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# Epidemiology of Potato Blackleg in Warm Climate

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**Abstract:** Potato (*Solanum tuberosum* L.) planted in warm climate in September, in Egypt, showed no above ground symptoms of blackleg. The harvested crop showed severe tuber collapse similar to that being produced by the soft rot disease. Tentative identification of the isolated bacteria revealed no affiliation to the soft rot bacterium *Erwinia carotovora* ssp. *carotovora*. Verification of identity was made by PCR that showed its close similarity, to *E. carotovora* ssp. *atroseptica* at 119 bp, compared to an authentic Dutch isolate PD 4202. The present study suggested that the quality of water phase in the field soil may play an important role in rot progress at the time of harvest. Tonicity, cation(s) content and pH of the water phase of soil, suspending the bacterial inoculum, were found important in this regard. The rot increased under hypertonic stress (10 $g^{-1}$ NaCl) was attributed to a greater proliferation of bacteria on the expense of nutrients withdrawn from tuber cells. Further increase (20 $g^{-1}$ NaCl) in tonicity, however, decreased tuber rot, indicating negative correlation between the aggressiveness of strain to potato tubers and their osmotic tolerance. Pathogen suspended in hypotonic solution produced greater rot possibly due to greater invasion by bacteria and/or maximization of the intercellular spaces following cell turgidity. The monovalent and divalent cations at approximately similar osmotic strength decreased the rot symptoms. This effect may be attributed to their effect on a group of depolymerases with different optimal conditions and/or their effect on changing optimal pH for pectinolysis. The tuber rot was recorded over a wide range of pH. Further investigations are needed to study in depth other edaphic factors related to epidemiology of blackleg disease in warm climate.

**Key words:** *E. atroseptica*, blackleg warm climate, water quality, osmosis, pH

## INTRODUCTION

Blackleg disease caused by (*E. carotovora* ssp. *atroseptica*) and soft rot caused by (*E. carotovora* ssp. *carotovora*) are found wherever potatoes are grown. The first is widespreading in wet soil and relatively low, (18-19°C) temperatures. The second is favoured by higher temperatures above 25-30°C, mostly in heaped potato under improper storage conditions (Oliveira *et al*., 2003). Recently, it has been observed that the harvested winter crop, planted in warm climate in September, is subject to a tuber collapse similar to that being produced by the soft rot disease. Characterization of bacteria associated with this symptoms revealed that the case is not produced by *E. cartovora* ssp. *carotovora.*

The objectives of this study were undertaken to document the occurrence of blackleg disease in Egypt grown under warm condition and report factors effecting the epidemiology of disease.

## MATERIALS AND METHODS

**Source of isolates and verification of identity:** Five isolates recovered from rotted tubers during isolation trials made on Crystal Violet Pectate (CVP) medium (Cupples and Kelman, 1974) were used in this investigation. Production of acid from maltose, α-methylglucoside production of reducing substances, from sucrose and growth at 36°C (Schaad *et al*., 2001) were used for tentative species differentiations. Verification of identity was made by PCR (Smid *et al*., 1995) using an authentic Dutch isolate of *E. carotovora* ssp. *atroseptica*, PD 4202. The specific primer had the following sequence of bases:

(SR1cR) : 5 AGA CTC TAG CCT GTC AGT TTT-3
(SR3f) : 5 GGT GCA AGC GTT AAT CGG AAT-3

**Inoculum preparation:** The selected isolates in concern were inoculated in accurately measured volumes (100 $mL^{-1}$ flask) of nutrient broth medium and incubated for 4 days at 25°C. The cultures were centrifuged at 4500 rpm, for 10 min and the harvested bacterial cells was resuspended in 50 mL NaCl solution(s),with different tonicity, ranging from 0-20 $g^{-1}$ NaCl. The inoculum density was photometrically standerdized for different isolates to give $10^8$ cfu $mL^{-1}$ at 560 nm (Gomah and Mahmoud, 2007).

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For monovalent and divalent cationic studies, the sediment(s) was resuspended in 50 mL/isolate of NaCl (0.15 M), $CaCl_2$ (0.04 M), $MnCl_2$ (0.03 M) and optically standardized as described before. The calculated molar concentration of the afore-mentioned chlorides gives approximately similar cationic strength. Different pH treatments were prepared in phosphate buffered solutions to give a pH ranging from 5.8-8.0.

**Inoculation techniques:** Standardized Spunta cv. tubers (55 mm in diam.) were surface sterilized by flaming. A hole was made in the tuber lengthwise with a sterilized cork borer and the bacterial suspension (0.5 mL/hole) was pipetted in (Gomah and Mahmoud, 2007). The tubers were kept covered with polyethylene sheets for one hour to facilitate diffusion of the liquid and invasion of the bacteria through the cut holes. The latter were closed with their respective removed cylinders and sealed with sterilized paraffin wax. Incubation was made for 4-8 days at 25°C in plastic bags for each composite replicate (5 tubers/rep.) of a given treatment. The check treatment was prepared in the same way except the bacterial suspension that was replaced by sterile water. Inoculation trials were repeated twice. A completely randomized design was used.

