Infectivity and Survival of Soft-Rot Bacteria in Chinese Cabbage

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ABSTRACT

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The population of *Erwinia carotovora* var. *carotovora* in inoculated plant tissues of Chinese cabbage *(Brassica pekinensis)* was influenced by the presence of other microorganisms. The infection potential of the soft-rot bacterium was correlated with the relative proportion of soft-rot bacteria and other bacterial cells instead of the total soft-rot bacteria population level in the inoculum dosage. The rotting capacity of *E. carotovora* var. *carotovora* isolates was high, but decreased when the inoculum included other bacteria. *Erwinia carotovora* var. *carotovora* was not detected in soil samples from fields where Chinese cabbage had not been cultivated or while plants were in the seedling stage following first planting. It was barely detectable in a field where there had been one previous cabbage crop.

Additional key words: Brassica pekinensis.

Bacterial soft-rot, caused by *Erwinia carotovora* var. *carotovora* (Jones) Dye, is one of the most destructive diseases of Chinese cabbage [*Brassica pekinensis* (Lour.) Reprecht.] in the tropics. Initial infection usually occurs on the outer petioles that are in contact with soil at the "wrapping" stage of the plants. Further decay in the host tissues advances rapidly after the initial lesions (11).

Erwinia carotovora var. *carotovora* and *E. aroideae* are widely distributed in the soil in Japan (5, 7, 9, 10, 11). However, studies in the United States (1) and Europe (3, 8) have indicated that the primary inoculum of *E. carotovora* does not come from the soil but from vegetatively propagated materials.

At the Asian Vegetable Research and Development Center (AVRDC), severe soft-rot infection was observed in a field in which only sugarcane had been grown for more than 20 years. However, no *E. carotovora* could be detected in such soil before cabbage was planted, nor in the cabbage seeds. This suggested that the soft-rot bacteria might be present at undetectable population levels. This paper reports on both the detection of *E. carotovora* var. *carotovora* in field soils and the infectivity of the bacterium in pure and mixed inocula.

MATERIALS AND METHODS

Several of the media reviewed recently by Cupples and Kelman (1) were tested for isolation of soft-rot bacteria. Two media and the plant tissue method were chosen to grow soft-rot bacterial populations for study and to measure the pathogen's rotting capacity, respectively.

The modified Drigalski's medium (BTB) has been used in Japan (5). Although this medium is not specifically selective, it allows differentiation between soft-rot *Erwinia* and other soil microorganisms by colony morphology. *Erwinia carotovora* produced golden colonies with surrounding halos on this medium. Tests designed by Graham (4) were used as a routine procedure to identify *Erwinia carotovora* var. *carotovora*. A fluorescent *Pseudomonas* sp. commonly associated with soft-rot *Erwinia,* both in diseased plant tissues and in soil samples, produced small blue circular colonies on the same medium.

The crystal violet pectate (CVP) medium formulated by Cupples and Kelman (1) also was used to detect soft-rot bacteria. In dilution plate counts, CVP generally yielded higher colony count totals than the modified Drigalski's medium. However, the colonies on BTB could be identified conclusively as *E. carotovora* var. *carotovora,* whereas CVP did not distinguish between *E. carotovora* var. *carotovora* and others such as the *Pseudomonas* sp. By using the spot-plate technique (2), the polypectate activity of the microorganisms was determined on this medium. When CVP was used with BTB, the estimations and enumerations of the soft-rot *Erwinia* population in soil and plant materials were made with greater confidence.

The plant tissue method was used to test the rotting capacity. Sections of Chinese cabbage petioles placed in moistened dishes were gently punctured with a flamed needle to make wounds and then inoculated with a unit volume (0.01 ml) of inoculum. Total bacterial and soft-rot *Erwinia* populations of each inoculum were determined by dilution plate counts on CVP and BTB, respectively. The rotting capacity of the inoculum dosage was calculated by dividing the number of rotted petiole sections by the total of at least 25 inoculated sections.

We conducted a series of experiments to detect the *Erwinia* populations in soil and in plant tissues and to determine the infection potential of *E. carotovora* var. *carotovora* as the plant pathogen causing soft rot of Chinese cabbage.

To enumerate soft-rot *Erwinia* populations in field soil,

three samples were taken from the upper 10-cm at each location. Twenty grams of each sample were suspended in 80 ml of sterile water in 500-ml Erlenmeyer flasks. The soil suspension was shaken thoroughly and allowed to settle for 1 minute. Ten milliliters of the supernatant liquid was pipetted into 90 ml of sterile water, and a dilution series was made. One-tenth of a milliliter of each dilution was pipetted onto the CVP and BTB, and also onto the wounded petiole sections.

