A Selective Medium for Isolation of Corynebacterium nebraskense From Soil and Plant Parts

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ABSTRACT

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A selective agar medium (CNS) was developed for isolating *Corynebacterium nebraskense*, the causal agent of Goss's wilt, from corn tissue and soil. Selectivity depended on a combination of nalidixic acid, polymyxin B sulfate, lithium chloride, cycloheximide, and Bravo 6F®. Recovery of *C. nebraskense* on CNS from pure culture was equal to the recovery on a nutrient broth-yeast extract medium (NBY). CNS medium was quantitatively and qualitatively superior to other selective media developed for *Corynebacterium* spp. From soil, *C. nebraskense* generally could be isolated as low as 10^3 colony-forming units per gram of soil. More than 99% of the total recoverable soil bacteria on NBY could not grow on CNS. Some coryneform and coccoid soil bacteria grew on CNS. In addition to *C. nebraskense*, high efficiencies of plating on CNS were noted for some phytopathogenic *Corynebacterium* spp.; however, *C. sepedonicum* and *C. insidiosum* did not grow. Soil and airborne fungi were unable to grow on CNS.

Additional key words: Goss's wilt, *Zea mays*.

Corynebacterium nebraskense, causal agent of Goss's bacterial wilt and blight of corn, is a persistent and economically important corn pathogen in Nebraska (5,26). There is evidence that the primary source of inoculum is from overwintering corn residue (18). However, when *C. nebraskense* was incorporated in soil as infested corn residue or as a cell suspension, survival generally was about 3 wk under moist conditions, as determined by inoculating plants with soil extracts (18). Populations of *C. nebraskense* have not been monitored quantitatively. A selective medium would be useful for quantitative recovery and monitoring of the pathogen in the field.

The rationale for developing selective media for Gram-positive bacteria often takes advantage of differential growth of bacteria in the presence of antibiotics (eg, polymyxin B) (19) or slow-oxidizing agents (eg, potassium dichromate, sodium azide) (12,14,15). Cationic or anionic agents and various dyes and salts generally are not useful for the selection of Gram-positive bacteria (1,7,8,13).

Few media have been developed for isolating pathogenic corynebacteria. Most use slow-oxidizing agents as the basic selection factor (4,16,20). *Corynebacterium* spp. pathogenic to animals are routinely isolated on media containing 0.04% potassium tellurite (2). One medium useful for phytopathogenic corynebacteria is that of Snieszko and Bonde (20). They found that potassium dichromate added to medium "4-m-1" at a final concentration of 50 μg/ml inhibited Gram-negative bacteria, including *Erwinia carotovora*, and facilitated the isolation of *C. sepedonicum* from tubers in advanced stages of decomposition. Kado and Heskett (12) developed selective medium D2 for all species of *Corynebacterium*, including phytopathogens. Selectivity probably was caused by alterations of surface components and cell membrances by lithium chloride and polymyxin B, respectively.

Recently, Schneider and Grogan (R. G. Grogan, *personal communication*) developed a medium for isolating *C. michiganense* from tomato seed and vegetative tissue. Among other ingredients, they found that the fungicide Bravo 6F® (tetrachloroisopthalonitrile) selectively suppressed fungi and some bacteria.

We report here a medium for isolating *C. nebraskense* from diseased corn, corn residue, and soil; it is selective for *C. nebraskense* without loss of viability and is simple to prepare and use. The medium also was compared with other selective media developed for Gram-positive bacteria with respect to quantitative and qualitative isolation of *C. nebraskense*.

MATERIALS AND METHODS

Bacterial strains.—*Corynebacterium* spp. and strain designations are listed in Table 1. Strain CN74-1 of *C. nebraskense* was used to evaluate selective media. This strain was originally isolated in 1974 from field corn (*Zea mays*) near Grant, Nebraska, and is highly virulent on sweet corn (C. V. Golden Cross Bantam). Other strains of *C. nebraskense* used were CN76-1, 298, and CN18-6, which had high, moderate, and low virulence, respectively, for sweet corn and also were distinguishable by bacteriocin production or sensitivity (10) or plasmid DNA content (D. C. Gross and A. K. Vidaver, *unpublished*).

