# Soft Rot Disease Severity Is Affected by Potato Physiology and *Pectobacterium* taxa

**Maria del Pilar Marquez-Villavicencio,** Department of Plant Pathology, University of Wisconsin-Madison, 1630 Linden Dr., Madison, WI 53706; **Russell L. Groves,** Department of Entomology, University of Wisconsin-Madison; and **Amy O. Charkowski,** Department of Plant Pathology, University of Wisconsin-Madison

## Abstract

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*Pectobacterium* species cause disease worldwide in many crop and ornamental plants, including potato. A new *Pectobacterium* subspecies, *P. carotovorum* subsp. *brasiliensis* was recently described in Brazil and later found in the United States, Israel, and South Africa. Its virulence traits and host range remain unknown. A comparison of three taxa commonly found on potato showed that both *P. carotovorum* subsp. *carotovorum* and subsp. *brasiliensis* are more aggressive in causing tuber and stem soft rot than *P. atrosepticum*. Also, despite bacterial growth inhibition in vitro of *P. carotovorum* subsp. *carotovorum* and *P. atrosepticum* strains by *P. carotovorum* subsp. *brasiliensis*, this new subspecies and *P. carotovorum* subsp. *carotovorum* are able to co-colonize in the same infected tissue. Both subspecies were motile in lesions. Pathogenesis assays showed that host ranges of all three overlap, but are not identical. The host ranges of individual strains of *P. carotovorum* subsp. *carotovorum* and subsp. *brasiliensis* are limited, whereas *P. atrosepticum* can macerate many plant species in addition to potato. There was high variability in virulence assays with potato tuber; thus physiological factors were investigated. Tuber size, maturity, and field location had significant effects on susceptibility to soft rot, with larger, more mature tubers being more susceptible.

The enterobacterial plant pathogen *Pectobacterium* (formerly *Erwinia carotovora*) causes soft rot diseases in monocot and dicot host plants in at least 35% of angiosperms (28). In potato, *Pectobacterium* causes wilt, soft rot, and blackleg and affects plant health during field production and storage (39,40). Tuber soft rot and aerial stem rot often occur after plants are wounded, and tuber soft rot is promoted by low oxygen conditions (6,29). In contrast, blackleg is considered a tuber-borne disease, with the bacterial pathogen causing an inky black decay on the lower part of the potato stem (40). Copper sprays may be used to prevent infection of wounded plant stems and leaves, but once the plant is colonized, there is no chemical control available for this pathogen (11). Resistance genes active against *Pectobacterium* have been found in multiple host species, but their sequences and mechanisms remain unknown (23–25,33,43,45,50,53,57). No currently grown commercial potato variety has an effective level of resistance to soft rot, stem rot, or blackleg.

*Pectobacterium* pathogenesis has been studied for over a century (19). To promote rot, soft rot pathogens employ a wide range of plant cell wall degrading enzymes to disrupt and metabolize plant cells (1,48). Additional virulence determinants also have been described as contributing to bacterial invasion, establishment, multiplication, and host resistance evasion. These include the flagellar system (36), putative phytotoxins (3), quorum-sensing system (26,41,44), efflux pumps (51), the type III secretion system (16,54), and plant antimicrobial resistance systems (27). Conducive environmental factors are also critical for the infection process, such as water availability, low oxygen levels, and optimal temperatures for bacterial growth (40).

The *Pectobacterium* genus has been divided into four species: *P. atrosepticum*, *P. carotovorum*, *P. betavasculorum*, and *P. wasabiae* (12). A novel *Pectobacterium* taxon associated with monocot hosts is likely to be a separate *Pectobacterium* species or subspecies (56). Of these, *P. atrosepticum*, *P. carotovorum*, and *P. wasabiae* are commonly found on potato. The species *P. carotovorum* has been further divided into subspecies, with *P. carotovorum* subsp. *carotovorum* and *P. carotovorum* subsp. *brasiliensis* most commonly found on potato (9,21,28,52). *P. carotovorum* subsp. *carotovorum* is typically associated with stem and tuber soft rot (40), although a subtype also causes blackleg (8); *P. carotovorum* subsp. *brasiliensis* causes stem rot, tuber soft rot, and blackleg (9); and *P. atrosepticum* causes blackleg (40). *P. carotovorum* is considered a broad-host-range pathogen, and this species has been isolated from a wide range of plant species. In contrast, *P. atrosepticum* appears to be mainly a potato pathogen, although it has been isolated from other hosts, such as sunflower (2) and pepper (46). *Pectobacterium* taxa may be distinguished and classified with multi-locus sequence analysis (MLSA) (21,28), but no simple assay is available to quickly and accurately identify or differentiate *Pectobacterium* species.

The genes that contribute to differences in symptom development and host range among *Pectobacterium* species remain unknown. A recent comparative genomic study among *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *brasiliensis,* and *P. atrosepticum* strains found that these taxa share 77 to 81% of their genes (13). Many lineage-specific genes were identified and account for 18% (*P. atrosepticum*), 11% (*P. carotovorum* subsp. *carotovorum*), and 13% (*P. carotovorum* subsp. *brasiliensis*) of these genomes. The lineage-specific genes include those homologous to regulatory, plant cell wall degrading enzymes, toxin, and hemagglutinin-encoding genes, and some of these genes may explain differences in host range and virulence among these strains.

Corresponding author: Amy O. Charkowski
E-mail: amyc@plantpath.wisc.edu

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Frequent interactions are likely to occur among *Pectobacterium* taxa since multiple species are commonly isolated from the same field or even the same diseased plant (15,21,55). The importance of cooperation or competition of *Pectobacterium* species with each other is unknown. However, these pathogens produce several antimicrobial compounds such as carotovoricin (37), bacteriocin (14,49), and carbapenem (32,38), suggesting that competition with each other and with other opportunistic microorganisms occurs.

Sanitation, exclusion, and plant resistance are used to control soft rot (4). In potato, plant resistance is not yet widely employed due to the complexity of breeding selection. Screening for resistance to *Pectobacterium* is complicated by the large variation in plant responses observed in both field and laboratory trials. Prevention of *Pectobacterium* diseases remains challenging due to the widespread distribution of this pathogen and its latent infection of tubers, weeds, and rotation crops (40). Resistance to tuber soft rot has been associated with high calcium levels and pectin methylation of plant cell walls (34,35), and is inversely associated with high water content (40). Other physiological sources of susceptibility in tubers have been little explored.

The objective of the current study was to describe trait differences among different *Pectobacterium* taxa that infect potato. For this study, we focused on the three taxa for which genome sequences are available (3,13) and for which multiple strains that have been classified by MLSA into taxa are available (21,28), and we examined differences in aggressiveness on potato stems and tubers, competition between strains in infected tissue, and host range. We also gained insight into physiological differences, such as tuber size, that affect tuber resistance to soft rot.

## Materials and Methods

**Bacterial strains, plasmids, and growth media.** Bacterial strains and plasmids used in this study are listed in Table 1. Most of the strains differ in plant host and geographical areas from which they were isolated. Bacterial strains were grown in Luria-Bertani agar (LBA) or liquid medium at 37°C, with the exception of *P. atrosepticum* strains, which were grown at 30°C. If required, antibiotics were used at the following concentrations: chloramphenicol, 50 μg/ml; spectinomycin, 50 μg/ml; and kanamycin, 50 μg/ml.

