

## RAPID SCREENING OF PHYTOPATHOGENIC *Erwinia* sp. OF TWO POTATO VARIETIES (SPUNTA AND DESIREE) FROM ALGERIAN AGRICULTURAL FIELDS

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### ABSTRACT

**Rapid screening of phytopathogenic *Erwinia* sp. of two potato varieties (Spunta and Desiree) from Algerian agricultural fields.** Isolation, phenotypic identification and *in vitro* phytopathogenicity screening of *Erwinia* sp. from agricultural field of two potato varieties (Spunta and Desiree) in Algeria. The current study aims to isolate, identify and screen phytopathogenic isolates of *Erwinia* sp. causing potato diseases. The techniques presented in this study for isolation and characterization of phytopathogenic *Erwinia* sp. are conventional methods that are used in this field of research. Seven phytopathogenic bacteria were recovered from potato tubers of two varieties (Spunta and Desiree). The phenotypic identification allowed characterizing typical colonies of *Erwinia* sp. on two semi-selective media: King's B and TCC media. *Erwinia* sp. formed characteristic colonies on King's B medium that were round, convex and representing creamy color. While, *Erwinia* sp. also developed specific colonies on TCC medium which were pale purple, circular, convex, even bulging; smooth and mucous. *In vitro* phytopathogenicity test on potato slices lead to screen the phytopathogenic isolate E5 characterized by highest rotten tissue zone of (2.33 ± 0.29 cm) and (2.33 ± 0.58 cm) toward Spunta and Desiree varieties, respectively. Followed, by isolate E4 characterized by rotten tissue zone of (1.83 ± 0.58 cm) and (2.17 ± 0.29 cm) toward Spunta and Desiree varieties, respectively; compared to their corresponding uninfected controls. The RTZ (Rotten tissue zone) evidently is proportional to the specific pathogenicity of *Erwinia* sp. isolates and the characteristic sensitivity of various varieties (Spunta and Desiree). Thus, make determining RTZ a rapid screening technique for the selection of the highest phytopathogenic isolates. This investigation provides valuable information for rapid screening (infected potato tuber) and characterization (isolation using semi-selective media) of pathogenic *Erwinia* sp. engendering potato disease compared to existing methods like infection of leaves or plants; and phytopathogenic *Erwinia* sp. identification through PCR amplification or *in situ* hybridation.

**Key words:** Desiree, *Erwinia* sp., phytopathogenicity, potato varieties, Spunta

### INTRODUCTION

The characterization of plant diseases is undoubtedly of major importance. Especially when it comes to dealing with a redoubtable pathogen as *Erwinia* sp. or worldwide important crop like potato. The three main factors constituting this branch include bacteria/plant pathogenicity mechanisms have received a great attention. Therefore, the study of these main factors could contribute directly to our understanding of all phenomena and interdependencies generated; and indirectly to the control and preservation of potato crops for a sustainable agriculture. *Erwinia* genus comprises species that are plant pathogens, non-pathogen, epiphytes, and opportunistic human pathogens (Borruso

*et al.*, 2017). Particularly, it consists of 15 species that are associated with plants as pathogens, saprophytes, or epiphytes (Lelliott & Dickey, 1984). Mainly, species causing the highest losses of fruit or vegetables include *Erwinia amylovora*, *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora*, *E. chrysanthemi* and *E. stewartii* (Waleron *et al.*, 2004). This genus is characterized by Gram-negative bacteria belonging to Enterobacteriaceae family (Aremu & Babalola, 2015). *Erwinia* and its subgeneric members usually are motile rods bearing peritrichous flagella and are able to ferment glucose leading to acid formations. Their fermentative pathway yields mixed acids and 2,3-butanediol. All are catalase positive and negative for exocellular cytochrome oxidase activity (Kado, 2006). Several

species (soft rot *Erwinia* spp.) were moved to two new genera as *Pectobacterium* and *Dickeya* (Nabhan *et al.*, 2013). Especially, pectinolytic *Erwinia* (*E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora* and *E. chrysanthemi*) attacks potato plants (Ladjouzi, 2006).

