# Bacteria and Yeast Associated with Sugar Beet Root Rot at Harvest in the Intermountain West

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**ABSTRACT**

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An undescribed wet rot of roots was observed in surveys of recently harvested sugar beet roots in Idaho and eastern Oregon in 2004 and 2005. Microorganisms isolated from 287 roots fell into the following groups: A (41% of strains), B (29%), C (17%), D (11%), E (2%), and F (1%). Groups A, B, C, and F were composed of bacteria while groups D and E were yeasts. Subgroup A1 (80% of group A strains) included *Leuconostoc mesenteroides* subsp. *dextranicum* strains and subgroup A2 (20%) contained *Lactobacillus* strains. Group B was dominated by subgroup B1 (92% of strains), which included *Gluconobacter* strains. When only one organism was isolated from rotted roots, strains from subgroup A1 were isolated most frequently. Group C was composed of enteric bacteria. Strain B322 of *L. mesenteroides* subsp. *dextranicum* caused the most severe rot on root slices and produced symptoms similar to those in harvested roots. Results suggest that *L. mesenteroides* subsp. *dextranicum* is among the first bacterial species to enter sugar beet roots, closely following fungal infections or entering directly through openings such as growth cracks. The bacterial rot leads to yield loss in the field but likely also leads to storage and factory-processing problems.

Additional keywords: acetic acid bacteria, fermentation, lactic acid bacteria

Bacterial vascular necrosis and rot in sugar beet (*Beta vulgaris* L.) has been attributed to *Pectobacterium betavasculorum* (Thomson et al.) Gardan et al. (syn. *Erwinia carotovora* (Jones) Bergey et al. subsp. *betavasculorum* Thomson et al.) (11,20,29,32) and *Pseudomonas* spp. (16,30). In the western United States, *P. betavasculorum* has been associated with root rot in sugar beet in Arizona, California, Idaho, Montana, and Washington (20,24,29,33,36). The bacterial rot in sugar beet can be variable from wet to dry (29). Resistance to *P. betavasculorum* has been identified (14,33,34) and incorporated into commercial cultivars. Several seed companies regularly screen germplasm to maintain resistance in commercial cultivars.

Recent field observations and isolations revealed that other bacteria also might play important roles in causing bacterial rot. In Oregon and Idaho, a previously undescribed root rot with wet symptoms that suggested the involvement of bacteria has been observed. Roots with advanced symptoms had a vinegar-like fermented smell, rotted out cavities, and viscous slime indicative of bacterial activity. Preliminary isolations revealed that the disease was associated not with *Pectobacterium* spp. but with a complex of other bacteria instead. Observations from a survey of root rots in the field and fungal isolations (*unpublished data*) suggested that fungal root rots were frequently but not always present with the wet rot symptoms. Roots in fields with heavy soils and excessive soil moisture also were observed to have the wet decomposition problem. Because filamentous fungi and oomycetes were not always associated with the wet symptoms suggestive of bacterial rot, this study focused on the bacteria always associated with these symptoms. Therefore, the objective of this study was to identify the bacteria possibly responsible for this wet root rot problem in sugar beet, as well as to establish their distribution and relative pathogenicity.

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## MATERIALS AND METHODS

**Survey.** An assessment was made of bacterial root rot in recently harvested roots delivered to piling grounds in Idaho and Oregon at the end of the 2004 and 2005 growing seasons. In all, 29 and 28 piling grounds were visited from southeastern Idaho (American Falls, southeastern Idaho; Magic Valley, south-central Idaho; and Treasure Valley, southwestern Idaho) to southeastern Oregon in 2004 and 2005, respectively. We collected 120 roots in 2004 and 167 roots in 2005 with wet rot symptoms suggestive of bacterial causality. To ensure an even sampling distribution, no more than 15 symptomatic roots were collected from a pile each year. Isolations were conducted by removing 10-by-10-mm pieces of internal root tissue from the margins between rotted tissue and white, healthy-appearing tissue. The pieces of tissue were surface sterilized, disinfested in 0.6% sodium hypochlorite for 60 s, and then rinsed in sterilized reverse osmosis water for 60 s. The surface areas of each tissue piece then were removed and a 2-by-2-mm piece was macerated in a drop of sterile well water. To isolate bacteria and yeasts, a loop of the suspension was streaked onto yeast extract-dextrose-calcium carbonate agar (YDC) and King's Medium B (KMB) and the culture incubated at 30°C (21). Representative colonies from each root were restreaked onto YDC after 3 to 7 days.

**Identification.** Organism strains initially were characterized and grouped based on colony morphology, color, gram stain reaction, cell shape, and size. To ensure that strains were grouped appropriately, carbon source utilization profiles were determined on one-third of the strains from each group using GN2 MicroPlates (Biolog Inc., Hayward, CA) and protocols recommended by the manufacturer for gram-negative enterics. Although designed for gram-negative bacteria, the Biolog plates gave reproducible profiles with gram-positive bacteria and yeast as well. When there was variability in carbon source utilization detected among members of a group, the remaining members of that group also were tested.