**Virulence assays in potato.** Nine commercial varieties that are widely grown in the United States and Canada, including russet (Goldrush, Russet Norkotah, and Russet Norkotah Colorado 8), red (Dark Red Norland, Red Norland, and Red La Soda), and white (Pike, Snowden, and Superior) varieties were used for tuber assays. Uniform size B potato tubers (3.8 to 5.7 cm diameter) obtained from the Lelah Starks Elite Foundation Seed Potato Farm were surfaced-sanitized for 10 min with 10% NaOCl, rinsed thoroughly, and allowed to air dry. Inoculum was prepared by growing bacterial strains overnight on LBA medium and then suspending the cells in sterile water. For each treatment, 20 tubers were stabbed between the bud and stem ends approximately 1.5 cm deep with a pipette tip, and 10 μl of a $10^8$ CFU/ml bacterial suspension was placed into the wound. Sterile distilled water was used to inoculate negative controls. The potato tubers were placed into plastic bags to maintain high humidity, randomized in plastic trays, and incubated for 3 days. Our preliminary experiments were completed at 20, 24, and 28°C, but all strains and varieties were only tested at 28°C. Based on our preliminary work, this inoculum level is the lowest that reliably causes tuber maceration under these conditions with WPP14, which is among the most aggressive *P. carotovorum* subsp. *carotovorum* strains in our collection (28,55). Diseased tubers were cut open, and macerated tissue was scooped out with a spatula and weighed. For assays in potato stems, potato plants (Russet Norkotah, Red Norland, Pike, and Superior) were grown from certified seed in a greenhouse under natural light. One-month-old potato plants were stab-inoculated with 10 μl of a $10^8$ CFU/ml bacterial suspension. Plants were placed into plastic bags and incubated at room temperature (22°C) for 2 days. Stems were sliced in half and lesion length was measured. For each experiment, 5 to 8 stems per strain were inoculated, and the experiment was performed twice. Means of each isolate in each potato variety were tested for homogeneity of variance, and based on those tests a nonparametric Kruskal-Wallis test was conducted. Means separation of each treatment was compared using a Dunn's multiple comparison test or a Mann-Whitney test.

**Examination of variation in resistance to soft rot among potatoes from the same cultivar.** To evaluate factors that might contribute to tuber susceptibility to soft rot disease, potato tubers

**Table 1.** Strains and plasmids used in this study

| Strains/plasmids | Relevant characteristics | References |
|---|---|---|
| *Escherichia coli* | | |
| DH5α | *supE44 ΔlacU169 (Δ80lacZΔM15) hsdR17 recA1EnA1 gyrA96 thi-1 relA1* | Clontech |
| *Pectobacterium* | | |
| *P. atrosepticum* | | |
| SCRI1043 | Isolated from potato in Scotland, sequenced strain | (3) |
| Eca6 | Isolated from potato in British Columbia | (7,28) |
| Eca31 | Isolated from potato in Wisconsin | (7,28) |
| *P. carotovorum* subsp. *brasiliensis* | | |
| BAA-417 (1692) (Ecbr212) | Type strain of *P. carotovorum* subsp. *brasiliensis,* isolated from potato in Brazil, sequenced strain | (9, Glasner, 2008 #4768) |
| 1692(p519DsRed.T3(DNT)) | $Ap^R$, 1692 carrying p519nDsRed.T3 | This work |
| Psp940 | Isolated from potato in Brazil | (28) |
| WPP1 | Isolated from potato in Wisconsin, 2001 | (28,55) |
| WPP165 | Isolated from potato in Wisconsin, 2001 | (28) |
| WPP501 | $Sp^R$ derivative of *Pcb* 1692 | This work |
| *P. carotovorum* subsp. *carotovorum* | | |
| Ecc380 | Isolated from potato in Oregon | (7,28) |
| WPP14 | Isolated from potato in Wisconsin, 2001 | (28,55) |
| WPP14(p519nGFP) | $AP^R$; WPP14 carrying p519nGFP | This work |
| WPP220 | Isolated from marigold in Wisconsin, 2005 | (28) |
| WPP236 | Isolated from burdock in Wisconsin, 2005 | (28) |
| WPP359 | $Cm^R$, *gfp::cm*-labeled WPP14 | H.-S. Kim (22) |
| Plasmids | | |
| pGEM®-T Easy | $Ap^R$, *lacZ′*, cloning vector | Promega |
| pHP45ΩSp | $Ap^R$, $Sp^R$/$Sm^R$, template plasmid carrying $Sp^R$ cassette | |
| pTALand_AD | $Ap^R$, 3.2-kb of AED0001215 and *Pcb* 1692 *hrpS* regions in pGEM®-T Easy | This work |
| pTALand_AD::*sp* | $Ap^R$, $Sp^R$, 5.2-kb of AED0001215 and *Pcb* 1692 *hrpS* regions in pGEM®-T Easy | This work |
| pVSV209 | $Kn^R$, *rfp*, transcriptional terminator-(*AvrII*, *Sal*I, *Stu*I)-promoterless $Cm^R$ and GFP | (10) |
| P519nDsRed.T3(DNT) | $Ap^R$, DsRed.T3(DNT) with *nptII* promoter | This work |
| p519nGFP | $Ap^R$, GFP with *nptII* promoter | (31) |

were harvested from four locations in four different fields that were planted with the same seed potato lot (Russet Norkotah Colorado 8). For each location, all tubers from five adjacent plants were harvested. The tubers from each individual plant were placed into plastic bags and then into a cooler. The soil adhered to the tubers at harvest was collected from the sample bags for analysis once the tubers were returned to the lab. Tuber inoculation was conducted as indicated above, but incubation was done at 22°C. Analysis of mineral content, including P, K, Ca, Mg, S, Zn, B, Mn, Fe, Cu, Al, and Na, in the soil adhered to tubers from each location was obtained from University of Wisconsin Soil & Plant Analysis Lab, Madison, WI. Pearson's correlation coefficient was determined for tuber size-macerated tissue, and significance was calculated using $P = 0.05$. To find significant differences in susceptibility in tubers among different fields and among locations within each field, a nonparametric Kruskal-Wallis test was conducted. Means separation of each treatment was compared using a Dunn's multiple comparison test ($P = 0.05$).

**Bacterial growth in potato tubers.** Bacterial growth curves in potato tubers were determined for the sequenced *Pectobacterium* strains *P. atrosepticum* (*Pa*) SCRI1043, *P. carotovorum* subsp. *brasiliensis* (*Pcb*) 1692, and *P. carotovorum* subsp. *carotovorum* (*Pcc*) WPP14. For some experiments, a *Pcc* WPP14 derivative, WPP359, and *Pcb* 1692 derivative, WPP501, carrying antibiotic resistance gene cassettes inserted into the chromosome were used. These strains were constructed by using allelic-exchange mutagenesis to insert antibiotic resistance gene cassettes into the intergenic region between *hrpS* gene and a convergently transcribed gene of unknown function (22). This allowed us to select and differentiate these two subspecies when they were co-inoculated into plants. We could not detect differences in virulence between wild-type strains and their chromosomally marked derivatives (data not shown). Inoculations with sterile water were used as a negative control. For bacterial inoculations, strains were grown overnight on LBA medium and were then suspended in sterile water to an approximate $OD_{600}$ of 0.2 ($1 \times 10^8$ CFU/ml). Surface-sanitized potato tubers (Red Norland) were stab-inoculated by placing 10 µl of a bacterial suspension containing $10^5$ bacteria into 1-cm-deep holes made with a 10-µl pipette tip. Each tuber was placed into an individual plastic bag, and the tubers were incubated at 28°C. Bacterial growth was monitored at 0, 1, 2, and 3 days after inoculation. For each tuber, potato tissue was recovered from the inoculation site (~0.5 g), weighed, and then homogenized in sterile water. Bacterial populations per gram of host were determined by dilution plating on LBA medium. For *Pcc*/*Pcb* co-inoculations, the medium was amended with either chloramphenicol (50 µg/ml) or spectinomycin (50 µg/ml). Five replicates were used to calculate means and standard errors. The experiment was repeated twice. One-way analysis of variance was determined for each time point, and means were separated with Tukey's multiple comparison test ($P = 0.05$).