Potato (*Solanum tuberosum* L.) is a perennial, herbaceous, dicotyledonous and a tuberous plant of the Solanaceae family (Boufares, 2012). It is cultivated in more than 150 countries and plays a key function in the world food system. Potato is ranked as the fourth most important crop in the world after rice, maize and wheat (Daoud & Doudou, 2017). In 2013, world potato production was estimated at 368.1 million tonnes, with a cultivated area of 19.4 million hectares, characterized by an average yield production of 18.9 tonnes / hectare. This, without including seedlings that account for 32.2 million tonnes (FAOSTAT, 2015). China was first rankst among the producing countries reaching 88.9 million tonnes (FAOSTAT, 2015). The demand for potatoes is increasing thus representing the leading crop market in terms of surface area and production (Chehat, 2008). The two varieties (Spunta and Desiree) were selected to investigate in this study as indicator models of phytopathogenicity because their widespread cultivation in Algeria. In addition, the varieties was conducted to test the effect of cultivar variability on potato sensitivity toward bacterial diseases (Hamad *et al.*, 2021). Indeed, there were 169 potato varieties registered in Algeria (Foudil, 2016). The varieties Spunta and Desiree were among the most cultivated. The Spunta variety was dominant; as more than 55% of the cultivated areas were composed of this variety (Kheddam *et al.*, 2017).

Numerous bacterial infections are occurring on the tuber and crop of potato. The following genera include these bacteria: *Ralstonia*, *Pectobacterium* (*Erwinia*), *Streptomyces* and *Clavibacter* (Latour *et al.*, 2008). Various species of *Erwinia* are known as potato pathogens such as *Erwinia carotovora* subsp. *atroseptica* (van Hall) Dye, *Erwinia carotovora* subsp. *carotovora* and *Erwinia chrysanthemi* (Pérombelon & Kelman, 1980). All three bacteria can cause tuber soft rot; *E. carotovora* subsp. *atroseptica* is the primary blackleg agent in cool areas, whereas in hotter regions *E. chrysanthemi* predominates (Hélias *et al.*, 2000b).

Bacterial pathogenic diseases is an important problem in potato production. Pathogenic bacteria may remain and spread in farming areas once introduced. In addition, its unintentional and unrecognized propagation are particularly essential in a contaminated or latent (asymptomatic) potato seed infection (Stevenson *et al.*,

2001). Bacterial phytopathogens proliferate quickly and cause significant loss and economic harm of production under favorable conditions for disease development (De La Fuente & Burdman, 2011). In agricultural production, more consideration has been devoted to bacterial infections (Rienzie *et al.*, 2021). It is challenging to control bacterial infections in potato farms. When a disease is transmitted to fields, it can survive and spread over lengthy period (Azhar & Wani, 2021).

*E. carotovora* subsp. *carotovora* and *Erwinia carotovora* subsp. *atroseptica* are quality, pathogens causing losses in the field and during storage (Latour *et al.*, 2008). Around 22% of potatoes are lost every year because of viral, bacterial, fungal and pestinal attacks on the tuber and potato plants, with over 65 million tons annually damaged, and 30–50% this enormous loss is caused alone by bacterial soft rot (Czajkowski *et al.*, 2011). It is difficult to estimate, but known to be huge, loss of agricultural production owing to bacterial soft rot. In 1980, the worldwide loss of potato crop due to soft rot was expected to range from US\$50 to US\$100 million (Pérombelon & Kelman, 1980). Blackleg, aerial stem rot, and stem wet rot are main bacterial foliar diseases caused by pectolytic erwinias, as well as bacterial soft rot in tubers (Van der Wolf & De Boer, 2007). Probably, bacterial soft rot and blackleg, are the most severe crop diseases causing productivity loss (Salem & Abd El-Shafea, 2018).

