Ecology and Epidemiology

Sugar Beet As a Symptomless Host for *Corynebacterium sepedonicum*

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ABSTRACT

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Corynebacterium sepedonicum (syn. *Clavibacter michiganense* subsp. *sepedonicum*) was recovered from within the roots of symptomless sugar beet plants that had grown in the field and from roots grown in soil that was artificially infested with *C. sepedonicum*. The sugar beet strains were identical to potato strains based on biochemical, pathological, and serological tests. Sugar beet strains caused ring rot symptoms of interveinal chlorosis and wilt of potato, eggplant, and tomato. Sugar beet was established as a symptomless natural host for *C. sepedonicum*.

Additional key words: bacterial diseases, bacterial ring rot, endophytes, latent infections.

Sugar beets contain a wide array of bacteria that not only survive in roots but also multiply after roots are harvested and stored (1,7). Research has been under way to determine whether these endophytes might be contributing to the loss of sucrose in the stored root. During the course of these investigations, 34 bacterial species from eight genera were isolated and assayed for their ability to hydrolyze sucrose. A coryneform bacterium that had the second highest capacity to hydrolyze sucrose was isolated from a greenhouse-grown sugar beet seedling. The isolate was identified as *Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt. & Burkh. (syn. *Clavibacter michiganense* subsp. *sepedonicum* (Spieck. & Kotth.) Davis et al). This finding was considered

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significant because of the implications it could have regarding the epidemiology of ring rot of potato.

C. sepedonicum causes ring rot of potato by colonizing the xylem vessels and causing wilt. Control of this disease is based primarily on the use of disease-free or certified ring-rot-free seed potatoes. All states in the United States that certify seed potatoes have a zero tolerance for ring rot, and any detected ring rot causes the rejection of a field or seed lot for certification. North Dakota State Seed Department records showed that nearly 50% of all ring rot cases in North Dakota's seed industry had no traceable inoculum source (N. C. Gudmestad, *unpublished*), suggesting that there are unknown survival mechanisms for *C. sepedonicum*.

In the study reported here, experiments were performed to demonstrate that *C. sepedonicum* can infect sugar beet roots and survive without causing symptoms. Implications for potato culture are discussed.

MATERIALS AND METHODS

Original isolation and pathogenicity. Untreated sugar beet (*Beta vulgaris* L.) seeds were planted in the greenhouse in nonsterile sandy loam. Twenty seedlings were harvested after 6 wk, and the leaves and petioles were removed. The hypocotyls and roots were surface disinfested by washing in detergent, submerging for 30 sec in 0.1% sodium hypochlorite (NaOCl), rinsing in sterile distilled water (SDW), submerging in 95% ethanol, and flaming off the ethanol. The root and hypocotyl tissue was assayed for bacteria in a laminar flow hood under aseptic conditions. The tissue was crushed and minced in a small amount of sterile 0.02 M potassium phosphate buffer (KPB) at pH 7.2. Samples of the homogenate were passed through 10-fold dilutions of KPB and plated on nutrient broth yeast extract (NYS) agar containing the following ingredients per liter: 8 g of nutrient broth (Difco), 2 g of yeast extract (Difco), 0.5 g of K_2HPO_4, 0.5 g of sucrose, 15 g of agar, and 1 ml of 1 M $MgSO_4 \cdot 7H_2O$ added after autoclaving. Isolated colonies were subcultured and incubated for 7–10 days at 23–25 C. Standard biochemical tests for the identification of plant-pathogenic corynebacteria were used to identify this original isolate (14). Confirmation of the identification was based on pathogenicity as suggested by Vidaver (16).

