG enzyme activity (Fig 8).

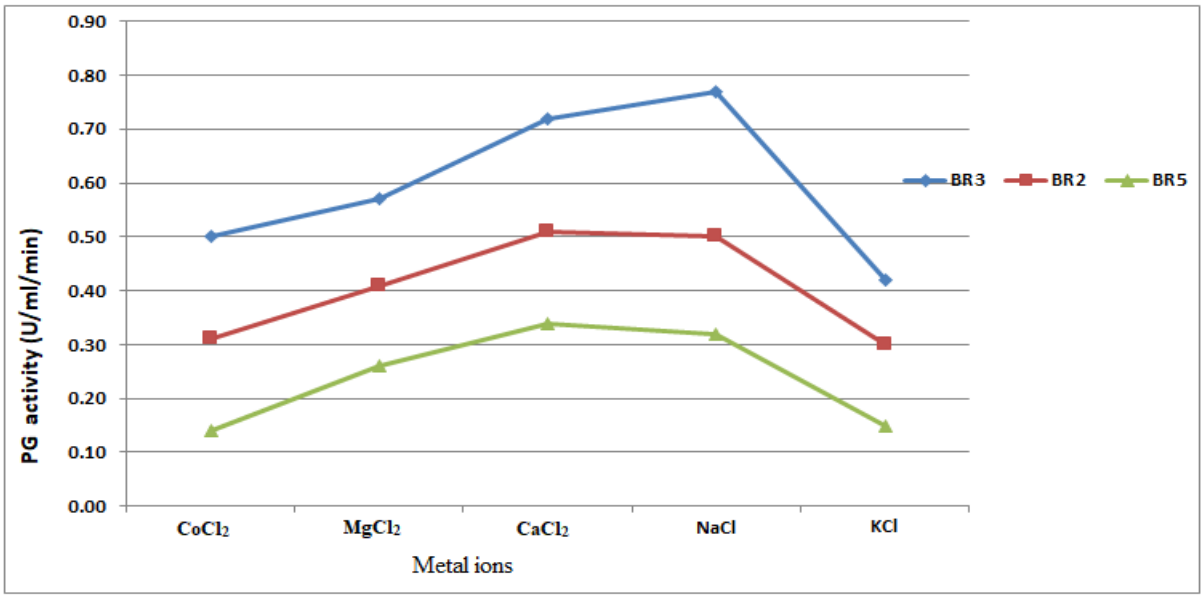

Fig.7. Effect of metal ions on the PG activity of *BR2*, *BR3* and *BR5* isolates