**Bacterial antagonism assays.** To determine if *P. carotovorum* subsp. *brasiliensis* could inhibit growth of other strains, for each *Pcb* strain, a single colony grown in LB agar medium was streaked across a lawn of *Pcc* WPP14 or *Pa* SCRI 1043. To prepare bacterial lawns, isolated colonies of each strain were resuspended in sterile water to an approximate $OD_{600}$ of 0.2 ($1 \times 10^8$ CFU/ml). One milliliter of the bacterial suspension was added to 15 ml of precooled (approximately 35 to 40°C) LBA medium. Plates were air-dried for 5 min and *Pcb* strains were streaked. Plates were incubated at 37°C or room temperature for 16 h, and the presence or absence of a halo was recorded. Three plates were used for each strain and the experiment was repeated twice.

**Bacterial competition assays.** In vivo competition between *Pcb* 1692 and *Pcc* WPP14 was determined by comparing the bacterial growth in potato stems of inoculations with each strain individually and to co-inoculation of these two strains. For this experiment, we used bacterial strains WPP359 and WPP501, which encode chloramphenicol or spectinomycin resistance, respectively, as described above. A competition index (CI) was used to measure the change in the population ratio of *Pcc* and *Pcb* strains after co-inoculation into potato stems. We defined CI as the output ratio of *Pcc* WPP14 to *Pcb* 1692 divided by the input ratio (1:1) of these two strains. One-month-old potato stems (Superior) ($n = 5$) were inoculated with a suspension of $5 \times 10^3$ CFU for single inoculations and a total of $5 \times 10^3$ CFU at a 1:1 ratio for co-inoculations. Inoculations with sterile water were used as noninoculated controls. Plants were placed into plastic bags and incubated in a growth chamber at 26°C for 3 days. Bacterial populations were monitored by destructive sampling at 1, 2, and 3 days after inoculation. At each sampling time, stems were cut, weighed, and ground with a sterile pestle in sterile polypropylene tubes (12 × 75 mm) with 1 ml of sterile water. Bacterial homogenates were dilution plated onto LBA medium plus appropriate antibiotics. Plates were incubated at 37°C for 24 h. Two independent biological replicates of the experiment were conducted. One-way analysis of variance was determined in each time point, and means were separated with Tukey's multiple comparison test ($P = 0.05$).

**Confocal laser scanning microscopy assays.** To determine bacterial localization and movement during soft rot infection in leaves, we used confocal laser scanning microscopy assays. For this experiment, we used a derivative strain of WPP14 that contains a p519nGFP plasmid that was previously used for microscopy in live tissue plants (*unpublished*). To differentially observe *Pcb* 1692 strain during co-infection with *Pcc* WPP14, we built a similar plasmid construct, but replaced GFP with a red fluorescent protein (DsRed.T3(DNT)). The DsRed.T3(DNT) gene and its RBS with an extra adenine was PCR-amplified from the pVSV209 plasmid (10) using primers (Integrated DNA Technologies, Inc., Coralville, IA) in Table 2 and fused to the *npt2* promoter of p519 plasmid using *Xba*I and *Eco*RI sites. This plasmid was electrotransformed into wild-type *Pcb* 1692. The stability of p519::GFP and p519nDsRed.T3(DNT) in *Pcb* 1692 and *Pcc* WPP14, respectively, was examined by monitoring bacterial populations in LB medium supplemented with or lacking antibiotics. Every 24 h after incubation, bacterial populations were enumerated by dilution plating, and 200 µl of the bacterial suspension was transferred to 2 ml of fresh medium. At least eight consecutive passages were done. The bacterial populations of *Pcb* 1692 (p519::DsRed.T3) and WPP14 (p519::GFP) started to decrease after the fifth passage. We observed around 10-fold decline in the bacterial population that carried the p519 plasmid after comparing bacterial count in LBA medium supplemented with antibiotics with counts in LBA medium only. To examine bacterial pathogenicity in potato, strains were inoculated at $5 \times 10^5$ CFU per site into 1-month-old potato stems

**Table 2.** Primers used in this study

| Primer | Sequence (5′→3′) | Restriction sites | Amplified region |
|---|---|---|---|
| P0900 | ACCCATAAAGCGCGTCTGATGGA | … | 1.5-kb of |
| P0901 | CGTACTCTGCGAAGCTTCCTACCCCAATCGGTAAACGGCGG | *Hin*dIII | *Pcb* 1692 *hrpS* |
| P0902 | GGAAGCTTCGCAGAGTACGAATGACGGCAGGCCGACTTTT | *Hin*dIII | 1.6-kb of |
| P0903 | GGTGAGCATAGCGACCATTTG | … | *Pcb* 1692 AED-003287 |
| P0904 | GCTCTAGAGCTAAGAAAGGAGATATACATATG | *Xba*I | 0.716-kb of |
| P0905 | CCGGAATTCCGGAAAAGGATCCCCGCTACAGGAACAGGTG | *Eco*RI | DsRed.T3(DNT) |
| P0906 | GCAGGTAGCTTGCAGTGGG | … | 0.874-kb of |
| P0907 | CTCAAGCTT AAAAGGATCCCCGCTACAG | *Hin*dIII | ntpII::DsRed.T3(DNT) |

(Superior) by wounding the plant stems and pipetting 10 µl of the bacterial suspension into the wound. For co-inoculations, a 1:1 mixture of cells at $5 \times 10^5$ CFU was used. Plants were placed into plastic bags and incubated in a growth chamber at 26°C. Soft rot symptoms in leaves appeared 2 to 3 days after inoculation, and these symptomatic leaves were cut from the petiole and used for confocal studies. Plant tissue (approximately 1 $cm^2$) was placed into glass-bottom dishes poly D-lysine coated (35 mm), 10 mm glass diameter (MatTek Corporation, MA, USA), and examined with a Nikon AR1 high-speed spectral confocal system (Nikon Instruments Inc., NY, USA) with fluorescent excitation laser lines of 500 to 550 nm for green fluorophores and 570 to 620 nm for red fluorophores at the W.M. Keck Laboratory for Biological Imaging (University of Wisconsin-Madison, School of Medicine). Elements Nikon software was used to acquire and observe images and Elements ND acquisition software was used for the time course video which was collected every 30 fds over a span of 30 s. The experiment was done twice with at least three whole leaves per treatment. We also attempted to examine stems, but were unable to obtain clear images due to the size and shape of the potato stems.

**Pathogenesis assays in different plant hosts.** To test pathogenesis in multiple plant species, we chose species that have been previously reported to be hosts of *Pectobacterium* (28). Some of the vegetables used for this assay were purchased in a local market, including green onion (*Allium fistulosum*), table beet (*Beta vulgaris*), radish (*Raphanus sativus*), asparagus (*Asparagus officinalis*), Romaine lettuce (*Lactuca sativa*), carrot (*Daucus carota*), celery (*Apium graveolens*), rapini (*Brassica rapa* subsp. *rapa*), sweet potato (*Ipomoea batatas*), and green beans (*Phaseolus vulgaris*). The rest of the plants were grown from seed in a greenhouse under natural light supplemented with sodium arc lights, including spinach (*Spinacia oleracea* 'Bordeaux'), lettuce (*Lactuca sativa* 'Buttercrunch'), Swiss chard (*Beta vulgaris* 'Fordhook Giant'), radish (*Raphanus sativus* 'Sparkler'), sunflower (*Helianthus annuus* 'Dwarf Sunspot'), carrot ('Little Fingers'), and corn (*Zea mays* 'Bodacious'). Plants grown from seeds were inoculated while still growing in pots. Plants ($n$ = 8 to 10) were stab-inoculated with 10 µl of a $10^8$ CFU/ml bacterial suspension, placed in a plastic tray, and covered by a plastic bag to maintain high humidity. Vegetables were incubated at room temperature (~22°C) for 3 days and then evaluated for the presence of soft rot symptoms. Sterile water was used for negative control inoculations.