Inoculum of the *C. sepedonicum* was prepared for a pathogenicity test as recommended (14). The bacteria were grown at 21–23 C for 18 hr in a 125-ml Ehrlenmeyer flask containing 50 ml of NYS broth in rotary shake culture (100 rpm) at room temperature. One milliliter was then transferred to three flasks each containing 50 ml of NYS broth and grown for 6 hr. The bacteria were collected from the three flasks by centrifugation at 12,000 *g* for 15 min and the pellet was suspended in 10 ml of one-half-strength NYS broth and adjusted to 10^6 colony-forming units (cfu) per milliliter from a standard curve that was made at 600 nm. Two tomato plants (*Lycopersicon esculentum* L. 'Rutgers') were inoculated at the two- to three-leaf stage with the sugar beet or a potato (*Solanum tuberosum* L.) strain of *C. sepedonicum*. The inoculation was done by inserting disposable 27-gauge syringe needles laden with 0.1 ml of inoculum into stems of tomatoes at two locations, one near the base and one in the axil of a leaf. The needles were left in place until the inoculum had absorbed into the stems.

Hemispheres of potato tuber tissue (potato balls), each with a single sprout about 7 mm long, were excised from tubers using a domestic melon ball scoop. Two potato balls were prepared from each of potato cultivars Norchip and Red Norland. Inoculum was prepared by growing the bacterium for 48 hr in NYS broth. The bacteria were collected by centrifugation, resuspended in half-strength NYS broth, and adjusted to 10^6 cfu/ml. A disposable 27-gauge syringe needle containing 0.1 ml of the inoculum was inserted into the base of the developing sprout. Two tomato plants and two each of the potato cultivars were not inoculated to serve as controls. Plants in all experiments were grown, inoculated, and incubated in a glasshouse at 18–26 C with supplemental light from high-pressure sodium lamps for a light period of 12–14 hr.

Field survey for naturally infected sugar beets. A survey of field-grown sugar beet roots was performed to determine whether the original isolation of *C. sepedonicum* from a sugar beet seedling grown in the greenhouse was a spurious event. The methods used here to extract and concentrate the bacterium from sugar beet were similar to those used by Miller (12) with potatoes. The enrichment of the bacterium in eggplant for subsequent isolation was similar to the bioassay used by Zielke and Naumann (19). Sugar beet roots were collected in July 1984 from 10 fields selected for their proximity to potato fields. The roots were stored in perforated plastic bags at 4–6 C (ideal storage conditions) for 2–7 mo while awaiting processing. Extracts were prepared from each of 128 roots by first washing each root in detergent then removing approximately 100 g of tissue. The epidermis was removed and the tissue was trimmed to 100 g and cut into pieces measuring about $1 \times 2 \times 5$ cm to facilitate homogenation in the Waring Blendor. Each cube was surface disinfested by being placed in 0.5% NaOCl for 2 min, followed by a rinse in SDW, and then into 95% ethanol followed by burning off of the ethanol. This disinfestation treatment was deemed effective because our tests indicated that *C. sepedonicum* did not survive the NaOCl exposure and flame-off of ethanol on root tissue that had been dipped in a suspension of the bacterium containing 10^4 cfu/ml.

The disinfested root pieces were then homogenized in a sterile, stainless-steel Waring Blendor containing 100 ml of sterile 20 mM KPB at pH 7.2. The homogenate was centrifuged twice in tubes that had been washed and then soaked in phosphate detergent overnight, followed by submersion in 95% ethanol for 30 min. The first centrifugation was for 15 min at 500 *g*, after which the supernatant was removed and centrifuged for 30 min at 12,000 *g*. Samples (20 μl) of pellets from the centrifugation were applied to wells of toxoplasmosis slides and examined for *C. sepedonicum* using indirect immunofluorescent antibody staining (IFAS). The IFAS was done with monoclonal antibodies formed against cell wall components of *C. sepedonicum* (3) and supplied to us by S. H. De Boer. Centrifuged pellets that were positive for *C. sepedonicum* according to the IFAS test and had at least 20 fluorescing bacterial cells per slide well were diluted with sterile 20 mM KPB as necessary to allow flow through the 27-gauge needle.