Selected strains from each bacterial group were submitted to Microbial ID (MIDI Labs, Newark, DE) for 16S rRNA sequencing (first 500 bp). The 16S rRNA gene was polymerase chain reaction (PCR) amplified from genomic DNA isolated

from pure cultures. The primers used were 16S universal primers 5F and 531R. Selected strains from each of the yeast groups were submitted to Microbial ID for sequencing of 28S rRNA variable D2 region (approximately 300 bp). The primers used corresponded to positions 3,334 and 3,630 in the *Schizosaccharomyces japonicus* large subunit rRNA gene. Sequence analysis was performed using MicroSeq (Applied Biosystems, Foster City, CA) microbial analysis software and database. Sequence comparisons also were conducted using BLASTn to search the GenBank database via the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov/blast).

**Root rot tests.** Two experiments were conducted in which strains of bacteria and yeast were inoculated into sugar beet root slices to determine pathogenicity. Because most of the strains isolated came from the bacterial subgroup (A1) containing *Leuconostoc* spp. or were found in association with strains in this subgroup, the first pathogenicity test was focused on representative strains of these organisms. In the experiment, there were 10 treatments, which included three bacteria strains (*Enterobacter cancerogenus* B139, *Gluconobacter asaii* B225, and *Leuconostoc mesenteroides* subsp. *dextranicum* B322) and a strain of yeast (*Pichia fermentans* B154) inoculated separately or in various combinations, along with a no-organism check. The combination treatments were chosen on the basis of the frequency of their occurrence in isolations from harvested roots. In the second pathogenicity test, a different selection of organisms was tested. These included bacterial strains *E. cancerogenus* B86, *G. asaii* B31, *Lactobacillus plantarum* B106, and *Pectobacterium betavasculorum* B20 and the yeast strain *Pichia fermentans* B9. In addition, *Pectobacterium betavasculorum* strain Ecb-3 BS114 from California (provided by Robert Lewellen, United States Department of Agriculture–Agricultural Research Service, Salinas, CA) was used for comparison. There were 17 treatments that included each organism inoculated alone, the organisms in various combinations, and the control.

Healthy-appearing roots of cvs. Beta 8600 and Beta 4199R were harvested in October and used in the first and second sets of experiments, respectively. The roots were washed free of soil and stored in the refrigerator at 4°C. Then, roots were immersed in 0.6% sodium hypochlorite for 1 min, rinsed in sterile reverse osmosis water, and allowed to air dry under sterile conditions in a laminar hood. All exterior portions of the root were removed. The roots were cut into cross-sectional slices 8 to 10 mm thick and 45 to 70 mm in diameter. A single slice was placed inside a 15-by-100-mm petri dish on sterilized (121°C for 30 min) filter paper moistened with sterile well water. A hole (2 mm in diameter by 3 mm deep) was made in the center of the slice with a sterile tooth pick. Bacteria, yeast, or both were applied to the hole using a sterile toothpick that had been dipped into a 48-h-old culture grown on YDC at 30°C. Slower-growing bacterial strains were cultured for several additional days prior to inoculation. The controls were mock inoculated with sterile toothpicks. The petri dishes containing inoculated root slices then were placed into plastic bags, arranged by block, and incubated at 30°C. The root slices for each block came from the same root. Thus, each experiment had a randomized complete block design with three replications per treatment. The diameter of rotted area was measured at both 24 and 48 h after inoculation. After the 48-h reading, streaks were made onto YDC from the outer edge of the rot and the cultures were incubated at 30°C to confirm the presence of all inoculated organisms and the absence of contamination. Both experiments were repeated once.

**Petiole rot test.** Bacterial strains (*E. amnigenus* B92, *E. cancerogenus* B86, *G. asaii* B31, *L. plantarum* B106, *Leuconostoc mesenteroides* subsp. *dextranicum* B322, *P. betavasculorum* B20, *P. betavasculorum* Ecb-3 BS114 (California strain), and *Pseudomonas fluorescens* B82) and yeast strains (*Candida oleophila* B14, *Pichia fermentans* B9, and *P. membranifaciens* B8) were evaluated for pathogenicity in two petiole rot tests. The experimental design was a randomized complete block with five replications per treatment. Each experiment was performed twice. Sugar beet cvs. C40, susceptible to bacterial necrosis and rot, and Crystal 217R, with some resistance to bacterial necrosis and rot, were used as hosts in the first and second experiment, respectively. Plants were grown from seed in 10.2-cm plastic pots with Sunshine Mix no. 1 (Sun Gro Horticulture, Bellevue, WA) which contains 70 to 80% Canadian sphagnum peat moss, perlite, dolomitic limestone, gypsum, and wetting agent. The plants were fertilized once a week with 20-10-20 general-purpose fertilizer (Scotts Co., Marysville, OH) at 100 ppm beginning 3 weeks after planting. The greenhouse conditions were set at 23°C day and 18°C night temperatures, with day length extended to 13 h with metal halide lamps (250 μmole $s^{-1}m^{-2}$ measured at plant top). The plants were inoculated at the eight-leaf growth stage using a 27-gauge needle dipped into a 24- to 48-h-old culture grown on YDC. The four largest petioles were punctured with the inoculation needle 2 to 4 cm from the crown.

