Research Report to Maine Potato Board Research Committee

Improving Strategies in Managing Blackleg and Soft Rot of Potato

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1. Population diversity of *Dickeya* and *Pectobacterium* spp. in the Northeastern states

Isolates of *Dickeya* and *Pectobacterium* spp. were obtained from plants and tubers of potato as well as surface water in the Northeastern states during 2015 and 2017. Genomic DNAs were extracted using FastDNA Spin Kit (MP Biomedicals, LLC). Genes of *gapA, recA, and dnaX* were sequenced and phylogenetic trees were established based on these sequences. *Dickeya dianthicola* slight variation depending on the geographic location. All four Maine isolates were identical base on *gapA* gene sequence and somewhat different from the isolates from other states (Figure 1). Total genomic DNAs of water samples were extracted and analyzed using next-generation sequencing. There were many groups of bacteria. Around 30% of the samples showed *Dickeya* and *Pectobacterium* spp. (Figure 2).

2. Screen for potential materials that inhibit the growth of *Dickeya* and *Pectobacterium* spp.

Antagonistic efficacy of select products was examined on agar plates, and minimal inhibition concentration (MIC) or effective concentration for 50% of growth inhibition (EC50) of each product determined by testing the antibacterial activity on chemical-amended agar media. Bacterial cultures were incubated for 24 hr in tryptic soy broth in a test tube, and the test chemical compounds were added as serial dilutions. Sterile distilled water was used for control. In results, streptomycin, copper sulphate, oregano essential oil, and Lifegard were effective in inhibiting both *D. dianthicola* and *P. parmentieri*.

3. Examine the effect of select products for disease control in the field

For in-field disease control, several treatments were applied, including Phostrol applied after emergence & 50% blossom, methyl salicylate for foliar application, copper sulphate for seed treatment, streptomycin for seed treatment, and Lifeguard for foliar application. Prior to planting and seed treatments, potato 'Lamoka' tubers were inoculated with *D. dianthicola* ME30 at 10^7 cfu/ml, using a vacuum pressure tank for 15 minutes at 0.8 bar. The tubers were air-dried before use. There were no significant differences among any of the above treatments. Streptomycin may help to enhance disease resistance and increase yield (Table 1).

4. Inter-species interaction and survival of blackleg pathogens

Cultures of *Dickeya* and *Pectobacterium* spp. were each incubated in tryptic soy broth at 28C, and their growth was measured using a chromatographic plate reader. In the field, *D. dianthicola* ME30 and *P. parmentieri* ME175 were inoculated to seed potato of 'Shepody', 'Lamoka', and 'Atlantic'. *Dickeya dianthicola* had the slowest growth comparing with other species (Figure 3). However, *D. dianthicola* had higher virulence than *P. parmentieri* (Figure 4). More importantly, the mixture of *D. dianthicola* and *P. parmentieri* resulted in the highest disease incidence. 'Shepody' was tolerant but 'Lamoka' was susceptible to both *D. dianthicola* and *P. parmentieri* (Figure 4). When Dickeya dianthicola was treated with 500 uM of $CuSO_4$ for 1 hour or 50 uM of $CuSO_4$ for 2 hours, the bacterium stayed dormant for longer than 60 days in the viable but non-culturable state.

5. Varietal tests

To screen potato germplasm on the resistance to blackleg and soft rot, 370 clones from a diploid hybrid population of *S. phureja-S. stenotomum* were examined. Potato tubers were inoculated by poking a 1-cm deep hole and add a 15 µl tryptic-soy-broth based bacterial suspension of *P. parmentieri* strain ME175, *D. dianthicola* strain ME30, or tryptic soy broth only. Tubers were incubated 3 days at 28^{o}C in a

moist plastic chamber, then cut transversally through the inoculation points, and lesions were measured. Lesion size (cm^2) was analyzed using JMP. There were significant differences among clones (Figure 5).