**Statistical analysis.** All statistical analysis was conducted with GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA).

## Results

**Aggressiveness of *P. atrosepticum* and *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* on commercial potato varieties.** Significant differences (Dunn's multiple comparison test, $P < 0.0001$) were observed in aggressiveness among and within *Pectobacterium* taxa inoculated into potato tubers (Table 3). Results from multiple strains and multiple varieties were combined to examine each group as a whole. *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* were not significantly different in aggressiveness in potato tubers, but were significantly different from *P. atrosepticum* (Fig. 1A) (Dunn's multiple comparison test, $P < 0.0001$). The negative controls did not show tuber soft rot symptoms in any of the treatments. *P. carotovorum* subsp. *carotovorum* and subsp. *brasiliensis* were similar in aggressiveness, except in Red La Soda, in which subsp. *brasiliensis* was significantly more aggressive (Dunn's multiple comparison test, $P < 0.0001$). *P. atrosepticum* was significantly ($P < 0.05$) less aggressive in all potato varieties (Dunn's multiple comparison test, $P < 0.0001$), except for in Russet Norkotah, which was among the most resistant of the varieties tested. The sequenced strains (*Pa* SCRI1043, *Pcb* 1692, and *Pcc* WPP14) were also assayed in Yukon Gold tubers at 20°C. No statistical differences in maceration were found between *Pcc* WPP14 and *Pcb* 1692. *Pa* SCRI1043 caused significantly less maceration (Dunn's multiple comparison test, $P < 0.0001$) when compared to *Pcc* WPP14 and *Pcb* 1692, and it was not statistically different from the negative controls (data not shown).

Within the *Pcb* clade, there were differences in aggressiveness among strains in all potato varieties except for three, Dark Red

**Table 3.** Relative virulence of *Pectobacterium* strains on stab-inoculated potato tubers[y]

| | Potato varieties[z] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Red | | | White | | | Russet | | |
| | DRN | RNL | RLS | SWN | SUP | PIKE | CN8 | RNK | GDR |
| *Pcb* | | | | | | | | | |
| Pcb1692 | 6.26 a | 4.41 a | 4.58 a | 1.95 ab | 0.80 a | 0.73 ab | 3.13 ab | 0.40 a | 0.64 a |
| Psp940 | 4.98 a | 4.36 a | 4.24 a | 4.00 b | 3.3 b | 1.25 b | 3.53 a | 2.15 b | 0.67 a |
| WPP165 | 6.62 a | 4.12 a | 4.20 a | 1.86 a | 1.75 ab | 0.62 a | 2.57 ab | 0.22 a | 0.59 a |
| WPP1 | 4.58 a | 1.83 b | 3.17 a | 1.23 a | 0.98 a | 0.58 a | 1.20 b | 0.14 a | 0.43 a |
| NC | 0 b | 0 c | 0 b | 0 c | 0 c | 0 b | 0 c | 0 c | 0 b |
| *Pcc* | | | | | | | | | |
| WPP14 | 5.74 a | 4.04 a | 2.44 ab | 1.65 a | 0.99 a | 0.52 a | 3.38 a | 0.51 a | 0.36 a |
| Ecc380 | 4.77 a | 1.94 a | 0.68 b | 0.97 a | 0.32 a | 0.49 a | 0.94 a | 0.28 a | 0.25 a |
| WPP236 | 5.77 a | 4.47 a | 2.49 a | 1.11 a | 2.16 a | 0.86 a | 2.68 a | 0.12 a | 0.30 a |
| WPP220 | 5.89 a | 2.91 a | 3.33 a | 1.84 a | 1.38 a | 0.71 a | 1.81 a | 0.54 a | 0.43 a |
| NC | 0 b | 0 b | 0 b | 0 b | 0 b | 0 b | 0 b | 0 b | 0 b |
| *Pa* | | | | | | | | | |
| Eca31 | 0.34 a | 0.04 ab | 0.43 a | 0.60 a | 0.10 a | 0.14 a | 0.17 a | 0.10 a | 0.11 a |
| SCRI1043 | 0.44 a | 0.14 b | 0.28 a | 0.44 ab | 0.13 a | 0.18 a | 0.11 a | 0.13 a | 0.14 a |
| Eca6 | 0.64 a | 0.03 a | 0.15 a | 0.22 b | 0.15 a | 0.18 a | 0.05 ab | 0.11 a | 0.13 a |
| NC | 0 b | 0 c | 0 b | 0 c | 0 b | 0 b | 0 b | 0 b | 0 b |
| *Pcb* | 5.61 A | 3.68 A | 4.05 A | 2.26 A | 1.70 A | 0.79 A | 2.60 A | 0.72 A | 0.58 A |
| *Pcc* | 5.54 A | 3.34 A | 2.23 B | 1.40 A | 1.20 A | 0.64 A | 2.20 A | 0.36 AB | 0.34 A |
| *Pa* | 0.47 B | 0.07 B | 0.29 C | 0.42 B | 0.13 B | 0.17 B | 0.11 B | 0.12 B | 0.13 B |
| NC | 0 C | 0 C | 0 D | 0 C | 0 C | 0 C | 0 C | 0 C | 0 C |

[y] Macerated potato tissue data from *Pectobacterium* strains that belong to the *Pcb*, *Pcc*, and *Pa* multilocus sequence analysis (MLSA) clades (21,28) after inoculation into 9 commercial potato varieties. Significant differences are shown among means within each column from each clade. Lowercase letters represent comparisons among strains within a MLSA clade. Uppercase letters denote comparisons among MLSA clades. Means with the same letter are not significantly different according to Dunn's multiple comparison test at $P = 0.05$.

[z] Means were calculated from macerated tissue weight of 20 tubers (size b) that were assayed in 2 independent experiments. DRN: Dark Red Norland, RNL: Red Norland, RLS: Red La Soda, SNW: Snowden, SUP: Superior, CN8: Russet Norkotah Colorado 8, RNK: Russet Norkotah, GDR: Goldrush Russet. *Pcb*: *Pectobacterium carotovorum* subsp. *brasiliensis*, *Pcc*: *Pectobacterium carotovorum* subsp. *carotovorum, Pa*: *Pectobacterium atrosepticum*. NC: negative control.

Norland, Red La Soda, and Goldrush Russet, in which no significant differences were found. In the case of *Pcc* strains, no significant variation was found, except in Red La Soda. No significance difference in aggressiveness was found among *Pa* strains except in Red Norland and Snowden. Collectively, *Pcb* and *Pcc* strains were more aggressive in red varieties compared to russets and white varieties. We were surprised to find that all three taxa were significantly more aggressive on Dark Red Norland than on Norland since Dark Red Norland is a selection of Norland (Mann-Whitney's multiple comparison test, $P < 0.0001$). Similarly, *Pcb* and *Pcc* strains were significantly more aggressive on the Russet Norkotah Colorado 8 selection than they were on Russet Norkotah (Mann-Whitney's multiple comparison test, $P < 0.0001$). We used a subset of the varieties to test whether *P. atrosepticum* was more aggressive on stems, as a tradeoff for being less aggressive in tubers, but our data did not support this hypothesis (Fig. 1B). *P. atrosepticum* was always less aggressive than *P. carotovorum* subsp. *carotovorum* and subsp. *brasiliensis* on stems. In some cases, *P. carotovorum* subsp. *brasiliensis* was more aggressive than either *P. atrosepticum* or *P. carotovorum* subsp. *carotovorum* on both stems and tubers.