Plants were rated both 1 and 2 weeks after inoculation using a scale from 0 to 9. where 0 = healthy looking plant, 1 = slight discoloration around the inoculation point, 2 = discoloration extends half way up petiole, 3 = discoloration extends three-quarters of the way up the petiole, 4 = inoculated petioles dead, 5 = adjacent petioles starting to show wilting, 6 = half of the adjacent petioles dead, 7 = three-quarters of the adjacent petioles dead, 8 = only center whorl still green, and 9 = dead plant. The scale was not used categorically but was used as a guide to generate fractional ratings. Fresh weight of tops clipped immediately above the crown was determined 2 weeks after inoculation. Isolations were conducted on any petiole rated 2 or higher by streaking onto YDC and incubating cultures at 30°C.

**Data analysis.** Proc Univariate (SAS; SAS Institute Inc., Cary, NC) was used to test the normality of the data. Bartlett's test was utilized to test for homogeneity of variance between experiments. Data from multiple repetitions of an experiment were pooled when possible and analyzed using Proc GLM (SAS), and Fisher's protected least significant difference was used for mean comparisons.

## RESULTS

**Root symptoms.** Recently harvested roots collected from sugar beet piles exhibited rot (Fig. 1) on the side of the root 45% of the time and on top of the root 21% of the time. The roots were almost completely rotted 34% of the time, making it impossible to establish the area of rot initiation. Roots without extensive rot mimicked symptoms we would associate with fungal rots. Roots with advanced rot symptoms had a vinegar-like fermented smell, rotted-out cavities, and viscous slime indicative of bacterial activity.

**Bacterial and yeast groupings and species identification.** Bacteria and yeast strains isolated from harvested sugar beet roots were categorized into six groups (bacteria in groups A, B, C, and F and yeasts in groups D and E). Group A (Table 1) comprised non-spore-forming gram-positive strains that formed white, slow-growing colonies on YDC. Subgroup A1, comprising 80% of the strains in group A, had spherical-shaped cells and similar carbon source utilization (Biolog) profiles. Strains B268 and B322 from this subgroup were determined to be *L. mesenteroides* subsp. *dextranicum* based on analysis of 16S rRNA sequence (0.00% difference from the reference strain in the MicroSeq database). The subspecies designation, *dextranicum*, was supported by results from tests with Biolog plates in which L-arabinose utilization was negative and fructose, maltose, melibiose, sucrose, and trehalose utilization were positive (13). Subgroup A2, containing 20% of the strains in group A, were rod shaped and had similar Biolog profiles. Strains B100 and B106 from subgroup A2 were determined to be *Lactobacillus plantarum* based on 16S rRNA sequence (0.09% difference in MicroSeq analysis).

Bacterial group B was composed of gram-negative rods. Subgroup B1, comprising 92% of the strains in group B, formed pink colonies on YDC and had a similar Biolog profile. Strains B31, B71, B225, and B247 from subgroup B1 were determined to be *G. asaii* based on sequence analysis (0.21 to 0.42% difference from reference strain in MicroSeq database). Subgroup B2, comprising 8% of the strains in group B, formed slow-growing yellowish-orange colonies on YDC and had a similar Biolog profile. Strains B409 and B410 from subgroup B2 were 98 and 100% identical in 16S rRNA sequences to *Acetobacter lovaniensis* AB032351 in GenBank.

Group C also was composed of gram-negative rods. Subgroup C1, comprising 82% of the strains from group C, generally formed viscous, fast-growing, clear to white colonies on YDC and had similar Biolog profiles, but exhibited some variation in colony morphology. Strains from subgroup C1, identified on the basis of 16S rRNA sequence identity to strains in GenBank, included B92 (*Enterobacter amnigenus* AY579148, 99% identity), B86 (*E. cancerogenus* AY310730, 98%), and B122 (*Enterobacte*r sp. U39556, 98%). Also included in subgroup C1 were strains B208 and B139, identified as *E. cloacae* and *E. cancerogenus* based on 0.71 and 0.95% difference from the respective species reference in MicroSeq. Several Biolog profiles emerged from the remaining strains in group C. Strains from this remaining heterogeneous subgroup included B138 (0.85% difference from *Escherichia hermannii* in MicroSeq), B20 and B22 (97% identity to *Erwinia carotovora* [*Pectobacterium betavasculorum*] Z96091 in GenBank), and B194 (0.66% difference from *Serratia plymuthica* in MicroSeq). The two *P. betavasculorum* strains, B20 and B22, were positive for utilization of Tween-80 and N-acetyl-D-glucosamine in the Biolog tests, distinguishing them from *E. carotovora* subsp. *brasiliensis* (10).

