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Full Length Research Paper

Pathogenic variability within biochemical groups of *Pectobacterium carotovorum* isolated in Algeria from seed potato tubers

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One hundred *Pectobacterium carotovorum*, isolates, recovered from soft rot symptoms on potato tubers in Algeria and previously characterised taxonomically, were assessed in a half-tuber test for differences in ability to cause tuber rotting on two cultivars (Désirée and Bintje) respectively considered to be moderately and very susceptible to soft rot. A first trial at 20°C, involving the complete collection of isolates at two inoculum concentrations (2.10^5 and 2.10^7 cel. ml^{-1}), showed significant effects of inoculum dose, host cultivar and biochemical and molecular groups on pathogenicity. A significant interaction between pathogen groups and cultivars was also apparent. *P. carotovorum.* subsp. *atrosepticum* (*Pca*) was more pathogenic on cv. Bintje than on cv. Désirée, while the susceptibility of these two cultivars to *P. carotovorum.* subsp *carotovorum* (*Pcc*) was the opposite. Some *Pcc* isolates were non-pathogenic to both cultivars, and others were pathogenic on cv. Bintje but not on cv. Désirée. A second trial, conducted at 20 and 25°C with a high inoculum concentration (2.10^7 cel. ml^{-1}) of forty isolates representative from the collection, confirmed the previous findings, and showed a significant effect of temperature on pathogenicity. *Pca* isolates were more aggressive than *Pcc* isolates at both temperatures, but the difference was greater at 20°C. Our data therefore suggest that cultivar resistance rankings depend on the *Pectobacterium* subspecies considered, and should therefore be assessed separately for the various *Pectobacterium* subspecies.

Key words: Sensitivity, soft rot, aggressiveness, cultivars, resistance.

INTRODUCTION

Pectobacterium carotovorum subsp *carotovorum* (Vanhall) Dye and (*Pcc*), *Pectobacterium carotovorum* subsp. *atrosepticum* (*Pca*) (Jones) Dye (*Eca*) and *Pectobacterium chrysanthemi* (Burkholder) MacFadder and Dimmock (*Pch*) have a wide geographical distribution and are economically important pathogens. These bacteria can cause serious damage and are involved in soft rot diseases of several major crops, including potato, cucumber, tomato and carrot. (Pérombelon and Kelman, 1980; Stead, 1999; Farrar et al., 2000 Yishay et al., 2008). Soft rot on potato tubers can be a severe problem and affects potato industry, because of the long storage period between harvest and processing. Due to the vegetative mode of propagation of the plant, the inoculum concentration in the tubers seed is well correlated with the amount and severity of disease in the ware crop (Stead, 1999). Therefore, it is important to ensure that seed tubers must be pathogen-free to minimise the risk of disease occurrence in ware crops.

Infection by *Erwinia* usually results in extensive maceration and rotting of parenchymatous tissue in the organs affected due to the production of large amounts of proteases and pectic enzymes (Kotoujansky, 1987). In some cases, maceration is directly correlated with cell death (Garibaldi and Battman, 1971). The ability to cause soft rot varies among *Pcc* and *Pca* strains (Gregg, 1952; Johnson et al., 1989). It also depends on temperature, a factor essential for bacterial growth (Pérombelon and Kelman, 1980; Pérombelon, 2002). Disease potential is greatest when temperatures are in the range of (25 - 30°C). Temperature regimes may

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therefore determine the subspecies that will prevail when more than one of them is present in a region (Pérombelon and Salmon, 1995). Under cool and moist conditions, *Pca* is the main causal agent of blackleg, which originates from the mother tuber (Pérombelon and Kelman, 1987) and causes extensive decay of tubers when storage conditions are favourable, since the pathogen is capable of spreading rapidly from one tuber to another (Waterer and Pritchard, 1984). Under warmer and drier climates (Mediterranean or continental areas for instance), *Pcc* and *Pch* are commonly found and responsible for rotting of tubers under storage (Lumb et al., 1986; Cazelles et al., 1995).

Recently, Yishay et al. (2008) observed relationship between pathogenicity and genetic diversity among *P. carotovorum* subsp. *carotovorum* isolates which reveals a co-evolutionary specialization trend in the interaction between this pathogen and its hosts.

Several procedures have been described to determine the rate of maceration of tissue caused by *Pectobacterium* species and variation in the pathogenicity of these isolates (Smith and Bartz, 1990). Soft rot is routinely assessed by quantifying the decay of inoculated tubers through the measurement of lesion diameter (De Boer and Kelman, 1978), the volume of the rotted cavity in a half-tuber test (Ibrahim et al., 1978; Priou, 1992; Rabot et al., 1994), or the weight of rotted tissue (Bourne et al., 1981). Another possibility is to monitor the concentration of volatile metabolites (Waterer and Pritchard, 1984). A number of potato cultivars and Potato hybrids, assessed for susceptibility to rotting by *P. carotovorum*, proved more resistant to *Pcc* than to *Pca* (Lapwood et al., 1984; Carputo et al., 2000).