In numerous potato cultivars in western Algeria, soft rot symptoms were reported in potato plants (Benada *et al.*, 2018). When potato resistance is compromised, bacteria tend to cause infections by acting as opportunistic agents. Tubers and stems are afflicted with these latent infection (Pérombelon & van der Wolf, 2002; Raimi *et al.*, 2017). The formation of a water-soaked zone on the tuber surrounding lenticels or eyes is the first symptom of soft rot. When one of these lesions was cut, it was noticed fragile the tissue (Koepsell, 1978). Soft rot symptoms of potato tubers are shown as watered lesions, which become gradually soft (mushy, disintegrated and discolored). The tissue becomes dark, creamy and slim in the infected lesions and finally decomposed (Rahman *et al.*, 2012); plant emergence was reduced, plants were weak, curled and the leaves drooped, similar to wilt disease or water deficiency, and ultimately yellowing symptoms appeared (Rosenzweig *et al.*, 2016). While, blackleg symptoms appeared when *Erwinia* predominated in decomposing mother tubers, penetrate the stems, and proliferate in xylem vessels under favorable climatic circumstances (Pérombelon & van der Wolf, 2002).

## MATERIALS AND METHODS

**Research Site.** The study was carried out during April 2019. Characterizing symptomatic potato tubers belonging to two varieties: Spunta (white) and Desiree (red). Seven samples were collected from three fields at Sirat of the Mostaganem department. Figure 1 and Table 1 showed the geographical location of the three potato fields.

**Collection of Potato Tubers.** Potato tubers showing typical symptoms were chosen. The infected parts were characterized by creamy to dark brown, granular consistency, spongy appearance (Benada *et al.*, 2018) and releasing characteristic disagreeable odor (Rosenzweig *et al.*, 2016).

**Isolation.** Isolation of *Erwinia* sp. from infected tuber tissues was performed using the modified methods described by Pérombelon & van der Wolf (2002) and Lacroix & Vézina (2003). Briefly harvested potato

tubers were washed to remove soil excess. Then, the tubers were cut longitudinally into two identical parts using a sterile blade. Samples of 1 g were taken from the unhealthy areas and were macerated in sterile mortar. A suspension was prepared in 0.9% of sterile physiological water with constant stirring for 30 min. From this suspension, decimal serial dilutions were made ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$ ). Bacteria were isolated on a semi-selective King's B medium. They were purified by successive transplantations on a modified CCT (*Cycloheximide Crystal violet Thallium nitrate*) medium.

**Phenotypic Identification.** Colonies presenting typical characteristics of *Erwinia* sp. formed on Petri dishes containing semi-selective King's B and CCT media were described for establishing their phenotypic aspects (Lacroix & Vézina, 2003).

### Phytopathogenicity Test

**Preparation of Bacterial Inocula.** Pre-culture of each bacterial isolate was prepared from a colony streaked



Figure 1. Geographical representation of sampling potato fields (field 1, 2 and 3).

Table 1. Represents the GPS locations of potato fields

Fields	GPS location
Field 1	35°46'49.8"N 0°12'15.9"E
Field 2	35°47'24.7"N 0°10'37.4"E
Field 3	35°48'17.0"N 0°11'34.7"E

on Petri plate containing King's B medium, followed by incubation at 30 °C for 24 h. These pre-cultures served to prepare bacterial suspensions in 5 mL of physiological sterile water (Hélias *et al.*, 2000a). These last constituted the bacterial inocula used forming approximately  $1.5 \times 10^8$  CFU/mL equivalents to 0.5 McFarland standard (Hajimehdipoor *et al.*, 2010). The McFarland method was designed to estimate bacterial concentrations by means of a turbidity scale (absorbance) which consists of a series of tubes previously calibrated, and with an optical density produced by the precipitation of barium sulphate. This absorbance is compared to bacterial suspensions (Eduardo *et al.*, 2018).

**Preparation of Plant Material and Inoculation.** To verify the phytopathogenicity of the bacterial isolates in *in vitro* trials, potato slices were infected according to the modified method described by Priou & Jouan (1996). Potato tubers were washed before hand and soaked in ethanol (10%) for 5 min. Then, rinsed with sterile water and dried. Each tuber was cut longitudinally into several identical slices and placed in Petri dishes containing sterile filter paper. Wells (5 × 5 mm) were made in the centers of the potato slices. Then, they were filled with 50 µL of each bacterial inoculum. Petri dishes containing infected slices were incubated at 30 °C for 48–72 h. Development of typical symptoms were monitored visually and the phytopathogenicity of isolates were estimated through measurement the rotten tissue zone (RTZ) formed on potato slices (in cm).