Two bacterial strains placed in group F both were analyzed for 16S rRNA sequence. Strain B82 had 0.77% difference from *Pseudomonas fluorescens* C in the MicroSeq database. Strain B231 had 100% identity to *P. putida* AB180734 in GenBank.

The yeast were easily distinguished from the bacteria by their larger cell size and yeasty smell. Although groups D and E both included strains with a fast white growth habit on YDC, they were distinguished by unique Biolog profiles. Based on analysis of 28S rRNA sequences, strains B8 and B147 from group D had 99% identity to *Pichia membranifaciens* AY497668 in GenBank, whereas strain B9 had 0.00% difference from *P. fermentans* in MicroSeq and strains B150 and B154 were 100% identical to *P. fermentans* DQ377652 in GenBank. Sequences of strains B14 and B176 in group E were 100% identical to *C. oleophila* U45793 and *C. quercitrusa* AY529522 in GenBank, respectively.

**Survey results.** Overall, strains of bacteria and yeast were isolated individually from 48% of the samples, with strains in subgroup A1 being the most frequent (46%). When strains from A1 were isolated in association with other organisms, they were in combination with strains from groups B and C. When group B strains were isolated, 89% of the time they were in combination with other bacteria or with yeast. When group C strains were isolated, 67% of the time they were found with other bacteria or with yeast. When group D strains were isolated, 97% of the time they were associated with bacteria.

Most of the piling grounds in the American Falls area on the eastern end of the southern Idaho growing area had few beet roots, if any, with bacterial rot; only 5% of the bacteria or yeast strains came from this area. Magic Valley and Treasure Valley yielded 47 and 48% of all the strains collected, respectively. Beet roots with group A bacteria were found in most of the piling grounds in Magic Valley (49% of group A isolates) and Treasure Valley (48% of group A isolates). In the Magic Valley, 27% of the group A strains were from the Gooding pile, while the Hepworth, Kenyon, Cranney, Adelaide, and Filer piles had 14, 10, 9, 9, and 7%, respectively. In the Treasure Valley, 28% of the group A strains were from the Weiser pile, while the Reverse, Payette, Bowmont, and Mora piles had 17, 14, 13, and 9%, respectively. Group B strains were distributed throughout the Magic Valley (55% of isolates) and Treasure Valley (38%). In the Magic Valley, the piles with the most group B strains were Filer, Minidoka E/W, Hepworth, Gooding, Jerome, Cranney, Hobson, and Adelaide with 15, 13, 11, 8, 8, 8, 8, and 8%, respectively. Group C strains were found in many piling grounds in the Magic Valley (34% of strains) and Treasure Valley (66%), but some piles were hot spots. In the Magic Valley, the primary location for the group C strains was the Gooding pile (48% of strains from this valley). In the Treasure Valley, the primary locations were the Reverse, Mora, and Bowmont piles with 20, 20, and 10%, respectively.

Yeast strains in groups D and E were distributed between the Magic Valley (38% of strains) and Treasure Valley (54%). Piles with the most group D and E strains in Magic Valley were Cranney, Adelaide, and Hepworth with 26, 21, and 16%, respectively. The Treasure Valley locations with the most group D and E strains were Weiser, Reverse, Bowmont, Mora, and Payette with 22, 15, 11, 11, and 11%, respectively.

**Root rot tests.** In the first set of experiments, *Leuconostoc mesenteroides* subsp. *dextranicum* strain B322 inoculated alone caused more root tissue to rot than any of the other bacteria and yeast inoculated individually (Table 2). The wet rot symptoms produced were similar to those seen in the field. The amount of rot caused by the other organisms was not significantly different from the noninoculated check. When the organisms were tested in combination with *L. mesenteroides* subsp. *dextranicum*, the combination with *G. asaii* was not different from *L. mesenteroides* subsp. *dextranicum* alone whereas the other combinations tested resulted in less rot, indicating that the bacteria or yeast inoculated with *L. mesenteroides* subsp. *dextranicum* were inhibiting or competing with it.