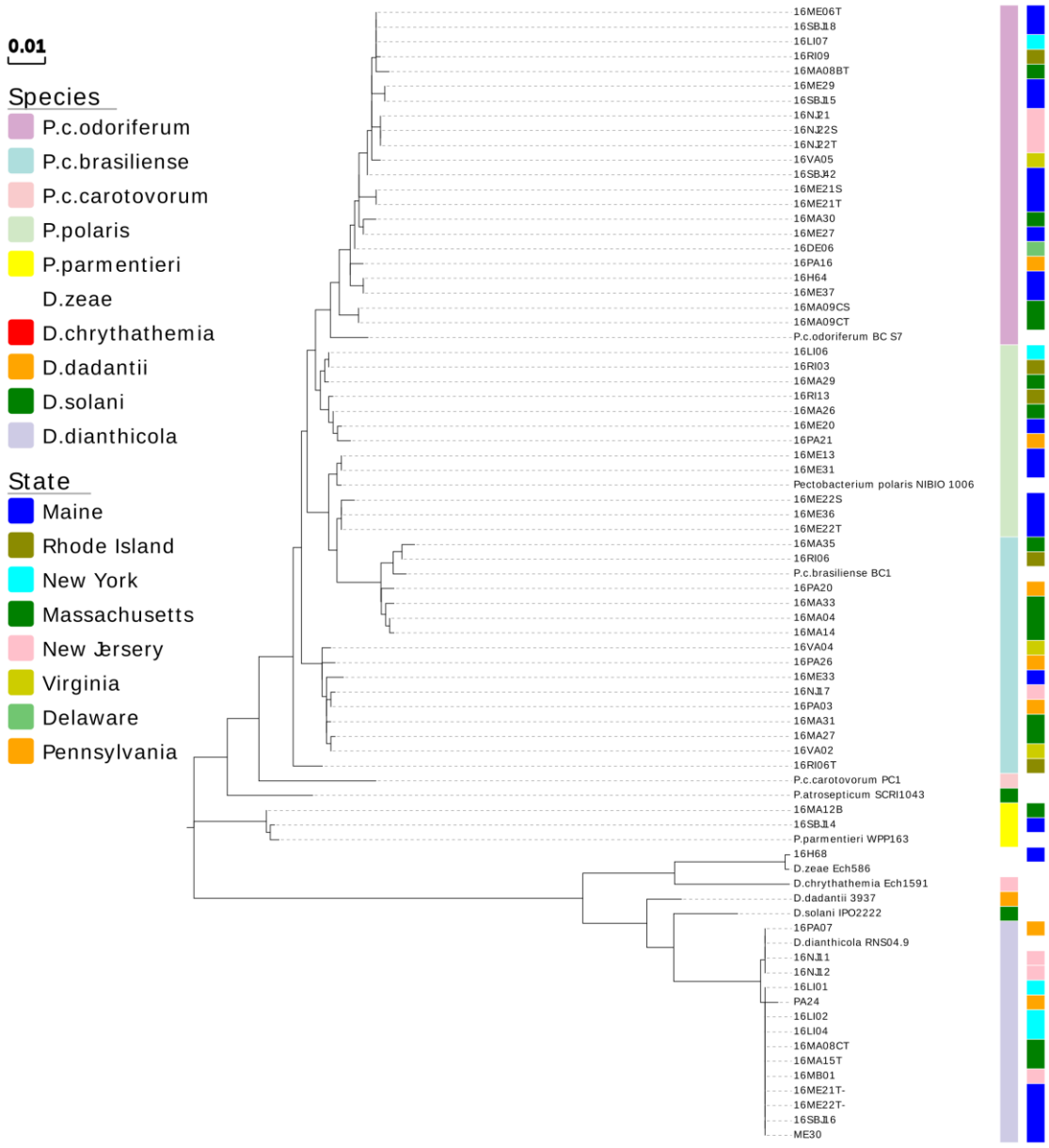

Figure 1. Phylogenetic trees of Dickeya and Pectobacterium spp. isolated from Northeastern states based on *gapA* gene sequence. On the right side of the tree, two columns of color labels were used, with the left column representing bacterial species and the right column representing states where the bacteria were isolated.