Media and additives.—The medium routinely used for growing cells was a nutrient broth yeast (NBY) extract medium (23) that contained, per liter: 8 g of Bacto-nutrient broth (Difco Laboratories, Detroit, MI 48232), 2 g of Bacto-yeast extract (Difco), 2 g of K_2HPO_4, 0.5 g of KH_2PO_4, 0.25 g of $MgSO_4 \cdot 7H_2O$, 5 g of glucose, and 15 g of Bacto-agar (Difco). The glucose and magnesium sulfate were added after sterilization from sterile stock solutions. The medium had a pH of 7.2 after autoclaving.

The *C. nebraskense* selective (CNS) medium was prepared by adding the following to 1 L of sterile NBY cooled to 50 C: 25 mg of nalidixic acid (freshly solubilized in 0.1 M NaOH, 10 mg/ml); 32 mg of polymyxin B sulfate (8,000 USP units/mg, 10 mg/ml fresh stock; Sigma Chemical Co., St. Louis, MO 63178); 40 mg of cycloheximide (10 mg/ml stock); 10 g of LiCl; and 0.0625 ml of Bravo 6F® (Diamond Shamrock Chemical Co., Cleveland, OH 44114) diluted 1:50 in sterile water from the original stock solution (53% tetrachloroisopthalonitrile). The CNS medium had a pH of 6.9 after autoclaving.

The yeast-extract-dextrose selective (YDS) medium (R. W. Schneider and R. G. Grogan, *unpublished*; R. G. Grogan, *personal*

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communication) contained, per liter: 29 g of dextrose, 10 g of Bacto-yeast extract, 5 g of LiCl, 20 ml of glycerol, and 20 g of Bacto-agar. After sterilizing and cooling YDS to 50 C, the following were added, per liter: 6 g of K_2HPO_4 (autoclaved separately in 10 ml of deionized water); 50 mg of cycloheximide; 25 mg of polymyxin B sulfate (8,000 USP units/ml, 10 mg/ml of fresh stock); 80 mg of potassium dichromate; and 20 mg of Bravo 6F (2 ml of a suspension of Bravo 6F, previously diluted 0.185 ml with 9.815 sterile deionized water, was added). The medium had a pH of 7.45 after autoclaving.

Medium D2 was prepared according to Kado and Heskett (12). Yeast-extract-dextrose-calcium carbonate agar (YDC) was prepared by Dowson's method (6) except that the glucose was autoclaved separately and ultrafine precipitated calcium carbonate (Mallinkrodt Chemical Works) was used.

The medium used for production of bacteriocins by *C. nebraskense* (MBAL) (10) was the modified Burkholder's agar of Nelson and Semeniuk (17) without peptone.

Additions to CNS test media generally were incorporated at levels close to the minimal inhibitory concentration for *C. nebraskense* (Table 2). These included: Protector-3L® (2-[thiocyanomethylothio] benzothiazole; Agway, Inc., Box 1333, Syracuse, NY 13201); sodium azide; potassium dichromate; potassium tellurite (Difco); and the nonionic surfactants Bio 88® (Alkyl polyethoxy ethanol and free fatty acids; Kalo Laboratories, Inc., Kansas City, MO 64137); polyethylene glycol 600 MO (Armak Co., McCook, IL 60525); Trydet TFA-11® (Polyoxyethylene tallate ester; Emery Industries, Inc., Los Angeles, CA 90054); and Plyac® (A-C polyethylene and octyl phenoxypolyethoxy ethanol; Hopkins Agriculture Chem. Co., Madison, WI 53701). Several antibiotics (erythromycin, tetracycline, dihydrostreptomycin, kanamycin, gentamicin, neomycin, chloramphenicol, carbenicillin, and ampicillin) were tested at various concentrations for usefulness as selective agents.