**Tuber weight, field location, and harvest site within a field are correlated with soft rot susceptibility.** We found variation among tubers within each variety when assessing tuber susceptibility to soft rot. To analyze whether tuber weight or harvest site was correlated with susceptibility to soft rot, we collected tubers from four sites within four different fields planted with the same seed lot. There were significant positive relationships between soft rot susceptibility and tuber weight in all fields tested: field 1: r(209) = 0.29, $P < 0.0001$; field 2: r(202) = 0.53, $P < 0.0001$; field 3: r(202) = 0.34, $P < 0.0001$; and field 4: r(159) = 0.39, $P < 0.0001$, indicating that larger tubers tend to be more susceptible to soft rot. A representative graph is shown in Figure 2. The fields were planted 2 weeks apart, with fields 2 and 4 planted on 7 and 8 May and fields 1 and 3 planted on 19 and 20 May. When tubers were harvested for this experiment, the vines on fields 2 and 4 were dead due to sprays with a vine-killing chemical several days prior to our harvest. The vines on fields 1 and 3 had been sprayed with a vine killer, but were still green when sampled. Tubers harvested from

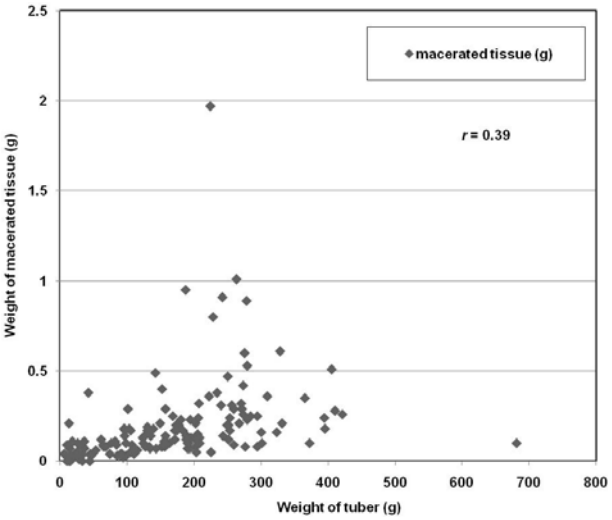


**Fig. 2.** Tuber weight is significantly correlated with susceptibility to soft rot. Representative data from one field are shown. Potato tubers (Russet Norkotah Colorado 8) were harvested from field 4 in Wisconsin in 2009, individually weighed, and then inoculated with $10^6$ bacteria of *Pcc* WPP14. Macerated tissue was weighed after 2 days of incubation at 22°C. Pearson's correlation coefficient ($r$) is shown.

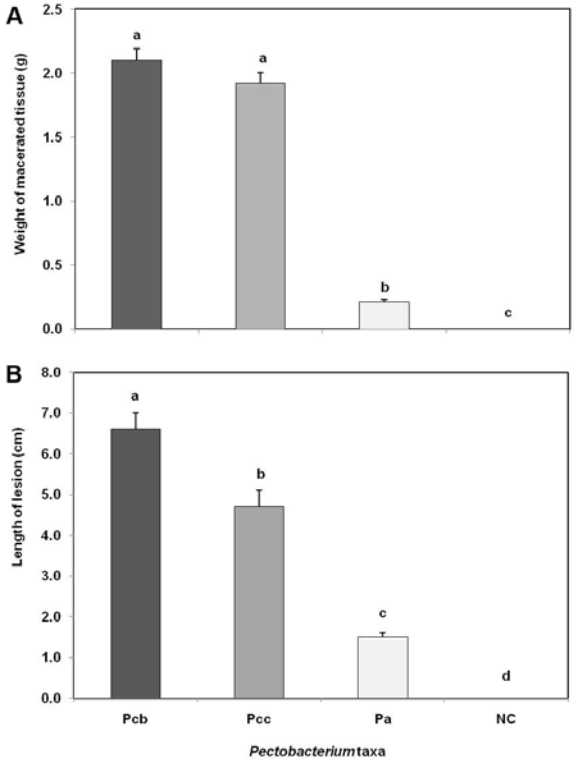


**Fig. 1.** Relative virulence of *Pectobacterium* taxa on inoculated potato tubers and stems. Bars represent the mean of the weight of combined macerated potato tissue data from all potato varieties from each *Pectobacterium* taxa **(A)** and the combined data of the length of lesion produced in potato (Superior) from all bacterial strains in each *Pectobacterium* taxon from two independent experiments **(B)**. Means with the same letter are not significantly different according to Dunn's test, $P = 0.05$. Error bars indicate standard error. NC: negative control.

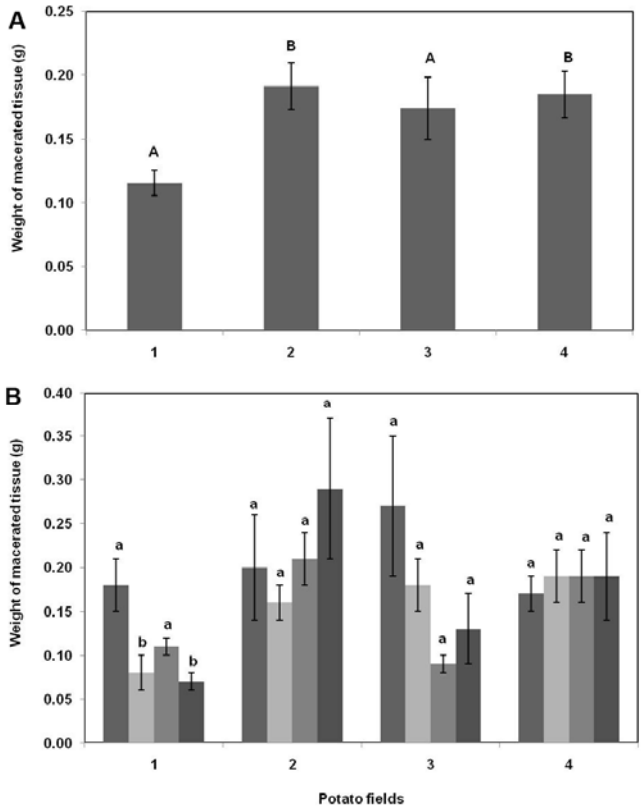


**Fig. 3.** Tubers harvested from different fields and locations differed in susceptibility to soft rot. Potatoes tubers (Russet Norkotah Colorado 8) were harvested from four different potato fields (1, 2, 3, and 4) **(A)**, and from four different sites within each field (indicated by columns in each potato field) **(B)**, in Wisconsin in 2009. A bacterial suspension of *Pcc* WPP14 containing $10^6$ bacteria was inoculated in the 160 to 210 tubers harvested per field. Macerated tissue was weighed after 2 days of incubation at 22°C. Bars represent the average of macerated tissue recovered from inoculated tubers. Error bars represent standard error. Means with the same letter are not significantly different according to Dunn's multiple comparison test, $P = 0.05$.

fields 1 and 3 were less susceptible to tuber soft rot, and in one of four fields, location of harvesting influenced susceptibility to soft rot (Fig. 3). There was no correlation between soft rot susceptibility and any of the soil mineral characteristics measured, including calcium, which ranged from 430 ppm to 1,530 ppm among the 16 sites tested.