Inoculation of eggplants (*Solanum melongena* L 'Black Beauty') in the two- to three-leaf stage was done by inserting into the base of the stem one needle laden with 0.1 ml of inoculum and one needle into the stem at a leaf axil. The needles were removed after the inoculum had disappeared. Control eggplants were inoculated with sterile 20 mM KPB. The inoculated plants were harvested 4–6 wk after inoculation to reisolate the bacterium. The petioles were removed and the stem was surface disinfested by flaming off ethanol. This technique was effective because the bacterium was not detected in dilution plates of homogenates of stems that had been surface inoculated with 10^6–10^8 cfu of *C. sepedonicum* per milliliter. The stems were homogenized in 30 ml of sterile KPB in a sterile tissue homogenizer flask. The homogenate was centrifuged as above and the pellet was examined with IFAS microscopy. The pellets that were IFAS positive were diluted and plated on NYS agar. Colonies of *C. sepedonicum* that developed were identified initially by colony characteristics, Gram stain, and cell morphology. Confirmation of identity was based on a positive reaction with IFAS and subsequent pathogenicity testing on eggplants. Pure IFAS-positive cultures were removed from agar culture plates, suspended in sterile 20 mM KPB, adjusted to 10^6 cfu/ml, and inoculated into eggplants as before. Control plants were inoculated with sterile KPB.

Pathogenicity of *C. sepedonicum* strains from field-grown sugar beet. Six strains of *C. sepedonicum* that were recovered from field-grown sugar beets were tested for pathogenicity in potato and eggplant. Five strains of known *C. sepedonicum* from potato that were randomly selected from our collection of cultures from potato-growing regions of North America were used as positive controls. Designated as NDCS-N1, NDCS-OFF, NDCS-S-1, NDCS-AS-1, and SDB-CS3R, they are listed in Table 1 along with the six sugar beet strains. Inocula were prepared by growing *C. sepedonicum* in NYS broth for 48 hr. The bacteria were collected by centrifugation and resuspended in half-strength NYS broth and

adjusted to 10^6 cfu/ml. Sterile half-strength broth was inoculated as a control. Three eggplants and two potato plants were inoculated with each strain. After inoculation and symptom development, extracts of stem tissues were diluted and plated on NYS agar to recover *C. sepedonicum* at the completion of the experiment. The identification of apparent *C. sepedonicum* colonies was confirmed by Gram stain, colony characteristics, and the IFAS test using monoclonal antibodies.

Artificial infection of sugar beets by *C. sepedonicum*. Sugar beet seeds were planted in 26-cm clay pots containing pasteurized sandy loam soil that had been amended with homogenized tubers showing ring rot symptoms. Three of the pots each contained 1% (v/v) homogenized tubers and three contained 5% (v/v); three pots were not amended. When the sugar beet roots were 200–500 g in size, they were harvested and 100-g root samples were assayed for *C. sepedonicum* as described above. The homogenate was gravity filtered through Miracloth and then through Whatman 1 filter paper, and the filtrate was centrifuged at 4,500 *g* for 1 hr. The filtrate was discarded and the pellet was resuspended in a small amount of distilled water and examined for *C. sepedonicum* using IFAS microscopy (3). Positive IFAS pellets were diluted and plated on NYS agar. Slow-growing bacterial colonies that conformed to *C. sepedonicum* based on Gram reaction and cell morphology were increased and inoculated into tomato seedlings as described above.

Serological testing. One culture each of *C. michiganense*, *C. insidiosum* (McCullock) Jensen, *C. nebraskense* Shuster et al, and *C. betae* Keyworth et al (Table 1), along with six field-grown sugar beet strains and one greenhouse sugar beet strain of *C. sepedonicum*, were tested serologically using antisera produced against potato strains of the bacterium. Rabbit polyclonal *C. sepedonicum* antiserum to a potato strain was used in immunodiffusion tests, and monoclonal antiserum 9A1 was used in immunofluorescence. The monoclonal antiserum used was the equivalent antiserum from hybridoma cell line 5. This monoclonal line did not cross-react with several other unidentified endophytic bacterial species of potato and other *Corynebacterium* spp. (3). It has been shown not to cross-react with bacterial endophytes of potato or other plant pathogenic corynebacteria (3; N. C. Gudmestad, *unpublished data*). Immunofluorescence was conducted as described by De Boer and Weiczorek (3) using 48-hr cultures grown on NYS agar. Three 10-fold serial dilutions of the strains were used and examined using an Olympus BH-2 microscope with an ultraviolet light source.