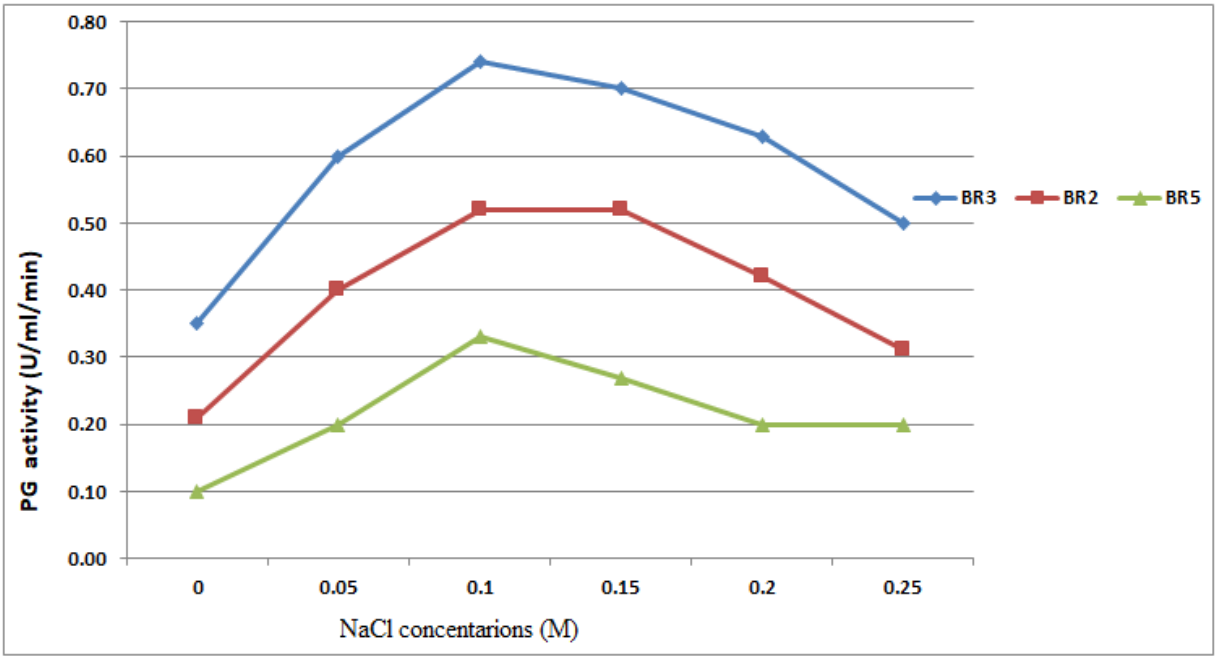

Fig.8. Effect of NaCl concentrations on the PG activity of *BR2*, *BR3* and *BR5* isolates

DISCUSSION

R. solanacearum isolates were isolated from different infected potato cultivar tubers collected from fields, markets, and cold storage facilities in El-Behiera governorate. The bacterial isolates' morphological, physiological, and biochemical features were studied (Popoola *et al.,* 2015; Kumar *et al.,* 2017), and the results indicated that all five isolates showed the characteristics of *R. solanacearum*.

The results obtained from inoculation tests performed on aerial stems of Spunta cultivar indicated that the infection with *R. solanacearum* isolates caused various degrees of wilting. Isolates of *R. solanacearum* were reported by other investigations to differ in their virulence on potato cultivars (2008, EL-Gayar, 2003; Khairy *et al.,* 2021). Data indicated that all isolates of *R. solanacearum* were virulent; however, the degree of virulence differed between isolates (Khairy *et al.,* 2021). The isolates No. BR3and BR4 were highly virulent, while the isolate BR2 was moderately virulent and the isolates BR1 and BR54 were weakly virulent according to the scale constructed by He *et al*., (1983). Data also showed that different degrees in susceptibility of potato cultivars to *R. solanacearum* isolates. Nicola cultivar was the most resistant, while Spunta cultivar was the most susceptible to the infection with isolates of *R. solanacearum*. Mondial cultivar proved to be moderately susceptible to the infection with the test isolates (Khairy *et al.,* 2021).

All isolates were able to produce polygalacturonase (PG) enzymes with different rates and differed activities. This was in agreement with previous studies (González and Allen, 2003, Schell, 2000 and Tariq and Lati, 2012).

pH is one of the main factors affecting on the active site of enzyme by creating an ionic balance. If there was no balance of ions, the 3D-shape of active site on enzyme structure would be disturbed so that the substrate could not be bounded in to the enzyme. Such conditions would lead to a decrease in the activity of enzymes (Srinivas and Panda, 1999). In this study, the optimum polygalacturonase (PG) activity was observed at pH 5, 5.5 and 6.0 in isolates *BR5, BR3 and BR2*, respectively, these findings were in agreement with several studies (Ofuya, 1984, Saarilahti *et al.,* 1990, Maisuria *et al;* 2010; Kothari and Baig, 2013). Moreover, The highest activity of polygalacturonase enzyme was observed after an incubation time at 2.5 and 2 h in isolates *BR3, BR*5and *BR2*, respectively, then the activity was decline these findings were supported by Mathew *et al.* (2008)

Data showed that the highest activity of the PG enzyme was at30 to 40 ºC, 30 and 35 ºC in isolates *BR3, BR2*and *BR5*, respectively, these results was harmony with (Kothari and Baig, 2013, Berber and Çetinkaya, 2020 and Almulaiky *et al.,* 2020). It was also observed that the lowest activity of the PG enzyme was at 50 ºC in all tested isolates. Incompatible temperature would be caused denaturation of enzymes. Denaturation of the enzyme was due to the release of covalent and hydrogen bond in the structure of the enzyme protein. The liberation of these bonds results in the disintegration of the enzyme protein folds that caused enzyme inactivation (Reece *et al.,* 2011)

