Bacterial Populations on Basal Lettuce Leaves and in Soil from under Lettuce Plants

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ABSTRACT

Populations of total green fluorescent bacteria and total pectolytic bacteria on lower healthy leaves from 7- to 8-week-old lettuce plants grown on nonridged organic soil reached 4.9×10^4 and 2.7×10^3 cells/cm^2 of leaf tissue respectively. The corresponding populations on senescing leaves were 1.3×10^4 and 3.8×10^3 cells/cm^2 of leaf tissue. On lettuce grown on ridges the populations of all bacteria were 10- to 100-fold less. After a rain, counts of total pectolytic bacteria on healthy leaves taken from 3- to 5-week-old plants reached 6.1×10^4 cells/cm^2 of leaf surface. Populations on older healthy leaves and senescing leaves were not altered by rain. In soil from under mature nonridged lettuce, the populations of total green fluorescent and total pectolytic bacteria reached 5.3×10^4 and 2.9×10^5 cells/g of oven dry soil, respectively. The corresponding populations in ridged soil were 1.1×10^4 and 8.3×10^5 cells/g of oven dry soil. Usually less than 50% of the pectolytic bacteria isolated from healthy and senescing leaves produced soft rot symptoms within 48 hours on wounded detached lettuce leaves. On healthy and senescing leaves from ridged and nonridged lettuce, the populations of pathogenic green fluorescent bacteria were variable and usually less than 4×10^2 cells/cm^2 of leaf surface. In soil from under ridged and nonridged lettuce the populations of pathogenic pectolytic bacteria were either undetectable or less than 3.1×10^4 cells/g of oven dry soil. Populations of pathogenic green fluorescent bacteria were either undetectable or less than 4.0×10^3 cels/g of oven dry soil.

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Additional key words: pectolytic bacteria, green fluorescent pseudomonads, soft rot bacteria.

The most common soft rot bacteria encountered on decaying vegetables are species of *Erwinia* and green fluorescent pseudomonads. Soft rot bacteria, which produce pectolytic enzymes (16), also are associated frequently with the exterior surfaces (11, 19) and internal tissues of healthy vegetables (9). These bacteria commonly are isolated from soils apart from a host (4, 7, 8, 10). Although the exterior surfaces of healthy vegetables are known to harbor bacteria, the role of soft rot bacteria as part of the normal surface flora is not well understood. Investigations on the enumeration and characterization of soft rot bacteria associated with the surfaces of healthy vegetables under field conditions would provide a better understanding of the epidemiology of bacterial decays under field and market conditions. Because of the frequent occurrence of lettuce decays in the field (2) and in transit, storage, and under market conditions (13), it appears that soilborne soft rot bacteria become established on healthy lettuce leaves. Under favorable field or storage conditions, surface populations of the soft rot bacteria may increase rapidly and cause decay of lettuce tissue. Since soft rot bacteria commonly are associated with decaying lettuce under field conditions in New York (2), and because of their association with lettuce bottom rot (12), this investigation was undertaken to determine the presence of soft rot bacteria on healthy and senescing lettuce leaves under field conditions. Similar determinations were made from soil samples taken from under maturing lettuce plants. This paper reports the results of these investigations.

MATERIALS AND METHODS.—During the summer of 1973, healthy and senescing leaves from lettuce plants *(Lactuca sativa* L. 'Minetto') and soil samples were collected from two farms (organic soil) in Oswego County, New York. Both locations have a long history of lettuce bottom rot (12). On one farm, lettuce was grown on conventional flat beds (nonridged) and on the other farm was grown on raised beds 10 cm (4 in) high and 17 cm (7 in) wide (ridged).

Leaf samples were taken from plants 3-8 weeks old. Because sections of large fields were planted at weekly intervals, it was possible to collect leaf samples from lettuce plants of different ages growing in the same field. A sample consisted of 35-40 leaves taken at random from plants of the same age. The lowest healthy green leaf was taken from each plant sampled. Lower senescing leaves (yellowing leaves) were collected from 7- and 8-week-old plants. Leaf samples were transported to the laboratory in plastic bags and stored at 1 C. All samples were assayed within 24 hours by first brushing the leaves free of organic soil and then by punching out one 2.85 cm^2 disk with a sterile cork borer from the proximal half of each leaf that was in contact with the soil. Disks from thirty-five leaves were placed in 100 ml of sterile distilled water in 500-ml Erlenmeyer flasks and shaken for 60 minutes on a Burrell Wrist-Action shaker. Appropriate dilutions were made from the leaf washings.