Immunodiffusion tests were conducted as described by De Boer (2) using culture fluid from pure cultures grown in NYS broth for about 2 wk. Culture fluids from a potato strain (NDCS-AS-1) of *C. sepedonicum* and a strain obtained from De Boer (2) were used as positive controls. Tissue homogenate from healthy seed tubers was used as a negative control.

Physiological and biochemical tests. Six field-grown sugar beet strains of *C. sepedonicum* were compared with regard to color and physiological and biochemical characteristics with 3 cultures of *C. sepedonicum* from the American Type Culture Collection (ATCC), 23 potato strains of the bacterium from various geographic origins, and 4 other species of *Corynebacterium* from ATCC (Table 1). Colony color was rated on a white background according to the Methuen Handbook of Color (10). Motility was determined in stab-inoculated, soft nutrient agar. The utilization of urease, rhamnose, trehalose, mannose, melibiose, cellobiose, xylose, maltose, glycerol, and sucrose was determined after 2 wk of incubation at room temperature with the Minitek system (BBL Microbiology Systems, Cockeysville, MD, 21030) and of lactose, mannitol, and melezitose according to Dye and Kemp (4). The production of H_2S, gelatinase, and caseinase was according to Dye and Kemp (4). Lipase production with Tween 60 or 80 was according to Sierra (15). Esculin hydrolysis was determined with Minitek after 2 wk of incubation at room temperature. Growth on a medium containing nalidixic acid, polymyxin B sulfate, and cycloheximide (CNS) was according to Gross and Vidaver (5). Growth on a tetrazolium medium was according to Kelman (8). Indigoidine production was indicated by the production of violet-blue color at the edge of colonies.

RESULTS

Original isolation and pathogenicity. A coryneform bacterium that was isolated from the hypocotyl tissue of a single symptomless sugar beet seedling from the greenhouse was identified as *C. sepedonicum*. It was a Gram-positive, slow-growing, nonmotile, pleomorphic rod that formed colorless to cream-colored colonies on NYS agar. The identity of this sugar beet strain of *C. sepedonicum* was confirmed when it caused ring rot symptoms on potato and tomato after stem inoculation. The symptoms were identical to those caused by potato strains of *C. sepedonicum*. Symptoms began on tomato 2 days earlier after inoculation with the potato strain than with the sugar beet strain. Uninoculated potato and tomato plants remained healthy.

Field survey for naturally infected sugar beets. Of 128 sugar beet roots from 10 fields assayed for *C. sepedonicum*, 49 roots or 38% with representatives from each of the 10 fields were positive for *C. sepedonicum* when examined with IFAS microscopy (Table 2). The number of positive reacting bacteria ranged from 1–2 to 40–60 per slide well. Of these 49 samples, 29 with at least 20 bacteria per well were used to inoculate eggplants. One eggplant developed ring rot symptoms after inoculation with the 29 centrifuge pellets. The bacterium was isolated on dilution plates from the single symptomatic eggplant. The bacterium also was isolated from centrifuge pellets of stem extracts from five inoculated but symptomless eggplants. Identification was confirmed by Gram stain, colony morphology, and IFAS microscopy. Eggplants that were inoculated with sterile distilled water remained healthy and

