

Lognormal Distribution of Bacterial Populations in the Rhizosphere

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ABSTRACT

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Rhizosphere bacterial populations were estimated on 43 sets of up to 60 entire root systems of young greenhouse and field-grown plants (potato, sugar beet, barley, tomato, or radish). Total aerobic bacterial populations, total fluorescent bacterial populations, and populations of inoculated plant growth-promoting strains of *Pseudomonas putida* or *P. fluorescens* generally approximated a lognormal distribution as determined by several graphic and statistical tests of normality (Rankit diagrams, Shapiro-Wilk tests, and Kolmogorov tests). Total bacterial populations and populations

of fluorescent pseudomonads varied by a factor of 10 to 50 within a given set of root systems. Seed piece or seed populations of inoculated rhizobacterial strains A1 or SH5 varied by a factor of 4-34, respectively, whereas rhizosphere populations of these strains on potato or sugar beet varied by a factor of 100-1,000. Populations of these inoculated strains of *Pseudomonas* on seed or seed pieces also approximated a lognormal distribution.

Rhizosphere bacteria may influence plant growth directly or indirectly, as exemplified by those altering nutrient availability, soil structure, composition of rhizosphere microflora, or producing phytotoxic metabolites. The ability of a bacterial strain to colonize, or establish a large population size in the rhizosphere, is a critical factor determining its importance as a root associate. Colonization, as such, is commonly determined by quantifying population size (hereafter referred to as populations) of rhizosphere bacteria at one or more points during the growth of host plants.

Quantitative studies of rhizosphere bacterial populations must address the distribution of these populations among individual plants. Although bacterial cells are discrete units, representation of large bacterial populations by a continuous distribution is a convenient approximation that facilitates mathematical and statistical analysis. The finding that the continuous lognormal distribution approximates populations of foliar epiphytic bacteria has provided insight into the epidemiology of these bacteria and a basis for the development of appropriate sampling techniques (12). A distributional analysis of rhizosphere bacterial populations is prerequisite to an understanding of the dynamic processes determining these populations.

Populations of rhizosphere bacteria are often estimated by gently shaking the entire root system or a portion thereof to remove loosely adhering soil, placing it in a known volume of sterile water or buffer solution, shaking vigorously for a given period of time, and dilution plating the washings on appropriate agar media (15). In some cases, samples are composed of more than one root system (5,23), a soil core sample, or a portion of known fresh weight or length selected at random from a single root system (7,18,20,21). Bacterial populations are generally expressed in terms of colony-forming units (cfu) per gram of root, per centimeter of root length, or per gram of soil.

Large variabilities in rhizosphere bacterial populations among root systems sampled within a single agricultural field have been reported (27) and must be considered in studies designed to quantify and compare rhizosphere populations among plants or treatments. Such studies have traditionally used standard analysis of variance procedures for mean separation of rhizobacterial

populations, relying upon several assumptions for their validity. Among these assumptions are the independence of the variances and means, normal distribution of experimental error terms, homogeneity of variances and additivity of treatment and error effects (8,9).

This study was initiated to quantify the variability and distribution of rhizosphere bacterial populations among individual plants of five agricultural species, and to test the first two assumptions of the standard analysis of variance procedures listed above. The logarithmic transformation (base 10) of bacterial population data was considered in testing these assumptions. Of special interest was the variability and distribution of rhizosphere populations of specific plant growth-promoting rhizobacterial strains used as seed inoculants in field and greenhouse experiments (6,21,31).

MATERIALS AND METHODS

Bacterial strains and inoculum preparation. *Pseudomonas putida*-*P. fluorescens* strains A1, B10, E6, and SH5 are plant growth-promoting strains of sugar beets or potatoes as determined from replicated field trials carried out in California and Idaho over a 3-yr period (6,21,31). Spontaneous mutants of these strains, selected on the basis of their stable resistance to rifampicin (Sigma Chemical Company, St. Louis, MO) at $100 \mu\text{g}\cdot\text{ml}^{-1}$ were used as inoculum in these studies.

Inoculum for seed or seed piece treatment was prepared as described previously (19,31) and used to coat sugar beet (*Beta vulgaris* L. convar. *crassa* (Alef.) J. Helm 'USH11') or tomato (*Lycopersicon esculentum* L. 'Bonnie Best') seed or potato (*Solanum tuberosum* L. 'White Rose') seed pieces. Single-eye potato tuber pieces were cut to standard size (mean fresh weight of 8.7 ± 1.2 g), allowed to dry for 2 hr, then shaken in dry bacterial inoculum until evenly covered. The average amount of powdered inoculum on each potato piece was 0.54 g.