In the second set of experiments, involving a different selection of organisms, significant rot was associated with Idaho and California strains of *Pectobacterium betavasculorum* (Table 3). Inoculations with *G. asaii* and *Lactobacillus plantarum* alone resulted in lower amounts of rot compared with the *P. betavasculorum* strains. The *G. asaii* and *Pichia fermentans* combination resulted in significantly higher severity of rot than *G. asaii* alone. Both *P. fermentans* or *Enterobacter cancerogenus* caused very little rot after 48 hr when inoculated alone. In both sets of experiments, isolations from rotted tissue confirmed the inoculated bacteria and

**Table 1.** Bacteria and yeast isolated from sugar beet root rots in 2004 and 2005 from recently harvested roots throughout southern Idaho and southeastern Oregon

| Group[y] | Genera[z] | Number of isolates | | | Total (%) |
|---|---|---|---|---|---|
| | | 2004 | 2005 | Total | |
| A | *Lactobacillus*, *Leuconostoc* | 25 | 136 | 161 | 41 |
| B | *Acetobacter*, *Gluconobacter* | 53 | 61 | 114 | 29 |
| C | *Enterobacter*, *Escherichia*, *Pectobacterium*, *Serratia* | 23 | 43 | 66 | 17 |
| D | *Pichia* | 11 | 34 | 45 | 11 |
| E | *Candida* | 4 | 4 | 8 | 2 |
| F | *Pseudomonas* | 1 | 1 | 2 | 1 |
| Total | … | 117 | 279 | 396 | … |

[y] Strains were characterized and grouped based on morphology, gram stain reaction, and carbon source utilization profiles.

[z] Identification based on analysis of selected strains from each group for 16S rRNA sequence for bacteria and 28S rRNA sequence for yeast.

yeast were present and no contamination was evident.

**Petiole rot test.** Strains of *Pectobacterium betavasculorum* from Idaho and California could infect sugar beet line C40 through petioles, but none of the other bacteria and yeast isolates were pathogenic (Table 4). In test 1, the *P. betavasculorum* strain from Idaho, B20, was more aggressive than the California strain, Ecb-3 BS114, based on petiole rot ratings and fresh top weight; however, in the second test, they were not significantly different.

Similar results were obtained with cv. Crystal 217R, which is partially resistant to *P. betavasculorum* (Table 5). The Idaho strain, however, had higher petiole rot ratings after 2 weeks and caused reduced fresh weight compared with the *P. betavasculorum* strain from California. Isolations from diseased tissue of both sugar beet cultivars confirmed the presence of *P. betavasculorum*.

## DISCUSSION

Given the reported predominance of *P. betavasculorum* as the causal agent of bacterial rot in sugar beet, we were surprised that only a few isolates of this organism were found associated with rotted roots in our survey. Instead, a previously unreported bacterial complex was found to be involved. The primary bacterial rot organism was the lactic acid bacterium *Leuconostoc mesenteroides* subsp. *dextranicum*, which caused the most rot in the pathogenicity tests on root tissue and was among the group of strains most frequently isolated individually. In the petiole inoculation tests, however, *L. mesenteroides* subsp. *dextranicum* failed to cause disease. Other lactic acid, acetic acid, and enteric bacteria, along with yeast, frequently were found associated with the rotted roots but were less aggressive in rotting root tissues individually in pathogenicity tests.

*Leuconostoc* is a genus of gram-positive bacteria found in fermenting vegetables, dairy products, manure, wine, and sugar factories (4,7,8,13,18,22,35). Lactic acid bacteria (LAB) are inhibitory to other bacteria because of their tendency to reduce pH and produce organic acids, hydrogen peroxide, and bacteriocins during their growth (25). When only one bacterium was isolated from a sugar beet root, they usually were strains from the subgroup containing *L. mesenteroides* subsp. *dextranicum*. This observation fits with the literature that indicates that *L. mesenteroides* usually are among the first organisms to start fermentation. *Leuconostoc* spp. are important in the initial phase of the fermentation of wheat silages but they eventually are superseded by other bacteria (1). They are characteristically less acidophilic than other bacteria, because their growth is curtailed at a pH of 5.4 to 5.7 (1).

In addition to the fermentation of wheat silage, LAB also are mainly responsible for the fermentation of vegetables such as cabbage, carrot, and beet; however, the indigenous LAB flora varies as a function of the quality of the raw material, temperature, and harvesting conditions (2,9,12). For example, sauerkraut production typically relies on a sequential microbial process that involves heterofermentative and homofermentative LAB, generally involving *Leuconostoc* spp. for the first group and *Lactobacillus* and *Pediococcus* spp. for the second (12). *L. mesenteroides* predominates in the initial phase of the fermentation process of sauerkraut and many vegetables but dies off rapidly when the pH reaches 4.0 and lower (12,15). Our results on sugar beet agree with this scenario, because strains from subgroup A1 containing *L. mesenteroides* subsp. *dex-*