Media evaluation procedures.—Strain CN74-1 of *C. nebraskense* was grown in NBY broth for 12 hr at 25 C in shake culture and adjusted turbidimetrically to 1 to 3×10^8 colony-forming units (CFU) per milliliter. Serial dilutions were made in 12.5 mM of potassium phosphate buffer, pH 7.2. Soil samples included a sandy loam, pH 7.6, from Fremont, Nebraska, and a clay loam, pH 7.5, from West Point, Nebraska. Nine ml of 12.5 mM potassium phosphate buffer was added to a 1-g soil sample and then mixed for 15 min on a rotary shaker (250 rpm). To this, 1 ml of the appropriate dilution of *C. nebraskense* was added, the mixture was serially diluted, and then 0.1 ml was distributed with a glass spreader onto test media. Depending on the experiment, three to six replicate plates were prepared for each dilution and treatment. After 4 to 8 days of incubation at 25 ± 3 C, test media were evaluated for recovery of *C. nebraskense* and for number and types of soil bacteria or fungi. The bacterium was incorporated in sterile

TABLE 1. Growth of phytopathogenic corynebacteria on *Corynebacterium nebraskense* selective medium

Corynebacterium spp.	Strains[a]	Average recovery[b] (%)
C. nebraskense	CN74-1, CN76-1, 298, CN18-6	93
C. michiganense	1379, 13-3, 15-2, 156-2	85
C. betae	CB101, CB102A	89
C. oortii	CO101	75
C. tritici	NCPPB 1857, CT102	79
C. rathayi		
Group 1	CR1	68
Group 2	CR101	0
C. flaccumfaciens		
Group 1	ATCC 6887	130
Group 2	Neb #21, 2AdK, CV4	17
C. poinsettiae		
Group 1	#13	65
Group 2	#1	29
C. insidiosum	239, M1B	0
C. sepedonicum	CSca, #30	0
C. iranicum	NCPPB 2253	0
C. fascians	6D21, CF101	0

[a]Strains were received from M. P. Starr, International Collection of Phytopathogenic Bacteria, University of Calif., Davis; E. Echandi, North Carolina State University; C. I. Kado, University of Calif., Davis; National Collection of Plant Pathogenic Bacteria, Herpenden, England; American Type Culture Collection, Rockville, MD; F. L. Lukezic, Penn State University; and S. A. Slack, University of Wisconsin, Madison.

[b]Recovery from sterile phosphate buffer. CNS = *C. nebraskense* specific medium; NBY = nutrient broth yeast medium. Average recovery = (no. colonies recovered on CNS × 10^2)/(no. colonies on NBY). Figures are calculated from average number of colonies per plate, three plates per strain. Log phase cultures, at about 10^8 colony-forming units per milliliter, served as the initial populations.

TABLE 2. Effects of various components in *Corynebacterium nebraskense* selective medium on the recovery of strain CN74-1 of that pathogen from soil

Component added to or deleted from CNS[a]	Concentration (mg/ml or % [v/v])	Colonies per plate[b]		Reduction[c] of soil bacteria (%)
		C. nebraskense	Soil bacteria	
None[d]	...	51.8 ± 9.5	40.8 ± 10.0	99.3
Polymyxin B sulfate[e]	0.032 mg/ml	71.7 ± 10.1	55.4 ± 16.5	99.1
Nalidixic acid[e]	0.025 mg/ml	56.8 ± 9.1	58.7 ± 9.8	99.0
Lithium chloride[e]	10 mg/ml	36.2 ± 8.1	65.3 ± 14.6	98.9
Cycloheximide[e]	0.04 mg/ml	50.5 ± 6.7	36.5 ± 8.7	99.4
Bravo 6F®[e]	1.3×10^{-4}%	55.0 ± 8.2	77.0 ± 13.5	98.7
Potassium dichromate[f]	0.05 mg/ml	60.0 ± 5.6	34.7 ± 5.9	99.4
Sodium azide[f]	0.002 mg/ml	63.7 ± 8.7	39.0 ± 10.3	99.4
Potassium tellurite[f]	3.1×10^{-5}%	0.0	7.0 ± 2.5	99.9
Protector 3L®[f]	3.1×10^{-4}%	5.0 ± 2.9	20.3 ± 7.8	99.7
Bio 88®[f]	1.25×10^{-3}%	44.5 ± 11.8	28.4 ± 10.5	99.5
Plyac®[f]	2.5×10^{-3}%	57.7 ± 15.1	33.4 ± 13.0	98.4
PEG 600 MO[f]	0.1%	47.0 ± 8.9	76.0 ± 12.1	98.7
Trydet TFA-11®[f]	1.0%	55.0 ± 9.8	58.0 ± 9.6	99.0

[a]CNS = *C. nebraskense* specific medium.

