

## Diversity of *Corynebacterium nebraskense* Strains Causing Goss's Bacterial Wilt and Blight of Corn

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### ABSTRACT

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The bacterium *Corynebacterium nebraskense*, first isolated in 1969 from a localized area, was detected in essentially all the corn (maize) growing areas of Nebraska and in bordering states by 1979. The 85 strains collected over this decade were classified by bacteriocin and bacteriophage typing into eight groups; most strains fell into one of four groups. Except during 1969, the strains had no apparent correlation with either the year of isolation or geographic source.

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Goss's bacterial wilt and blight of corn, which is caused by *Corynebacterium nebraskense*, was first observed in south central Nebraska in 1969 (15,19). The disease has since spread to new areas

within and outside the state (15,21).

Researchers evaluated inbred corn lines and commercial hybrids between 1969 and 1979 (2,5-7,16) and discovered varying degrees of resistance within existing germ plasm. However, the resistant hybrids often yield 10-15 bu/acre (9-13 hl/ha) less than susceptible hybrids that are free of the disease. Growers are thus not encouraged to plant resistant varieties unless the disease has reduced crop yield. The large and increasing acreage of susceptible varieties may have contributed to the spread of the disease.

*C. nebraskense* is a bacterium that shows little heterogeneity in standard

nutritional and biochemical tests (8,15,19). We show, however, that *C. nebraskense* strains fall into several distinct groups. We used both bacteriophage and bacteriocin typing to determine whether pathogen distribution was correlated with either the year of isolation or geographic source. These convenient techniques are frequently used to trace foci of infection and to differentiate strains of bacteria that are otherwise indistinguishable (11,14).

## MATERIALS AND METHODS

**Bacteria.** The 85 strains (Table 1) used in this study were collected over a span of 11 yr from Nebraska and bordering states (Fig. 1). Specimens with either leaf or wilt symptoms were obtained on periodic surveys throughout each growing season by the authors, county extension agents, cooperating plant pathologists from bordering states, and farmers. Characteristic discrete and discontinuous, water-soaked lesions parallel to the veins were considered diagnostic leaf symptoms (15).

Before 1971, samples were taken from different locations within a county and occasionally within the same field. In 1971 and later, only single isolates were

obtained from the first occurrence within each county.

The etiological agent was confirmed by isolation on a nutrient broth yeast extract medium for characteristic orange-pigmented colonies (19) and by patho-

genicity tests with Golden Cross Bantam sweet corn (18). Virulence of freshly isolated strains ranged from 0.5 to 4.5 on a five-point rating scale (18). Bacterial cultures purified by sequential colony picking were subsequently lyophilized

