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OCCURRENCE OF *Pectobacterium carotovorum* STRAINS ISOLATED FROM POTATO SOFT ROT IN MOROCCO

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Abstract – *Pectobacterium carotovorum* subsp. *carotovorum, Pectobacterium astrosepticum* and *Pectobacterium chrysanthemi* are the soft rot tuber of potatoes pathogens (*Solanum tuberosum*). The aim of this study was to determine the occurrence of these pathogens in Moroccan regions producing potatoes. Fifty three isolates of *Pectobacterium* were isolated on medium Crystal Violet Pectate. The comparison of their bacteriological characteristics with standard strains allowed us to conclude that all the isolates belonged to the *Pectobacterium.* With regard to phenotype characteristics, the variability that was found included 32 typical *Pectobacetrium carotovorum* subsp. *carotovorum*, 3 typical *Pectobacterium atrosepticum*, and 18 atypical *Pectobacterium carotovorum* subsp. *carotovorum*. Three strains of the atypical group; showed that the biochemical properties overlap among the *Pectobacterium carotovorum* and *Pectobacterium chrysanthemi*. These data were needed molecular characterization. However, the PCR amplification of total genomic DNA of 53 isolates with the two primers Y1/Y2 and P143/P145 yielded an amplified fragment of the expected size (434 bp) only with Y1/Y2, indicated that all the isolates collected and tested belonged to the *Pectobacterium carotovorum* species. On the basis the pathogenicity tests, these strains revealed that they were pectinolytic, and showed differences in aggressiveness against potato and leaves of tobacco.

Key words: Morocco, *Solanum tuberosum*, *Pectobacterium carotovorum,* soft-rot disease, isolation, phenotypic characteristics, variability, PCR, Hypersensibility Response, aggressiveness.

INTRODUCTION

The potato is already an integral part of the global food system with its production reaching a record of 325 million tons in 2006-2007. Since its independence in 1956, Morocco's potato production has expanded strongly, rising from approximately 150,000 tons in 1961 to a record 1.56 million tons in 2006 (19). Currently, the potato is Morocco's third largest crop, with its production concentrated along the northern Atlantic coast, south of Casablanca and in the mountainous regions as well. This production gradually decreased however, due to the infections brought on by various plant pathogens such as *Pectobacterium* presented by *Pectobacterium carotovorum* subsp. *carotovorum* (10) (former name *Erwinia carotovora* subsp. *carotovora*) (*Pcc*), *Pectobacterium atrosepticum* (*Pca*) (10) (former name *Erwinia carotovora subsp. atroseptica*), and *Pectobacterium chrysanthemi (Pch)* Dickeya spp. (28) (former name *Erwinia chrysanthemi*. (1, 13). This bacteria is associated with soft rot of potato and is widespread in potato fields all over the world and favors moisture and moderate temperature (3, 12, 22). *Pca* is particularly

Abbreviations: PCR: Polymerase Chain Reaction; ***Pa:*** *Pectobacterium atrospticum;* ***Pcc:*** *Pectobacterium carotovorum* subsp. *Carotovorum;* ***Pch:*** *Pectobacterium chrysanthemi ;* **HR:** Hypersensibility Reponse; **PCWDE:** Plant Cell Wall Degrading Enzymes; **CVP:** Crystal Violet Pectate.

damaging to all potatoes but, especially to potatoes that are mainly restricted to a cool temperate climate and thus *Pca* is considered an economically important pathogen for potatoes (33). *Pcc is* distributed in temperate climates as well as tropical zones, while *Pch* is a serious problem in warmer and subtropical climates (14, 24). The infection by *Pectobacterium* induces non-emergence, wilting/chlorosis, desiccation and blackleg, (3, 29). *Pectobacterium* are opportunistic pathogen, they are Gram negative, non spore forming, facultative anaerobic, fimbrea and pili are present in cells strains of (*Pcc)* and (*Pch)* (21, 26). These bacteria are characterized by the production of large quantities of extracellular plant cell wall-degrading enzymes (PCWDEs), which are factor of virulence and are responsible for tissue maceration and indirectly cell death (21). Several methods have been employed to isolate *Pectobactrium*, the one of the most effective being Crystal Violet Pectate Medium (CVP).To distinguishes among *Pectobacterium*, the most commonly used tests are the microbiological, biochemical,and pathogenicity methods (6), but some differences were reported to regarding their characteristics. Moreover, the methods based on fatty acid profiles, serology and molecular test have been developed to characterize their differences (30). In Morocco, they need more information on distribution and biodiversity of *Pectobacterium* species inducing soft rot of potato. In the present study, the objectives were (i) the evaluation occurrence of *Pectobacterium* sp. in the tubers of potatoes presenting soft rot symptoms in different Moroccan regions, (ii) characterization of Moroccan *Pectobacterium* strains on the basis of microbiological, biochemical, pathogenicity tests and by molecular methods based on PCR (iii) evaluation and comparison the aggressiveness of the isolates based on the pathogenicity tests.