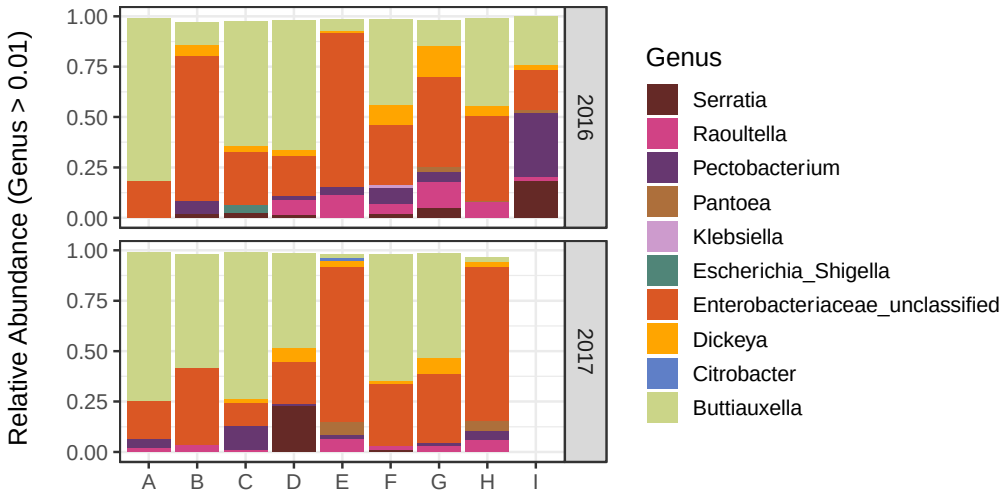

Figure 2. Relative sequence abundances on genus level in family *Enterococcaceae* in water samples.

Table 1. Chemical treatments for blackleg control

treatment	Emergence/15	Yield (lb)	StdDev
Phostrol after emergence & 50% blossom	14	18.1	3.4
Methyl salicylate, foliar application	13	22.1	4.5
Copper, seed treatment	14	21.3	1.5
Streptomycin, seed treatment	13	24.3	3.4
Lifeguard, foliar application	13	19.2	2.2
Non-treated	14	21.3	5.2

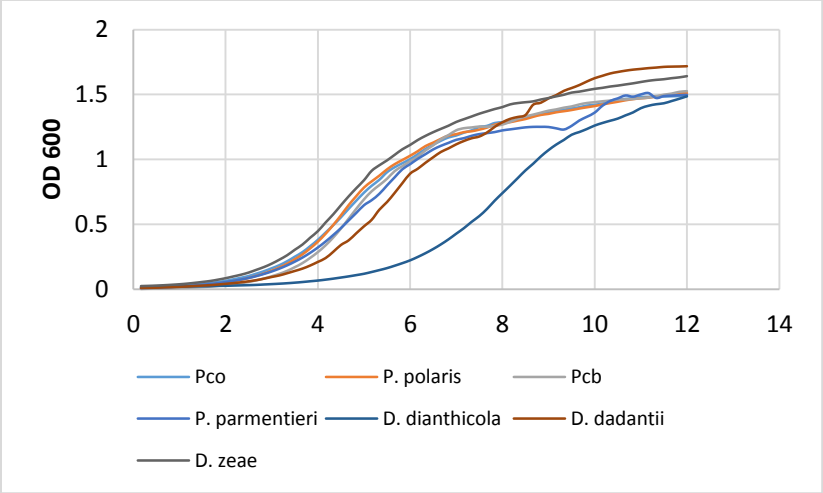

Figure 3. Growth responses of *Dickeya spp. and Pectobacterium spp.* in tryptic soy broth at 28C.