TABLE 1. Species and strains of *Corynebacterium* used in this study

Species/ Strain	Isolated by	Obtained from	Origin
	Potato Strains of C. sepedonicum		
NDCS-N-1	N. Gudmestad	N. Gudmestad	North Dakota
NDCS-S-1	N. Gudmestad	N. Gudmestad	North Dakota
NDCS-AS-1	N. Gudmestad	N. Gudmestad	North Dakota
NDCS-OFF	N. Gudmestad	N. Gudmestad	North Dakota
INM	A. Bishop	S. Slack	Idaho
NDH-1B	N. Gudmestad	N. Gudmestad	Minnesota
CsMT	D. Gross	A. Vidaver	Montana
SDB-R1	S. H. De Boer	S. H. De Boer	British Columbia
SDB-R2	S. H. De Boer	S. H. De Boer	British Columbia
SDB-R3	S. H. De Boer	S. H. De Boer	British Columbia
SDB-R4	S. H. De Boer	S. H. De Boer	British Columbia
SDB-R5	S. H. De Boer	S. H. De Boer	British Columbia
SDB-CS3R	S. H. De Boer	S. H. De Boer	British Columbia
SDB-CS3NM	S. H. De Boer	S. H. De Boer	British Columbia
SDB-CS3M	S. H. De Boer	S. H. De Boer	British Columbia
NDCS-EA1	N. Gudmestad	N. Gudmestad	North Dakota
NDCS-COL-1B	N. Gudmestad	N. Gudmestad	Minnesota
CSSD-1	L. DeBuhr	N. Gudmestad	South Dakota
CSSS-43	S. Slack	S. Slack	Wisconsin
CSSS-20	S. Slack	S. Slack	Wisconsin
CSSS-44	S. Slack	S. Slack	Wisconsin
CSSS-14	S. Slack	S. Slack	Wisconsin
CSCA-1	Unknown	A. Vidaver	California
		Sugar Beet Strains	
NDCSSB-5	G. Nielsen	W. Bugbee	North Dakota
NDCSSB-57	G. Nielsen	W. Bugbee	North Dakota
NDCSSB-65	G. Nielsen	W. Bugbee	North Dakota
NDCSSB-100	G. Nielsen	W. Bugbee	North Dakota
NDCSSB-101	G. Nielsen	W. Bugbee	North Dakota
NDCSSB-109	G. Nielsen	W. Bugbee	North Dakota
	American Type Culture Collection (ATCC)		
C. sepedonicum	···	ATCC	9850
C. sepedonicum	···	ATCC	33113
C. sepedonicum	···	ATCC	33111
C. michiganense	···	ATCC	14456
C. betae	···	ATCC	13437
C. nebraskense	···	ATCC	27822
C. insidiosum	···	ATCC	10253

were negative for fluorescing bacteria when tested with IFAS. *C. sepedonicum* was not recovered in dilution platings of extracts from control plants. The five cultures that were isolated from the symptomless eggplants induced symptoms in eggplant after stem injection (Table 2). Therefore, pathogenic isolates of *C. sepedonicum* were recovered from 6 of 128 sugar beets (4.7% recovery) that were collected from 4 of the 10 fields that were sampled.

Pathogenicity of strains from field-grown sugar beet. Each of the six sugar beet strains and the five potato strains of *C. sepedonicum* caused ring rot symptoms on the two potato and three eggplants after inoculation. Symptoms of chlorosis, wilt (Fig. 1A), and unilateral leaf growth (Fig. 1B) on eggplant were typical ring rot symptoms. The control plants remained healthy. *C. sepedonicum* was reisolated from the affected plants and identified according to colony characteristics, Gram stain, and positive IFAS reactions with monoclonal antibodies. This experiment confirmed our conclusion from the first inoculation experiment that the sugar beet strains of *C. sepedonicum* caused symptoms on potato and eggplant and that these symptoms were identical to those caused by potato strains.

Artificial infection of sugar beet roots. When sugar beets were grown in pasteurized soil that was amended with homogenized ring rot tubers, a positive IFAS test was obtained from one of the three assayed roots of each amendment treatment. No positive IFAS was found in roots from the nonamended check. The *C. sepedonicum* that was isolated from the dilution plates caused wilt of tomato after stem inoculation.

