APPLIED MICROBIOLOGY, Dec. 1974, p. 987-991
Vol. 28, No.6

Printed in U.S.A.

Agricultural Plants and Soil as a Reservoir for *Pseudomonas aeruginosa*

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Received for publication 15 August 1974

Pseudomonas aeruginosa was detected in 24% of the soil samples but in only 0.13% of the vegetable samples from various agricultural areas of California. The distribution of pyocin types of soil and vegetable isolates was similar to that of clinical strains, and three of the soil isolates were resistant to carbenicillin. *Pseudomonas aeruginosa* multiplied in lettuce and bean under conditions of high temperature and high relative humidity (27 C and 80–95% relative humidity) but declined when the temperature and humidity were lowered (16 C, 55–75% relative humidity). The results suggest that soil is a reservior for *P. aeruginosa* and that the bacterium has the capacity to colonize plants during favorable conditions of temperature and moisture.

Pseudomonas aeruginosa is currently considered one of the most frequent causative agents of hospital-associated infections and therefore has been the subject of many epidemiological investigations. Studies on the sources and vehicles by which the organism is transferred to patients have previously been limited to the immediate environment of patients inside the hospital. Such implicated sources include sinks, solutions and creams, hands of personnel, and inhalation and resuscitation equipment (4, 5, 9, 12, 13, 16).

Little attention was given to natural sources contributing to disease until Kominos et al. (10) found high counts of *P. aeruginosa* in raw vegetables in the hospital kitchen and suggested that these foods could act as primary vehicles for introducing the organism to patients. Their studies were motivated from the findings of Shooter et al. (15, 16), who found that foods and salads in hospital kitchens contained *Escherichia coli*, *P. aeruginosa*, and *Klebsiella* species and showed that patients could acquire these bacteria in their intestinal flora. Ornamental potted plants (J. J. Cho, S. K. Green, M. N. Schroth, and S. D. Kominos, Phytopathology **63**:1215, 1973) were also found to harbor *P. aeruginosa*, indicating that plants may serve as a natural reservoir in nature.

Significant decrease of *Pseudomonas* infection in burn patients was noted by S. D. Kominos (unpublished data) following the elimination of fresh vegetables from the diet. The pyocin types of *P. aeruginosa* from vegetables were distributed similarly to those isolated from clinical specimens.

The finding that consumption of vegetables in hospitals could be a means by which patients contract *P. aeruginosa* suggested a study to determine whether agricultural soils act as natural reservoirs of this bacterium. It also seemed important to investigate whether food plants are commonly colonized by *P. aeruginosa* from soil in the field, or if this might occur during harvest, or during a later processing stage, before reaching the consumer.

This report described the occurrence of *P. aeruginosa* in agricultural soils and plants and the capacity of clinical isolates to survive in plants during various environmental conditions.

MATERIALS AND METHODS

Field isolations. Soil and vegetable material were sampled at five randomly selected sites from 58 different locations in various agricultural areas of central California during the summer of 1973. Soil samples from each field were collected to a depth of 30 cm, combined, and thoroughly mixed. Fifty grams of each soil sample was placed in 100 ml of sterile distilled water (pH 7.2) and shaken for 1 h at 300 rpm. A portion (0.5 ml) of each soil suspension was then added to 4.5 ml of broth consisting of acetamide and salts (17). Tenfold dilutions to 10^{-4} were made, and after 48 h of incubation at 42 C, 0.1 ml of each dilution was plated on King medium B (8) with 0.03% cetrimide added (KBC) (2), and the plates were incubated at 42 C.

The soils sampled in counties 1 to 5 were a sandy loam with pH values varying between 7.0 and 7.6. The soils sampled in county 6 were a loamy sand with a pH of 7.3. The irrigation water in counties 1, 2, 4 and 6 was from wells and in counties 3 and 5 from reservoirs. The water was delivered to the fields by pipes and was determined to be free of *P. aeruginosa* in all cases (30 samples) by the previously described isolation techniques. Fertilization of the fields was by inorganic fertilizers.