**Rot assessment:** Transverse cut, passing through the hole, was made perpendicular to the long axis of the tuber and the rot dimensions was determined in cm. The volume of rot in $cm^3$ was determined according to Gomah and Mahmoud (2007). The following equation was used:

$$\text{Rot } (cm^3) = \Pi \times Lx\left[\frac{\text{diam.}}{2}\right]^2$$

Where:
Π = 3.14
L = Length of rot (cm)
diam. = Diameter of rot (cm)

## RESULTS

**Identification of isolates:** The isolates selected from CVP plates produced acid from maltose α-aminoglucoside produced reducing sugars from sucrose and did not grow at incubation temperature about 36°C. These results suggesting that the bacterial identity may be *E. carotovora* ssp. *atroseptica*.

The isolates, tentatively identified as *E. carotovora* ssp. *atroseptica,* were further verified for identity on a molecular bases. Figure 1 shows a close similarity of the domestic isolates with a Dutch one PD 4202. Note the development of bands at 119 bp in the conventional PCR method used.

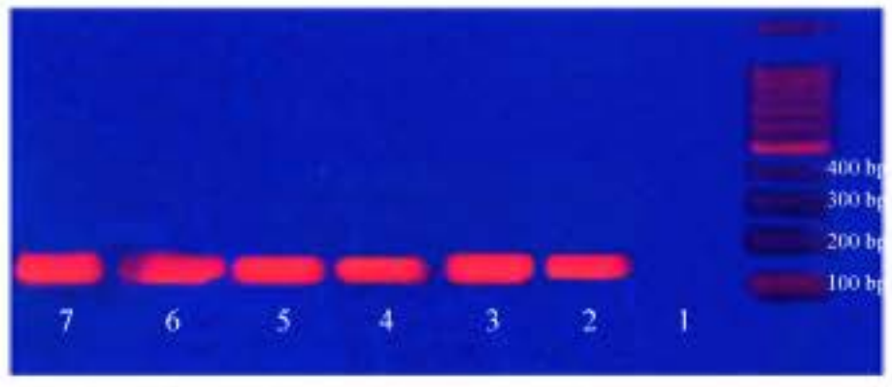


Fig. 1: Similarity of the domestic isolates with a Dutch one PD 4202. Note the development of bands at 119 bp in the conventional PCR method used

Table 1: Effect of osmosis on soft rot development

| | *Mean amount of rot $cm^3$ | | | | | |
|---|---|---|---|---|---|---|
| Treatments | Eca 1 | Eca 2 | Eca 3 | Eca 4 | Eca 5 | Mean ($cm^3$) |
| Hypotonic: (distilled water) | 4.2 | 4.7 | 5.6 | 4.7 | 3.2 | 4.48 |
| Isotonic (8.5 $g^{-1}$) | 0.0 | 3.2 | 4.5 | 4.5 | 5.1 | 3.46 |
| Hypertonic | | | | | | |
| 10.0 $g^{-1}$ | 8.7 | 10.0 | 10.7 | 10.7 | 4.7 | 8.96 |
| 15.0 $g^{-1}$ | 7.0 | 7.0 | 7.2 | 6.2 | 8.6 | 7.20 |
| 20.0 $g^{-1}$ | 8.0 | 8.6 | 5.6 | 5.6 | 5.6 | 6.84 |

$LSD_{0.05}$ = 2.1, *Mean of 25 tubers (for each trial; two trials were considered)

**Effect of osmosis on rot development:** Table 1 shows the effect of tonicity on tuber rot produced by *E. carotovora* ssp. *atroseptica*. The bacteria suspended in a hypotonic solution showed a considerable variation in rot development, with an average mean of 4.48 $cm^3$. The isotonic treatment (3.46 $cm^3$), though the noticeable variation in rot produced, did not show any significant difference compared to the hypotonic ones.

Bacteria suspended in solutions with different hypertonic potentials caused a significant rot increase over the hypotonic and isotonic ones. It is observed, however, that increasing the osmotic strength from 10 to 20 $g^{-1}$ had a deleterious effect on the bacterial growth, thus significantly decreased rot symptoms.

**Effect of initial pH values on rot development:** Table 2 shows that increasing the initial pH values of the suspending solution(s) of bacteria from 4.6 (distilled water) to 7.0 (saline) did not cause significant change in rot produced. The percentage of rot decrease produced by saline treatment, however, did not exceed 9% compared to that produced by the check treatment.