[b]Average number per plate from six plates and standard deviation. Numbers are × 10^{-4} colony-forming units per gram of artificially infested soil.

[c]Reduction = 100 − (no. bacteria recovered with the test medium/g soil × 10^2)/(total no. soil bacteria/g soil recovered on NBY). The average number of bacteria recovered on nutrient broth yeast (NBY) agar from 1 g (dry wt) of field soil was 6×10^7.

[d]CNS prepared by adding to NBY: 0.032 mg/ml of polymyxin B sulfate, 0.025 mg/ml of nalidixic acid, 10 mg/ml of LiCl, 0.04 mg/ml of cycloheximide, and 1.3×10^{-4}% (v/v) of Bravo 6F.

[e]Deleted

[f]Added

soil (autoclaved at 121 C for 20 min) to serve as the reference population of recoverable *C. nebraskense*.

Isolation from natural sources.—After development of the CNS medium, natural sources were tested for *C. nebraskense* recovery. Systemically-infected corn plants, in the milk-kernel stage, were collected along with rhizosphere soil samples from different geographic locations. Various plant parts (leaves, kernels, husks, cob, and pith) were ground in a mortar with phosphate buffer and plated onto NBY and CNS. The rhizosphere soil sample was assayed on CNS as described above. Also, 1 g of dry corn leaf residue showing discrete Goss's wilt lesions was ground in phosphate buffer, and dilutions were plated onto NBY and CNS.

Characterization of bacterial isolates from CNS.—The ability of a CNS bacterial isolate to produce typical Goss's wilt symptoms on sweet corn was determined. Plants were grown to the two- or three-leaf stage and then inoculated by the needle-puncture method of Vidaver (22). Symptom development was observed after 7 days. Control plants were inoculated with sterile phosphate buffer by the same procedure.

Colony shape, form, texture, and pigmentation initially were used to differentiate *C. nebraskense* (24) from soil bacteria. Selected isolates from CNS then were checked for Gram-stain reactions (21). Finally, selected orange and yellow Gram-positive isolates were tested for production of *C. nebraskense* bacteriocins CN1 or CN2 (10) on MBAL medium by the procedures of Vidaver et al (25) and also for pathogenicity by plant inoculations as described above.

RESULTS

Evaluation of additives to NBY medium for selection of C. nebraskense.—Of the media tested, CNS medium was best for isolation of *C. nebraskense* from soil (Table 2). More than 99% of the soil bacteria, as determined by comparison with plate counts on NBY, did not grow on the medium; no fungi grew. When either nalidixic acid, lithium chloride, or Bravo 6F was omitted from the medium, the ratio of *C. nebraskense* to soil bacteria fell below 1.00 (Table 2). Polymyxin B appeared to be optional since actinomycetes and other bacteria that were not inhibited by LiCl or nalidixic acid were fully suppressed by Bravo 6F.

Various additives to CNS either did not increase selectivity for *C. nebraskense* or, at the effective concentration, substantially decreased recovery. In general, the higher the ratio of strain CN74-1 to soil bacteria (Table 2), the better the apparent selectivity. Despite the apparently greater recovery of *C. nebraskense* relative to soil bacteria with sodium azide, potassium dichromate, Bio 88, or Plyac (Table 2), no quantitative improvement on CNS was observed in repeated trials. Also, these components reduced growth rate, altered pigmentation, or affected other characteristics significant for pathogen detection. The two nonionic surfactants, Trydet TFA-11 and PEG 600 MO, did not inhibit *C. nebraskense*, but at high concentrations (Table 2) they decreased the selectivity of CNS. Overall, no synergistic effects were detected between additives or components of CNS. All strains of *C. nebraskense* were sensitive to the lowest concentration of antibiotics used (ranging from 1 to 50 μg per disk, depending on the antibiotic), except nalidixic acid.