**Statistical Analysis.** To establish the significance of phytopathogenicity results of *Erwinia* sp. isolates toward the two potato varieties (Spunta and Desiree), data were analyzed by one-way ANOVA, followed by Dunnett test compared to their corresponding controls. Results were considered statistically significant at ( $p < 0.001$ ) and

( $p < 0.005$ ). Then, for determining if the phytopathogenicity result differences of *Erwinia* sp. isolates between the two varieties were significant, the data were examined by two-way ANOVA, followed by Sidak's multiple comparison tests. Results were considered statistically significant at ( $p < 0.05$ ) and ( $p < 0.01$ ). Experiments were performed in triplicate and repeated three times with similar results. All results were statistically compared using Graph Pad Prism V. 6.0.

## RESULTS AND DISCUSSION

**Phenotypic Identification on King's B Medium.** A total of 7 *Erwinia* isolates (E1, E2, E3, E4, E5, E6 and E7) were recovered from all samples of infected potato tubers. After incubation at 30 °C for 24 h on King's B medium, characteristic colonies of *Erwinia* sp. were identified round, convex and representing creamy color (Figure 2). Lakhdar (2018) reported that *Erwinia* genus formed round, convex, creamy colonies on King's B medium.

**Phenotypic Identification on CCT Medium.** After incubation on CCT medium at 30 °C for 48, phenotypic aspect of *Erwinia* sp. isolates revealed typical colonies that were pale purple, circular, convex, even bulging; smooth and mucous. Their growth was slower than King's B medium (Figure 3).

**Phytopathogenicity Test.** After three days of inoculation, characteristic symptoms were observed on tubers of the two potato varieties (Spunta and Desiree). They were small water-soaked spots on the surface. Rapidly these spots enlarged and the vegetal tissue was decomposed. The soft and blister-like areas were formed on the surface. Black rot lesion spread on rotting

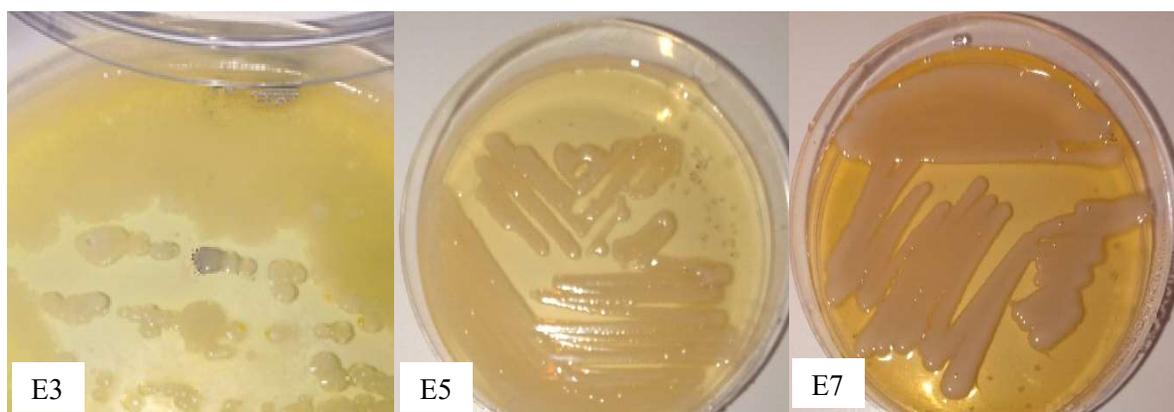


Figure 2. Macroscopic aspect of *Erwinia* sp. isolates on King's B medium.

tuber for some isolates, including isolate E7 on Sunta and Desiree varieties, and isolate E3 on Spunta variety (Figure 4). Similar results were reported by Elhalag *et al.* (2020) that the most frequently observed symptoms of collected potato tubers samples were slimy, wet, black rot lesion spreading on rotting tuber. Tuber tissues were distinctly macerated to a creamy consistency with a blacked color appearance.