Isolation from vegetable samples. In most instances, samples of plant material were taken from five different plants at five random sites per field so that a total of 25 plants was sampled from each field.

The following parts were assayed: tomato, one friut and one leaf per plant; lettuce, five outer leaves and the heart per plant; celery, three stalks (two from outside, one from inside) and the heart per plant; cauliflower, two leaves and the head of one plant (three different plants were sampled at each random site); and spinach, one leaf per plant (10 different plants were sampled at each random site).

The vegetable samples were sliced when necessary and placed individually into flasks containing phosphate-buffered (0.01 M) acetamide broth (pH 7.0).

Isolation and identification. All cultures were incubated for 48 to 96 h at 42 C to allow growth of *P. aeruginosa* while inhibiting saprophytic bacteria (7). Cultures grown in acetamide broth and on KBC agar which fluoresced with an ultraviolet lamp were suspected to be *P. aeruginosa*. Representative yellow or blue-green fluorescent colonies from each sample were isolated on King medium B and confirmed as *P. aeruginosa* by the characterization scheme of Gilardi (6).

Sensitivity of the isolation procedure. Samples of soil, a clay loam and a sandy loam with a pH of 7.0 and 7.2, respectively, were seeded with *P. aeruginosa* at a range of 1 to 10, 10 to 100, and 100 to 500 cells per 50 g of soil and processed as previously described. Lettuce, celery, and tomato fruits were similarly tested. Five replications were used per treatment. After 4 days of incubation, *P. aeruginosa* was recovered from all flasks seeded with *P. aeruginosa* at the 1 to 10 range. *P. aeruginosa* was not recovered from unseeded control flasks consisting of soil or vegetable material.

Inoculation of plants. Lettuce and bean plants were inoculated with *P. aeruginosa* to determine whether or not the bacterium multiplied in plant tissues and to determine how long it persisted under different environmental conditions. A clinical strain of *P. aeruginosa* (PA 8) grown overnight on King B slants was used in all experiments.

Leaves of lettuce plants (*Lactuca sativa* L., "Great Lake"), at the six-leaf stage, were infiltrated under vacuum with a cell suspension of 7×10^6 cells/ml of *P. aeruginosa*. This was done by placing the potted plants upside down in a vacuum chamber. The leaves were completely immersed in the inoculum, and the pots were held by a platform with aluminum foil placed across the soil surface to prevent soil loss. A vacuum of 710 mm of Hg was obtained, then released, at which time the leaves became thoroughly infiltrated with the inoculum, giving the leaves a water-soaked appearance. Control leaves were infiltrated with water. The plants were then grown in greenhouse chambers at 16 and 27 C, and at relative humidities fluctuating between 55 to 75% and 80 to 95%. The population of the bacterium was measured at 0, 1, 2, 3, 4, 6, 8, 10, 13, 16, 20, 25 and 30 days after inoculation by cutting one 16-mm disk from each of five infiltrated leaves per plant. The disks were rinsed three times in sterile distilled water to remove most of the bacteria adhering to the surface and then ground with a mortar and pestle in 5 ml of sterile distilled water. Three 0.1-ml portions from each dilution of a 10-fold dilution series were plated on KBC. Estimates of the bacterial populations were made after incubation at 42 C for 48 h. The above experiments were repeated five times, using three plants per treatment.

Bean plants (*Phaseolus vulgaris* L., "Pinto") were infiltrated when the primary leaves were approximately 9 cm long. The plants were infiltrated with 7×10^6 cells of bacteria per ml and maintained under the same environmental conditions as the lettuce plants. Two 5-mm disks were cut from primary leaves of each plant and then processed as in the lettuce experiments. The experiments were repeated twice with three plants per treatment.

To insure that any increase in bacterial population in the above experiment was a reflection of bacterial multiplication in the intercellular spaces of the plants and not on the plant surface, one disk from each of the previously described treatments was imprinted on KBC and removed. There was never more than five colony-forming units per disk except at the cut edges of the disks. As a further check to differentiate between multiplication of *P. aeruginosa* in the intercellular spaces and the leaf surface, the entire experiment with lettuce was conducted twice as previously described, with the exception that the leaf disks were immersed in 0.5% sodium hypochlorite for 1 min to surface sterilize them, then air-dried before plating on KBC. There was no apparent difference when comparing data from experiments where the disks were surface treated with sodium hypochlorite or washed with water.