Serological testing. Culture fluid from the greenhouse-grown and six field-grown sugar beet strains and a potato strain formed identical precipitin lines with antibodies produced against known pathogenic cultures of *C. sepedonicum* in double-diffusion tests. Precipitin lines did not form with culture fluids from *C. michiganense, C. insidiosum, C. nebraskense,* or *C. betae*. The seven sugar beet strains also reacted positively in IFAS with highly specific monoclonal antiserum that was prepared against a potato strain of *C. sepedonicum*. The phytopathogenic corynebacteria listed above did not react in the IFAS test.

Physiological and biochemical tests. The 6 sugar beet strains have physiological and biochemical characteristics that are identical with those of the 23 known potato strains and the 3 ATCC cultures of *C. sepedonicum* (Table 3). Four other species of *Corynebacterium* used in the test demonstrated characteristics that distinguished them from each other and from *C. sepedonicum*. All cultures tested were positive for esculin hydrolysis, catalase, acid from xylose, sucrose, and mannose and negative for caseinase; they were also nonmotile.

TABLE 2. Use of indirect immunofluorescent antibody staining (IFAS) to identify *Corynebacterium sepedonicum* recovered from field-grown sugar beets and prevalence of symptoms on eggplant after inoculation with IFAS-positive bacteria

Field[a]	Roots assayed (no.)	Roots (no.) IFAS-positive bacteria	Root extracts with IFAS-positive bacteria that induced symptoms in eggplants (no.)
APF	12	5	1
APF	16	7	
APF	10	4	
APF/PCP	14	4	
NAP	4	1	
APF	12	5	1
PCP	12	7	1
APF	12	4	
PCP	22	9	3
APF	14	3	
Total	128	49	6

[a]APF = sugar beets from field adjacent to potato field; PCP = sugar beets from field cropped previous year to potatoes; NAP = sugar beets from field not near any known potato field.

DISCUSSION

This report presents evidence that sugar beet can serve as a symptomless host in the greenhouse and in the field for the ring rot bacterium *C. sepedonicum*. This conclusion is supported by the recovery of the bacterium from surface-disinfested sugar beet roots that had grown in artificially infested soil and from sugar beet roots collected from four different fields. The sugar beet strain from the original sugar beet seedling reacted positively in double-diffusion and IFAS tests with antibodies produced against a potato isolate of the bacterium and caused typical ring rot wilt symptoms on potato and tomato. The six strains from field-grown sugar beets were identical in cultural characteristics and morphology to known strains of *C. sepedonicum* and reacted identically to potato strains in the physiological, biochemical, and serological tests. These strains caused wilt symptoms on potato and eggplant that were indistinguishable from those caused by known strains. The procedure we used to recover *C. sepedonicum* from field-grown sugar beets was similar to the bioassay used to detect latent ring rot infections and to recover low populations of this bacterium from potato tubers (12,18,19). Therefore, the long-held assumption that only potato is a natural host for *C. sepedonicum* (9,11) is not true.

Attempts were made to isolate *C. sepedonicum* from 190 roots that were collected from 19 randomly selected sugar beet fields. This trial failed to detect the bacterium when noncentrifuged root extracts were diluted and plated on nutrient agar (W. M. Bugbee, *unpublished data*). Recovery should not have been expected based on our subsequent experience with centrifuged extracts. The lack of recovery probably resulted from the low concentration of *C. sepedonicum* in comparison with other sugar beet bacterial endophytes in the crude root extract, together with the slow growth characteristics of the bacterium. The latter feature is a well-known problem that interferes with detection on culture plates because the