All symptomatic samples were belonging to cv. Scotch Hermes and cv. Scotch Cara and may indicate the susceptibility of the reported cultivar for soft rot and/or blackleg infection. Furthermore, the results of this current study highlight a concordance to results were reported by Marković *et al.* (2021) stated that two types of tissue decomposition could be notified for these diseases: the first group of isolates (Like E3, E4 and E7 for Spunta variety, and E4 and E7 for Desiree variety) produced cream-colored rotting tissue with dark brown margins in the zone between the healthy and decomposed tissue, and the second group (Such as isolates E5 for Spunta variety, and E3 and E5 for Desiree

variety) devastated the tissue causing cream-colored rotting but with no visible margins.

Measurement of necrotic area lengths showed sensitivity of the two potato varieties (Spunta and Desiree) to *Erwinia* sp. isolates (Figure 5). Higher susceptibility of Spunta was detected compared to Desiree. Phytopathogenic screening led to the selection of isolate E5 characterized by diameter of ( $2.33 \pm 0.29$  cm) and ( $2.33 \pm 0.58$  cm), followed by isolate E4 characterized by diameters of ( $1.83 \pm 0.58$  cm) and ( $2.17 \pm 0.29$  cm) Spunta and Desiree varieties, respectively.

The phytopathogenicity test is based on the observation of different symptoms caused after infection of potato tubers, in order to determine the level of pathogenicity of different pectinolytic strains as well as the sensitivity degree of various varieties (Ibrahim *et al.*, 1978; Priou & Jouan, 1992; Aiteche & Benzid, 2018). Rotten tissue diameters can vary up to 11-fold between the highest and the lowest pathogenic strains indicating clearly the variability of pathogenicity between tested strains. This variability, especially in the

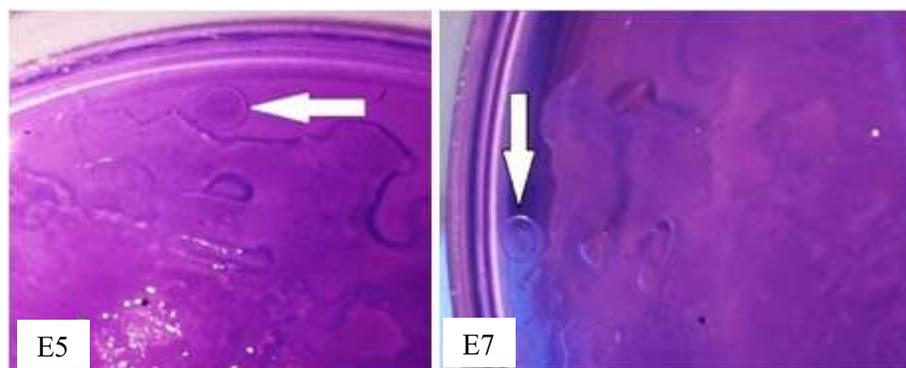


Figure 3. Macroscopic aspect of *Erwinia* sp. isolates on CCT medium (Arrows indicate the colony aspects of isolates E5 and E7).