Over the last years, the presence of *Pectobacterium* causing soft rot was detected in Algeria on seed potato imported from the Netherlands, the British Isles, Canada and France, as well as on seed stocks multiplied locally (Yahiaoui-Zaidi et al., 2003). A large biochemical and molecular diversity was detected among these isolates, which could be assigned taxonomically to *Pca*, a number of groups related to *Pcc*, and *Pectobacterium carotovorum* subsp. *odoriferum* (*Pco*) (Yahiaoui-Zaidi et al., 2003). The purpose of the present study is to investigate the pathogenic variability within the biochemical groups on the two potato cultivars Bintje and Désirée, focusing on 1) inoculum concentration, and 2) temperature dependence.

MATERIALS AND METHODS

Pathogenicity tests

Plant material

Tubers of potato (*Solanum tuberosum)* cultivars Bintje and Désirée selected for uniform size (ca. 40 mm) and not effected by soft rot causing bacteria used for pathogenicity tests. These Algeria (Désirée); both have been regarded as susceptible to soft rot (Pérombelon, 1979). Tubers were stored at 4°C from harvest until used and they were taken out of the cold storage and kept for 24 h at room temperature before inoculation.

Bacterial strains and growth conditions

Pectobacterium isolates used in this study were collected from rotted seed potato tubers in Algeria, during 1994 - 2000 and have been previously characterised taxonomically) (Yahiaoui-Zaidi et al., 2003). All isolates were maintained for extended periods as deep-frozen cultures (- 80°C) in Luria-Bertani (LB) medium (10 $g.l^{-1}$ tryptone, 5 $g.l^{-1}$ yeast extract and 10 $g.l^{-1}$ NaCl; pH 7.3 (Sambrook et al., 1989) supplemented with 30% glycerol. The type strains for *Pca* (CFBP 1526 -NCPPB 549, Graham), *Pcc* (CFBP 2046 -NCPPB 312, Jones), and *Pch* (CFBP 2048 -ICPB EC17, Burkholder), obtained from the French Collection of Phytopathogenic Bacteria (CFBP, INRA Angers, France) were used as standards.

Identification of PCR/RFLP groups

To assess possible relationships between pathogenicity and genetic diversity among *P. carotovorum* sp., isolates were assigned to RFLP groups after the analysis of the restriction pattern of the PCR products obtained with Y1 and Y2 Primers. The DNA from 40 µl samples was digested with *Alu*1 *Hae* II et *Hpa*II and *Sau*3a. Restriction profiles with all four enzymes were then combined to identify the unique RFLP groups According to Yahiaoui-Zaidi et al. (2003).

Pathogenicity tests on half tubers

Before inoculation, a bacterial suspension of each strain was prepared in sterile distilled water (SDW) from cultures on King's B medium and incubated at 26°C for 24 h. Initial suspension corresponding to 4.10^8 (cel. ml^{-1}) each isolate was prepared and further dilutions in SDW were made to obtain the concentrations of 4.10^6 (cel. ml^{-1}), then, 50 µl of each suspension were used as inoculum.The half-tuber method given by Ibrahim et al. (1978) was followed for the study of the pathogenic variability of the hundred *Pectobacterium* strains on the two cultivars. Tubers of each test cultivar were washed, surface-sterilised by dipping in 10% ethanol for 5 min, rinsed in SDW and air-dried overnight before cutting. Each tuber was cut from the rose end to the heel end in two roughly equal parts with a sterile knife. A hole (5 mm diameter x 5 mm depth) was drilled with a cork borer in the center of each half-tuber, which was then placed on tissue paper soaked with 100 ml of water in plastic containers and allowed to dry for one hour before inoculation. Each half-tuber was inoculated by depositing 50 µl of the previously prepared bacterial suspensions in the hole. For the control, SDW was used. The plastic boxes containing the inoculated tubers were covered with a plastic sheet sealed with a rubber band to maintain a saturating relative humidity. Disease development was scored after five days of inoculation in a dark moist chamber at 20°C. A second trial was conducted at 20 and 25°C with a high inoculum concentration (2.10^7 cel. ml^{-1}) of forty isolates representative from the collection. Disease development was scored after five days of inoculation at either 20 or 25°C. Symptoms were assessed visually, before all rotted tissue was removed with a spatula and the volume of water necessary to fill up the hole was recorded (Ibrahim et al., 1978). In each test, 10 half-tubers per cultivar were inoculated with each inoculum concentration of each strain.

Data analysis

The pathogenic ability of *Pectobacterium carotovorum* on each

cultivar was assessed as the means of the volume of water necessary to fill up the hole caused by rotting. Effects of cultivars, isolates (or groups thereof), temperature, and interactions between those parameters on pathogenicity were analysed by ANOVA using the GLM module of the SAS statistical package.