**Bacterial colonization in potato tubers.** To determine if *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum,* and *P. atrosepticum* differed in growth in potato tubers, one sequenced strain from each taxon was inoculated into potato tubers and bacterial growth was monitored. *P. atrosepticum* had significantly lower levels in potato tubers (Red Norland) 3 days postinoculation compared to *Pcb* WPP501 and *Pcc* WPP359 (Fig. 4) (Tukey's multiple comparison test, $P < 0.0001$). After 3 days, bacterial populations of *Pcb* WPP501 reached $1.9 \times 10^{10}$ CFU/g of tissue, *Pcc* WPP359 reached $1.3 \times 10^{10}$ CFU/g of tissue, and *Pa* SCRI 1043 reached an average $4.2 \times 10^{7}$ CFU/g of tissue. At 72 h postinoculation, initial symptoms of decay were observed in tubers inoculated with *Pcb* and *Pcc* strains but not in tubers inoculated with the *Pa* strain; thus substantial bacterial growth occurred prior to symptom development when *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* were inoculated into potato under these conditions. In contrast, *P. atrosepticum* populations declined after 48 h.

**In vitro and in planta growth inhibition of *Pcc* and *Pa* strains by *Pcb* 1692 strain.** *Pcb* 1692 encodes carbapenem, a beta lactam antibiotic (32,38). *Pcb* 1692 produced a halo of inhibition when streaked across lawns of *Pcc* WPP14 or *Pa* SCRI 1043, but none of the other *Pcb* strains tested inhibited WPP14 or SCRI 1043 (Fig. 5), and none of the *Pcc* or *Pa* strains tested inhibited WPP14 or SCRI 1043 (not shown).

To determine whether growth inhibition occurs in plants, *Pcb* 1692 was co-inoculated with *Pcc* WPP14 into potato stems. Both strains carried antibiotic resistance cassettes in a neutral region of their chromosome to allow differentiation between these two strains, and the derivative *Pcb* 1692 strain still inhibited *Pcc* WPP14 in culture (not shown). The competition indices were close to 1 at all three time points (day 1 = 0.98 ± 0.024; day 2 = 1.02 ± 0.031; day 3 = 0.99 ± 0.002); thus there was no detectable inhibition of *Pcc* WPP14 by *Pcb* 1692 in potato stems. Growth curves of single and co-inoculations of these strains showed no indication of inhibition or synergy when these strains were co-inoculated into potato stems (Fig. 6). A confocal microscopy observation of leaves with soft rot symptoms showed that *Pcb* 1692 and *Pcc* WPP14 coexist in the same location within leaves. No evidence of a patchy distribution, which would suggest differences in localization of the two strains within plants, was noted (Fig. 7). During the course of these observations, we also noted that both *Pcc* and *Pcb* strains were motile in symptomatic leaves and tubers.

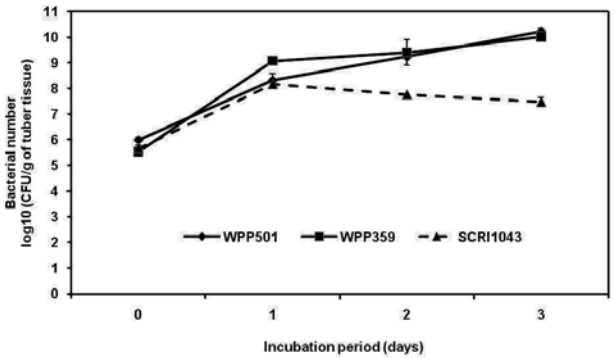


**Fig. 4.** Growth curves of *Pcc* WPP359, *Pcb* WPP501, and *Pa* SCRI1043 in potato tubers. Bacterial suspensions of approximately $10^5$ bacteria were stab-inoculated on potato tubers (Red Norland; $n = 5$), and tubers were incubated at 28°C. Bacterial populations were assayed daily by dilution plating a portion of the inoculated tissue. Error bars indicate standard error. The experiment was performed twice with similar results. This figure shows data from one experiment. One-way ANOVA was conducted in each time point. *: *Pa* is significantly different from *Pcc*; **: *Pa* is significantly different from *Pcc* and *Pcb*.

**Individual *Pa, Pcb,* and *Pcc* isolates cause soft rot symptoms in a limited range of plants.** To test if the host ranges of *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* differ, a range of plant species previously reported to be hosts of *Pectobacterium* (*Beta vulgaris*, *Lactuca sativa*, *Daucus carota*, *Apium graveolens*, *Brassica rapa, Ipomoea batatas*, *Spinacia oleracea*, and *Helianthus annuus*) were inoculated with multiple strains from each taxon. Four species, not yet reported to be *Pectobacterium* hosts in peer-reviewed journals to our knowledge, were also assayed (*Zea mays, Phaseolus vulgaris, Asparagus officinalis,* and *Allium fistulosum*). *Pcb* and *Pcc* strains caused soft rot symptoms in the same plant species ($n = 7$) (Table 4). Plants from Apiaceae (carrot and celery) and Brassicaceae (radish and rapini) families were susceptible to nearly all *Pcb*, *Pcc*, and *Pa* strains tested. None of the strains tested caused soft rot in table beets, spinach, asparagus, sunflower, or corn. Almost all *Pcb* strains (except WPP165) and all *Pcc* strains tested caused soft rot in green beans and green onions, neither of which have been

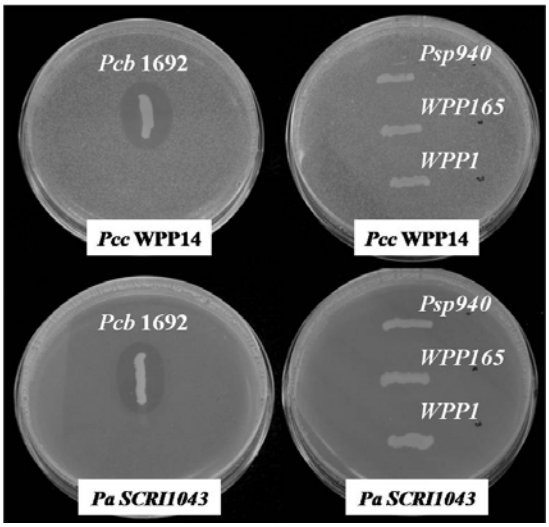


**Fig. 5.** *Pcb* 1692 has antibiosis against *Pectobacterium carotovorum* subsp. *carotovorum* and *P. atrosepticum* in LBA medium. *Pcb* strains were streaked across lawns of *Pcc* WPP14 and *Pa* SCRI1043 strains prepared in LBA medium (approximately $10^8$ CFU/ml). Plates were incubated at 37°C for 16 h. *Pcb* 1692: sequenced strain isolated from potato in Brazil; *Pcb* Psp940: *Pcb* strain isolated from potato in Brazil; *Pcb* WPP165: *Pcb* strain isolated from potato in Wisconsin; *Pcb* WPP1: *Pcb* strain isolated from potato in Wisconsin.

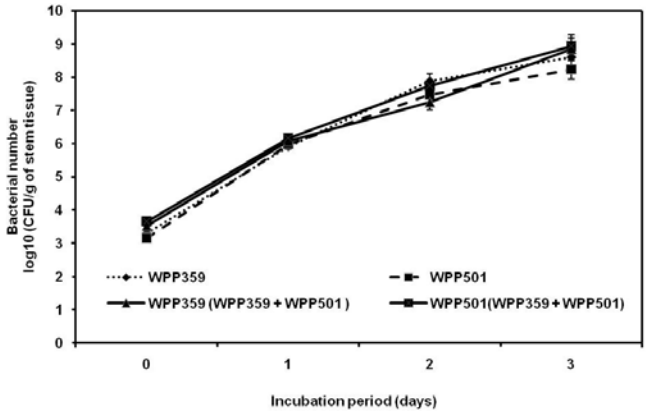


**Fig. 6.** Bacterial growth curve in potato stems of individual and co-inoculation of *Pcc* WPP359 and *Pcb* WP501. One-month-old potato stems (Superior; $n = 5$) were inoculated with a suspension of $5 \times 10^3$ CFU for single inoculations and at a total of $5 \times 10^3$ CFU at a 1:1 ratio for co-inoculations. Plants were placed into plastic bags and kept at 26°C for 3 days. This figure presents data from one of two biological replicates; similar results were observed for both replicates. No significant differences among bacterial number were found in each time point according to one-way ANOVA analysis ($P = 0.05$).

previously reported as a host for *Pectobacterium*. We were surprised to find that *Pa* strains, which we had expected to have a narrow host range, caused maceration in green onions, Swiss chard, carrots, celery, radish, rapini, and beans (Fig. 8). All *Pa* isolates caused maceration in radish leaves, but only Eca6 caused symptoms in leaves and roots.