Comparison of CNS with other selective media.—From sterile soil, *C. nebraskense* (Fig. 1) was recovered well (>95%) on CNS, YDS, and D2 media. Medium D2 was markedly inferior to CNS and YDS in recovery of *C. nebraskense* from natural soil (Fig. 1). In our repeated trials, less than 30% of the add *C. nebraskense* population could be recovered from soil or medium D2 in contrast to more than 90% recovery on CNS or YDS. In particular, soil bacilli grew well on medium D2, rapidly spreading across the plate

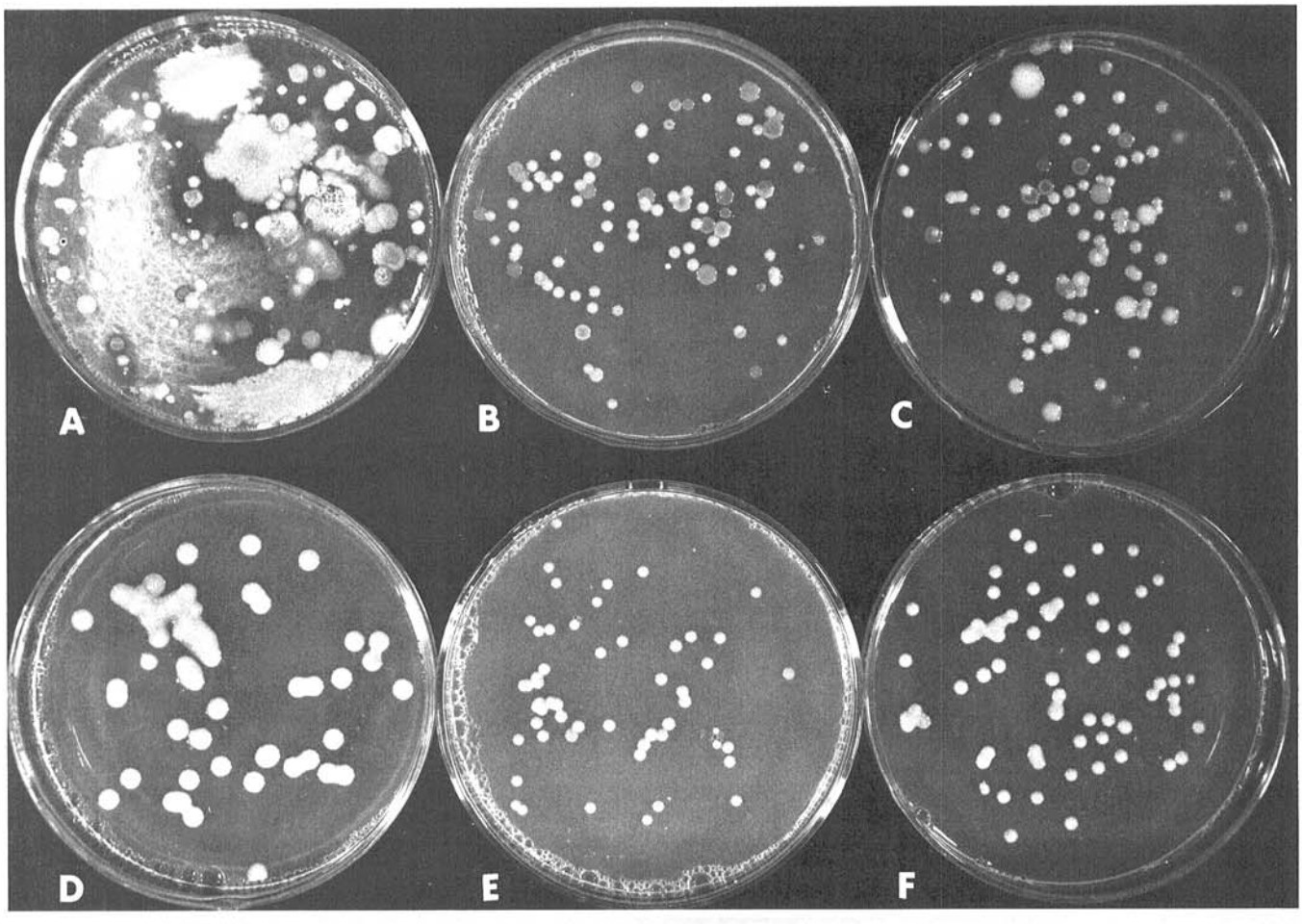

Fig. 1. Comparison of D2 (A,D), YDS (B,E) and CNS (C,F) media for growth and selection for *Corynebacterium nebraskense*, strain CN74-1. Plates A, B, and C compare the selectivity for *C. nebraskense* from nonsterile soil. Plates D, E, and F show the isolation of *C. nebraskense* from sterile soil. Approximately 5×10^5 CN74-1 cells were added per gram of soil.