Pyocin typing. Strains of *P. aeruginosa* were differentiated by the method of Darrell and Wahba (3) as modified by Zabransky and Day (18). A pyocin producing isolate of *P. aeruginosa* was defined as a certain type by the inhibition produced against 11 indicator strains of *P. aeruginosa*.

Sensitivity testing. Antimicrobial susceptibility to carbenicillin was determined by the disk diffusion method (1).

RESULTS

Pseudomonas aeruginosa was recovered from 24% (14 out of 58) of the soil samples (Table 1), and of these positive samples 71% (10 out of 14) were isolated from county 1. Soils in which tomatoes were grown yielded the highest frequency of isolation; *P. aeruginosa* was present

TABLE 1. *Isolation of Pseudomonas aeruginosa from soil and plant material*

County	Crop	No. of samples positive/total		
		Soil	Foliage	Fruit and flowers
1	Tomato	10/17	1/425	0/425
	Onion	0/1		
2	Lettuce	0/12	0/200	
	Celery	1/9	1/175	
	Cauliflower	0/3	0/45	
	Potato	0/1		
	Cabbage	0/1		
	Broccoli	0/1		
	Spinach	0/1	0/50	
	Onion	0/1		
3	Tomato	0/4	0/100	
	Corn	0/1		
4	Tomato	1/2	0/50	0/50
5	Tomato	0/1	0/25	
	Garlic	0/1		
6	Cotton	1/1		
	Corn	1/1		

in 45% (11 out of 24) of the tomato fields. Only 11% (1 out of 9) of the celery soils contained *P. aeruginosa*. The bacterium was not recovered from lettuce (0 out of 12) or cauliflower fields (0 out of 3).

Of the remaining fields assayed, representing nine different crops in five counties, *P. aeruginosa* was only detected in one cotton and one corn field, both from county 6. Twenty-nine percent of the soils that were positive for *P. aeruginosa* contained more than 100 *P. aeruginosa* cells/g (dry weight) of soil (Table 2).

Pseudomonas aeruginosa was recovered only twice from plant material collected from 43 fields (Table 1). It was isolated from one tomato leaf from a field in which *P. aeruginosa* was detected in the soil and from one celery plant in a field where *P. aeruginosa* was not isolated from soil, even though a total of 10 random soil samples were taken, instead of the usual 5 (Table 1).

Table 3 shows the pyocin types of *P. aeruginosa* isolated from soil and plants. Type D-2 and B-7 were the most frequently recovered, but nontypeable strains of *P. aeruginosa* were also commonly isolated from soil. Other types occasionally recovered were D-5, D-9, K, X-1 and X-10. The two types isolated from plants were B-7 from tomato and S from celery. Of these 34 isolates, 22 were sensitive to carbenicillin, 9 exhibited intermediate resistance, and 3 were resistant.

Moderately high temperatures and humidities appeared to favor colonization and survival of *P. aeruginosa* within plant tissue (Fig. 1). At 27 C and 80–95% relative humidity *P. aeruginosa* multiplied within the plant tissue and caused a rot of the leaves. The leaves began to yellow 2 to 3 days after inoculation, concomitant with bacterial multiplication. The bacterial population reached its highest level shortly before the leaves wilted (8 to 10 days). Water-infiltrated plants maintained under the same conditions but not inoculated with the bacterium began to yellow approximately 13 days after treatment because of the unfavorable effect of high temperature and humidity on lettuce growth.