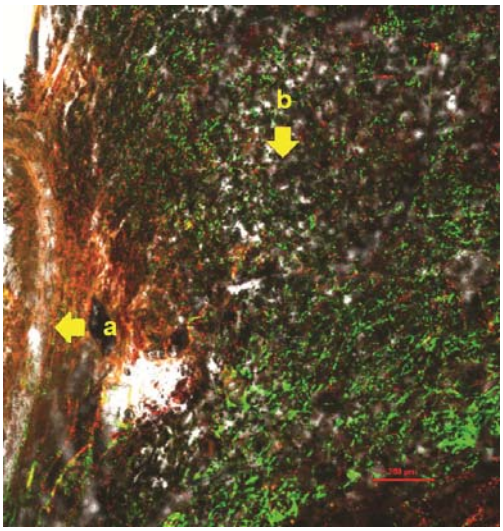


**Fig. 7.** Potato leaf with soft rot symptoms visualized using confocal microscopy shows *Pectobacterium carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* in the same location. One-month-old potato stems (Superior) were coinoculated with a suspension of $10^5$ CFU of *Pcc* WPP14(p519nGFP) and *Pcb* 1692(p519nDsRed.T3) at 1:1 ratio. The plants were placed into plastic bags and kept at 26°C. Tissue samples were taken for microscopy after 2 to 3 days of incubation and this figure shows one representative leaf. Bar: 200 µm. Merged image of green and red colored bacterial populations, labeled with one of two fluorescent proteins (GFP or DsRed.T3(DNT)), respectively. (a) Leaf vein (vascular tissue); (b) spongy mesophyll.

## Discussion

Genomes for three representative strains from *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* and *P. atrosepticum* taxa have been sequenced, but many of their basic biological characteristics remain unexplored. For this work, we focused on traits that impact breeding for resistance to *Pectobacterium* and also searched for differences in pathogenicity among these strains that may eventually be tied to genetic differences. *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* were more aggressive on potato tubers and stems than *P. atrosepticum*. We found that tuber size and maturity affect the susceptibility of potato tubers to soft rot, which complicates analysis of tuber resistance to maceration. We also found that *Pcc* and *Pcb* strains can co-colonize potato stems, even though *Pcb* 1692 inhibits growth of *Pcc* in culture. Although our assays were lab-based, they suggest that characterization of *P. carotovorum* and *P. atrosepticum* as broad and narrow host range pathogens, respectively, may be incorrect. We previously found that WPP14 and WPP165, two of the strains tested in this work, cause stem rot under field conditions and in four assays of five were indistinguishable in level of aggressiveness (21), similar to our finding here that *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* were indistinguishable in most of the assays performed. The lack of aggressiveness of *P. atrosepticum* on potato in our laboratory assays is not consistent with its importance as a plant pathogen in some locations, but does fit our observation that we routinely isolate *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum*, but have yet to isolate *P. atrosepticum*, from potato tubers and stems exhibiting signs of soft rot, stem rot, and blackleg in Wisconsin.

**Table 4.** Results from host range assays with *Pectobacterium*

| | | ***Pectobacterium* spp. clades**[x] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ***Pcb*** | | | | ***Pcc*** | | | | ***Pa*** | | | |
| **Plant family** | **Plant species** | **Pcb1692** | **Psp940** | **WPP165** | **WPP1** | **WPP14** | **Ecc380** | **WPP236** | **WPP220** | **Eca31** | **SCRI1043** | **Eca6** | **NC** |
| Alliaceae | *Allium fistulosum* (green onion) stems from harvested plants | + | – | + | + | + | + | + | + | – | – | + | – |
| Amaranthaceae | *Beta vulgaris* var. *cicla* (Swiss chard) 'Fordhook Giant' petioles from unharvested plants | + | + | + | + | + | + | + | + | – | + | + | – |
| | *B. vulgaris* (beets) roots from harvested plants | – | – | – | – | – | – | – | – | – | – | – | – |
| | *Spinacia oleracea* (spinach) 'Bourdeaux' leaves from unharvested plants | – | – | – | – | – | – | – | – | – | – | – | – |
| Asparagaceae | *Asparagus officinalis* (asparagus) modified stems from harvested plants | – | – | – | – | – | – | – | – | – | – | – | – |
| Asteraceae | *Helianthus annuus* (sunflower) 'Dwarf Sunspot' stems from unharvested plants | – | – | – | – | – | – | – | – | – | – | – | – |
| | *Lactuca sativa* (Romaine lettuce) leaves from harvested plants | – | – | – | – | + | + | + | + | – | – | – | – |
| | *L. sativa* (lettuce) 'Butter crunch' unharvested leaves | – | – | – | + | + | – | + | + | – | – | – | – |
| Apiaceae | *Daucus carota* (carrots) 'Little Fingers' roots from unharvested plants | + | + | + | + | + | + | + | + | +[y] | +[y] | – | – |
| | *D. carota* (carrots) roots from harvested plants | – | – | + | – | + | + | + | + | – | – | – | – |
| | *Apium graveolens* (celery) petioles from harvested plants | + | + | + | + | + | + | + | + | + | + | + | – |
| Brassicaceae | *Raphanus sativus* (radish) 'Sparkler' leaves from unharvested plants | + | + | + | + | + | + | + | + | +[y] | +[y] | +[y] | – |
| | *R. sativus* (radish) roots from harvested plants | + | + | + | + | + | + | + | + | – | – | + | – |
| | *Brassica rapa* subsp. *rapa* (rapini) stems from harvested plants | + | – | + | – | + | + | + | + | + | + | + | – |
| Convolvulaceae | *Ipomoea batatas* (sweetpotato) roots from harvested plants | + | + | + | + | + | + | + | + | – | – | – | – |
| Fabaceae | *Phaseolus vulgaris* (green beans)[z] fruit from harvested plants | + | + | – | + | + | + | + | + | + | – | – | – |
| Poaceae | *Zea mays* (corn)[z] 'Bodacious' stems from unharvested plants | – | – | – | – | – | – | – | – | – | – | – | – |

[x] *Pcb*: *P. carotovorum* subsp. *brasiliensis, Pcc*: *P. carotovorum* subsp. *carotovorum, Pa*: *P. atrosepticum.*
[y] Similar results were obtained in leaves with a lower inoculum concentration ($10^5$ CFU/inoculation).
[z] Not previously reported as a *Pectobacterium* host.

*P. carotovorum* subsp. *brasiliensis* was first reported by Duarte et al. 2004 (9) as a blackleg pathogen that is more aggressive than *P. atrosepticum*. We found that *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* do not differ from each other in their aggressiveness on potato tubers when all data were combined, and that both are more virulent than *P. atrosepticum.* There was variation among *Pcc* and *Pcb s*trains tested, and when data from individual potato varieties were examined, *Pcb* strains were more aggressive in one of the varieties tested. We hypothesized that *P. atrosepticum* would be more aggressive on stems as a tradeoff for being less aggressive on tubers, but instead we found that under lab conditions at the temperature used, *P. carotovorum* is more aggressive on both stems and tubers. This greater aggressiveness may account, in part, for the higher prevalence of *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* in the field compared to *P. atrosepticum* in Wisconsin.