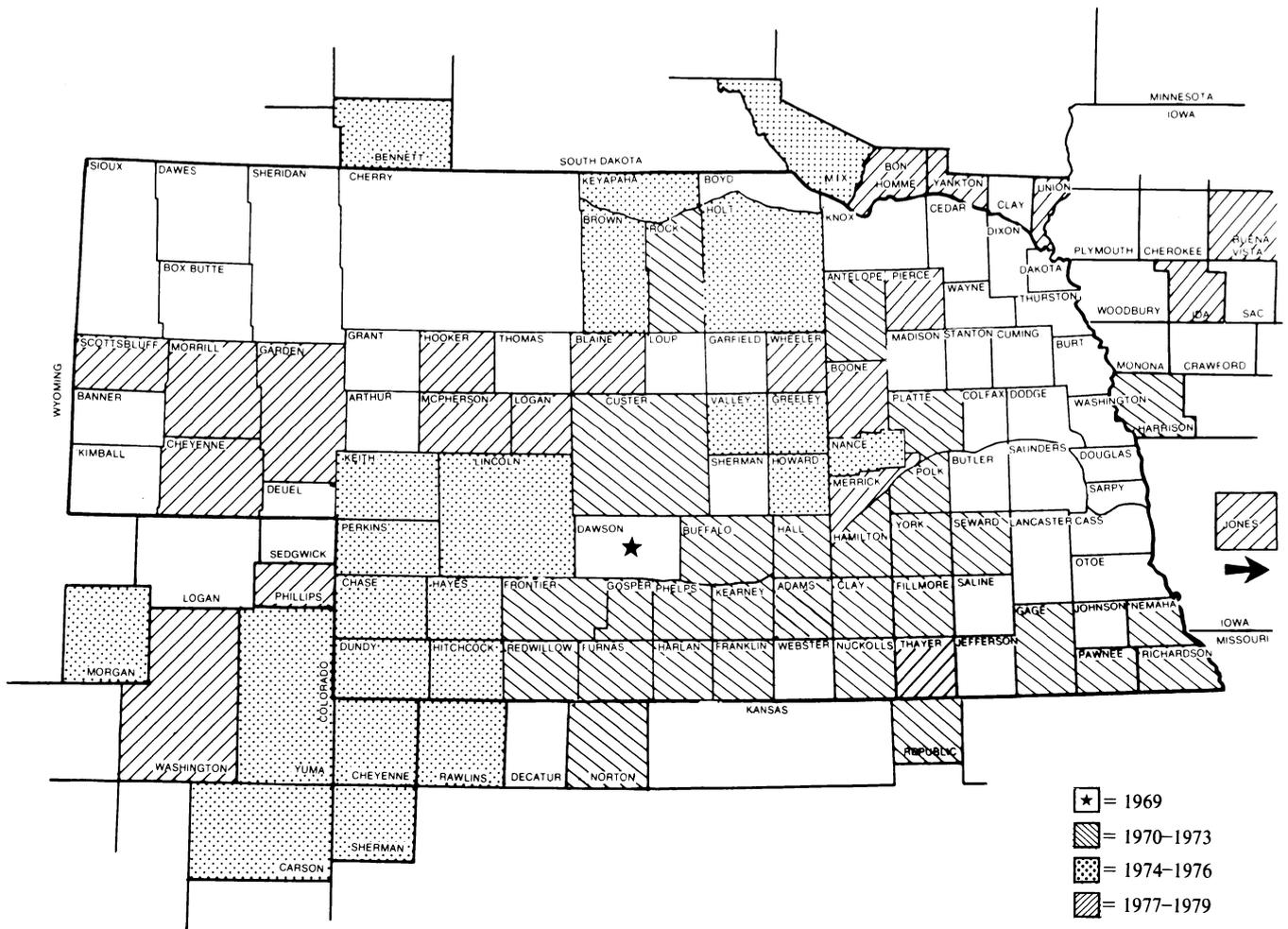
**Table 1.** Grouping of *Corynebacterium nebraskense* strains by phage sensitivity and bacteriocin production

Strain	Year of isolation	Geographic source (county)	Lysis by phage at RTD <sup>a</sup>					Phage group	Bacteriocin group
			CN8	CN11	CN77	CNRH	CNX		
CN18-6 <sup>b</sup>	1970	Dawson	-	-	-	-	+	A	1
CN18-1 <sup>b</sup>	1969	Dawson	+	+	+	+	+	B	1
CN37-1 <sup>c</sup>	1972	Phelps	+	+	+	-	+	C	1
CN18-2 <sup>c</sup>	1970	Dawson	+	-	+	+	+	D	1
721-L <sup>c</sup>	1971	Gosper	+	-	+	+	-	E	1
CN9-1 <sup>c</sup>	1973	Buffalo	+	+	-	+	+	F	1
K293E <sup>b</sup>	1971	Buffalo	-	-	-	-	+	A	2
NTA <sup>b</sup>	1971	Unknown	+	+	+	+	+	B	2

<sup>a</sup> Routine test dilution.

<sup>b</sup> Strains equivalent in both bacteriophage and bacteriocin reaction to CN18-6 were CN81-1, CNK-1, CN74-1, CNC-1, K293T, CN15-1, CN44-1, CNK-2, CN28-1; those equivalent to CN18-1 were CN4-1, CN30-1, CNN-1, CN18-3, CN38-3, CN38-2, CN38-1, CN18-4, CN49-1, CN68-1, CN18-5, CN37-2, CN38-4, 39, 172, 173, 721-S; those equivalent to K293E were CN4-2, CNSD-1, 313, CN76-1, CN76-2, CN50-1, CN50-2, CN51-1; those equivalent to NTA were HD363, 298, 311, 716, CN38-6, CN62-1, CN72-1, and CN48-1. Seventeen strains tested for phage sensitivity after completion of bacteriocin testing were typed into phage group A or B. Eighteen strains collected in 1977 and 1978 were lost after pathogenicity tests and bacteriophage typing into phage group A or B.