MATERIALS AND METHODS

Samples and Pectobacterium strains isolation

In the temperate seasons, one hundred samples were collected over a range of potatoes cultivars (*Solanum tuberosum* cv. desirée) , they presented the symptoms of soft rot from various geographic Moroccan regions (Casablanca,Gigou, Mdakra, Taoujtat, Eljadida, Mohammedia, Ghribga, Ouad zam, AinHallouf, Beni mellal, Berrachid, Souk el Arba, Sidi Kacem, Taza, Sidi Aissa, Mnasra, Tit Millil, Oulad Dris, El Attaouia, Sidi Slimane) (Fig. 1), the area plots of potato varied between 1 and 10 hectar. The potato tubers were first washed with running tap water, and the surface disinfected with 0.5% Sodium Hypochloride for 5 min, then washed three times with sterile deionized water and finally air-dried. Each sample was mixed using a sterile blender and 20 g of the homogenate were transferred in 10 mL phosphate buffer (pH 7) and incubated under agitation (120 rpm), at 27°C for 24h in anaerobic conditions. In order to promote the growth of *Pectobacterium atrospeticum,* it is essential to add 1 mL of double Polypectate Enriched Medium (DPEM) (5, 24). All treated samples were serially diluted up to 10^{-6} and spread (0.1 mL) over the surface of the selective diagnostic medium Crystal Violet Pectate (CVP) (14). Plates were incubated at 27°C for 24 to 72 h and *Pectobacterium* were recognized on CVP medium as it induced round pits, 2-3 mm in diameter and presented whitish grey coloration (23, 25). The colonies showing the characteristic of *Pectobacterium* were isolated and grown in pure culture on LPGA medium (yeast extract 5 $g.L^{-1}$(Difco), peptone 5 $g.L^{-1}$(Difco), glucose 10 $g.L^{-1}$(Difco) and agar (Merck) 15-17 $g.L^{-1}$ pH 7.2 ± 0.2).

Figure 1. Morrocan localisation map of sampling

Bacteriological characterization

In order to identify *Pectobacterium*, the strains were grown at 27°C for 24h on agar plates and they were tested for Gram staining, catalase, oxidase, nitrate production, reductase activity, pectinolytic activity on Sutton medium, and absence pigmentation of the strains in the King B medium (Difco) (6). Identification of confirmed *Pectobacterium* isolates to species and subspecies was conducted on the basis of the following physiological characteristics: indole production from tryptophan, acid production from (lactose, trehalose, α-methyl-glucoside, and melibiose), growth in 5% NaCl, growth on nutrient agar at 37°C, production of reducing substances from sucrose and lecithinase activity (8, 26). All tests were carried out at 27°C for 24h and compared with the standard strains.

Pathogenicity tests

In order to evaluate the ability of the strains to induce hypersensitive response (HR), the tests were performed with tobacco leaves (*Nicotiana tabacum* L.) (15). Bacterial suspensions of 53 strains at cell densities of about 5.0 x 10^8 CFU.mL^{-1} were infiltrated into the mesophyl leaf through a pin puncture with a 1 ml hypodermic syringe without a needle. The plants were kept at room temperature and examined for the Hypersensitivity Response (HR) 24 h after incubation (15). The ability of strains to macerate potato tissue (soft rot symptom) was also tested on 7-8 mm thick slices from tubers of potatoes (*Solanum tuberosum* cv. désirée). Two slits deep of 5 mm were made in the slices potato and filled with 20 µL of 10^8CFU.mL of each isolate; control tubers were treated with 20 µL of sterile water. In order to compare the aggressiveness between the strains of this collection, 8 strains (P206C3, P603AH1, P126AT1, P206C2, P606Sd2, P507CH1, P106F1 and P116C2) were taken as model to evaluate these differences in aggressiveness. These strains, for the first time, could be placed in two groups corresponding to gelatine degradation. The strains (P106F1, P116C2, P507CH1 and P606Sd2) designing the group I, were more able to degrade the gelatine than the strains of the group II (P206C2, P126AT1, P603AH1 and P206C3). The relative aggressiveness was evaluated by comparison with the phenotypic characteristics of the symptoms caused by the strains in tobacco leaves and in potato slices; and also examined by measuring the amount of macerated tissue of potato slices and its diameter.