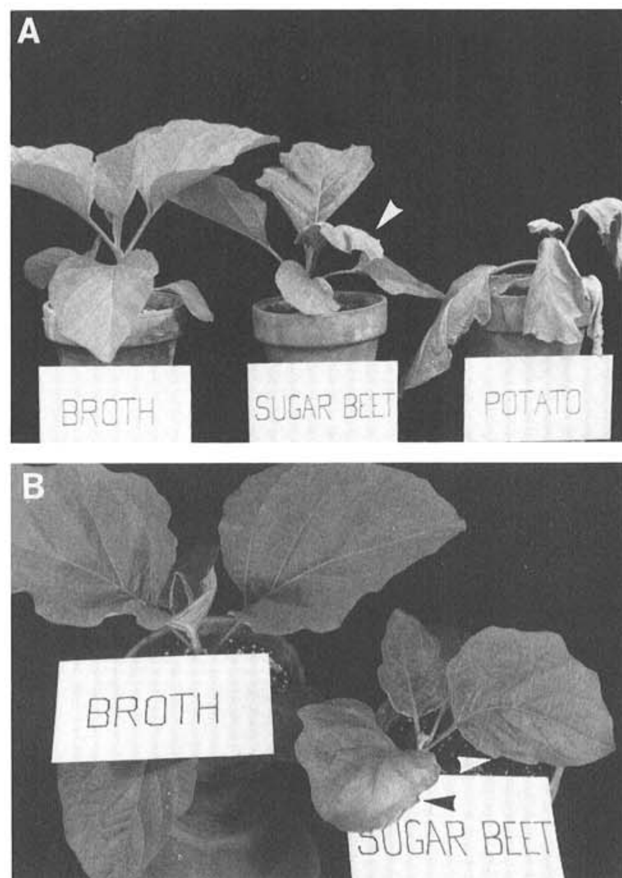

Fig. 1. Symptoms of **(A)** chlorosis, wilt (arrow), and **(B)** unilateral leaf growth (arrows) on eggplants that were inoculated with sugar beet or potato isolates of *Corynebacterium sepedonicum*. Controls were inoculated with sterile broth.

slow-growing *C. sepedonicum* is overgrown by other bacteria. Detection of sugar beet roots that contained *C. sepedonicum* was facilitated by concentrating the bacteria from root extracts by centrifugation and by labeling the target bacteria using the IFAS technique. Miller (12) and Zielke and Naumann (19) have concentrated *C. sepedonicum* in plant extract by centrifugation to increase the effectiveness of the extract as inoculum for inoculation into eggplant when attempting to detect latent infections of this bacterium in potato tubers. The use of the monoclonal antibody that has specificity for *C. sepedonicum* (3) aided the identification of the pellets that could be used for stem injection and subsequent confirmation of pathogenicity.

Eggplant and tomato have been used extensively in bioassays for ring rot diagnosis and in research of the disease (11,12,17–19). Ring rot symptoms on these plants will establish pathogenicity of Gram-positive isolates during diagnosis (11,16–18) and allow comparison of virulence of isolates (17) and detection of latent and mild infections in tubers (12,19). In the results reported herein, symptoms developed on one eggplant when eggplants were separately inoculated with 29 IFAS-positive bacterial pellets that were prepared from sugar beet roots. It has been shown that as few as 10 cells per milliliter will initiate symptoms when inoculated into stems of eggplant cultivar Black Beauty (19). Other research states that 2×10^3 was the minimum number of cells per milliliter required to initiate symptoms on Black Beauty and that 10^4 cfu/ml was necessary for symptom development if the inoculum was mixed with tuber tissue (17). The suppressive effect of tissue on inoculum may have partially accounted for the absence of symptoms in our experiments when eggplants were inoculated with centrifuged extracts from sugar beet roots. The presence of bacterial endophytes known to inhabit sugar beet roots (1,7) also could have contributed toward the inhibition of symptom development.

The absence of symptoms in our experiments prompted the attempt to isolate *C. sepedonicum* from the inoculated but symptomless eggplants. The bacterium was isolated from five symptomless eggplants, and symptoms did develop on all eggplants after reinoculation with 10^6 cfu/ml. Even though symptoms of chlorosis and wilt developed, they were mild and eggplants seldom died. Unilateral growth of leaves occurred as described and illustrated by Zielke and Naumann (18). In addition, potato plants infected with a sugar beet strain developed symptoms, but the wilt never progressed to the point where the entire plant wilted and eventually died. These results suggest that *C. sepedonicum* strains from sugar beet are less virulent than potato strains on all hosts that were tested. Subsequent virulence testing that compared sugar beet and potato strains has confirmed this hypothesis (6). Low virulence also may have accounted for the absence of symptoms when eggplants were inoculated with IFAS-positive bacterial pellets from sugar beet roots that had grown in ring rot field soil. Recent work has confirmed that eggplant cultivar Black Beauty is the most suitable host for a bioassay to detect *C. sepedonicum* and that low virulence can be expressed as a delay or absence of symptoms depending on the strain that is used (17).