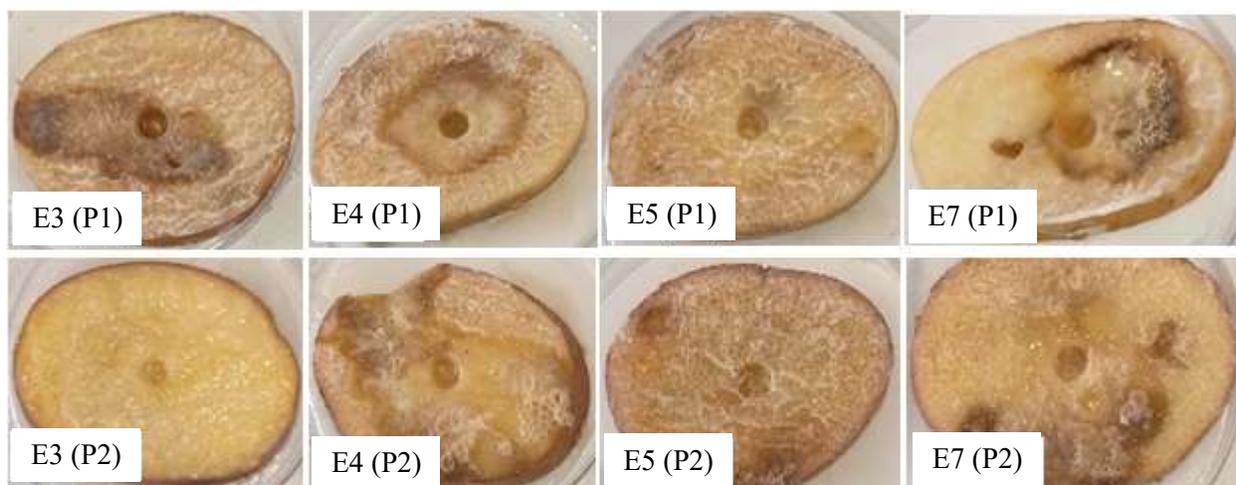


Figure 4. Pathogenicity test of *Erwinia* sp. isolates on potato varieties (P1: Spunta, P2: Desiree, E: *Erwinia* sp.).

*E. carotovorum* species, has been described by many investigations (Priou & Jouan, 1992; Yahiaoui-Zaidi *et al.*, 2003).

The white variety (Spunta) was more sensitive than the red variety (Desiree). This low tolerance could be explained by the Spunta variety deficiency in the synthesis of a number of secondary compounds such as glycolaloids (-solanine and -chaconine) compared to Desiree variety. Indeed, these secondary metabolites are involved and play a significant role in the resistance against various pathogens and pests (Ginzberg *et al.*, 2009). In addition, Andrivon *et al.* (2003) mentioned also that the resistance of potato varieties to soft rot agents was correlated to their content of phenolic compounds and other bioactive substances.

Comparison of the phytopathogenicity results between the two varieties by two-way ANOVA followed by Sidak's multiple comparison tests revealed

significant differences among two isolates (E2 and E3) (Table 2). This mean that globally phytopathogenicity of *Erwinia* sp. depended on potato variety and *Erwinia* sp. isolates.

Characterization of phytopathogenic bacteria infecting potato fields, especially *Erwinia* sp., seemed to be of a great importance for a better understanding of different aspects related to the plant pathogenicity phenomenon. Phenotypic identification of bacterial isolates revealed characteristic colonies of *Erwinia* sp. on King's B and CCT media. They were round, convex and creamy-white on King's B medium and pale purple, circular, convex, even bulging; smooth and mucous on CCT medium. Colony morphology for *Erwinia* spp. varies depending on the type of media on which they are cultivated. In general, the colonies were white and smooth. They may be domed, shining, mucoid-type colonies with radial striations or may appear smooth with