Molecular characterization

DNA extraction

DNA extraction was carried out on 700 µl of the bacterial suspension (10^8 CFU.mL^{-1}), using the method of Li & Boer (1995). Samples were centrifuged at 12000g/10min at 4°C. The pellet was frozen at -20°C and incubated for 10 min suspended in 500 µL of TE Buffer pH 8 (10 mM, Tris. Cl, 1mM EDTA, pH 8). Fifty microliters of 250 mM EDTA, pH 8, 50 µL of 14% SDS and 10 µl proteinase K at 10 mg. mL^{-1} were added before incubation for 1h at 56°C. An equal volume of 7.5 M ammonium acetate was added to separate cellular fragments from the DNA by a centrifugation at 12000g for 30 min at 4°C, and 0.8 ml isopropanol -20°C was added to 0.8ml of clear supernatant, incubated for 30 min at -20°C and centrifugated at 12000g for 30 min. The pellet was washed with the ethanol at 70% and precipitated with a second centrifugation at 12000g/20 min at 4°C. Finally the DNA pellet was dried at room temperature and resuspended in 50µl TE Buffer pH 8, the DNA samples were stored at -20°C. Gel electrophoresis was performed by using 0.8% agarose gels with 1X TBE Buffer containing 0.5 µg.mL^{-1} ethidium bromure (9).

PCR amplification

PCR of the *pel* gene was amplified using pelY primers, Y1 and Y2, specific to *Pectobacterium carotovorum* selected from a pectate lyase-encoding gene of the Y family (4, 9). PCR was performed in a 20 µL final solution containing 50 ng of genomic DNA, 2 mM of $MgCl_2$, 4 µL of PCR buffer 5X, 1 mM of each dNTPs, 1U Taq polymerase from Promega and 0.4 µM final concentration of each primer: Y1 (5'TTA CCG GAC GCC GAG CTG TGG CGT3') and Y2 (5'CAG GAA GAT GTC GTT ATC GCG AGT3') (4). Amplification was performed under the following conditions; the initial cycle of denaturation was at 95°C for 1 min and the second step was performed by running 35 cycles (1 min at 94°C, 1 min at 65°C, 1.5 min at 72°C), and an additional cycle of 7 min at 72°C for an extension period. PCR using the primers P143 (5'CAC CCG TGG TTG TAA AAC ACG TTT3') and P145 (5'GCG GTT TCC TGC TCT GTG GTT3') (7) was performed in a 20 µL final solution containing 50 ng of genomic DNA, 2 mM of $MgCl_2$, 4 µL of PCR buffer 5X, 1 mM of each dNTPs, 1U Taq polymerase from Promega and 1µM of final concentration of each primers, the amplification was performed under the following conditions, the initial cycle of denaturation was at 95°C for 5 min, the second steps was performed by running 35 cycles (30s at 94°C, 30s at 55°C, 45s at 72°C) and an additional cycle of 7 min at 72°C for extension period.

Detection of amplified PCR-DNA products

An analysis of PCR products was realised by electrophoresis in 1.5% agarose. A standard 1 Kb DNA ladder was included on each gel as well as positive and negative controls.

RESULTS

Phenotypic tests

To evaluate the distribution of *Pectobacterium carotovorum* in some Moroccan areas, samples were carried out from different Moroccan regions covering the area of local production**.** Fifty three isolates formed recognized colonies on CVP medium (Table1) and cavities indicating the degradation of polypectate (31). These 53 isolates were transferred on King B medium for growth and purification. The characteristics of all the isolates were consistent with those described for the *Pectobacterium*, and were identified as *Pectobacterium* according the biochemical and physiological tests when compared with standard characters as shown in Table 2. The different isolates were: Gram negative, catalase activity; none showed oxidase activity, and fermented glucose. Furthermore, they could reduce nitrate and growth in anaerobic-aerobic conditions. In addition, they were able to induce soft rot in potato slices and maceration on tobacco leaves. Thus, according to table 2, we showed the distribution of the 7 groups (*Pcc*^{T,} *Pca*^{T}, *Pcc*^{a}, *Pcc*^{b}, *Pcc*^{c} *Pcc*^{d} and *Pcc*^{e}) in the sampling cities. The group (*Pcc*^{T}) corresponded to 32 isolates of total collection, was constituted by typical strains. These isolates that were able to produce acid from different sugars was examined. Negative results were obtained with maltose α-methyl (D) glucoside, sorbitol and D-arabitol. Positive results were obtained with trehalose, lactose and melibiose. The isolates grew at 36±37°C and showed the inability to form reduced substances from sucrose. The group (*Pca*^{T}) corresponding to 3 isolates were described to typical *Pca* on the basis of the acid production