It is interesting to note that increasing the initial pH from 4.6 to 6.6 showed a tendency for greater decrease in the amount of rot produced. However, increasing the pH to 8.0 caused a significant increase in rot by 27.0%.

**Effect of cations on rot development:** Table 3 shows the effect of cations, with different valencies, in the form of chlorides, on rot development.

Table 2: Effect of initial pH values on rot development

| Treatments | *Mean amount of rot ($cm^3$) Eca 1 | Eca 2 | Eca 3 | Eca 4 | Eca 5 | Mean | Rot decrease |
|---|---|---|---|---|---|---|---|
| **Control (DW)** | | | | | | | |
| pH 4.6 | 4.8 | 4.4 | 4.8 | 4.9 | 4.4 | 4.7 | |
| **Saline** | | | | | | | |
| pH 7.0 | 4.4 | 3.5 | 4.5 | 4.5 | 4.4 | 4.3 | 9.0 |
| pH 5.8 | 4.5 | 4.5 | 3.0 | 3.5 | 3.8 | 3.9 | 17.0 |
| pH 6.6 | 3.4 | 3.2 | 4.0 | 4.5 | 4.0 | 3.8 | 19.0 |
| pH 7.4 | 4.2 | 3.5 | 4.5 | 4.4 | 4.5 | 4.2 | 11.0 |
| pH 8.0** | 6.3 | 5.8 | 6.0 | 5.8 | 6.3 | 6.0 | 0.0** |

$LSD_{0.05} = 4.6$, *DW: Distilled Water,

$$\text{Rot decrease} = \frac{\text{Rot DW} - \text{Rot T}}{\text{Rot DW}} \times 100$$ , **Rot increase 27.7%

Table 3: Effect of cations with different valencies on rot development

| Treatments | *Mean amount of rot ($cm^3$) Eca 1 | Eca 2 | Eca 3 | Eca 4 | Eca 5 | Mean | Rot decrease |
|---|---|---|---|---|---|---|---|
| Control (DW) | 5.0 | 5.2 | 5.7 | 5.7 | 3.8 | 5.1 | |
| $Na^{+}Cl^{-}$ (0.15 M) | 4.2 | 4.6 | 3.8 | 4.4 | 4.2 | 4.6 | 17.6 |
| $Ca^{++}Cl^{-}_{2}$ (0.04 M) | 2.1 | 3.2 | 3.0 | 3.1 | 2.5 | 2.8 | 45.1 |
| $Mn^{++}Cl^{-}_{2}$ (0.03 M) | 3.0 | 3.0 | 2.8 | 2.6 | 2.5 | 2.8 | 45.1 |

$LSD_{0.05} = 2.2$, $\text{Rot decrease (\%)} = \frac{\text{Mean rot control} - \text{Mean rot treat}}{\text{Mean rot control}} \times 100$

The monovalent sodium chloride (0.15 M) treatment did not cause significant decrease in rot compared to distilled water. The percentage of decrease in rot following the monovalent sodium chloride treatment was 17.6% .

The divalent calcium chloride treatment (0.04 M), with an approximately isotonic strength, significantly decreased the rot symptoms. The percentage of rot decrease was 45.1%, compared to 17.6 for sodium chloride. Similar trend was observed in manganese chloride treatment.

## DISCUSSION

It is shown in this study that tuber collapse of a harvested winter potato crop at certain districts in Egypt is not caused by *E. carotovora* ssp. *carotovora,* though planting is being made in hot season in September and October. The isolated bacteria produced acid from maltose, α-methylglucoside and produced reducing sugars from sucrose which are remarkable for *E. carotovora* ssp. *atroseptica* (Schaad *et al*., 2001). Polymerase Chain Reaction (PCR) of the tentatively identified domestic isolates revealed close similarity, at 119 bp, with an authentic *E. carotovora* ssp. *atroseptica*, PD 4202, isolate (Smid *et al*., 1995; Tath *et al*., 2001).

It seems probable that the high soil temperature(s) at the time of planting (in September) is not favoruable for the blackleg development, early in the season. Infection may take place at later stages when cooler temperatures and wet conditions are prevailing. The disease is reported to be favoured by moist soil and relatively low temperatures, lower than 18-19°C (Smadja *et al*., 2004). Other epidemiological factors as lentilcle infections, growth cracks, injuries at the harvest and poor suberization, the degree of cultivar maturity may also be involved (Seif El-Yasal, 1984; Perombelon, 1992; De Boer, 2002). Therefore, the late season infection under favourable epidemiologically sustainable conditions may suggest tuber losses encountered. The thermodependence of bacterial growth and enzymatic activities implicated in pathogenicity of two *E. carotovora* subspecies was indicated by Smadja *et al*. (2004). Their results are in agreement with ecological data implicating *E. carotovora* ssp. *atroseptica* in disease when the temperature is below 20°C. The optimal temperature for pathogenicity appears to be different from the optimal growth temperature but seems to be a compromise between this temperature and temperatures at which lytic activities are maximal.