Soil samples were collected at random from the same fields that leaf samples were collected. Samples of 400 g were collected from the top 2.5 cm (1 in) of soil beneath 30 plants each at least 6 m (20 ft) apart. The samples were collected at a distance of 2-7 cm (1-3 in) from the base of the plants. The soil was transported to the laboratory in plastic bags and stored at 1 C. All samples were assayed within 24 hours. Each sample was mixed thoroughly and assays were made from duplicate subsamples equivalent in weight to 2 g oven dry soil (100 C for 12 hours). These samples were suspended in 200 ml of water in 500-ml Erlenmeyer flasks and shaken on a Burrell Wrist-Action shaker for 30 minutes. A series of dilutions then was made and each one assayed, but usually only dilutions of 10^3-10^6 were used.

All leaf and soil dilution samples were plated simultaneously on three different media. Because soft rot bacteria generally produce pectolytic enzymes, the ability to hydrolyze pectin was utilized as a tool to enumerate potential soft rot bacteria. The mineral pectin medium described by Hankin et al. (5) was utilized to detect the total population of pectolytic bacteria. The medium described by Sands et al. (14) was used to detect pectolytic green-fluorescent pseudomonads. Because it later was found that this medium did not always give a positive response for known pectolytic green fluorescent pseudomonads in this study, the medium then was used to determine the total populations of green-fluorescent pseudomonads only. Nutrient agar (Difco) was used as a nonselective medium to determine total bacterial populations.

Plates were inoculated by a spread plate procedure instead of the conventional pour plate method because pectolytic enzyme synthesis of pseudomonads is believed to require aerobic conditions (5). A 0.1 ml aliquot of each dilution was added to each plate which was then rotated on a petri dish turn table (Fisher Scientific Co.) and the aliquot was spread evenly over the surface of the medium with a glass rod. Four or five replications were made per treatment. Assay plates were incubated in the dark for 48-72 hours at 27 C. The number of colonies of bacteria per plate were recorded as the number of propagules per cm^2 of leaf tissue or per g of oven dry soil. On the mineral pectin medium all pectolytic colonies from plates with 10-50 such colonies were isolated and streaked onto a second plate. Green-fluorescent pseudomonads from leaf and soil assays were treated similarly using the medium of Sands et al. (14). The plates were incubated for 24-48 hours at 27 C prior to making pathogenicity tests.

The pectolytic bacteria and green-fluorescent pseudomonads were stab inoculated onto disinfested lettuce leaves to determine the percentage of these bacteria that were pathogenic. The healthy lettuce leaves were disinfested and maintained in casserole dishes as previously described (12). A wire grating was placed over each leaf, and the leaves were stab-inoculated at 2.5 cm (1 in) intervals on a square pattern so that 25-30 colonies could be tested per leaf. The leaves were incubated at 27 C

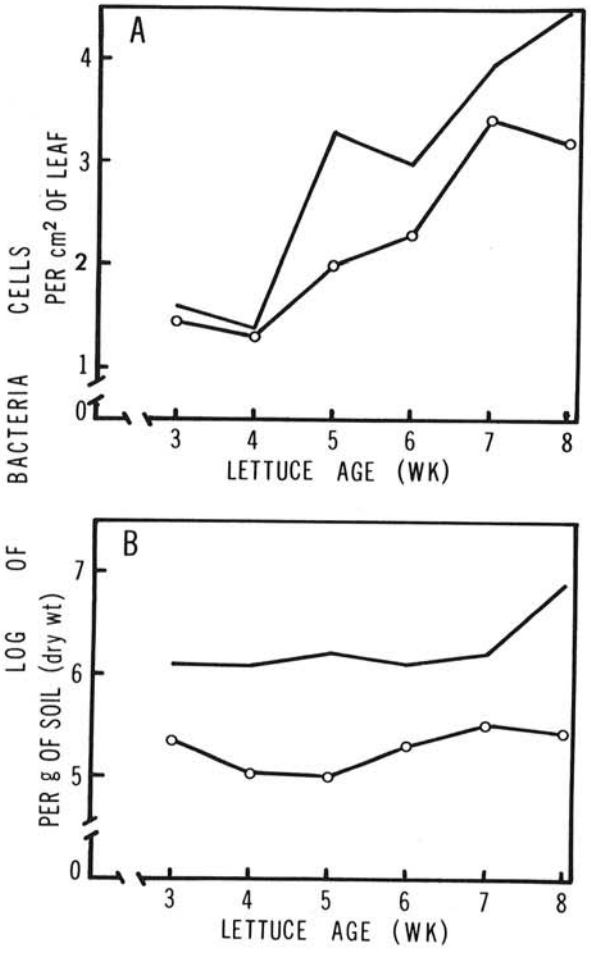

Fig. 1-(A,B). Relative populations of total bacteria (———) and total pectolytic bacteria (—o—) in nonridged lettuce fields. **A)** Populations on lower leaves of lettuce plants at different ages (population levels of total pectolytic bacteria also shown in Table 3). **B)** populations in soil taken from under plants of various ages.