TABLE 3. Recovery on *Corynebacterium nebraskense* selective medium of *C. nebraskense* (CN74-1) from different soils[a]

Sample no.[b]	*C. nebraskense* added (CFU/g[c] soil)	Sandy loam (Colonies recovered)		Clay loam (Colonies recovered)	
		C. nebraskense	Soil bacteria	*C. nebraskense*	Soil bacteria
A	74.0×10^5	...	...	85.0 ± 6.0	10.0 ± 3.0
B	72.3×10^4	73.0 ± 5.2	36.0 ± 3.5	79.7 ± 7.2	113.0 ± 14.8
C	68.3×10^3	69.3 ± 2.5	>300	64.7 ± 9.3	>1,000
D	57.7×10^2	90.0 ± 25.0	>3,000	15.7 ± 7.4	>10,000

[a]Figures are average number per plate from three plates and the standard deviation. The average number of bacteria recovered on nutrient broth yeast agar from 1 g (dry wt) of sandy loam field soil was 9×10^7 and for clay loam, 6×10^7.
[b]After addition of *C. nebraskense* to soil and phosphate buffer (final volume 10 ml), sample A was diluted 10^{-3}; B, 10^{-2}; C, 10^{-1}; and D undiluted.
[c]CFU = number of colony-forming units per gram.

and inhibiting the growth of *C. nebraskense*. *Bacillus* species did not grow on either CNS or YDS. Also, some fungi grew on D2 but not on CNS or YDS.

On CNS and YDS, little or no difference in recovery of *C. nebraskense* was detected (Fig. 1). However, the growth rate was faster, the orange pigmentation was more intense, and colony morphology and texture (24) were more typical for *C. nebraskense* on CNS than on YDS. Repeated comparisons of CNS with YDS showed no quantitative difference in number and type of bacteria that grew on these media. Thus, overall growth characteristics of *C. nebraskense* on CNS resembled those on NBY.

No growth of *C. nebraskense* occurred on YDC medium after nalidixic acid, LiCl, Polymyxin B sulfate, Bravo 6F, and cycloheximide were added at the concentrations used in CNS. However, individual components, except lithium chloride, enabled growth of *C. nebraskense*. Therefore, the same components in a different medium apparently produced different results.

Recovery of C. nebraskense from soil.—*C. nebraskense* was isolated from sandy and clay loam in similar numbers (Table 3). In sandy loam, quantitative recovery of *C. nebraskense* CN74-1 occurred above 10^2 cells/g. In clay loam, the same strain was quantitatively recovered only above 10^3 cells/g, due to higher populations of other bacteria. The recovery was essentially the same if sampled immediately or after 10 min. Both soil types lacked resident orange-pigmented bacteria capable of growing on CNS.

Recovery of C. nebraskense from naturally infected tissue or corn residue.—Corn plants showing systemic symptoms of Goss's wilt were collected from different fields. Isolations on CNS from all plant parts (leaves, kernels, husks, cob, and pith), yielded *C. nebraskense*; generally, no other bacteria grew on the medium. In contrast, soil bacteria from the plant's rhizosphere lacked the colony morphology or pigmentation associated with *C. nebraskense*. Randomly chosen Gram-positive soil isolates from CNS (64 were tested) did not cause Goss's wilt on sweet corn or produce either bacteriocin CN1 or CN2.

Dry corn residue had a population of 6×10^5 CFU of *C. nebraskense*/g dry leaf tissue (Fig. 2) when plated on CNS. The pathogen was not recovered on NBY. *C. nebraskense* constituted only 0.43% of the total bacterial population from debris capable of growing on NBY (Fig. 3) but 33% of the total bacterial population growing on CNS. Six randomly selected isolates were verified as *C. nebraskense* by pathogenicity tests on sweet corn and by bacteriocin production.