Experimental design. In greenhouse experiments, sugar beet or tomato seeds or potato seed pieces were planted into 75-mm-diameter ceramic pots containing an unsterilized field soil (Hesperia fine sandy loam) collected from Shafter, CA. Pots were handwatered or subirrigated with half-strength Hoagland's solution and thinned to one plant per pot after emergence. Plants were grown for 2-3 wk under greenhouse conditions (18-27 C) before sampling. One greenhouse experiment was composed of 49 plants and 32 experiments (data sets) of 10 plants each.

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Field studies were carried out during 1982 or 1983 in experimental field plots (potato and sugar beet) or in commercial fields (radish, [*Raphanus sativus* L.] or barley [*Hordeum vulgare* L.]) located near Bakersfield, Ventura, Davis, Visalia, or Moss Landing, CA. Fields with light sandy loam soils and exhibiting homogeneous moisture and soil conditions were selected as sampling sites in these studies. However, because of the extremely wet 1983 winter, the potato trial in Bakersfield was flooded for 1 wk immediately following emergence. All sets of field data were composed of from 40 to 60 samples collected within a diameter of 15 m in an agricultural field. Each sample comprised the entire, recovered root system of a single plant.

Sampling procedures. Field-grown seedlings were harvested with approximately a 10-cm-diameter core of surrounding soil, thereby avoiding mechanical damage to the root system. Samples with visibly damaged feeder roots were discarded. Root systems and surrounding soil were placed in plastic bags and transported back to the laboratory in an ice chest. The time from sampling to plating ranged from 4 to 24 hr among experiments.

Entire root systems were retrieved from field or greenhouse samples with sterile forceps. A diligent attempt was made to retrieve all roots from each sample. Roots were shaken gently to remove all but the most closely adhering soil, and placed in 25 ml of washing buffer (0.1 M phosphate buffer, pH 7.0, supplemented with 0.1% (w/v) peptone [Difco Laboratories, Detroit, MI]), and shaken (200 rpm) for 30–60 min. Previous studies have indicated that no significant difference in numbers of bacteria present in root washings were detected from 20 min to 2 hr of shaking (J. E. Loper, unpublished). Serial dilutions were carried out to 10^{-8} and plated in duplicate on appropriate agar medium. Total fluorescent pseudomonad populations on seed, seed pieces, or roots were estimated on King's Medium B (KBM) amended after autoclaving with sterile solutions of cycloheximide ($150 \mu\text{g}\cdot\text{ml}^{-1}$, Sigma Chemical Co.), dichlorane (Botran®, active ingredient $100 \mu\text{g}\cdot\text{ml}^{-1}$) and benomyl (Benlate®, $100 \mu\text{g}\cdot\text{ml}^{-1}$). After 4 days incubation at 27 C, colonies of fluorescent bacteria were enumerated on KBM plates under ultraviolet irradiation (366 nm). Inoculated bacterial populations were estimated on KBM supplemented with rifampicin at $100 \mu\text{g}\cdot\text{ml}^{-1}$ and incubated under the same conditions. Total aerobic bacterial populations were estimated on 20% tryptic soy agar (Difco Laboratories) after 7 days of incubation at 21 C.

Plate counts of inoculated bacterial strains of total fluorescent pseudomonads on KBM varied in direct proportion with dilution. However, plate counts of total aerobic bacteria on 20% tryptic soy agar did not decrease proportionately with dilution, presumably due to in vitro antibiosis occurring among the bacterial colonies on the plate itself (22). To address this problem, dilutions were carried out until <20 colonies were detected on each plate, and total aerobic bacterial populations were estimated from counts of these plates. Although the population estimates are based on few colonies per plate, duplicate plate counts were made for each sample and the potentially large influence of in vitro antibiosis on the accurate estimation of rhizosphere populations was minimized.

Root mass was estimated by retrieving each root system from the washing buffer after dilution plating was completed. In most cases, root mass was estimated on a dry weight basis. However, the radish and barley root systems were too small to allow accurate measurement of dry weight. Fresh weight of the fibrous barley root systems, and root length of the unbranched radish root systems were recorded.

Data analysis. Rhizosphere populations of inoculated strains and of fluorescent pseudomonads were estimated from dilution plates with densities ranging from five to 300 colonies per plate. The contribution of each individual plate count to the calculated mean (cfu per root system) was proportional to its dilution from the original root washings. Rhizosphere populations of total aerobic bacteria were estimated directly from duplicate plates with densities of <20 colonies per plate. Bacterial populations were expressed as cfu per gram or centimeter of root tissue, per root system (sample), and as the logarithms (base 10) of these values.