We were surprised to find that Dark Red Norland, a selection of Red Norland with a darker red skin, was more susceptible to soft rot that its parent variety. Similarly, the Colorado 8 selection of Russet Norkotah, which is more resistant to Verticillium wilt, was more susceptible to soft rot than Russet Norkotah. We controlled for variation as carefully as we could by using only B size tubers from the same farm. It is possible that physiological differences account for our results, but also possible that the somatic changes that altered skin color and Verticillium resistance also affect soft rot resistance in these two lines.

Although we controlled for variation in the tuber assays as carefully as possible, tuber to tuber variation was still found. This variation cannot be attributed to genetic differences, since potatoes are vegetatively propagated, and is therefore likely to be due to physiological differences among tubers. For example, when tubers were inoculated with multiple *Pcc* or *Pcb* strains, we noted individual tubers were resistant or susceptible to all of the strains that were inoculated. If physiological differences that affect tuber susceptibility can be identified and controlled, farmers may be able to reduce the incidence of soft rot. To begin to address this question, tubers from a single seed lot that was planted into different fields were harvested from four locations in each of four fields and inoculated with *Pcc* WPP14. Soft rot susceptibility was significantly correlated with tuber weight, with smaller tubers being more resistant to soft rot. It was also correlated with tuber maturity, with the more mature tubers being more susceptible. However, more mature tubers tend to have better developed periderm and thus resist injuries that can inoculate bacteria into the tuber flesh.

However, neither tuber weight nor harvest date can account for the variation we saw within varieties in our tuber experiments since we controlled for these variables. Tuber maturity may still be a factor, since tubers develop at different times under potato plants and some of the tubers examined may be older than others, even though they are the same size. We examined soil that was adhered to the collected tubers, with the hypothesis that variation in soil characteristics that affect plant nutrition might account for differences in susceptibility. We found no correlation with any soil mineral data and soft rot susceptibility. With regard to soft rot on tubers, either *Pcc* or *Pcb* strains are useful for screening for plant


**Fig. 8.** Symptoms of soft rot caused by strains of *Pectobacterium.* Plants were inoculated with approximately $10^6$ CFU of bacterial cells, covered with a plastic bag, and incubated at room temperature (~22°C) for 3 days. **A,** Swiss chard, **B,** carrots, **C,** green onions, **D,** green beans, **E,** radish roots, **F,** radish leaves. 1: Eca31, 2: SCRI1043, 3: Eca6, NC: negative control (inoculations with sterile water).

resistance, but tubers of a similar weight and age should be assayed when comparing varieties to each other.

Production of antibiotics such as carbapenem and multiple bacteriocins suggests that *Pectobacterium* species use multiple strategies to compete with other microbes or with each other (40). In this study, we found that only *Pcb* 1692 (isolated from Brazil) was able to inhibit growth of *Pcc* WPP14 and *Pa* SCRI1043 in culture. This finding suggests that this in vitro phenotypic trait is strain-specific rather than taxon-specific. Genome analysis of the three *Pectobacterium* lineages showed that only *Pcb* 1692 encodes a gene responsible for the production of the carbapenem antibiotic 1-carbapen-2-em-3-carboxylic acid (13); however, whether this is the biological explanation of the antagonistic effect we observed is not known. Since no inhibition of *P. carotovorum* subsp. *carotovorum* by *P. carotovorum* subsp. *brasiliensis* was detected after co-inoculation into stems, we did not pursue the cause of the effect observed in culture. We cannot rule out that competition among *Pectobacterium* strains might occur as maceration progresses and nutrient availability decreases in other potato tissue where similar arrays of metabolic profiles are used, in other plant hosts, or during saprophytic growth in soil or water. Mixed infections of *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* and *P. wasabiae* are common; thus our co-colonization results reflect what is found in potato fields (21,55).

Many bacterial plant pathogens are motile, and flagellar genes contribute to virulence either through taxis toward plants or while the pathogen is inside the plant (5,18,47). In *P. atrosepticum* and *P. carotovorum* subsp. *carotovorum*, motility also contributes to virulence and appears to be co-regulated with other virulence factors (17,30,36). The exact role and the contribution to different stages of the infection process are still unknown. We observed that *P. carotovorum* is motile in infected potato leaves and tubers, suggesting that motility may not only be required for successful invasion of the tissue but also used during later stages of diseases. Biogenic mixing may be important on very large scales. For example, sea creatures are proposed to be responsible for some ocean mixing (20). Whether biogenic mixing is also important on very small scales, such as whether *Pectobacterium* motility contributes to virulence by increased mixing of pectolytic enzymes in diseased plants, is an intriguing question.

Due to advances in DNA sequence analysis, multiple *Pectobacterium* subtypes have recently been described (8,9,56), and a previously described species, *P. wasabiae*, is now known to be common on potato (21,42). Unfortunately, it is typically not possible to determine from work published prior to this decade which *Pectobacterium* taxon was isolated from any particular host; thus the host range of each *Pectobacterium* taxon remains obscure. Part of the motivation for obtaining multiple *Pectobacterium* sequences is to identify the genetic basis for host range differences among *Pectobacterium* species. To test the hypothesis that *P. carotovorum* subsp. *brasiliensis* has a wide host range and to identify useful experimental hosts that vary in susceptibility to *Pectobacterium* taxa, we inoculated multiple strains into numerous crop species. We found that *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* and *P. atrosepticum* differ in experimental host range and identified lettuce, sweet potato, radish, and beans as useful species for host range studies. Of these, Romaine lettuce may be useful for examining genomic differences between *P. carotovorum* subsp. *brasiliensis* and subsp. *carotovorum* that affect host range, and sweet potato may be useful for exploring genomic determinants of host range in *P. carotovorum* versus *P. atrosepticum*. We cannot rule out that the lack of virulence in some hosts tested was due to nonconducive environmental conditions for these particular hosts or the particular variety being tested having resistance to *Pectobacterium.* However, our data suggest that although the genus *Pectobacterium* has a wide host range, individual strains are limited in which hosts they can infect. Some plant taxonomic groups, such as the order Poales and the family Asparagaceae, have never had a report of disease caused by *Pectobacterium*. Our data provide some additional support that plant species in these groups are resistant to *Pectobacterium*.

We were surprised to find that, although *P. atrosepticum* is unable to efficiently macerate potato tubers, it causes maceration symptoms on green onions, Swiss chard, carrots, celery, radish, rapini, and green beans at the same inoculum levels and under the same incubation conditions used for potato. Our data did not support the hypothesis that *P. atrosepticum* is a narrow host range pathogen compared to *P. carotovorum*. Therefore, using these two species as a model for comparison of narrow and broad host range pathogens may be incorrect. Recently, *P. atrosepticum* was reported to be the causal agent of head rot disease on sunflowers in Turkey (2), which provides support for the hypothesis that it has a broad host range. The lack of reports of this species on other hosts could be due to the relative difficulty of isolating it compared to other *Pectobacterium* species and the likely presence of multiple *Pectobacterium* species on diseased host plants. Molecular detection methods capable of detecting and differentiating all *Pectobacterium* species directly from infected plants have not yet been developed, but would be useful for determining how often *P. atrosepticum* is found in nonpotato hosts as well as whether multiple *Pectobacterium* species are commonly found on other crops.

## Acknowledgments

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