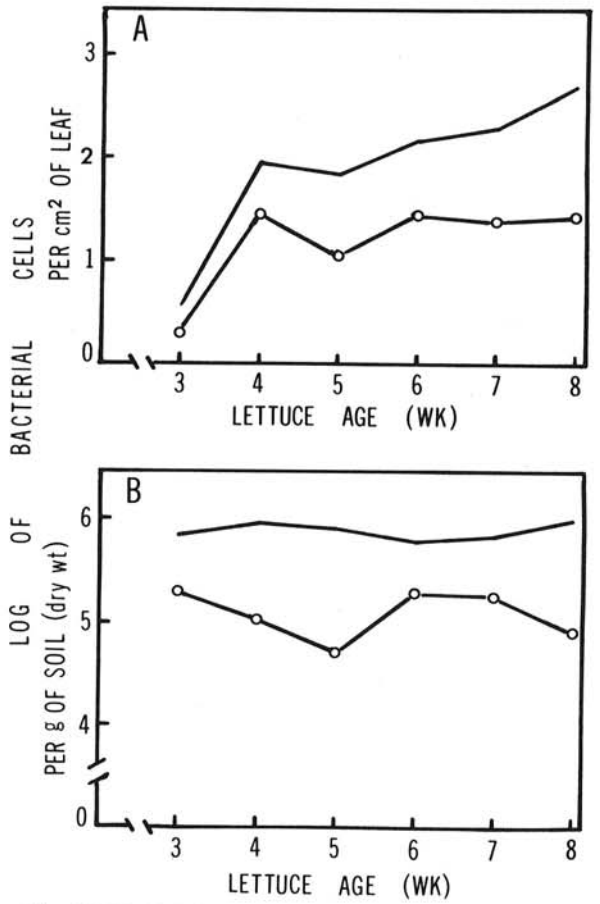

Fig. 2-(A,B). Relative populations of total bacteria (———) and total pectolytic bacteria (—o—) in ridged lettuce fields. **A)** Populations on lower leaves of lettuce plants at different ages. **B)** Populations in soil taken from under plants of various ages.

under moist conditions and observed every 24 hours up to 4 days. Isolates producing large expanding lesions were considered pathogenic. Isolates that produced browning at the point of inoculation or small brown or black lesions that did not expand were considered nonpathogenic.

RESULTS.—The populations of total bacteria and total pectolytic bacteria were greatest on leaves of older plants, greater on senescent than on healthy leaves, and greater on leaves from nonridged lettuce than from ridged lettuce (Fig. 1, 2). In all observations, the populations of total bacteria and total pectolytic bacteria increased on lower leaves as the plants matured.

On nonridged lettuce (Fig. 1-A) there was a 10^3-fold increase in total bacteria on the lower leaves from 3- to 8-week-old lettuce plants. This was paralleled by a 10^2-fold increase in total pectolytic bacteria. Assays of soil samples collected from under nonridged lettuce revealed a more stable population as the lettuce matured (Fig. 1-B). Soil samples from under 8-week-old plants showed nearly a 10-fold increase in total bacteria compared to the 7-week samples (Fig. 1-B). The population of total pectolytic bacteria in the soil also remained fairly constant at 10^5 cells/g of oven dry soil and increased only slightly as the lettuce matured (Fig. 1-B).

Leaf samples from ridged lettuce (Fig. 2-A) also yielded higher counts of total bacteria as plants matured; however, the 10^2-fold increase was less than observed on nonridged lettuce leaves. Total pectolytic bacteria on ridged lettuce also increased, but reached a final population on mature lettuce that was 10^2-fold less than corresponding populations on nonridged lettuce. On both ridged and nonridged lettuce, counts of total bacteria on senescing leaves reached 10^5 cells/cm^2 of leaf tissue. Both total and pectolytic bacteria in soil collected from under ridged lettuce remained relatively constant.