When plants were maintained at high temperature and low humidity, the population of *P. aeruginosa* remained almost constant for about 15 days before slowly decreasing (Fig. 1). A slight yellowing of the infiltrated leaves was observed after 10 to 15 days. Control plants infiltrated with water showed no symptoms

TABLE 2. *Population of Pseudomonas aeruginosa isolated from soil and plant material*

County	Crop	No. positive	No. of samples with indicated colony counts[a]		
			10^0–10^1	10^1–10^2	10^2–10^3
1	Tomato (soil)	10	2	4	4
1	Tomato (leaf)	1[b]			
2	Celery (soil)	1	1		
2	Celery (stalk)	1[b]			
4	Tomato (soil)	1	1		
6	Cotton (soil)	1	1		
6	Corn (soil)	1		1	

[a] Per gram of dry weight.
[b] Count not determined.

TABLE 3. *Pyocin types of Pseudomonas aeruginosa isolated from soil and plant material*

Crop	No. of isolates typed	Pyocin types								
		B-7	D-2	D-5	D-9	K	S	X-1	X-10	NT[a]
Tomato (soil)	28	7	10	1		1		3	1	5
Tomato (leaf)	1	1								
Cotton (soil)	1				1					
Corn (soil)	2	1								1
Celery (soil)	1		1							
Celery (stalk)	1						1			

[a] NT, Nontypables; no inhibition of indicator strains.

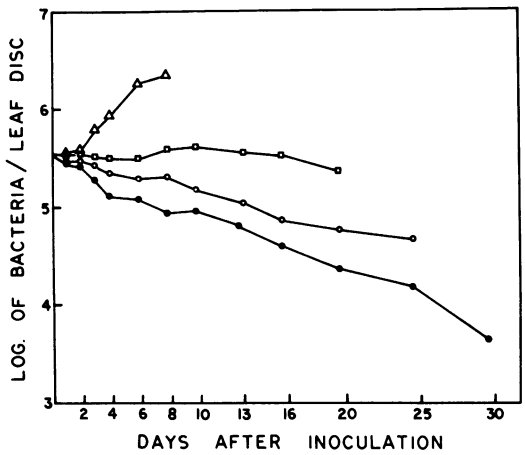

FIG. 1. *Growth curve of P. aeruginosa in leaves of lettuce plants at different environmental conditions: ●, 16 C, 55–75% relative humidity; ○, 16 C, 85–95% relative humidity; □, 27 C, 55–75% relative humidity, △, 27 C, 85–95% relative humidity. Each point represents 15 replications.*

during the experiment. The population of *P. aeruginosa* decreased after infiltration to plants that were grown at 16 C, although the rate of decrease was considerably lower when plants were maintained at a high relative humidity (Fig. 1). At this low temperature, there was no evidence of injury to leaves infiltrated with bacteria or water when the plants were incubated at either high or low humidity.

The survival and multiplication of *P. aeruginosa* within bean tissues (Fig. 2) under different environmental conditions were similar to that in infiltrated lettuce leaves. The leaves wilted after inoculation with bacteria when grown at high temperature and high humidity. Otherwise, there were no symptoms of injury.

DISCUSSION

Pseudomonas aeruginosa was recovered from 24% (14 out of 58) of the California soils tested, which suggests that it is fairly common soil-borne bacterium in agricultural soils. That these soils are a natural habitat for the bacterium is supported by the facts that there was no history of organic fertilizers being used on the fields, no pasturing of animals, and irrigation water was free of the bacterium. Although various textbooks cite soil as a common source for the bacterium, we, as well as Ringen and Drake (14), could find little published data to support the statements. Furthermore, Ringen and Drake (15) found *P. aeruginosa* in only 3 of 100 soil samples and concluded that "its natural habitat, in part at least, is human feces and sewage." Our greater percentage of detection of *P. aeruginosa* from soil is presumably a reflection of improved isolation techniques.

We have no explanation for the sporadic occurrence of *P. aeruginosa* in the different localities. It did not appear to be related to soil type, pH range, or fertilization practices. It was surprising that such a high percentage of soil was found to contain *P. aeruginosa* when considering the relatively few samplings per field. Many microorganisms are not evenly distributed throughout soil and occur in clumps or pockets of soil associated with a food base. This may be the case with *P. aeruginosa*, and thus its occurrence in soils could well be even more prevalent than indicated by our detection methods.