**Table 2.** Rot of sugar beet root slices by bacteria (*Enterobacter cancerogenus* B139, *Gluconobacter asaii* B225, and *Leuconostoc mesensteroides* subsp. *dextranicum* B322) and yeast (*Pichia fermentans* B154) isolated from harvested sugar beet collected from southern Idaho and southeastern Oregon

| | Diameter of rotted area (mm)[z] | |
|---|---|---|
| **Bacterial and yeast species** | **24 h** | **48 h** |
| *E. cancerogenus* | 3 de | 4 c |
| *G. asaii* | 5 cde | 12 bc |
| *L. mesenteroides* subsp. *dextranicum* | 15 a | 32 a |
| *P. fermentans* | 2 e | 3 c |
| *L. mesenteroides* + *E. cancerogenus* | 8 cd | 16 b |
| *L. mesenteroides* + *G. asaii* | 14 ab | 30 a |
| *L. mesenteroides* + *P. fermentans* | 8 cd | 12 bc |
| *L. mesenteroides* + *G. asaii* + *E. cancerogenus* | 6 cde | 9 bc |
| *L. mesenteroides* + *G. asaii* + *P. fermentans* | 10 bc | 16 b |
| Noninoculated check | 2 e | 2 c |
| *P* | <0.0001 | 0.0004 |
| LSD ($P \leq 0.05$) | 5 | 12 |

[z] Results presented are means of two experiments each with three replications. Means followed by the same letter are not significantly different based on Fisher's least significant difference (LSD).

**Fig. 1.** Wet rot symptoms associated with the bacterial rot found in Idaho sugar beet.

*tranicum* strains were the primary strains isolated individually, making it likely that strains from this subgroup initiated the rot process in sugar beet. Pathogenicity rot tests in this study showed that *L. mesenteroides* subsp. *dextranicum* caused the most rot in sugar beet roots but suffered when in competition with enteric bacteria and yeast. The *Lactobacillus plantarum* strains, acetic acid bacteria, and enterics isolated in this study likely entered root tissues after *Leuconostoc mesenteroides* subsp. *dextranicum*, because they were found more frequently in combination with and caused significantly less rot than *L. mesenteroides* subsp. *dextranicum* individually.

Rot tests indicated that *G. asaii* did not inhibit rot when combined with *L. mesenteroides* subsp. *dextranicum.* Thus, this strictly aerobic acetic acid bacterium (23) seems to not make compounds deleterious to *L. mesenteroides* subsp. *dextranicum* or compete for substrate. *G. asaii* is known to oxidize ethanol to acetic acid (23,27) and prefers sugar-enriched environments whereas a closely related acetic acid bacterium, *Acetobacter*, prefers alcohol-rich environments and was found less frequently.

Representative isolates from the enteric bacteria (group C strains) and yeast (group D and E strains) did reduce the amount of rot when combined with *L. mesenteroides* subsp. *dextranicum*, indicating that there was inhibition or they were in competition for substrate. Previous research indicates that, if yeast cell number is high or at least equal to the cells of lactobacilli, growth of lactobacilli is inhibited (28). The *Pichia* and *Candida* spp. isolated from the beet roots are known to be lactate-assimilating yeasts; however, they lack the ability to ferment all sugars because of their low alcohol tolerance (5,6,17,19,31). Thus, the organisms found in sugar beet rot likely were the same as those found in vegetable fermentations, and the succession of species occurring during the rotting process are likely to be similar as well.

Bacteria in the Enterobacteriaceae (group C) represented only a small minority of the strains isolated from rotted sugar beet roots. Among them were strains of *P. betavasculorum.* One strain from Idaho generally was more aggressive than the California strain in rotting root and petiole tissues. For this reason, seed companies that screen germplasm for resistance to *P. betavasculorum* have incorporated Idaho strains from this study into their screening programs.

The economic impact of bacterial root rot extends beyond yield losses in the field, because losses during storage and processing occur as well (3,26). Severe processing (root slicing problems and clogged filters) and quality problems occur because of rot and the formation of slimy microbial polysaccharides (7,26). Microorganisms isolated from sugar beet were collected from different parts of Finland to study their slime production (3,26). Among the isolates chosen for further study on the basis of their high viscosity or monosaccharide composition were *L. mesenteroides*, *Rah-*

**Table 3.** Rot of sugar beet root slices by bacteria (*Enterobacter cancerogenus* B86, *Gluconobacter asaii* B31, *Lactobacillus plantarum* B106, and *Pectobacterium betavasculorum* B20) and yeast (*Pichia fermentans* B9) isolated from harvested sugar beet collected from southern Idaho and southeastern Oregon[y]