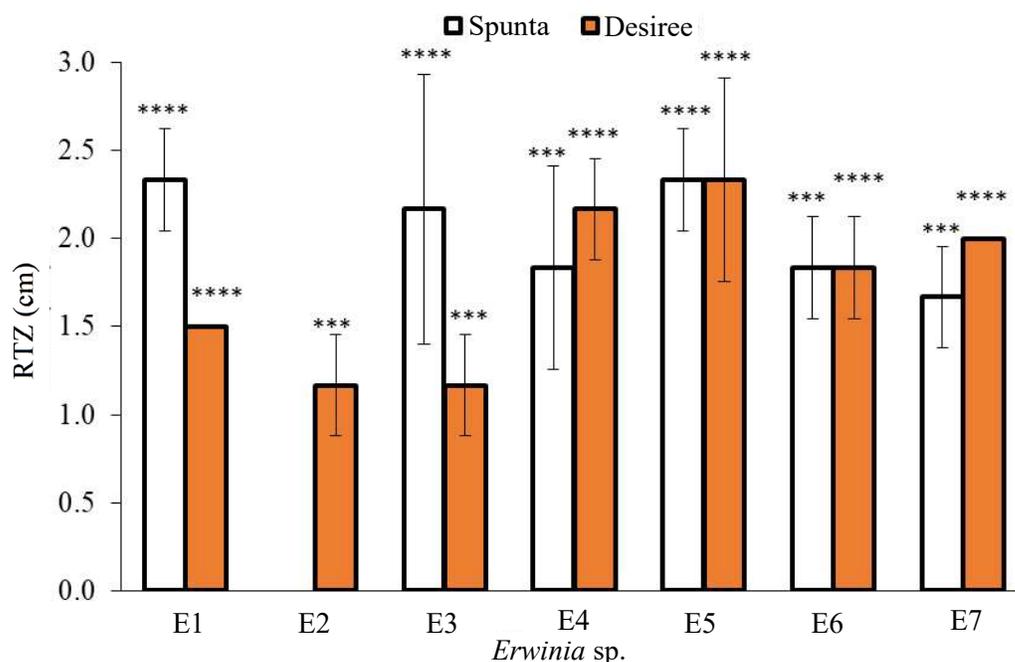


Figure 5. Phytopathogenicity test of *Erwinia* sp. isolates against two potato varieties (RTZ: rotten tissue zone; \*\*\*\*: significant difference at  $p < 0.001$ ; \*\*\*: significant difference at  $p < 0.005$ ).

Table 2. Two-way ANOVA followed by Sidak's multiple comparison test

Isolates	Adjusted P value	Summary
E1	0.0687	ns
E2	0.0042	**
E3	0.0179	*
E4	0.899	ns
E5	>0.9999	ns
E6	>0.9999	ns
E7	0.899	ns

ns= not significant; \*= significant difference at  $p < 0.05$ ; (\*\*\*) significant difference at  $p < 0.01$ .

entire edges. Craters may be formed around the colonies on some media. Also, pigments can be produced by a number of species; ranging from cream, pale yellow to light pink (Holt *et al.*, 1986).

After 48 h of incubation on King's B medium, macroscopic characterization of *E. carotova* colonies showed characteristic small, whitish, domed and mucous colonies (Benada, 2019). Moreover, after 72 h of incubation on CCT agar, colonies of *E. amylovora* were pale purple, circular, strongly convex or even domed, then become smooth and mucous (LNPV, 2005; CIPV, 2016). Some *Erwinia* species are complex taxons, composed of strains having a wide variety of phenotypic, biochemical, environmental and genetic characteristics (Toth *et al.*, 2003; Yap *et al.*, 2004).

*In vitro* phytopathogenicity test revealed high sensitivity (RTZ > 10 mm) of the two potato varieties (Spunta and Desiree) to *Erwinia* sp. isolates according to categorizing symptom scale proposed by Pédrón *et al.* (2021). Rotten tissue zone can be attributed to the secretion by *Erwinia* sp. isolates of secondary metabolites acting as phytopathogenicity factors and damaging potato plant tissue. Mainly, pectinolytic *Erwinia* caused typical symptoms of blackleg or tuber soft rot secreted effective cell wall degrading enzymes responsible for tissue maceration (Pritchard *et al.*, 2012). It was obvious that RTZ would have a proportional relationship with the specific pathogenicity of *Erwinia* sp. isolates, and also proportional to the characteristic sensitivities of different varieties (Sunta and Desiree). Thus, if the pathogenicity of *Erwinia* sp. isolates or the sensitivity of potato variety increases, the RTZ will also increase.

A higher susceptibility to Spunta was compared to Desiree. Similar results were reported by Benada (2019) reported that the Desiree variety was the most resistant to *E. carotova* subsp. *carotova* among six potato varieties (Spunta, Kondor, Desiree, Laura and Margaritte). At temperatures 15–25°C, the Spunta variety produced high quantities of pectinases, notably endopolygalacturonic transaminase, thus induced more symptoms (Pérombelon, 1994; Smadja *et al.*, 2004). This finding was also confirmed by the study reported by Rabot *et al.* (1994) which described 8 potato cultivars, among Desiree variety was moderately susceptible to soft rot caused by *P. atrosepticum* strains. This investigation had additionally shown that this variety had also a low susceptibility to blackleg disease. In another study, Allefs *et al.* (1996) showed that cultivar Desiree was the most susceptible among 12 potato varieties. Moreover, evaluation of the response of 14 potato