RESULTS

Biochemical characteristics

Tests for pectolytic activity, growth at 37°C, acid production from methyl α-D-glucoside, production of reducing substances from sucrose, utilisation of melibiose and citrate, showed that all isolates had the characteristics of soft rot *Pectobacterium*. The collection included 40 typical *Pcc* isolates, which grew at 37°C and did not utilise α-methylglucoside or produce reducing substances from sucrose, and 14 typical *Pca* isolates, which did not grow at 37°C, utilised α-methylglucoside and produced reducing substances from sucrose. The remaining isolates were clustered into seven groups and named as (*Pcc1, Pcc2, Pcc3, Pcc4, Pcc5, and Pcc6*) which were genetically similar or identical to the *Pcc* type strain but did not produce all the typical biochemical or physiological responses of this subspecies and one atypical *P. c.* subsp *odorifera* (*Pco1*)

Molecular typing

The RFLP analysis undertaken with the 99 *Pectobacterium* isolates by digesting the 434-bp amplified fragment with the four restriction enzymes revealed the presence of 12 different profiles (RFLP groups: 1, 3, 4, 8, 9, 10, 12, 14, 22, 25, 26 and 27) demonstrating polymorphism among the various isolates. Three of these RFLP groups have not been previously described and were assigned numbers 25–27. The RFLP groups 1, 8, 9 and 22 were the most frequent in the collection.

Pathogenicity of *Pectobacterium* isolates

Among the number of *P. carotovorum* tested, *most of them* were pathogenic to both cultivars Bintje and Désirée at (2.10^7 cel. ml^{-1}). However, some of the *Pcc* strains failed to induce symptoms on the two cultivar, others caused rotting on cv. Bintje but not on cv. Désirée.

The visual aspect of symptoms in both cultivars was different between *Pca* and *Pcc*: the former group produced a dark brown slimy necrosis surrounded by a black ring, while soft rot caused by the latter group was light brown and drier. These symptoms were similar to those caused by the *Pca* and *Pcc* reference isolates, respectively. Moreover, *P. c.* subsp *odorifera* (*Pco*) strain showed a high level of aggressiveness; rot aspect was intermediate between *Pca* and *Pcc* isolates. In all cases, control plants did not exhibit any symptoms.Twenty five of the 85 typical and atypical *Pcc* isolates entirely failed to cause rotting At 2.10^5 cel. ml^{-1} on both Bintje and Désirée, and the volume of rotted tissue produced by the remaining isolates remained small (0.1 - 0.9 ml).This result is summarized in a Table 1.

Pathogenicity differences between groups

As expected, aggressiveness was significantly high at the higher inoculum concentration (2.10^7cel. ml^{-1}) for all isolates and on both cultivars (Figure 1). Overall, *Pca* and*Pco* isolates were significantly more aggressive than *Pcc* isolates: the volumes of rotted tissue caused by *Pca* isolates at an intermediate inoculum concentration (2.10^5 cel. ml^{-1}) were nearly the same as those caused by *Pcc* isolates at 2.10^7 cel. ml^{-1}.

Significant aggressiveness differences between and within RFLP groups were also observed (Figure 2). Interestingly, the four isolates belonging to RFLP group 25 recently described were more aggressive than the reference *Pcc* strain CFPB 20-46 at the same concentration (two fold difference in the mean rot volume at 2.10^7 cel. ml^{-1}. Two of these isolates were obtained from seed of cvs Désirée and Timate multiplied in Algeria, and the other two were isolated from Dutch seed lots of cvs Désirée and Diamant. *Eca* isolates obtained from Algerian seed lots also were generally more aggressive than the reference strains CFPB 15.26 and 86.20 on the two cultivars. The aggressiveness of isolates collected from Dutch seed lots was similar to that of the corresponding type strains.

Cultivar Bintje proved more susceptible than cv Désirée to *Pcc,* whereas the opposite was true for *Pca* and *Pco*. This effect was more pronounced at 20°C than at 25°C for the last two subspecies (Table 2).

ANOVA analysis of the rot volumes after inoculation of the two cultivars with 40 isolates representative of the collection at 20 and 25°C showed highly significant effects of temperature on disease severity, as well as a highly significant temperature pathogen interaction (Table 3). Analyses conducted separately for each bacterial group revealed that *Pca* isolates were the most aggressive, particularly at 20°C. Inoculation at 25°C of the two cultivars with an inoculum of 2.10^7 cel. ml^{-1} resulted in similar amounts of rot with *Pcc* and *Pca* isolates.

DISCUSSION

Our results confirmed that the half-tuber inoculation method is suitable for assessing the aggressiveness variability among *Pectobacterium* isolates. The differences in disease produced by the isolates tested here were consistent with those observed by other workers (e.g. Jones, 1910; Johnson et al., 1989; Smith and Bartz, 1990).They were also related to the taxonomic diversity

Table 1. Half tubers inoculation of cvs Bintje and Désirée at 20°C with *Pectobacterium* isolates with 2.10^6 and 2.108 cel. ml^{-1}.