Table 1. Origins and references of isolates used in this study

isolates	Location	Source	Year of Sampling	PCR products	Morrocan city
P116SK1	Morocco	Potato	2006	434bp	Sidi kacem
P116SK2	Morocco	Potato	2006	434bp	Sidi kacem
P116SK5	Morocco	Potato	2006	434bp	Sidi kacem
P116Sd1	Morocco	Potato	2006	434bp	Sidi banour
P116Sd2	Morocco	Potato	2006	434bp	Sidi banour
P126AT1	Morocco	Potato	2006	434bp	Elattaouia
P126AT2	Morocco	Potato	2006	434bp	Elattaouia
P126AT3	Morocco	Potato	2006	434bp	Elattaouia
P126AT5	Morocco	Potato	2006	434bp	Elattaouia
P126AT6	Morocco	Potato	2006	434bp	Elattaouia
P126OD1	Morocco	Potato	2006	434bp	Oulad driss
P126TH1	Morocco	Potato	2006	434bp	Touahra
P126TH3	Morocco	Potato	2006	434bp	Touahra
P116C2	Morocco	Potato	2006	434bp	Casablanca
P206C3	Morocco	Potato	2006	434bp	Casablanca
P206C2	Morocco	Potato	2007	434bp	Casablanca
P106F1	Morocco	Potato	2006	434bp	Fes
P126MN1	Morocco	Potato	2006	434bp	Mnasra
P126MN2	Morocco	Potato	2006	434bp	Mnasra
P126MN5	Morocco	Potato	2006	434bp	Mnasra
P126SI1	Morocco	Potato	2006	434bp	Sidi issa
P126SI2	Morocco	Potato	2007	434bp	Sidi issa
P507BM1	Morocco	Potato	2007	434bp	Beni mellal
P507BM2	Morocco	Potato	2007	434bp	Beni mellal
P307k1	Morocco	Potato	2007	434bp	Kénitra
P307k2	Morocco	Potato	2007	434bp	Kénitra
P303K1	Morocco	Potato	2003	434bp	Kénitra
P303K4	Morocco	Potato	2003	434bp	Kénitra
P306K1	Morocco	Potato	2006	434bp	Kénitra
P507K8	Morocco	Potato	2007	434bp	Kénitra
P507K12	Morocco	Potato	2007	434bp	Kénitra
P303TJ1	Morocco	Potato	2003	434bp	Taoujtat
P303TJ2	Morocco	Potato	2003	434bp	Taoujtat
P303TJ3	Morocco	Potato	2003	434bp	Taoujtat
P507CH1	Morocco	Potato	2007	434bp	Chtouka
P507OZ1	Morocco	Potato	2007	434bp	Oued Zem
P507AH1	Morocco	Potato	2007	434bp	Ain halouf
P507AH2	Morocco	Potato	2007	434bp	Ain halouf
P606MN1	Morocco	Potato	2006	434bp	Mnasra
P603TM3	Morocco	Potato	2006	434bp	Titmellil
P603TM5	Morocco	Potato	2003	434bp	Titmellil
P603TM1	Morocco	Potato	2003	434bp	Titmellil
P303T1	Morocco	Potato	2003	434bp	Taza
P603AH1	Morocco	Potato	2003	434bp	Ain halouf
P606Sd2	Morocco	Potato	2006	434bp	sidikacem
P606Sd5	Morocco	Potato	2006	434bp	sidikacem
P606Sd1	Morocco	Potato	2006	434bp	sidikacem
P206A2	Morocco	Potato	2006	434bp	Gigou
P303 MN1	Morocco	Potato	2003	434bp	Mnasra
P303MN4	Morocco	Potato	2003	434bp	Mnasra
P303MH4	Morocco	Potato	2003	434bp	Mohammedia
P303MH5	Morocco	Potato	2003	434bp	Mohammedia
P303MH6	Morocco	Potato	2003	434bp	Mohammedia

Table 2. Results of the identification according to the biochemical and physiological tests conducted on the 53 isolates from the Morrocan regions