cultivars to four potato diseases leads to conclude that the Desiree cultivar was the most susceptible to dry rot (Acuña *et al.*, 2004). Furthermore, the aggressiveness of *E. carotova* susp. *carotova* was confirmed in different potato varieties (Spunta, Kondor, Desiree, Laura and Margaritte) that showed significant differences between strains of the same variety and different varieties. The variation between strain virulence and variety susceptibility was significant ( $p < 0.05$ ). Particularly, among 20 *Erwinia* isolates tested, the strain MAI was highly virulent on the Spunta variety compared to the other strains (Benada, 2019).

There were few studies focused on phytopathogenicity of *Erwinia* spp. in western Algeria. Plant pathogenic prokaryotes, like other plant pathogens, are influenced by a variety of factors, including local climate, host plant distribution, pathogen dispersal ability, occurrence of animal vectors, pathogen adaptability to local conditions, pathogen capacity to infect new host plants, and local cultivar resistance (Kudela, 2009). *E. carotovora* was becoming a concern because of the environmental conditions in the eastern areas of Algeria. Therefore, temperature and the availability of water are two major influences on bacterial infection development. Structural and physiological modifications increased incidence and severity of diseases that can be a consequence of these variables when temperature and relative humidity are highest. Statistical analysis demonstrated a substantial impact of various factors on *Erwinia* spp. population dynamics and soft rot development on the surface of wounded potato tubers including temperature (10, 15 and 20 °C), relative humidity (86, 96 and 100%) and initial bacterial inoculum ( $10^5$ ,  $10^7$  and  $10^9$  CFU/mL) (Moh *et al.*, 2012).

Bacterial strains can considerably influence the *in vitro* pathogenicity test. Various *Erwinia* isolates displayed typical signs of soft rot with varied degrees of aggression in treated potato tubers (Elhalag *et al.*, 2020). *Erwinia* isolates with high levels of extracellular plant cell wall-degrading enzyme synthesis were highly effective in infecting potatoes (Pérombelon & Kelman, 1980). The lack of *E. carotovora* subsp. *carotovora* pathogenicity in potato cv Bintje and Desiree cultivars might be attributed to its deficiency to generate an amplification product using primers (Y1 and Y1) (Yahiaoui *et al.*, 2003). Therefore, there was an urgent need to combine phenotypic identification on selective media, and specific and sensitive polymerase chain reaction (PCR) assay for the rapid, accurate detection of *Erwinia* spp. symptomatic potato tuber/plant samples in western Algeria.

## CONCLUSION

Bacterial infections caused by *Erwinia* species are probably among the most spreader potato diseases. This current study has described a simple method for isolation and identification of these bacteria from two potato varieties (Spunta and Desiree) using phenotypic characterization on two semi-selective media (King's B and TCC). Moreover, a rapid screening technique of phytopathogenic isolates through reproducing symptom on potato slices was reproduced in *in vitro* trials that allowed comparison and determination of the highest phytopathogenic isolates. The RTZ (Rotten tissue zone) seemed to be proportional to the specific pathogenicity of *Erwinia* sp. isolate, and the characteristic sensitivity of various varieties (Sunta and Desiree). Thus, as the pathogenicity of *Erwinia* sp. isolate or the sensitivity of potato variety increases, the RTZ will increase. The techniques presented in this study for isolation, identification and characterization of phytopathogenic *Erwinia* genus were conventional methods used in this field of research. The rapid isolation and characterization of phytopathogenic *Erwinia* sp. isolates by colorimetric method using new selective media seemed to be very promising, especially for the differentiation between *Erwinia* species. Also, more advanced techniques exist, such as *in situ* hybridization and PCR amplification. Studies and inventories of phytopathogenic bacteria featured a great importance, allowing to better understanding of different aspects related to the phytopathogenicity such as pathogens distribution, diagnosis, and the phytopathogenicity evolution.

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