N°	Ssp	MIBb	MIDb	MIBa	MIDa	N°	Ssp	MIBb	MIDb	MIBa	MIDb
*1	Pcc	0.75	0.60	0.10	0.10	54	*Pcc*^{nt2}	1.27	1.20	0.40	0.80
*2	*Pcc*	1.04	1.08	0.30	0.10	55	*Pcc*^{nt2}	1.00	1.10	0.30	0.05
*3	*Pcc*	0.05	0.05	0.10	0.10	56	*Pcc*^{nt2}	0.90	1.09	0.05	0.05
4	*Pcc*	1.05	0.52	0.05	0.05	57	*Pcc*^{nt2}	1.00	0.05	0.15	0.05
5	*Pcc*	0.40	0.25	0.05	0.05	58	*Pcc*^{nt3}	1.10	0.90	0.05	0.05
6	*Pcc*	0.90	0.84	0.05	0.05	59	*Pcc*^{nt}	1.02	1.55	0.60	0.80
7	*Pcc*	0.05	0.05	0.05	0.05	60	*Pcc*^{nt3}	1.10	0.91	0.30	0.05
8	*Pcc*	0.05	0.05	0.05	0.05	*61	*Pcc*^{nt}	1.09	0.90	0.05	0.05
9	*Pcc*	0.60	0.20	0.05	0.05	*62	*Pcc*^{nt3}	0.94	0.30	0.15	0.05
10	*Pcc*	1.52	1.00	0.75	0.05	*63	*Pcc*^{nt}	0.05	0.05	0.05	0.05
11	*Pcc*	1.40	0.32	0.70	0.05	64	*Pcc*^{nt3}	1.00	0.40	0.60	0.05
12	*Pcc*	1.04	0.20	0.10	0.05	65	*Pcc*^{nt}	0.40	0.05	0.05	0.05
13	*Pcc*	0.88	0.48	0.05	0.05	66	*Pcc*^{nt3}	1.30	1.00	0.90	0.05
*14	*Pcc*	1.60	0.60	0.25	0.05	67	*Pcc*^{nt3}	0.90	0.05	0.05	0.05
*15	*Pcc*	0.50	0.40	0.05	0.10	68	*Pcc*^{nt}	0.05	0.05	0.05	0.05
*16	*Pcc*	1.22	0.90	0.05	0.05	69	*Pcc*^{nt3}	0.20	0.20	0.05	0.05
*17	*Pcc*	1.30	1.10	0.20	0.05	70	*Pcc*^{nt}	0.22	0.05	0.05	0.05
*18	*Pcc*	1.10	0.90	0.05	0.05	71	*Pcc*^{nt3}	0.40	0.40	0.05	0.05
19	*Pcc*	1.10	1.60	0.43	0.50	72	*Pcc*^{nt}	1.30	0.71	0.45	0.05
20	*Pcc*	0.90	0.40	0.05	0.05	73	*Pcc*^{nt3}	1.40	0.72	0.05	0.05
*21	*Pcc*	1.65	1.00	0.50	0.05	74	*Pcc*^{nt3}	1.20	0.91	0.80	0.05
*22	*Pcc*	1.50	0.60	0.30	0.05	75	*Pcc*^{nt}	1.39	0.20	0.80	0.25
*23	*Pcc*	1.20	0.70	0.20	0.12	76	*Pcc*^{nt3}	1.32	1.07	0.20	0.40
24	*Pcc*	1.10	0.51	0.40	0.30	77	*Pcc*^{nt}	0.71	0.35	0.40	0.05
25	*Pcc*	1.10	0.60	0.30	0.20	78	*Pcc*^{nt3}	0.71	0.05	0.05	0.05