RESULTS AND DISCUSSION

Detection of Erwinia carotovora var. carotovora in plant tissues.—We examined soil samples from a field shortly after plowing under diseased Chinese cabbage plants and detected only traces of *E. carotovora*. This indicated that the soft-rot *Erwinia* population declined very rapidly in field soils. When petiole sections of Chinese cabbage were inoculated with bacterial inoculum prepared directly from diseased plant tissues and incubated for different time periods in the laboratory, the proportion of *Erwinia carotovora* bacteria to the total bacterial population in the petiole sections (detected by CVP and BTB) decreased as the incubation time increased (Table 1).

Infection potential.—The infection potential, as used in this paper, is defined as the infectivity of an inoculum, and was estimated by the percentage of petiole sections that rotted when inoculated with a volume (0.01 ml) of freshly prepared inoculum. The term is proposed because we found that the rotting capacity of *E. carotovora* var. *carotovora* was correlated with the relative proportion, rather than the numbers, of the soft-rot bacterial cells in an inoculum from a natural source. The inoculum sources of the present study were prepared either by grinding diseased tissues or by suspending a soil sample; however, the weight of the samples were standardized. In our case, 5 g (fresh weight) of diseased cabbage tissues or 20 g (wet weight) of soil samples were added to either 95 ml or 80 ml of sterile water, respectively, to make up the inoculum.

The population of *E. carotovora* var. *carotovora* was detected by plating the same inoculum on BTB medium, and total bacterial population was estimated on CVP. The results indicated that infection potential of an inoculum was greater when the proportion of soft-rot cells to the total bacterial population was higher and was not correlated closely with the total number of *E. carotovora* var. *carotovora* cells in the inoculum (Table 2).

The infection potential of this pathogen was high when the inoculum contained only *E. carotovora* var. *carotovora* isolates (Fig. 1). An inoculum containing less than 100 soft-rot bacterial cells caused 5% soft rot in 24 hours and 60% in 48 hours. The disease developed rapidly after initial infection in susceptible Chinese cabbage plants.

A gram-negative fluorescent *Pseudomonas* sp. frequently was present in cabbage tissue infected by soft rot. Experiments were conducted in which the nonsoft-rot bacteria were mixed with the soft-rot pathogen in various proportions of relative concentrations. The capability of soft-rot production was affected by the presence of this bacterium (Table 3). The polypectate hydrolytic activity also was affected. At an inoculum dosage of 10^6-cells in 0.01 ml, the soft-rot bacteria rotted 100% of the petiole sections in 24 hours, by only 63% when 10^8-cells of the nonsoft-rot bacteria were included in

TABLE 1. Relative proportions of *Erwinia carotovora* var. *carotovora* recovered from petiole sections of Chinese cabbage inoculated with an inoculum freshly prepared from diseased tissues

Original[a] inoculum (%)	Days after inoculation[b]		
	2	3	4
94[c]	16[c]	9	0
94	7	10	8
94	23	23	5
93	9	1	3
62	33	7	6

[a]The original inoculum was freshly prepared from soft-rot infected Chinese cabbage tissues.
[b]Infection of petiole sections resulted from inoculation with 0.01 ml of the original inoculum.
[c]Proportion of *E. carotovora* var. *carotovora* (as a %), detected on BTB medium, to total bacterial population detected on CVP. Mean of three samples.

TABLE 2. Rotting capacity and relative pectolytic activity of *E. carotovora* var. *carotovora* (ECC) with inoculum prepared from diseased cabbage tissues

ECC[a]	ECC, % of total bacterial cells[b]	Reaction on CVP[c]	Soft-rot production[d] (%)
1.0×10^4	62	6/32	67
2.5×10^6	14	5/32	31
3.5×10^6	7	21/32	0
1.2×10^7	88	20/32	33
1.2×10^8	91	32/32	90

[a]Detected on BTB medium.
[b]Total bacterial cells determined on CVP medium.
[c]Number of pectolytic-positive reactions per 32 spot-plates.
[d]Percentage of 32 petiole sections which rotted.