	Standard strains			**Isolates collection**						
Tests	***Pcc***	***Pca***	***Pch***	***Pcc^T***	***Pcc^a***	***Pcc^b***	***Pcc^c***	***Pcc^d***	***Pcc^e***	***Pca^T***
Microbiological tests										
Staining Gram	GN	GN	GN	GN	GN	GN	GN	GN	GN	GN
Catalase activity	+	+	+	+	+	+	+	+	+	+
Oxidase activity	-	-	-	-	-	-	-	-	-	-
Pectinolytic activity	+	+	+	+	+	+	+	+	+	+
Fermentation/oxidation	+	+	+	+	+	+	+	+	+	+
Gelatine degradation	+	+	+	+	-	+	+	+	+	+
Pigment production	-	-	v^b	-	-	-	-	-	-	-
Nitrates reduction	+	+	+	+	+	+	+	+	+	+
Biochemical tests										
Activity lecithinase	-	-	+	-	-	-	-	-	-	-
Indole Production	-	-	v	-	-	-	-	-	+	-
Acid production from:										
Lactose	+	+	v	+	+	+	+	-	+	+
Trehalose	+	+	-	+	+	+	+	+	+	+
α-□methyl glucoside	-	+	-	-	-	-	-	-	-	+
D-arabitol	-	-	-	-	-	-	-	-	-	-
Sorbitol	-	-	-	-	-	+	+	-	-	-
Melibiose	+	+	+	+	+	+	+	+	+	+
SRS	-	v	v	-	-	-	-	-	-	-
Growth at 37°C	+	-	+	+	+	+	-	+	+	-
Number of isolates				32	9	2	1	3	3	3

Standard *Pcc* 1322, *Pca* 1323, *Pch* 1324 SRS Reducing substances from sucrose GN Gram negative (*100) Symbols: + = 80% or more of strains positive; - = 20% or less of strains positive
v = strains variable; v^b = developing dark brownish to blue colonies easily distinguishable from other *Pectobacterium* sp [36]
Pcc^{T}Typical isolates of *P.carotovorum. carotovorum,* ***Pca^T*** Typical isolates of *P. atrosepticum*
Pcc^c isolates had all the characteristics of *E. carotovora carotovora* but not a growth ability at 37°C and produced acid from sorbitol
Pcc^b isolates had all the characteristics of *Pcc* but produced acid from sorbitol
Pcc^d isolates had all the characteristics of *Pcc* but produced acid from lactose
Pcc^e isolates had all the characteristics of *Pcc* but produced indole from tryptophan
Pcc ^{a} isolates had all the characteristics of *Pcc* but inability to hydrolysed gelatine

from α-methylglucoside and were not able to grow at 37°C. Eighteen isolates appeared as atypical of *Pcc;* the group (*Pcc^c)* corresponded to 1 isolate, had all the characteristics of *Pcc^T,* but was not able to grow at 37°C and produced acid from sorbitol**.** The group (*Pcc^b*) corresponding to 2 isolates also produced acid from sorbitol. Three atypical *Pcc* strains designated the group (*Pcc^d*) could produce acid from lactose. Nine isolates designated (*Pcc^a*) had also all the characteristics of *Pcc* but showed the inability to hydrolysed gelatine. Finally, 3 atypical strains designated (*Pcc^e*) had all the characteristics of *Pcc^T* but could produce indole from tryptophan. Finally**,** in this study no isolates showed lecithinase activity. The main bacterial phytopathogens affecting the potato in the area tested are *P. carotovorum.* According to figure 2, the strains *Pcc^T* were homogeneously represented in the whole sampling area, *Pca^T* were only localized on the spots of the collect on the south-east of Casablanca. The group *Pcc^c* was found in the spot of collect on the North and the North East of Casablanca. The strains *Pcc^b* were found in the same spot of collect on the North East of Casablanca. The 3 atypical *Pcc* strains designated *Pcc^d* were also found in the different spot of collect and 9 isolates designated *Pcc^a* one more time these strains only co- localise on one spot. Finally, 3 atypical strains designated *Pcc^e* were found in the spot of collect on the west of Casablanca.