Characteristics of bacterial isolates from CNS.—Soil and debris yielded bacteria on CNS that were either Gram-positive or Gram-

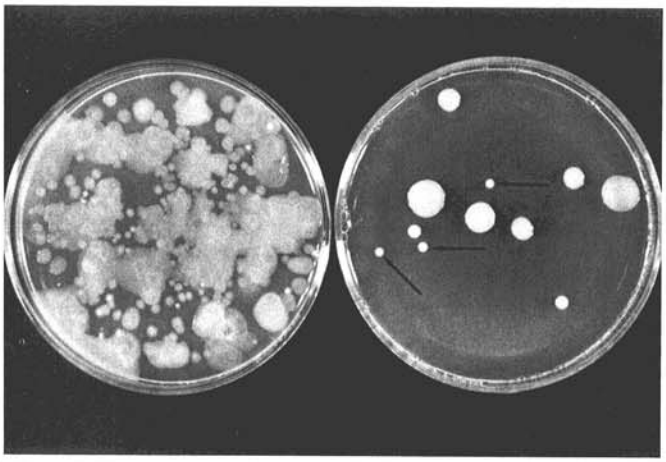

Fig. 2. Comparison of nutrient broth yeast extract medium (NBY) (left) and *Corynebacterium nebraskense's* selective medium (CNS) (right) for selection of *C. nebraskense* from naturally infested corn leaf residue. Orange *C. nebraskense* colonies were visible (arrows) on CNS medium after 7 days' growth at 25 C. The population of *C. nebraskense* in the dry leaf tissue averaged 6×10^5 colony-forming units per gram as determined on CNS medium (right). Total recoverable bacteria were 1.4×10^8 colony-forming units per gram on NBY medium (left).

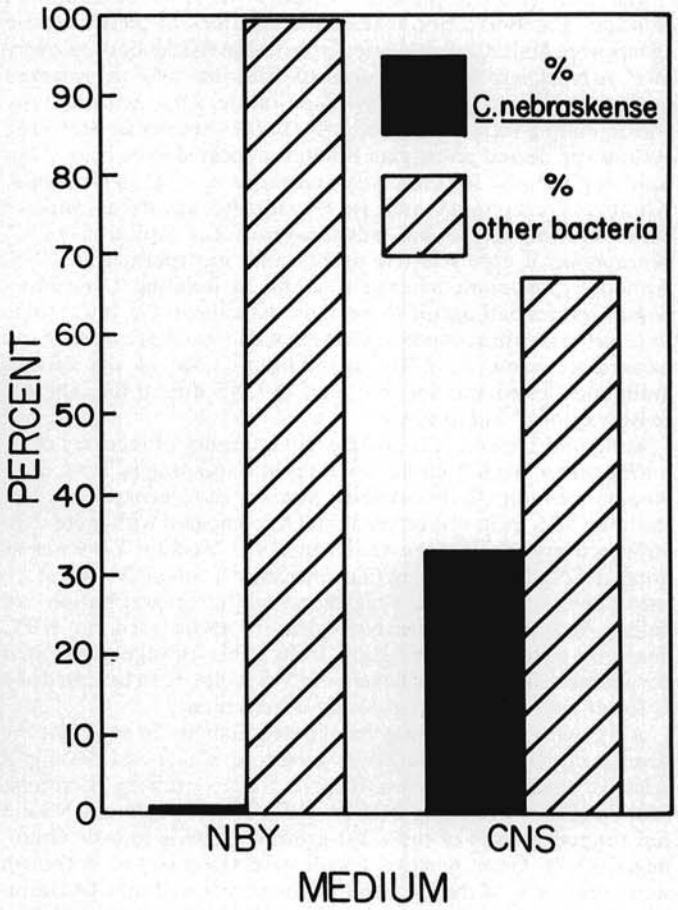

Fig. 3. Percentage of *Corynebacterium nebraskense* relative to total number of bacteria isolated from naturally infested corn leaf residue. Total recoverable bacteria were determined separately for nutrient broth yeast (NBY) extract medium and for *C. nebraskense* selective (CNS) medium.

variable. Gram-negative bacteria did not grow on CNS. More than 90% of the isolates were pleomorphic, medium-to-short rods, characteristic of coryneform bacteria similar to *Corynebacterium* or *Arthrobacter* spp. (3) when examined by phase contrast microscopy. Some coccoid bacteria also grew on CNS; these were similar to *Micrococcus* spp. in cell size and morphology, as well as colony pigmentation and morphology.