26	*Pcc*	0.90	0.68	0.10	0.30	*79	*Pcc*^{nt4}	1.20	1.28	0.71	0.60
27	*Pcc*	0.90	0.30	0.10	0.10	80	*Pcc*^{nt4}	0.90	1.56	0.70	0.80
28	*Pcc*	1.10	0.90	0.30	0.05	81	*Pcc*^{nt4}	1.39	1.30	0.60	0.70
*29	*Pcc*	1.08	0.80	0.10	0.10	82	*Pcc*^{nt4}	0.89	0.05	1.00	0.05
*30	*Pcc*	1.30	0.80	0.75	0.25	*83	*Pcc*$^{nt'}$	0.05	0.05	0.05	0.05
*31	*Pcc*	0.80	0.60	0.10	0.10	*84	*Pcc*^{nt5}	0.77	0.52	0.05	0.05
*32	*Pcc*	0.90	0.30	0.05	0.05	*85	*Pcc*^{nt6}	0.89	1.30	0.60	0.50
*33	*Pcc*	1.19	0.70	0.10	0.10	*86	*Pco*^{n7}	1.69	2.29	0.70	0.70
*34	*Pcc*	1.30	0.80	0.15	0.05	*87	*Pca*	2.00	3.42	0.80	1.90
*35	*Pcc*	1.00	0.50	0.40	0.25	*88	*Pca*	2.24	3.11	1.09	1.50
*36	*Pcc*	1.10	1.10	0.05	0.15	89	*Pca*	2.81	3.28	1.40	1.60
37	*Pcc*	1.00	0.90	0.15	0.15	*90	*Pca*	2.56	3.29	1.23	1.90
38	*Pcc*	0.80	0.71	0.45	0.05	*91	*Pca*	2.90	4.10	1.72	200
*39	*Pcc*	0.80	0.40	0.30	0.05	*92	*Pca*	3.62	4.20	2.39	2.20
40	*Pcc*	0.75	0.60	0.30	0.15	*93	*Pca*	3.72	4.20	2.10	2.50
41	*Pcc*^{nt1}	0.60	0.20	0.10	0.05	94	*Pca*	3.05	3.62	1.10	1.60
*42	*Pcc*^{nt2}	0.84	0.60	0.20	0.05	95	*Pca*	2.40	2.91	2.09	0.90
*43	*Pcc*^{nt2}	1.50	1.10	0.60	0.50	96	*Pca*	2.70	2.89	1.95	1.00
44	*Pcc*^{nt2}	1.50	1.01	0.80	0.10	97	*Pca*	2.45	2.96	1.00	1.10
45	*Pcc*^{nt2}	1.20	0.90	0.50	0.05	98	*Pca*	2.20	2.52	1.60	1.20
*46	*Pcc*^{nt2}	0.90	0.80	0.05	0.30	99	*Pca*	2.40	2.60	1.50	1.00
47	*Pcc*^{nt2}	1.30	1.30	0.15	0.80	*100	*Pca*	2.38	2.91	1.00	0.80
*48	*Pcc*^{nt2}	1.10	0.72	0.40	0.40	86 20	*Pca**	2.31	3.39	1.30	1.50
*49	*Pcc*^{nt2}	0.60	0.52	1.60	0.80	15 26	*Pca*^{T}	2.82	3.01	1.50	1.30
50	*Pcc*^{nt2}	2.00	1.60	0.70	0.60	91 8	*Pcc**	0.90	1.19	0.05	0.05