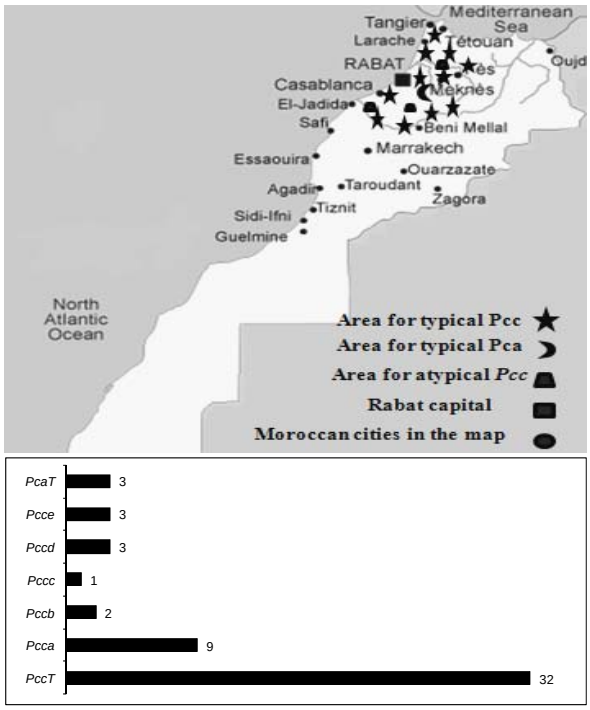

Figure 2. Geographic repartition in Morocco of the 7 groups of strains in the area sampling

PCR results

To confirm the microbiological and biochemical identification, 53 Moroccan *Pectobacterium carotovorum* isolates were further tested by PCR. The PCR amplification of total genomic DNA was based on two couples, the specifics primers Y1/Y2 for identification of *Pectobacterium carotovorum*, and the specific primers P143/P145 for identification of *Pch.* The amplification of 50 total genomic DNA extracted from the six groups (Pcc^T, Pca^T, Pcc^a, Pcc^b, Pcc^c, Pcc^d) were successfully amplified, they were induced an amplified fragment of 434 bp with the primers Y1/Y2 (Fig. 3, Table 1). According to biochemical tests, the remaining 3 atypical strains from the last group (Pcc^e) showed characteristic of *Pch*, they produced indol from tryptophan. However, the PCR amplification was performed by the two couples of primers P143/P145 and Y1/Y2. The result yielded an amplified fragment only with Y1/Y2 that confirmed their appurtenance of *P. carotovorum.*

Pathogenicity tests

Results of the pathogenicity tests showed that 53 *P. carotovorum* strains in this study induced HR on tobacco leaves; the infiltrated leaf area showed the first signs of tissue collapse and the zone became necrotic within 48 h. These tests showed also soft rot symptoms on the slices of potato after 24h. Control tobacco leaves and the slices of potato inoculated with water remained healthy. The appearance of symptom was documented by photography (Fig. 4).

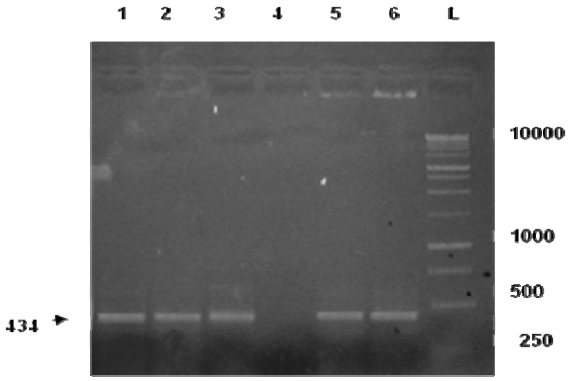

Figure 3. Typical example: Gel electrophoresis in 1.5 % agarose of PCR product from genomic DNA with primers Y1/Y2 of CVP derived isolates. Lane L, DNA ladder; lane 4, negative control; lane 5, positive control and lanes 1, 2, 3 and 6 isolates of the collection. The arrowhead indicates the 434 bp amplified fragment

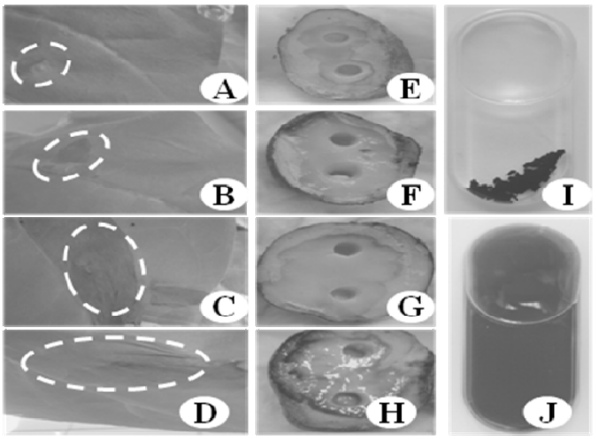

Figure 4. Comparison of Hypersensitive Response (HR) induction on Tobacco and soft rot symptom in potato slices by the strains of group I* (A, B, E and F), the isolates of group II** (C, D, G and H), and phenotypic characteristics of gelatin degradation by the group I phenotypic characteristics of gelatin degradation by the group J. *isolates of group I (P206C2, P126AT1, P603AH1 and P206C3), **isolates of group II (P106F1, P507CH1, P606Sd2 and P116C2). Bacteria were infiltrated into tobacco leaves and infected potato slices as described in text.