Growth of phytopathogenic Corynebacterium spp. on CNS.—All strains of *C. nebraskense, C. michiganense, C. betae, C. tritici,* and *C. oortii* plated at high efficiencies on CNS with only a moderate decrease in growth rate (Table 1). In general, recoveries of 65% or greater on CNS, in comparison with NBY, were considered to be close to maximum. Variation ranged from 65 to more than 100%, and this was attributed to inherent sampling or plating errors. In contrast, strains of *C. flaccumfaciens, C. poinsettiae,* and *C. rathayi* generally grew poorly on CNS with considerable variation in colony size and number. Our strains of *C. insidiosum, C. sepedonicum, C. iranicum,* and *C. fascians* did not grow on CNS.

DISCUSSION

The CNS medium enables recovery of *C. nebraskense* from infected fresh tissue, dry corn residue, or soil. In these cases, more than 99% of the total noncoryneform bacterial flora can be eliminated. Total recovery (~ 100%) of *C. nebraskense* added to soil was achieved for strains that differed in plasmid DNA content or bacteriocin production (Gross and Vidaver, *unpublished*). The virulence of four strains did not change detectably after growth on CNS. Although adequate colony growth required 3 to 4 more days on CNS than on NBY, other qualitative characteristics such as colony shape, texture, and pigmentation on CNS remained consistent with growth on NBY.

The basic selective ingredients include nalidixic acid, lithium chloride, and Bravo 6F. When concentrations of these selective agents were higher, effects varied from loss in efficiency of recovery of *C. nebraskense* with LiCl, toxicity with Bravo 6F to a marked reduction in growth rate with nalidixic acid, without any corresponding increase in selectivity. In the presence of Bravo 6F, cycloheximide and polymyxin B sulfate appeared to be helpful but were not essential for selectivity and recovery of *C. nebraskense.* Nonionic surfactants and slow-oxidizing agents at various concentrations were not advantageous for isolation of *C. nebraskense* if core selective agents were incorporated in CNS. Although potassium tellurite is useful in isolating *Corynebacterium* species pathogenic to humans and animals (2), it was toxic to *C. nebraskense* at concentrations that allowed the remaining soil bacteria to grow on CNS. In addition, none of the various antibiotics tested was incorporated in CNS due to the inherent sensitivity of *C. nebraskense.*

Kado and Heskett (12) obtained an efficiency of recovery of *C. michiganense* from bacterial mixtures and soil ranging from 27 to 80% on medium D2. In our tests, recovery of *C. nebraskense* was less than 30% from soil on medium D2, compared with more than 90% recovery of *C. nebraskense* on CNS. Medium YDS was as good as CNS for recovering *C. nebraskense* from soil. However, *C. nebraskense* on CNS had a higher growth rate; pigmentation and colony morphology were equivalent to those seen on NBY, enabling easier differentiation from other orange-pigmented corynebacteria. An added benefit of CNS is that it can be stored at 4 C for at least 1 mo without losing effectiveness.

Although *C. nebraskense* has limited viability in soil (18), the diverse microbial soil flora was used to evaluate and develop a selective medium. *C. nebraskense* is a slow-growing bacterium (doubling time averaging 2.25 hr: NBY, 25 C). Medium CNS did not support growth of most fast-growing bacteria (mostly Gram-negative) or Gram-positive bacilli or actinomycetes. Although more than 90% of the bacterial population in soil may be Gram-positive (3,9,11), only some coryneform bacteria and *Micrococcus* spp. were isolated on CNS. Not all coryneform bacteria will grow on CNS, however; certain strains of *C. sepedonicum* and *C. insidiosum* did not grow on CNS. Modifications in type or concentration of selective agents might enable growth of these species on an NBY base, which by itself supported profuse growth of all strains of all the species tested.

CNS medium should prove useful in routine isolations of *C. nebraskense* from fresh, infected tissue. More importantly, CNS shows potential as an epidemiologic tool for quantitatively monitoring populations of *C. nebraskense* throughout the corn-growing season. The medium also is expected to be useful in monitoring primary inoculum populations in residue, soil, or water.

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