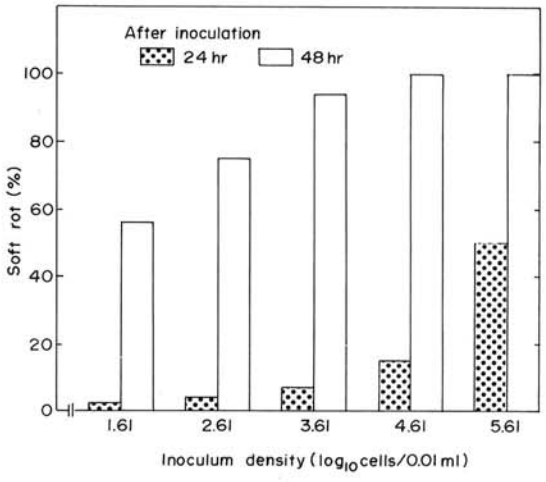

Fig. 1. Soft-rot infection on 25 petiole sections induced by inoculation through wounds with indicated number of cells from a pure culture of *Erwinia carotovora* var. *carotovora*.

TABLE 3. The rotting capacity of *E. carotovora* var. *carotovora* (ECC) when mixed in different proportions with a fluorescent *Pseudomonas* sp. commonly found in diseased tissues of Chinese cabbage and soil samples from cabbage fields

ECC: *Pseudomonas*[a]	Soft-rot production, %[b] 24 hours	48 hours	Reaction on CVP[c]
6 : 0	100	100	23/25
6 : 5	100	100	25/25
6 : 6	100	100	15/25
6 : 7	88	100	15/25
6 : 8	63	63	10/25
5 : 8	33	33	0/25
4 : 8	0	0	0/25
3 : 8	0	0	0/25
0 : 8	0	0	0/25

[a]Proportion of two bacteria in $\log_{10}$.
[b]Percent of petiole sections which rotted.
[c]Number of pectolytic-positive reactions per 25 spot-plates.

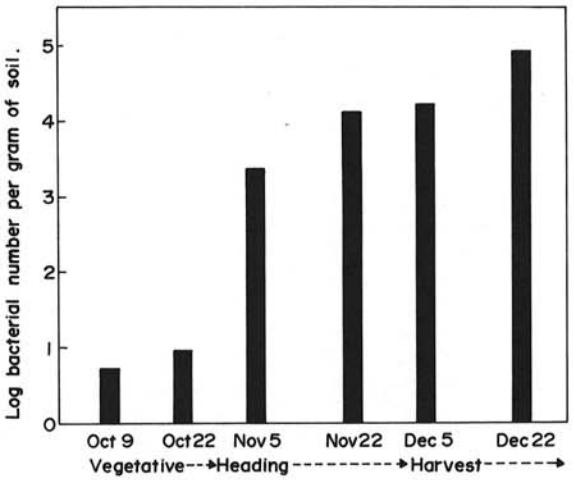

Fig. 2. Seasonal increase of *Erwinia carotovora* var. *carotovora* populations in soil samples taken during the growth of Chinese cabbage following one previous cabbage crop.

the inoculum. When the combination of soft-rot and nonsoft-rot bacteria was 10^4 and 10^8 cells in the inoculum, respectively, no soft-rot production was noted after 48 hours of incubation.

The results further confirmed that the infection potential of *Erwinia* is influenced by other soil microorganisms.

Detection in soil.—Although it has been reported that the soft-rot pathogen of Chinese cabbage can be detected readily in the soil by BTB and the carrot-slice technique (6, 10, 11), we found it was difficult to detect such bacteria in soil samples, by both media (BTB and CVP) and the plant tissue method. When Chinese cabbage had not been cultivated in a field, no *E. carotovora* var. *carotovora* was found. *Erwinia carotovora* var. *carotovora* also was not detected in soil samples from fields in which the first crop of Chinese cabbage was at the seedling stage. But soft-rot infection of the cabbage crop was observed frequently at later stages of the plant growth. This apparently indicates that in tropical soils where the winter temperatures seldom drop below 20 C, *E. carotovora* var. *carotovora* may be present in soils at an undetectable population level by our technique or methods.

However, *E. carotovora* var. *carotovora* was isolated from soil in which Chinese cabbage had been the previous crop. In this field, the soft-rot bacterial population began increasing at the head-forming stage and then remained at 10^5 cells/g of soil (wet weight) until the end of that cropping season (Fig. 2). In the Lishan mountain area of Taiwan, where Chinese cabbage has been cultivated each year for the last 10 years, only low populations of soft-rot bacteria could be detected in soil samples. The maximum soft-rot incidence was 10 to 20% of the plants in the fields.

The present investigation indicated that *E. carotovora* var. *carotovora* may be present in the tropical soils even in the absence of host crops, but at undetectable population levels. However, such populations increase to detectable levels during the growth of Chinese cabbage. Additionally, the infection potential is greatly influenced by other soil microorganisms. Whether the low population in soils also is affected by other microorganisms remains to be determined.

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