| | Diameter of rotted area (mm)[z] | | |
|---|---|---|---|
| **Bacterial and yeast species** | **Exp 1, 24 h** | **Exp 2, 24 h** | **48 h** |
| *E. cancerogenous* | 6 fgh | 6 efg | 7 j |
| *G. asaii* | 13 bcde | 4 fg | 19 d |
| *L. plantarum* | 11 cdefg | 4 fg | 15 f |
| *Pectobacterium betavasculorum* Ecb-3 BS114 | 11 cdefg | 27 b | 23 c |
| *P. betavasculorum* B20 | 26 a | 14 cd | 26 b |
| *Pichia fermentans* | 7 efgh | 5 fg | 6 k |
| *E. cancerogenus* + *P. fermentans* | 8 defgh | 8 cde | 8 i |
| *G. asaii* + *E. cancerogenus* | 10 cdefg | 7 defg | 8 i |
| *G. asaii* + *L. plantarum* | 13 bcde | 7 defg | 16 e |
| *G. asaii* + *P. fermentans* | 14 bcd | 23 b | 26 b |
| *L. plantarum* + *E. cancerogenus* | 11 cdefg | 5 fg | 11 h |
| *L. plantarum* + *P. fermentans* | 15 bc | 10 def | 12 g |
| *Pectobacterium betavasculorum* B20 + *E. cancerogenus* | 12 cdef | 15 c | 19 d |
| *P. betavasculorum* B20 + *G. asaii* | 5 gh | 10 cdef | 8 i |
| *P. betavasculorum* B20 + *L. plantarum* | 24 a | 13 cde | 19 d |
| *P. betavasculorum* B20 + *Pichia fermentans* | 19 b | 52 a | 40 a |
| Noninoculated check | 2 h | 2 g | 2 l |
| *P* | <0.0001 | <0.0001 | <0.0001 |
| LSD ($P \leq 0.05$) | 7 | 8 | 1 |

[y] Strain Ecb-3 BS114 of *Pectobacterium betavasculorum* from California was included in the study for comparison. Results are means of two experiments, each with three replications. Means separation was performed on $\log_{10}$-transformed data using Fisher's least significant difference (LSD; $P = 0.05$) but nontransformed means are presented.

[z] Means followed by the same letter are not significantly different based on Fisher's LSD ($P = 0.05$). The data for the two experiments at 24 h could not be pooled due to variance heterogeneity.

**Table 4.** Results of inoculation of petioles of sugar beet cv. C40 (susceptible to *Pectobacterium betavasculorum*) with bacteria (*Enterobacter amnigenus* B92, *E. cancerogenus* B86, *Gluconobacter asaii* B31, *Lactobacillus plantarum* B106, *Leuconostoc mesenteroides* subsp. *dextranicum* B322, *P. betavasculorum* B20, and *Pseudomonas fluorescens* B82) and yeast (*Candida oleophila* B14, *Pichia fermentans* B9, and *P. membranifaciens* B8) isolated from harvested sugar beet roots collected from southern Idaho and southeastern Oregon[y]

| | Test 1[z] | | | Test 2[z] | | |
|---|---|---|---|---|---|---|
| **Bacterial and yeast species** | **DR, 1 week** | **DR, 2 weeks** | **FW (g)** | **DR, 1 week** | **DR, 2 weeks** | **FW (g)** |
| *C. oleophila* | 0 d | 1 c | 31 a | 1 b | 1 b | 19 d |
| *E. amnigenus* | 0 d | 1 c | 22 c | 1 b | 1 b | 20 cd |
| *E. cancerogenus* | 0 d | 0 d | 22 c | 1 b | 0 c | 20 cd |
| *G. asaii* | 0 d | 1 c | 25 bc | 1 b | 0 c | 20 cd |
| *Lactobacillus plantarum* | 0 d | 0 d | 29 ab | 1 b | 1 b | 20 cd |
| *Leuconostoc mesenteroides* subsp. *dextranicum* | 0 d | 0 d | 28 ab | 1 b | 1 b | 26 a |
| *Pectobacterium betavasculorum* Ecb-3 BS114 | 4 b | 5 b | 12 d | 4 a | 9 a | 3 e |
| *P. betavasculorum* B20 | 5 a | 8 a | 6 e | 4 a | 9 a | 4 e |
| *Pichia fermentans* | 1 c | 1 c | 23 c | 1 b | 0 c | 23 b |
| *P. membranifaciens* | 0 d | 1 c | 29 ab | 1 b | 0 c | 20 cd |
| *Pseudomonas fluorescens* | 0 d | 0 d | 31 a | 1 b | 1 b | 22 bc |
| Noninoculated check | 0 d | 0 d | 22 c | 0 c | 0 c | 20 cd |
| *P* | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| LSD ($P \leq 0.05$) | 1 | 1 | 5 | 1 | 1 | 3 |

[y] Strain Ecb-3 BS114 of *P. betavasculorum* from California was included in the study for comparison. Means followed by the same letter are not significantly different based on Fisher's least significant difference (LSD; $P = 0.05$).

[z] Disease ratings (DR) were conducted on a scale from 0 to 9, where 0 = healthy plant and 9 = dead plant. FW = fresh weight.