Zielke and Naumann (19) have shown that the percentage of eggplants that develop symptoms increases if they are inoculated with bacteria freshly isolated from eggplants with symptoms. This enrichment procedure apparently accounted for our success in causing symptoms on eggplants after they were inoculated with strains that were recovered from symptomless eggplants.

There was no apparent association between potato fields and the prevalence of infected sugar beet roots. This lack of association is supported by earlier work where a *Corynebacterium* sp. was isolated from sugar beet roots from a field where potatoes had never been grown (7), leading to the speculation that the bacterium may be seedborne in sugar beet. This hypothesis is supported by the recovery of the bacterium in dilution plates from a 6-wk-old sugar beet seedling that had grown in nonsterile soil in the greenhouse. The probability is remote that a culturable level of this slow-growing bacterium could have developed in the seedling from a soil source within 6 wk; however, a culturable population might have developed if it was seedborne. The possibility that *C. sepedonicum* is seedborne in sugar beet is currently being investigated.

The recovery of potato strains of *C. sepedonicum* from sugar beets that were grown in soil artificially amended with ring-rot-infected potato tubers demonstrates another possible mechanism

TABLE 3. Biochemical and physiological comparison of 6 strains of *Corynebacterium sepedonicum* from sugar beet with 23 strains from potato, 3 strains from American Type Culture Collection (ATCC), and 4 other species of *Corynebacterium* from ATCC

	C. sepedonicum			*Corynebacterium*			
Test	Potato	Sugar beet	ATCC	*michiganense*	*betae*	*nebraskense*	*insidiosum*
Color	Pale yellow 3A3[a]	Pale yellow 3A3	Pale yellow 3A3	Wax yellow 3B5	Naples yellow 3B7	Golden yellow 5B7	Curry yellow 4C8
Growth on[b]							
CNS	–[c]	–	–	+	+	+	+
TTC	–	–	–	+	+	–	+
Utilization							
Acetate	+	+	+	+	+	+	+
Fumarate	+	+	+	+	–	+	+
H_2S production	v	–	v	+	+	+	+
Indigoidine production	–	–	–	–	–	–	+
Gelatinase	–	–	–	+	–	–	–
Lipase							
Tween 80	–	–	–	–	+	–	–
Acid production from							
Lactose	–	–	–	+	+	+	+
Maltose	v	v	v	+	+	+	+
Mannitol	+	+	+	+	+	–	+
Glycerol	v	v	v	+	+	+	+
Melezitose	–	–	–	–	+	–	–
Trehalose	–	–	–	+	+	w	w
Rhamnose	–	–	–	–	+	–	–
Melibiose	–	–	–	+	+	w	w
Cellobiose	v	v	v	+	+	+	+

[a]Color code from Methuen Handbook of Color (10).
[b]CNS = growth medium containing cycloheximide, nalidixic acid, and polymixin B sulfate (5); TTC = growth medium containing tetrazolium chloride (8).
[c]– = negative, + = positive, v = variable, and w = weak.

by which sugar beet may become infected with the bacterium. The evidence that is presented here would strongly suggest that sugar beets aid in the survival of the bacterium. Nelson (13) has shown the ability of this bacterium to persist in infected potato stems in the soil. In North Dakota, sugar beet and potato frequently follow each other in the crop rotation sequence, thus providing either crop the opportunity to become infected with the bacterium that resides in the crop residue. Current ring rot control recommendations suggest that potatoes not be grown in fields of a previously infected crop (11). If future field research shows that sugar beet debris is an inoculum source for potato ring rot, this recommendation may need to be expanded in our production area to include fields previously cropped to sugar beets.

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