Table 1. Contd.

51	*Pcc*^{nt2}	1.10	0.29	0.35	0.05	20 46	*Pca*^{T}	1.20	1.15	0.43	0.40
52	*Pcc*^{nt2}	1.30	1.21	0.10	0.50	0.48	*Pch*^{T}	0.05	0.05	0.05	0.05
53	*Pcc*^{nt2}	0.90	1.09	0.40	0.50						

MIB: mean of 10 inoculations of cultivar Bintje; MID: mean of 10 inoculation of cultivar Desiree. (a) at 2.10^6 and(b) at 2.10^8 cel. $ml^{-1.}$ *Pcc* ^{nt} :non typical (1 to 6) and P*co*^{nt7} *Pectobacterium. carotovoum*. subsp. *odorifera* inable to grew at 37°C. *Pca** 86.20: *P. c.* subsp. *atrosepticum*; *Pcc** 91.8 : *P. c.* subsp. *carotovorum*; *Pch*: *P. chrysathemi*. * Bernard Jouan, National Institute of Agronomic Research, Rennes, France, personal collection. *Pca*^{T} 15.26, *Pcc*^{T} 20.46 and PchT 20.48 (Strains type from CFBP: French Collection of Phytopatogenic Bacteria, INRA, Angers, France).

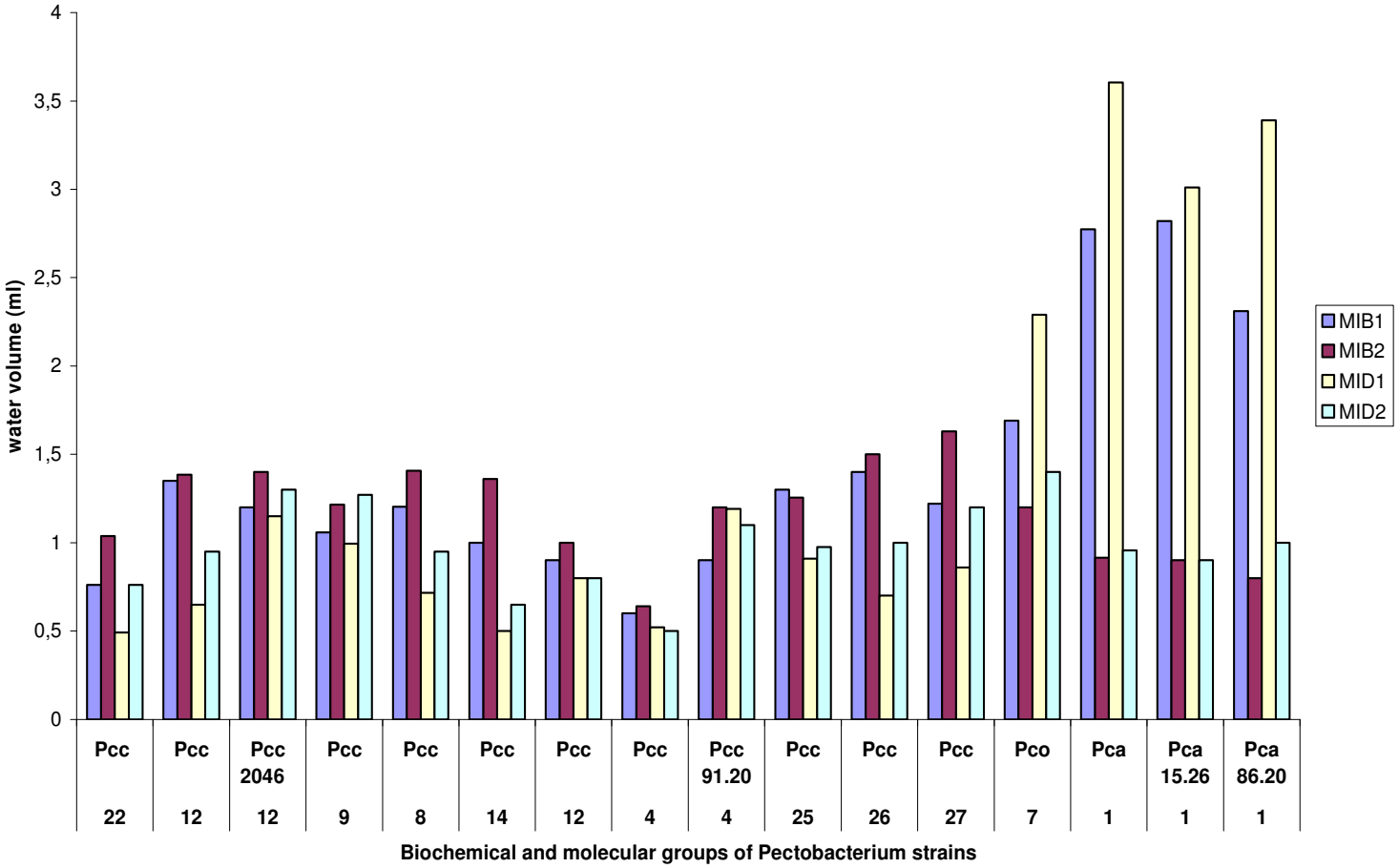

Figure 1. Pathogenic variability within biochemical and molecular groups of *Pectobacterium carotovorum* sp. on two cultivars Bintje and Désirée inoculated with (2.10^7cel. ml^{-1}), and incubated at 20 and 25°C.

existing within this collection isolated from various cultivars and derived from populations of different geographic origins.

At relatively moderate inoculum concentrations (2.10^5 cel. ml^{-1}), many *Pcc* isolates from our collection failed to induce rotting, whereas all the *Pca* isolates proved pathogenic (Table 1). The higher aggressiveness to potato tubers of *Pca* was confirmed at higher inoculum concentrations, confirming previous observations about aggressiveness differences between *P. carotovorum* subspecies (Lapwood et al., 1984). Interestingly, the unique *Pco* isolates from our collection were also highly pathogenic on potato tubers. This observation confirmed the diversity existing within the *Pcc* isolates. The pathogenic variability observed within the *Pcc* isolates on the two cultivars extends earlier observations (Jones, 1910; Johnson et al., 1989; Smith and Bartz, 1990). Disease failed to develop in both cultivars Désirée and Bintje inoculated with some *Pcc* isolates. The failure of some *Pcc* isolates to cause tuber rot could be explained