On ridged and nonridged lettuce, apparently because of the dry conditions, the total populations of green-fluorescent bacteria were low on leaves from 3- to 6-week-old plants (Tables 1, 2). On mature nonridged lettuce, populations as high as 4.9×10^4 cells/cm^2 on leaf tissue and 5.3×10^4 cells/g in soil samples were detected. On ridged lettuce, populations reached 3.0×10^3 cells/cm^2 on mature leaves and 1.1×10^4 cells/g in soil from under maturing plants.

During July and August 1973 the lettuce fields in Oswego County generally were drier than usual. However, on one occasion samples were collected one day after a heavy rain. In this instance, populations of both total and pathogenic pectolytic bacteria increased 10- to 100-fold on leaves not touching the ground (Table 3). The populations on the lower leaves of older plants were not greatly altered.

The results of the pathogenicity tests were variable and a low percentage of the total pectolytic bacteria and total green-fluorescent pseudomonads from leaf and soil samples were able to decay lettuce tissue (Tables 1, 2, 3). The greatest number of pathogenic pectolytic and green-fluorescent bacteria were associated with older healthy leaves in contact with the soil, senescing leaves and soil from under 6- to 8-week-old plants. Leaf and soil samples from nonridged lettuce yielded a higher percentage of the pathogenic green-fluorescent bacteria than corresponding samples from ridged lettuce (Tables 1, 2).

TABLE 1. Populations of total and pathogenic green-fluorescent pseudomonads on leaf and soil samples from a nonridged lettuce field

Samples	Age of lettuce[b]	Green Fluorescent Pseudomonads[a]	
		Total population	Pathogenic
Leaves	3	4.3×10^1	0
	4	2.5×10^2	0
	5	4.0×10^2	0
	6	2.0×10^1	0
	7	2.6×10^4	0
	8	4.9×10^4	2.0×10^3
	Senescing	1.3×10^4	0
Soil	3	0	0
	4	3.9×10^3	2.8×10^3
	5	0	0
	6	9.6×10^3	0
	7	2.1×10^4	1.1×10^3
	8	5.3×10^4	4.0×10^3

[a]Data presented as number of bacteria per cm^2 of leaf tissue or as number of bacteria per g oven dry soil.
[b]Weeks.

TABLE 2. Populations of total and pathogenic green-fluorescent pseudomonads on leaf and soil samples from a ridged lettuce field

Samples	Age of lettuce[b]	Green Fluorescent Pseudomonads[a]	
		Total population (no.)	Pathogenic (no.)
Leaves	3	0	0
	4	7.6×10^1	0
	5	5.0×10^1	0
	6	1.0×10^2	0
	7	3.2×10^3	4.0×10^2
	8	3.0×10^3	4.8×10^1
	Senescing	1.1×10^4	3.0×10^2
Soil	3	1.7×10^3	0
	4	1.0×10^3	0
	5	6.3×10^3	0
	6	6.8×10^3	5.0×10^2
	7	1.1×10^4	0
	8	6.0×10^3	4.3×10^2

[a]Data presented as number of bacteria per cm^2 of leaf tissue or as number of bacteria per g oven dry soil.
[b]Weeks.

The same situation occurred for pathogenic pectolytic bacteria (Table 3, Fig. 2). Populations on samples taken from 7-week-old and senescing leaves of lettuce grown on ridges were two and 50 colonies/cm^2 of leaf tissue, respectively (latter data not shown in Fig. 2).

DISCUSSION.—The trends in the number of total bacteria, total pectolytic bacteria and total green-fluorescent pseudomonads, indicate that conditions under mature lettuce plants favor bacterial development. This was most striking on leaves from nonridged lettuce. Contact of healthy leaves with procumbent senescing leaves and the soil apparently is a governing factor in altering bacterial populations. In nonridged lettuce fields healthy, senescent and decomposing leaves in contact

TABLE 3. Populations of pectolytic bacteria and pathogenic bacteria on leaf samples from a nonridged lettuce field

Age of lettuce[b]	Pectolytic bacteria[a]	
	Total population[d] (no.)	Pathogenic (no.)
3	3.0×10^1	0
3[c]	3.7×10^3	1.9×10^3
4	2.1×10^2	2.0×10^1
4[c]	5.3×10^3	1.8×10^2
5	1.7×10^2	0
5[c]	6.1×10^4	1.6×10^4
6	2.0×10^2	1.5×10^1
6[c]	4.3×10^2	4.1×10^1
7	2.7×10^3	6.0×10^2
7[c]	3.3×10^3	2.3×10^2
8	1.4×10^3	3.0×10^2
Senescing	3.8×10^3	1.3×10^3