Aggressiveness of strains

Variations in the relative aggressiveness among 8 strains (P206C3, P603AH1, P126AT1, P206C2, P606Sd2, P507CH1, P106F1 and P116C2) was evaluated by comparing the phenotypic characteristics of the symptoms caused in leaves of tobacco and in potato slices; and also examined by measuring the amount of macerated tissue and the diameter of the rotting zone surrounding the infect, focus artificially in

the slices of potato. However, there were significant differences in symptoms incited by the 8 isolates. The results of the HR test revealed that the isolates P206C3, P603AH1, P126AT1 and P206C2 were able to induced smaller zone of tissue collapse in the leaves of Tobacco (Fig. 4A, 4B) than those of P606Sd2, P507CH1, P106F1 and P116C2 induced larger zones (Fig. 4C, 4D). Interestingly, the strains P606Sd2, P507CH1, P106F1 and P116C2 induced HR more rapidly than the P206C3, P603AH1, P126AT1 and P206C2. The strains of the group I induced the first signs within 8h; in contrast with the strains of the group II showed the first signs of collapse after 12 h. The 8 strains caused a visible soft rot on potato slices 24 h after inoculation, but showed difference on phenotypic characteristics and variations of percent macerated tissue and its diameter. The percentage of macerated tissue varied among the isolates ranging from 10.06 %, for the isolate P206 C2, to 36%, and for the isolate P606Sd2. On the other hand, the diameters of macerated tissue varied among the isolates ranging from 3 mm for the isolate P206C2 and 5 mm for the isolate P606Sd2. The strains P606Sd2, P507CH1, P106F1 and P116C2 developed more aggressive symptoms of soft rot than the remaining strains after inoculation. P206C3, P603AH1, P126AT1 and P206C2 induced a creamy white soft rot (Fig. 4E, 4F), while the strains (P606Sd2, P507CH1, P106F1 and P116C2) induced light brown to black soft rot accompanied with nauseating odour (Fig. 4G, 4H). There was a significant positive correlation between the diameter and the percentage of the macerated tissue caused by these strains. Interestingly, a high link was observed between the ability of gelatine degradation (Fig. 4I, 4J), HR induction and phenotypic characteristics of soft rot potato slices according to the results (Table 3, Fig. 4) of the isolates. We can propose the sequence of increasing aggressiveness for the 8 isolates as following: P206C2< P126AT1 <P603AH1 <P206C3 <P106F1 <P116C2 <P507CH1< P606Sd2.

Table 3 Relationship between Hypersensitive Response (HR) induction, percent of macerate tissue and the diameter of soft rotting zone in 8 isolates

Isolates	Percent of macerate tissue (%)	Diameter of the macerate tissue (cm)	HR
P206C2	10,06 ± 2.56	3 ± 0.5	+
P126AT1	19,2 ± 3	3 ,16 ± 0.28	+
P603AH1	21,45 ±2.66	4 ± 0.86	+
P206C3	21,67 ±4.87	4 ± 0.86	+
P106F1	26,66 ±3.52	4,33 ± 0.28	++
P116C2	27,57 ±5	4,66 ± 0.86	++
P507CH1	31,3 ±2.87	5 ± 0.32	++
P606Sd2	36 ±4.23	5 ± 0.2	++

+ Positive reaction in the leaves of the tobacco plants infected; HR Hypersensitive Reaction Slices of potato incubated with 10^8CFU/ml with the different isolates 24 H at 27°C, the softened tissue was removed and the maceration is given in (%) of weight reduction ant its diameter is giving in (cm). The values shown are means of three different experiments. Each value indicates the mean of three replicates for each treatment.