<sup>c</sup> Strains CN18-2, CN9-1, and CN37-1 were lysed at 100 RTD by phages CN11, CN77, and CNRH, respectively. Strain 721-L was lysed at 100 RTD by phages CN11 and CNX.



**Fig. 1.** Distribution of Goss's bacterial wilt and blight of corn, 1969-1979. One inch (25.4 mm) represents approximately 53.5 miles (86 km). Jones County, Iowa, is about 200 miles (322 km) from the Nebraska border. Shading reflects the year of first occurrence.

(18) and restored as needed.

**Bacteriophage isolation and typing.** The phages CN8, CN11, CN77, CNRH, and CNX were isolated from soil by enrichment culture (20), with *C. nebraskense* CN18-5 as the propagating strain. Soils were obtained from various locations in different years from fields harboring the pathogen.

Phages were tested at a routine test dilution (RTD), which titered at 1 to 5 × 10<sup>5</sup> plaque-forming units per milliliter, and at 100 × RTD. The medium was a nutrient broth yeast extract agar (17) and the procedures were those described by Parker (14). The essential requirements of typing were met: stable reagents and standard type strains, an adequate number of differentiable strains, and

reproducibility (1,11). Although degrees of lysis were recorded, only positive and negative results are shown here (Table 1). Results represent two to four independent experiments.

**Bacteriocin typing.** The strains were typed by bacteriocin production (12), using *C. michiganense* 13-3 as indicator. Two media, nutrient broth yeast extract and modified Burkholder's agar that lacked peptone, were used to differentiate production of bacteriocins designated as CN1 (group 1) and CN2 (group 2). Results reported here are from two to 10 independent experiments, depending on the strains tested.

## RESULTS

**Geographic distribution.** In 11 yr, *C. nebraskense* spread several hundred miles in all directions from the localized area, Dawson County. The disease has been detected through isolation and pathogenicity tests in essentially all corn growing areas of Nebraska and in some counties of neighboring states (Fig. 1).

**Bacteriophage and bacteriocin typing.** The *C. nebraskense* strains were differentiated by their reaction to five lytic phages and their bacteriocin production pattern on two different media (Table 1). The initial three strains collected from three fields in Dawson County (Fig. 1) were indistinguishable and fell into phage group B, bacteriocin group 1. In 1970, one of four new strains from other counties was typed into phage group A.

By 1971, increased strain diversity was detected (Table 2) both by phage and bacteriocin typing. The rare phage types (C-F) were represented by single strains, all of which were collected in different years from 1970 to 1973. Strains collected since then have been typed into phage group A or B. However, no phage type

was correlated with any of the bacteriocin types, ie, the bacteriocin type was independent of phage. Stability of phage typing was very good in that none of 10 strains tested at a 7-yr interval showed any change in phage sensitivity.

Tests repeated within several weeks of each other were reproducible for both bacteriophage and bacteriocin typing. All strains except one from Perkins County were sensitive to one or more phages. Saprophytic or phytopathogenic corynebacteria of related and unrelated species were insensitive to these phages, except for a single strain (of three tested) of *C. insidiosum* that showed sensitivity to all the phages at the higher concentration tested (100 × RTD).

Bacteriocin production by *C. nebraskense* was prevalent but not diverse (12). Some strains of *C. nebraskense* produced CN1; others produced both CN1 and CN2. Bacteriocin was produced by *C. nebraskense* on two media (Fig. 2). Of particular interest was the isolation of two bacteriocin types from a single sample (Table 2).

No relation was found between phage or bacteriocin type and geographic source; eg, an Iowa strain was indistinguishable from a Buffalo County strain (Table 2). Limited experiments showed no correlation between virulence and either phage or bacteriocin type. Geographic sources and dates of isolation for all strains listed in Table 1 can be obtained from the senior author.

## DISCUSSION

Goss's bacterial wilt and blight of corn (maize) spread considerably since it was first observed in 1969. Once the disease occurred in a county, it reappeared in subsequent years if susceptible varieties were grown. It was sporadic, however, and might not be seen for several years. In Dawson County, where the disease was first detected, it was less frequent since 1974, probably because of the extensive planting of resistant varieties. Infested debris and seed can serve as primary sources of inoculum (15); infection occurred during hot (over 30 C) and damp weather. Because the western corn belt frequently has rapid shifts of temperature, wind (including blowing sand and soil), and precipitation (including hail), inoculum may be spread as favorable climatic conditions arise from such changes. Infection was encouraged by the continuing planting of susceptible corn hybrids.