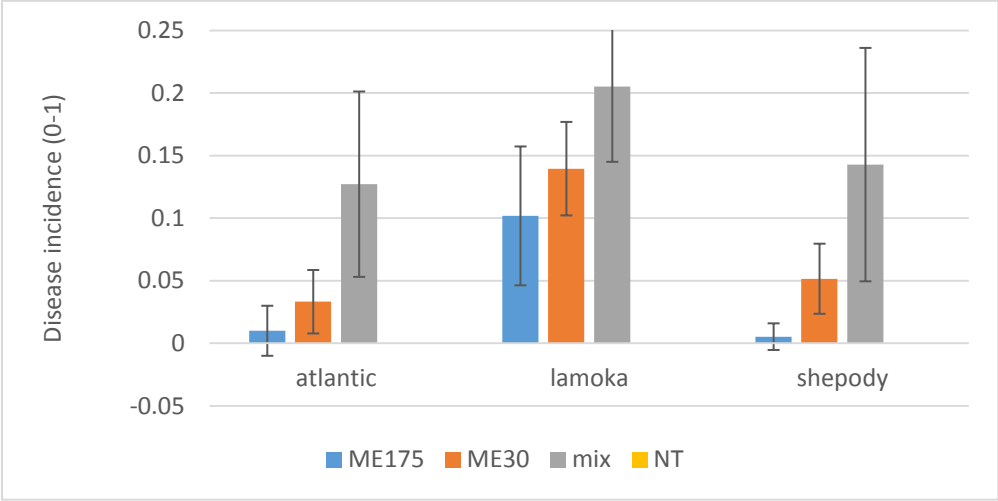

Figure 4. Blackleg of potato inoculated with *D. dianthicola* OR/AND *P. parmentieri* at Aroostook Research Farm, Presque Isle, 2018.

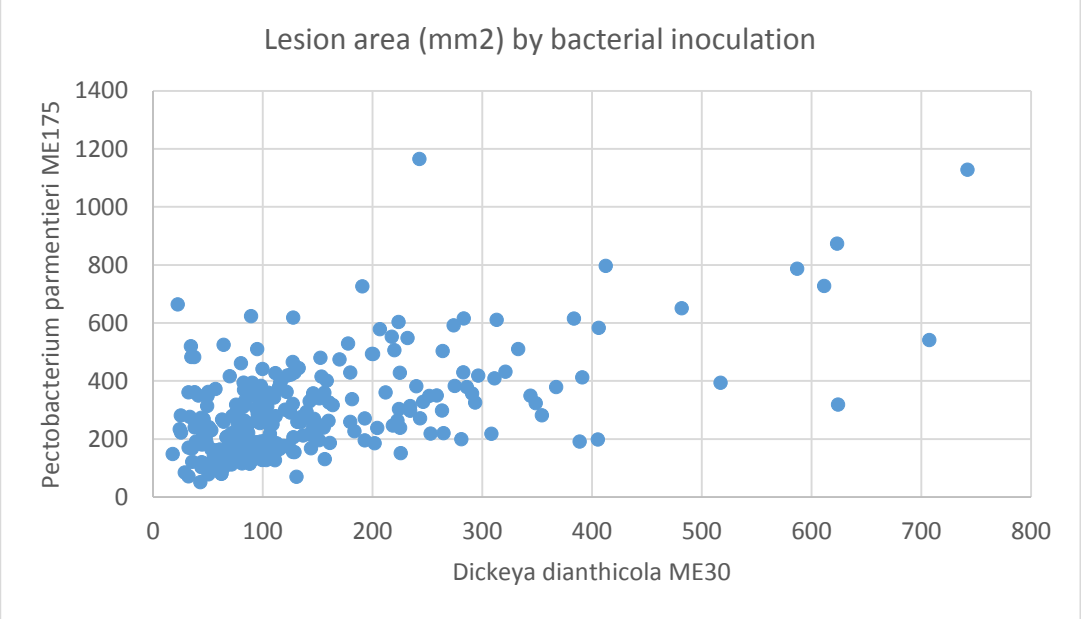

Figure 5. Responses of 370 potato clones to *Dickeya dianthicola* ME30 and *Pectobacterium parmentieri* ME175, 2018.

Summary

Dickeya dianthicola had diversified genetic background depending on the location, indicating the outbreak of blackleg may not have a single origin. *Dickeya* spp., *Pectobacterium* spp. and other bacteria have association, although the actual interaction was not known. *Dickeya dianthicola* was more virulent than *P. parmentieri*, but the mixture of the two species were more aggressive than each species was. Some potato germplasm can be used for future breeding resources for the resistance to blackleg.