**Table 5.** Results of inoculation of petioles of sugar beet cv. Crystal 217R (partially resistant to *Pectobacterium betavasculorum*) with bacteria (*Enterobacter amnigenus* B92, *E. cancerogenus* B86, *Gluconobacter asaii* B31, *Lactobacillus plantarum* B106, *Leuconostoc mesenteroides* subsp. *dextranicum* B322, *P. betavasculorum* B20, and *Pseudomonas fluorescens* B82, and) and yeast (*Candida oleophila* B14, *Pichia fermentans* B9, and *P. membranifaciens* B8) isolated from harvested sugar beet roots collected from southern Idaho and southeastern Oregon[y]

| Bacterial and yeast species | DR, 1 week[z] | | DR, 2 weeks | FW (g) |
|---|---|---|---|---|
| | Test 1 | Test 2 | | |
| *C. oleophila* | 0 c | 0 c | 0 d | 26 b |
| *E. amnigenus* | 0 c | 1 b | 1 c | 23 e |
| *E. cancerogenus* | 0 c | 1 b | 1 c | 24 d |
| *G. asaii* | 0 c | 1 b | 1 c | 24 d |
| *Lactobacillus plantarum* | 0 c | 1 b | 0 d | 27 a |
| *Leuconostoc mesenteroides* subsp. *dextranicum* | 0 c | 0 c | 0 d | 24 d |
| *Pectobacterium betavasculorum* Ecb-3 BS114 | 3 b | 4 a | 5 b | 13 h |
| *P. betavasculorum* B20 | 5 a | 4 a | 7 a | 8 i |
| *Pichia fermentans* | 0 c | 1 b | 0 d | 22 f |
| *P. membranifaciens* | 0 c | 1 b | 0 d | 27 a |
| *Pseudomonas fluorescens* | 0 c | 1 b | 1 c | 25 c |
| Noninoculated check | 0 c | 1 b | 0 d | 21 g |
| *P* | <0.0001 | <0.0001 | <0.0001 | <0.0001 |
| LSD ($P \leq 0.05$) | 1 | 1 | 1 | 1 |

[y] Strain Ecb-3 BS114 of *P. betavasculorum* from California was included in the study for comparison. Means followed by the same letter are not significantly different based on Fisher's least significant difference (LSD; $P = 0.05$). The data for the two experiments at 1 week could not be pooled due to variance heterogeneity. The fresh weight (FW) analysis was conducted on $\log_{10}$-transformed data but the untransformed means are presented.

[z] Disease ratings (DR) were conducted on a scale from 0 to 9, where 0 = healthy plant and 9 = dead plant.

*nella aquatilis*, and *E. amnigenus* (26). Of the microbes isolated from both sugar beet surfaces and interiors, 28% produced extracellular slimes (35% of these isolates were heteropolymer producers) (26). This result differs from data published by other authors, which suggest that 95% of polysaccharides produced by sugar beet spoilage organisms are homopolysaccharides—dextran or levan (26).

Sugar factories try to avoid the problems caused by microbial polymers by discarding spoiled beet roots (26). In addition, commercially available dextranase and levanase have been used to aid with filtration problems (26). However, according to the results obtained in the Finland study, sugar beet spoilage microbes can form many different types of heteropolysaccharides, which explains why the processing problems are not systematically avoided by the use of available degrading enzymes (26). The rot complex in Idaho contains some of the same bacteria as were found in the Finnish study; therefore, beet roots with this bacterial rot likely will lead to filtration problems in sugar factories.

The sugar beet piles that contained higher levels of this wet root rot were concentrated in growing areas that also contained fungal root rot problems (*data not shown*). However, fungal isolations revealed that not all roots associated with this wet rot were associated with fungi and, when fungi were isolated, the species varied (*data not shown*). Also, bacteria inoculated alone into root slices were able to produce wet rot symptoms similar to those seen in the field. In the field, when bacteria are initiating rot, the symptoms may mimic those of fungal infections because they likely closely follow fungal infections at times. Isolations suggest that cavities or cracks in the crown area may allow a direct entry point for bacteria and yeast. Additional research will be required to determine whether bacteria and yeast gain entry to the roots on their own, by invading areas with growth cracks or cavities in the crown area or invading areas damaged by fungal rots or pests. In tomato, *L. mesenteroides* subsp. *mesenteroides* previously was established as a primary postharvest pathogen but the mode of pathogenicity was not determined (8). Indigenous LAB undoubtedly are present on plant surfaces and in the soil (2,8,9,12); however, additional investigations will be required to determine whether flies may be vectoring the bacteria directly from confined animal feeding operations and if manure applications may be contributing to changes in bacterial populations in the soil. As crop rotations and agronomic practices continue to evolve in this growing area, control options for this wet root rot will be needed. To aid in the development of control options, the primary source of the bacteria and yeast should be investigated along with research into sustainable cropping practices.


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