The phage and bacteriocin typing lacked a discernible pattern for use in evaluating distribution by year of isolation or geographic source. Because the three strains from 1969 were indistinguishable, however, typing suggested a probable single infection source. The diversity of strains after 1969 suggests that multiple sources of inoculum were

**Table 2.** Phage and bacteriocin groups of *Corynebacterium nebraskense* in a representative year (1971)

Strain	Geographic source (county)	Phage group	Bacteriocin group
39	Dawson <sup>a</sup>	B	1
172	Harlan <sup>b</sup>	B	1
173	Harlan <sup>b</sup>	B	1
298	Dawson <sup>d</sup>	B	2
311	Pawnee <sup>c</sup>	B	2
716	Adams <sup>c</sup>	B	2
NTA	Location not specified	B	2
313	Harrison (Iowa) <sup>c</sup>	A	2
K293E	Buffalo <sup>c</sup>	A	2
K293T	Buffalo <sup>f</sup>	A	1
721-L	Gosper <sup>c</sup>	E	1

<sup>a</sup>Gothenberg, NE.

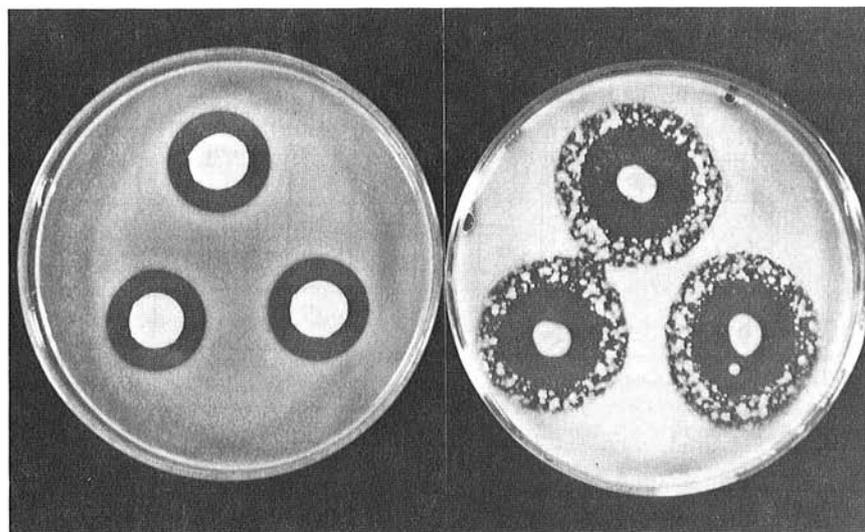
<sup>b</sup>Huntley, NE.

<sup>c</sup>Republican City, NE.

<sup>d</sup>Lexington, NE.

<sup>e</sup>Isolated farm.

<sup>f</sup>Different colony type, isolated from same sample as K293E



**Fig. 2.** Bacteriocin production by *Corynebacterium nebraskense*. *C. michiganense* 13-3 is the indicator in both plates, in which the *C. nebraskense* was spotted in triplicate. (Left) CN1 production on nutrient broth yeast extract. (Right) CN1 and CN2 production on modified Burkholder's agar. Note the double zone, with bacteriocin CN1 produced in the inner zone and CN2 in both the inner and outer zones.

present in different years or geographic areas, or both.

Phage sensitivity and bacteriocin production serve as useful tools for differentiating *C. nebraskense* strains that are otherwise relatively homogeneous in biochemical or bacteriological analyses (8,15,19) and plasmid analyses (13). This apparent homogeneity may reflect recent evolution of the pathogen and the relative homogeneity of the susceptible host plant. In contrast to *C. nebraskense*, just a few strains of either *C. michiganense* or an orange-pigmented coryneform pathogenic on wheat are separable into many bacteriocin types (3,9, R. R. Carlson and A. K. Vidaver, unpublished). Phage typing has also been useful in differentiating strains of the tomato canker pathogen *C. michiganense* (10) and, to a lesser extent, the alfalfa wilt pathogen, *C. insidiosum* (4).

Monitoring of *C. nebraskense* distribution in the western corn belt will continue. The research will attempt to discover whether bacteriophage and bacteriocin types remain the same as described here or whether new types emerge.

#### ACKNOWLEDGMENTS

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