It is established that the degree of deterioration of tubers is depending on a group of interrelated extrinsic and intrinsic factors affecting both the organ attacked and the invading bacteria. The depth of wounding, the low degree of suberization in the intercellular spaces and exhaustion of oxygen, which varied with temperature of respiring tubers and the calcium content of tubers are among the factors studied in tuber rot problems (McGuire and Kelman, 1984, 1986; De Boer, 2002; Dontsova and Bigham, 2005; Gloux *et al*., 2005).

Tubers inoculated with *E. carotovora* ssp. *atroseptica* under different osmotic strength caused considerable variation in rot produced. The rot increase under hypertonic stress may be attributed to greater proliferation of bacteria on the expense of nutrients withdrawn from the tuber cells (Gerges, 2007). However, the tendency of rot decrease by increasing the osmotic potential (from 10 to 20 $g^{-1}$ NaCl) is evidently disagree with the afore-mentioned speculation on the bacterium-inoculum increase. Therefore, additional factors either single or integrated may be involved. In this regard, Gloux *et al*. (2005) reported a negative correlation between the aggressiveness of strains to potato tubers and their osmotic tolerance. They added that the disruption of the gene encoding the major osmoprotectant uptake system highly enhanced bacterial virulence on potato tubers. Furthermore, it is shown in this study that the bacteria inoculated under hypotonic stress showed a tendency for greater rot compared to those introduced with isotonic solution. Such effect may be physically attributed to greater absorption of the hypotonic solution through the

cut surface that facilitates invasion of bacteria and/or maximizing the intercellular spaces following cell turgidity (Gerges, 2007). Retardation of pectinolytic activity as a result of salting out the enzyme, may be another factor inter-related to the reported decrease of rot in case of isotonic treatment (Moyo *et al*., 2003). The pectinolytic enzyme production by *Pseudomonas marginalis* was reported to be dependent on the interaction between temperature, pH and salt concentration, as well as the age of culture (Membre and Burlot, 1994).

The monovalent sodium ($Na^+$, 0.15 M) and the divalent calcium ($Ca^{++}$, 0.04 M) with approximately similar osmotic strength, in the form of chlorides decreased the rot by 17.6 and 45.1%, respectively. Similar decrease was found with manganese chloride ($Mn^{++}$, 0.03 M) at the same osmotic strength. This indicates that the used cations in the form of chlorides did not increase the amount of rot when incorporated with the bacterial suspension. The earliest record made on the inter-relation between cations and tissue maceration may be made by Bateman (1964). He reported that calcium, barium and magnesium inhibited tissue maceration by polygalacturonase whereas potassium and sodium did not significantly influence the process. McGuire and Kelman (1986) reported that the severity of tuber rot caused by *E. carotovora* ssp. *atroseptica* was inversely related to the concentration of tuber calcium. They reported that infiltration with solutions containing $Mg^{++}$ and $Sr^{++}$ also reduced the subsequent severity but not as effectively as $Ca^{++}$, however, these divalent cations proved more effective than $Na^+$ and $K^+$ (McGuire and Kelman, 1984; Conway *et al*., 1992).

The effect of cations on pectin degradation was studied *in vitro* as well in other investigators. Stack *et al*. (1980) reported four pectate depolymerases one of which depolymerize sodium polypectate in both endolyase manner at pH 8.5 in the presence of $Ca^{++}$ and an endohydrolase manner at pH 6.0, regardless the presence of cations. They reported as well a depolymerase with an exolyase activity over a wide pH range (5.0-10.0); requiring $Mn^{++}$ and pH 8.5 for optimum activity.

Accordingly, it is observed in this work that rotting is taking place over a wide range of initial pH values of the suspending solution of the pathogen, at either acidic or alkaline sides. The effect may indicate the presence of a group of depolymerases active over a broad pH range (5.0-10.0) but with optimum activity at pH 8.5 (Stack *et al*., 1980). The incidence of rot over a wide range of pH and the decrease of rot with different cations application may indicate an *in situ* effect of cations on a greater suberization of the surfaces in the cut holes used in inoculation.

## CONCLUSION

It could be concluded that the potato blackleg bacterium may infect potatoes, raised under warm conditions, late in the growing season when low temperatures and wet conditions are prevailing. The quality of water suspending the bacteria at the time of infection and/or the *in situ* effect has playing a significant role in rot development. The tonicity of the suspending solution, the variation in cation content and the initial pH values and their integrated effects may be important factors associated with tuber rot severity under certain circumstances. Further investigation are needed on the relationship between other edaphic factors and rot development.

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