To determine if the normal distribution described the probability

distribution of bacterial populations among individual root systems, estimates from a set of seedlings were ranked, assigned tabular rankit values (11), and plotted against these assigned rankit values. A rankit is defined as the average deviate for each rank order in a sample of N observations, drawn at random from a normal population with $\mu = 0$ and $\sigma = 1$ (4). By hypothesis, a sample drawn from a normal population should differ from tabular rankits (average deviates from a sample of the same size known to be normally distributed) only in scale and by random sampling error. Therefore, samples from a normally distributed population, when arranged in rank order and plotted against tabular rankit values, should approximate a straight line (13). The mean of these samples should correspond to a rankit value of zero and the mean plus or minus one standard deviation should correspond to rankit values of +1 or -1, respectively. The similar linear trend of population estimates in many independent rankit diagrams indicates a general agreement with the normal distribution.

Statistical procedures to test for normality relied upon the Shapiro-Wilk test (for data sets with ≤ 51 samples) (28) or the Kolmogorov test for goodness-of-fit (for data sets with > 51 samples) (30). The Shapiro-Wilk statistic W can be interpreted as the correlation between the spacing of the ordered data and that of the ordered expected values. If the data set is normally distributed, the W value will approach one; as the set departs from normality, W will decrease. P values, describing the probability with which a given W value could be calculated from a normally distributed data set of specified sample size, are given in Shapiro and Wilk (28). The Kolmogorov test statistic D can be interpreted as the deviation between the spacing of the ordered data and that of the ordered expected values. If the data set is normally distributed, the D value will approach zero. As the set departs from normality, D will increase. P values, again describing the confidence level with which one can reject the null hypothesis (thereby rejecting normality of the data set), are given by Stephens (30). A univariate procedure provided by SAS (Statistical Analysis Systems release 79.6, SAS Institute, Inc., Cary, NC) was used to obtain W values, D values and P values for all data sets. P values larger than 0.15 were represented as >0.15 when determined from the Kolmogorov D statistic. The General Linear Models procedure (SAS) was used to obtain F and P values of linear regression using the method of least squares.

RESULTS

Lognormal distribution of bacterial populations in the rhizosphere. *Graphic tests for normality.* The variability and distribution of total aerobic rhizosphere bacterial populations among a set of 53 barley seedlings is described in Fig. 1. When bacterial populations determined from each seedling are expressed as cfu per root system and plotted on a histogram, the population distribution is positively skewed (Fig. 1A). That is, relatively few root systems support high bacterial populations while many root systems harbor relatively low bacterial populations. However, when rhizosphere populations are transformed to \log_{10} values, a more symmetric distribution is observed (Fig. 1B).

The distribution of rhizosphere bacterial populations on this set of barley seedlings was further described as a rankit diagram (Fig. 2). The plot of untransformed population estimates against assigned rankits is curved, whereas the log transformed estimates approximate a straight line, indicating that total aerobic rhizosphere bacterial populations of this set of barley root systems are more closely approximated by a lognormal than a normal probability distribution.

Similar rankit diagrams were constructed for all data sets listed in Table 1. Rankit diagrams are presented here describing populations of fluorescent bacteria on a set of 52 field-grown radish seedlings (Fig. 3), and populations of the inoculated rhizobacterial strain A1 on a set of 60 field-grown potato root systems (Fig. 4). In all cases except one, logtransformed data were approximated by a straight line whereas untransformed data were not. The one exception was with estimates of inoculated strain A1 on root systems harvested from the flooded field in Bakersfield (unpublished).

Statistical tests for normality. Statistical analysis of these data sets by the Shapiro-Wilk test or Kolmogorov test indicated that untransformed population values were not approximated by a normal distribution (Table 1). That is, the null hypothesis stating that a normal distribution can describe bacterial counts was rejected. In contrast, the null hypothesis was not rejected when logtransformed values of these same data sets were analyzed by these tests (except in the data set describing populations of inoculated strain A1 on potato root systems from the flooded

Bakersfield plot). Similarly, the Shapiro-Wilk or Kolmogorov tests indicate that a lognormal distribution can describe the counts of bacterial inoculants, strains SH5 and A1, on sugar beet seed and potato seed pieces, respectively (Table 1). In all cases but one (potato seed piece experiment 1), the normality of untransformed values can be rejected. Population values in this data set are more closely approximated by a lognormal than by a normal distribution.