The optimum PG activity was observed with NaCl followed by $CaCl_2$ in BR3 isolate the opposite of what is in isolate BR5 and There was no obvious difference was observed in PG activity when using NaCl and $CaCl_2$ in BR2 isolate, while the inhibited of PG activity was observed with CO^{2+} and K^+ ions several studies (Manachini *et al*., 2005; Trindade *et al.,* 2016; Rahman *et al.,* 2019; Almulaiky *et al.,* 2020) were clarified that the effect of various metal ions on the activity of PG enzyme.

Results in Fig (8) revealed that the addition of sodium chloride increases the PG activity. The optimum NaCl concentration for PG activity at about 0.1 and 0.15 M. A higher concentration of NaCl (0.25 M) or lower concentration (0.05 M) decreased the PG enzyme activity (Mathew *et al.,* 2008, Berber and Çetinkaya 2020)

CONCLUSION

There is relationship was found between production and activity of polygalacturonase enzyme and virulence of some R. solanacearum isolates. In addition to some environmental conditions such as, pH, incubation temperature, incubation time, metal ions and concentrations of Na+ were affected on polygalacturonase enzyme activity, where, The optimum PG activity was observed at pH 5 and 5.5 and PG activity reached the highest level at 2 and 2.5 from incubation time, the maximum activity of PG was observed at 30 ºC and 40 ºC of incubation temperature, the optimum PG activity was observed with NaCL followed by CaCl2, The optimum NaCL concentrations for PG activity were 0.1 and 0.15 M.

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الملخص العربي

علاقة نشاط انزيم البولى جالاكتيورونيز بين عزلات العفن البنى ومرضيتها على اصناف البطاطس

اسيا رشاد عيد وسوسن صلاح الدين الشامى

بكتيريا Ralstonia solanacearum التي تسبب الذبول البكتيري في العديد من الأنواع النباتية، تنتج عددًا من الإنزيمات التي تعمل على تحطيم جدار الخلية النباتية والتي يُعتقد أنها عوامل مرضية. ومن بينها Endopolygalacturonase (PG)(PehA) واثنين من exo-PGs (pehB وpehC). تم عزل وتحديد R. solanacearum من درنات البطاطس المصابة، مما أسفر عن عزل خمس عزلات BR1هى و BR2 و BR3 و BR4 و BR5. تم تحديد الشدة المرضية للعزلات، وأظهرت النتائج أن العزلات اختلفت في قدرتها المرضية. وان العزلات BR3 و BR2 وBR5 لها مستويات مختلفة من المرضية على سيقان البطاطس للصنف المدروس(قوي، متوسط، ضعيف) على التوالي، لذلك تم اختيار هذه العزلات لتقييم قابلية أصناف البطاطس للإصابة بعزلات .R. solanacearum. اختلفت حساسية أصناف البطاطس للعزلات. كان الصنف Spunta هو الأكثر حساسية، بينما كان الصنف Nicola الأكثر مقاومة وكان الصنف موندیال حساسًا إلى حد ما. تم قياس نشاط إنزيم PG في العزلات الخمس. كانت عزلات BR3 و BR2 و BR5عالية ومتوسطة ومنخفضة النشاط على التوالي. لوحظ نشاط PG الأمثل عند الأس الهيدروجيني ٥ و٥,٥ ووصل نشاط PG إلى أعلى مستوى له عند ٢ و٢,٥ من وقت تحضين، ولوحظ ان اقصى نشاط PG عند ٣٠ درجة مئوية و٤٠ درجة مئوية من درجة، ولوحظ نشاط PG الأمثل مع كلوريد الصوديوم يليه $CaCl_2$.كان تركيز كلوريد الصوديوم الأمثل لنشاط PG عند حوالي ٠,١ و ٠,١٥ مول.

الكلمات الدالة: الزبول البكتيرى، انزيم، حساسة، نشاط.