Pyocin typing of *P. aeruginosa* showed that soil contained a variety of strains, many of which had the same pyocin production pattern as clinical strains. The most commonly isolated types of *P. aeruginosa* from clinical specimens, B-7 and D-2 (10), were also the types most frequently detected in soil. This suggests that soil constitutes a possible reservoir for strains of *P. aeruginosa* found in humans. The other types, D-5, D-9, K, X-1, X-10 and nontypables, have infrequently been recovered from clinical specimens (10).

Of particular importance was the isolation of three carbenicillin-resistant strains of *P. aeruginosa* from soil. Carbenicillin-resistant strains have also been isolated from vegetables

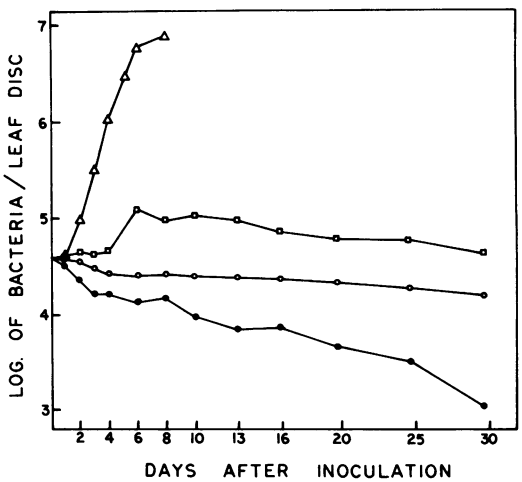

FIG. 2. *Growth of P. aeruginosa in leaves of pinto beans at various environmental conditions: ●, 16 C, 55-75% relative humidity; ○, 16 C, 85-95% relative humidity; □, 27 C, 55-75% relative humidity; △, 27 C, 85–95% relative humidity. Each point represents six replications.*

served to patients (S. D. Kominos, unpublished data). Such resistant strains may be selected through antibiotic therapy and thus lead to further complications of the infectious process. Lowbury et al. (11) demonstrated the emergence of highly resistant strains of *P. aeruginosa* following carbenicillin treatment of hospitalized patients.

Kominos et al. (10) showed that fresh vegetables commonly carried strains of *P. aeruginosa* and considered them to be a major vehicle for the dissemination of this bacterium. However, in this investigation, *P. aeruginosa* was only found twice in plants sampled in the field—from a tomato leaf (type B-7) and from a celery plant (type S). It was surprising that the recovery of *P. aeruginosa* from the celery plant was in a field where it was not detected in the soil. Most likely, however, it was present in soil but was not detected.

The rare finding of *P. aeruginosa* on vegetables in California, even though present in various soils, raises questions as to how the relatively high percentage of vegetables sampled by Kominos et al. (10) became colonized by *P. aeruginosa*. If plants are not widely colonized by *P. aeruginosa* in growing fields, as this study suggests, then contamination may occur during harvest, handling, processing, and transit. Vegetables may come in contact with soil, insects, humans, and other sources of *P. aeruginosa*, thereby acquiring the organism. Colonization would thus occur rapidly, if, during processing and transit, there were periods when temperature and moisture favored bacterial growth. This concept is strengthened by the findings of Kominos et al. (10), which showed that transported vegetables (especially tomatoes) contained large numbers of *P. aeruginosa*.

The incidence of *P. aeruginosa* in plants and soils from California and other states should be studied for several years, however, before concluding that this organism is an infrequent colonizer of plant materials in farm lands. The environment differs substantially among years, and, as with common bacterial diseases of California plants, there are years when conditions do not favor bacterial infection. The California climate, in general, would not seem to be conducive for *P. aeruginosa* to colonize plants because of the semiarid conditions where most crops are grown. Climates where a high humidity is common, with frequent rainfall, would appear ideal for *P. aeruginosa* invasion of plants. Sprinkler irrigation would also appear to favor *P. aeruginosa*, since splashing water could carry the bacterium to above-ground plant parts where they could enter the plant through water-congested areas. All the fields tested in this study were furrow irrigated and situated in an area where the average summer rainfall was negligible.

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