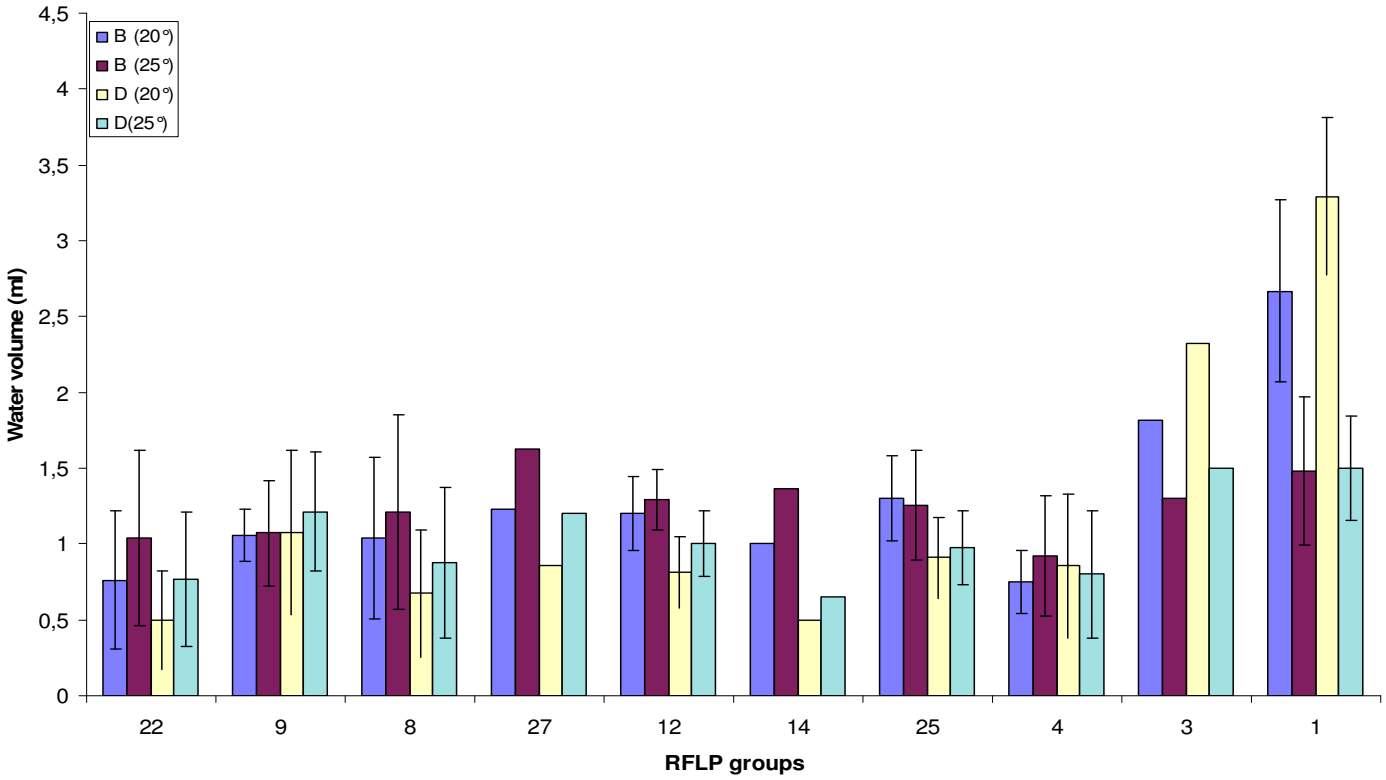

Figure 2. Pathogenic differences between 10 RFLP groups (1, 3, 4, 8, 9, 12, 14, 22, 25 and 27) of *Pectobacterium carotovorum* on two potato cultivars Bintje (B) and Désirée (D).

Table 2. Results of potato tubers inoculation, with 40 strains of *P. carotovorum* from Algeria.

Strain Number	Subsp	MIB_1	MIB_2 (ml)	MID_1	MID_2	RFLP groups	Years
1	*Pcc*	0.75	1.25	0.60	1.20	22	1995
2	*Pcc*	1.04	1.18	1.08	1.30	9	1995
3	*Pcc*	0.05	0.05	0.05	0.05	22	1995
14	*Pcc*	1.60	2.00	0.60	1.00	8	1995
15	*Pcc*	0.50	0.85	0.40	0.60	22	1995
16	*Pcc*	1.22	1.63	0.86	1.20	27	1995
17	*Pcc*	1.30	1.70	1.10	1.40	22	1995
18	*Pcc*	1.10	1.20	0.90	0.95	8	1995
21	*Pcc*	1.65	1.80	1.00	1.40	8	1995
22	*Pcc*	1.50	1.42	0.60	0.90	12	1995
23	*Pcc*	1.20	1.35	0.70	1.00	12	1995
29	*Pcc*	1.08	1.41	0.80	1.00	9	1994
30	*Pcc*	1.30	1.70	0.80	0.90	22	1994
31	*Pcc*	0.80	1.06	0.60	0.75	9	1994
32	*Pcc*	0.90	1.08	0.30	0.80	22	1994
33	*Pcc*	1.19	1.22	0.70	0.95	22	1994
34	*Pcc*	1.30	1.40	0.80	1.05	9	1995
35	*Pcc*	1.00	1.36	0.50	0.65	14	1995
36	*Pcc*	1.10	1.16	1.10	1.30	9	1995
39	*Pcc*	0.80	1.28	0.40	0.60	22	1996
42	Pcc^{at}	0.84	0.90	0.60	0.65	8	1996

Table 2. Contd.