DISCUSSION

In Morocco, *P. carotovorum* is well known as a pathogen of potato and it was detected in many other Mediterranean countries (14). To evaluate the distribution of *Pectobacterium* sp. in some Moroccan areas, samples were carried out from different Moroccan regions covering the area of local production of potato**.** In our study 53 isolates were collected. All isolates used in this study belong to *Pc*, PCR was carried out with two types of primers P143/P145 specific *Pch and* Y1/Y2 specific *Pc.* The result yielded an amplified fragment with Y1/Y2 that indicated their appurtenance of *P. carotovorum*. These isolates were commonly pathogenic to potato slices and to leaves of tobacco caused Hypersensitive Response (HR). This response has been documented in several *Pectobacterium* species; these bacteria possess *hrp* genes and ability to produce the harpins and the elicitors of HR (11, 15).With regard to phenotype characteristics, the variability was revealed between the isolates from different spot of collect. Seven phytopathogenic groups were recognized, it suggests the biochemical properties overlap among the isolates. *Pcc* presented by the typical group (Pcc^T and 5 atypical group Pcc^a, Pcc^b, Pcc^c, Pcc^d and Pcc^e) while the *Pca* presented only by 1 typical group (Pca^T). Phenotypic diversity among *Pcc* isolates may result from their broad host range, their presence in the soil and on the surfaces of plants contrasted to strains of *Pca* (2, 10, 33). The geographical distribution of *Pca* was systematic influenced by environmental conditions especially the temperature. They are more sensitive to the high temperatures than *Pcc* and *Pch* (24, 35). Indeed in Morocco, there is a temperate climate with relatively high temperatures during the vegetation period, *Pca* was often associated with potatoes and causing typical symptoms of blackleg on stems (5). Indeed in this study, *Pcc* form a more heterogeneous group than *Pca* (2, 10, 20**)** and it was previously reported by some report (8, 35). The attempts to find variation in aggressiveness among isolates revealed differences. Interestingly, the high correlation was observed between the ability of gelatine degradation and phenotypic characteristics of symptoms in the pathogenicity tests. In contrast, differences in pathogenicity of *Pc* isolates were previously reported (32). On the other hand, difference aggressiveness between the strains was documented by the rapidity of their pectinolytic enzymes expression and secretion. Further evidence that their role is important, is that avirulent mutants have been isolated that is either deficient or defective in the production or secretion of these enzymes (27). Pectolytic enzymes of *P. carotovorum* probably had the most important role in maceration by degrading pectic components of the plant cell wall and middle lamella, resulting in the separation of the cells (30). On the other hand, Kaewnum *et al* 2006 indicate that a functional pectate lyase plays a role in HR induction on certain plants *in Xanthomonas axonopodis* pv. *glycines* like many other soft rotting *Pectobacterium* sp.*;* it produced an assortment of degradative enzymes including Cellulase (Cel), Protease (Prt), Polygalacturonase (Peh), Pectin lyase (Pnl) and several isoforms of pectate lyase (Pel) (18). These extracellular enzymes are crucial to the virulence of this soft rot pathogen although Prt and Cel do not macerate plant tissues, but contribute to virulence by augmenting the action of pectinases (3, 16). However, inactivation of one of the Cells significantly reduced the maceration capacity of potato tissue compared to wild-type *Pcc* (34). In addition, several proteases with a minor role in pathogenesis have been described. Their contribution to virulence is not yet fully understood, but could involve provision of amino acids for biosynthesis of bacterial proteins or degradation of host proteins associated with resistance (33), thereby increasing the ability of the pathogen to colonize its host and may contribute to greater virulence (in the sense of causing greater host damage). These microbiological and biochemical tests accompanied with the PCR confirmation, showed the occurrence and the predominance of *P. carotovorum* in the samples of soft rot potato in this study. These data compared with the other Moroccan results (1) show that potato is often infected during these years by *P. carotovorum* and occasionally by *P. chrysanthemi*. Thus, in this study, it is well established that the *P. carotovorum* is the causal organism of soft rot potato in the temperates seasons in the various geographic Moroccan regions of the sampling (Fig. 1). These bacteria appear to be a highly biochemical and molecular heterogeneous bacterial species that varies greatly with regard to its aggressiveness and HR.

In conclusion, *P. carotovorum* is certainly one of the most important pathogen responsible for potato soft rot in Morocco all over the production area; it can cause significant losses during plant growth, harvest, transport and storage. The present study also shows that *P. carotovorum* can be transmitted on potato seeds. This indicates that naturally infested potato seeds can be a primary and important source of inoculum. Further studies will now be needed to analyse more in deep the molecular specific diversity and understand the reason of the specific localizations of atypical strains.

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