[a]Data presented as number of bacteria per cm^2 of leaf tissue.
[b]Weeks.
[c]Data taken after a rain.
[d]The data for total population of pectolytic bacteria prior to the rain also is presented in graphic form in Fig. 1-A. It is shown here for comparative purposes with population levels following the rain and population levels of pathogenic pectolytic bacteria.

with the soil are often moist. The restricted air movement and resulting high relative humidity (18) under maturing plants contribute to the maintenance of the moist conditions under these plants. This combination of moist environment and available substrate (senescing leaves) is favorable for bacterial growth. The lower populations of bacteria on leaves from ridged lettuce were associated with the drier conditions resulting from better air circulation under ridged plants. The rapid rise in bacterial populations on young healthy leaves of nonridged lettuce observed after a rain may have resulted from the large amounts of bacterial infested organic soil splashed onto the leaves.

The populations of total bacteria and total pectolytic bacteria in the soil from under plants remained fairly stable as plants matured. Under nonridged lettuce, a slight rise in the number of total bacteria and total pectolytic bacteria was detected under maturing lettuce. This rise probably represented the response to introduction of plant materials into the soil which temporarily stimulated the growth of the soil microflora. The less dramatic changes in numbers of bacteria on and under plants on ridged soil may be related to the drier conditions created by this cultural practice.

Green-fluorescent *Pseudomonas* spp. represent one of the predominant groups of bacteria in soils (3, 6). In this study, populations as high as 5.3×10^4 cells/g of soil were detected under mature lettuce plants. Sands and Rovira (14) isolated as many as 1.8×10^2 cells/g of green-fluorescent pseudomonads from a red-brown soil in Australia. The higher populations reported in the present study may have resulted from the relative conditions of higher moisture and organic matter in the New York organic soils sampled. Populations of total green fluorescent bacteria on leaf surfaces in the present study were as high as 4.9×10^4 cells/cm^2 of leaf. Green-fluorescent *Pseudomonas* spp. are known to be abundant on leaf surfaces of herbaceous plants (1), but little information is available on direct enumerations of this group of bacteria on leaf surfaces of vegetables.

The data presented in this study indicate that pathogenic green-fluorescent and pectolytic bacteria are present on healthy leaves and in the surrounding soil. However, the populations of the pathogenic bacteria appear low and variable as detected by the methods used. This may be due to other factors in addition to the dry conditions encountered during this study. Meneley and Stanghellini (9) isolated soft rot bacteria from healthy cucumbers by utilizing their ability to hydrolyze pectin and macerate potato and cucumber slices. They found that the bacteria produced soft rot on cucumber only after high-temperature (37 C) incubation of cucumbers or inoculation with *Pythium aphanidermatum*. It is possible that a higher percentage of the total pectolytic bacteria from lettuce leaves and soil may have produced soft rot symptoms on excised leaves if "activated" in a similar manner. Since soft rot bacteria commonly were found in *Rhizoctonia solani* infected plants (12) it is possible that this fungus provides conditions that stimulate the growth of soft rot bacteria.

Although the populations of pathogenic green-fluorescent pseudomonads and pectolytic bacteria detected in this study were low and the incidence of bacterial decay similarly was low, the potential of these low populations cannot be overlooked. Provided with proper conditions, such as free moisture and available nutrients, populations may reach significant levels in a short time. If wounding of leaves occurred, these organisms then would further be favored in a selective habitat. A low inoculum density of these soft rot bacteria in the proper location under favorable environmental conditions can be as destructive as a large population. This was revealed by Telneset (17) who showed that approximately 40 cells of *E. carotovora* in 0.5 ml of water on a wounded potato tuber caused as much decay as 4×10^6 cells when tubers were incubated for 72 hours at 27 C.

The combination of moist environment, organic soil and senescing leaves under maturing lettuce plants undoubtedly provides conditions suitable for the development of soft rot bacteria. When healthy tissue is wounded, or infected by other pathogens, the bacteria can enter and multiply under moist conditions. It has been shown in a previous study (12) that lettuce soft rot bacteria acting either alone on wounded lettuce leaves or in conjunction with *R. solani*, can cause a rapid decay of lettuce tissue. By controlling fungal pathogens with fungicides, and by using cultural practices that maintain dry conditions under lettuce plants to suppress bacterial development, the potential destruction of lettuce under field and market conditions may be reduced.

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