43	*Pcc*^{at}	1.50	1.51	1.10	1.15	25	1996
46	*Pcc*^{at}	0.90	1.00	0.80	0.80	12	1996
48	*Pcc*^{at}	1.10	1.00	0.72	0.80	25	1996
49	*Pcc*^{at}	0.60	0.64	0.52	0.50	4	1996
61	*Pcc*^{at}	1.09	1.40	0.90	1.09	8	1996
62	*Pcc*^{at}	0.94	1.14	0.30	0.60	8	1996
63	*Pcc*^{at}	0.05	0.05	0.05	0.05	8	1996
79	*Pcc*^{at}	1.20	1.40	1.28	1.50	9	1995
83	*Pcc*^{at}	0.05	0.05	0.05	0.05	22	1995
84	*Pcc*^{at}	0.77	1.20	0.52	1.05	22	1995
85	*Pcc*^{at}	0.89	0.90	1.30	2.00	9	1995
86	*Pco*^{at}	1.69	1.20	2.29	1.40	7	1996
87	*Pca*	2.00	0.60	3.42	1.00	1	1995
88	*Pca*	2.24	0.85	3.11	0.80	1	1996
90	*Pca*	2.56	0.90	3.29	0.90	1	1996
91	*Pca*	2.90	1.00	4.10	1.00	1	1996
92	*Pca*	3.62	1.30	4.20	1.20	1	1995
93	*Pca*	3.72	1.10	4.20	1.00	1	1995
100	*Pca*	2.38	0.65	2.91	0.80	1	1995
1526^{TS}	*Pca*	2.82	0.90	3.01	0.90	1	TS
8620	*Pca*	2.31	0.80	3.39	1.00	1	RS
2046^{TS}	*Pcc*	1. 40	1.15	1.30	1. 20	12	TS
918	*Pcc*	0.90	1.20	1.19	1.10	4	RS

MIB_1 and MIB_2: mean (ml) of ten inoculations on cultivar Bintje at 20 and 25°C respectively with 2.10^8 cel. ml^{-1}. MID_1 and MID_2: mean (ml) of ten inoculations on cultivar Desiree at 20 and 25°C respectively.^{TS} with 2.10^8 cel. ml^{-1}: Type strain of French Collection of hytopathogenic Bacteria, Angers, France. RS: Reference strain. The forty representative isolates (1 - 100) were previously described by Yahiaoui-Zaidi et al., 2003.

Table 3. ANOVA analysis of the rot volumes after inoculation of the two cultivars/ Effects of temperature on disease severity.

Cultivars	Temperature	Subsp	Temp/ Subsp	Var/Temp	
F values	24.58	26.94	198.83	222.34	7.58
P	0.0001	0.0001	0.0001	0.0001	0.006

by the lost of the pathogenicity due to the successive subculturing as reported by Priou (1992). However, in our tests, the pathogenicity of certain isolates differed between cultivars, and varied among isolates that were isolated at the same time. Thus, the loss of aggressiveness or failure to cause disease did not appear to have resulted from subculturing. Bartz (1980) obtained similar results, and suggested that some isolates collected from symptoms would not be likely to initiate disease in nature. The low aggressiveness of several isolates towards their cultivar of origin is not easily explained. Dickey (1981) observed that certain isolates of *P. chrysanthemi* were not pathogenic on their host of origin and suggested that such isolates may not have initiated the lesions from which they were obtained.Our results also suggest a differential susceptibility of potato cultivars Bintje and Désirée to the various *P. carotovorum* subspecies. Differences in the susceptibility of the two cultivars to rotting were evident when comparing their behaviour towards *Pca,* more pathogenic on Desirée than on Bintje, and, more pathogenic on Bintje than on Désirée. The behaviour of Algerian *Pca* isolates was intriguing, because according to Hélias et al. (2000) Bintje was known to be more susceptible to soft rot than Désirée. The fact that most Algerian isolates originated from Désirée, a popular cultivar in northern Africa, may explain their higher aggressiveness towards this cultivar.

The resistance of tuber tissue of cultivars Désirée to

soft rot caused by *Pca* isolates could be related to the presence of some phenolics compounds like anthocyanins as described by Wegener and Jansen (2007), in coloured potato.

As expected, we observed a significant effect of temperature on soft rot development (Table 2; Figure1), as well as a highly significant temperature pathogen interaction, consistent with previous data indicating that *Pcc* isolates have a higher optimum temperature for growth than *Pca* isolates (Pérombelon and Kelman, 1980). Certain differences in pathogenicity and aggressiveness among the taxons of the soft rot *Pectobacterium* have been related to the optimum growth temperature. Indeed, besides the genetic characteristics of the host cultivar and pathogen isolate, environmental conditions also condition largely the influences of the quantum of disease (Bain et al., 1990). Temperature exerts a differential effect on the aggressiveness of *Pectobacterium* taxa by regulating of their enzyme production (Kotoujansky, 1987, Pérombelon and Kelman, 1980; Pérombelon 1982). The inability of some isolates to induce disease can be explained by the absence of enzyme production, particularly pectate lyase (PL). In *P. chrysanthemi* for example the low production of PL causes a reduction of pathogenicity (Hugouvieux-Cotte-Pattat et al, 1992).

Overall, our data further strengthen the recommendation of Priou (1992) to use Désirée and Bintje as control cultivars to allow comparisons of pathogenicity tests between laboratories. The differential reaction of these two cultivars to Algerian *Pectobacterium* isolates, coupled with the genetic characteristics of the bacterial populations involved, provide a starting point for a more comprehensive study of pathogenic adaptation mechanisms in soft rot *Pectobacterium*. Yishay et al. (2008) reported that a clear decline in virulence of the monocot isolates towards the dicot hosts suggests these isolates are better adapted to monocot hosts.

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