A uniform distribution of *P* values calculated from a number of data sets of equal sample size would support the hypothesis that a population is normally distributed. The Shapiro-Wilk test for normality was performed on inoculated bacterial populations (expressed as cfu per root system and as \log_{10} [cfu per root system]) of 32 data sets, each comprised of 10 greenhouse-grown plants. A uniform distribution approximates the *P* values obtained from the logtransformed data but not those obtained from the untransformed data of the 32 data sets (Table 2). The null hypothesis could be rejected for only two of the 32 sets of transformed data values.

Rhizosphere bacterial populations of 32 greenhouse data sets and 10 field-collected data sets were approximated by a lognormal distribution as determined by several graphic and statistical tests. Other distributions related to the lognormal were not considered here and may also approximate these bacterial populations.

Quantitative variability of bacterial populations in the rhizosphere. Bacterial populations were highly variable within a given set of root systems (Table 3). Total aerobic bacterial populations varied 153-fold (ranging from 1.5×10^8 to 2.3×10^{10} cfu per root system) among barley root systems and 35-fold (ranging from 1.1×10^7 to 3.8×10^8 cfu per root system) among radish root systems. Fluorescent bacterial populations varied 56-fold (ranging from 2.5×10^6 to 1.4×10^8 cfu per root system) among barley root systems and 52-fold (ranging from 7.7×10^4 to 4.0×10^6 cfu per root system) among radish root systems. Variances of inoculated strains A1 (potato) and SH5 (sugar beet) were generally larger than those of total fluorescent pseudomonad populations. Populations of strain A1 varied 164-fold (ranging from 3.6×10^3 to 5.9×10^5 cfu per root system) among root systems harvested near Ventura. Populations of strain SH5 varied 569-fold (ranging from 7.9×10^3 to 4.5×10^6 cfu per root system) among root systems harvested near

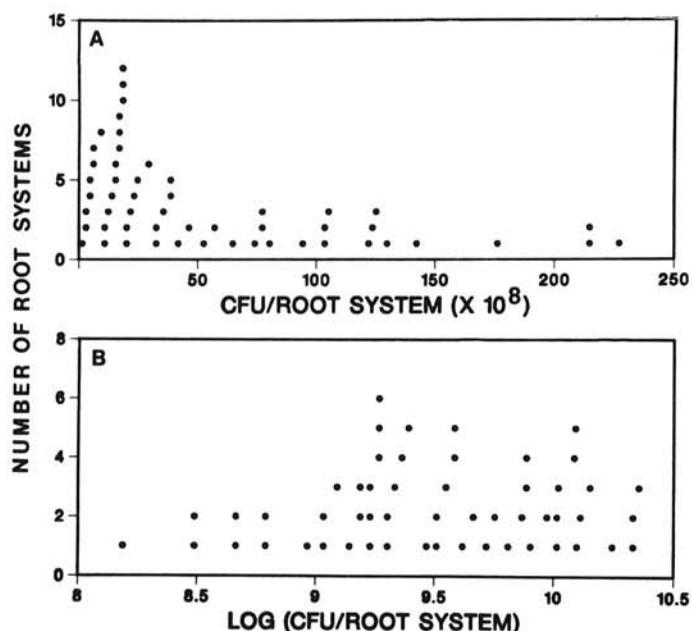


Fig. 1. Histogram of population size of total aerobic rhizosphere bacteria as determined from a set of 53 barley seedlings and expressed as **A**, cfu per root system or **B**, \log_{10} (cfu per root system). Rhizosphere populations of individual root systems are plotted against the number of plants with populations within increments of **A**, 10×10^8 cfu per root system, or **B**, 0.1 \log_{10} (cfu per root system).

TABLE 1. Normality of each of 11 data sets of rhizosphere bacterial populations as determined by the Shapiro-Wilk or Kolmogorov D tests

Data set	Sample size (n)	Shapiro-Wilk test ^a				Kolmogorov test ^b			
		Cfu per root system		\log_{10} (cfu per root system)		Cfu per root system		\log_{10} (cfu per root system)	
		<i>W</i>	<i>P</i> < <i>W</i>	<i>W</i>	<i>P</i> < <i>W</i>	<i>D</i>	<i>P</i> > <i>D</i>	<i>D</i>	<i>P</i> > <i>D</i>
Rhizosphere populations									
Total aerobic bacteria									
Potato-Bakersfield	50	0.80	<0.01	0.98	0.74
Barley-Visalia	53	0.20	<0.01	0.08	>0.15
Radish-Moss Landing	50	0.70	<0.01	0.97	0.49
Fluorescent pseudomonads									
Potato-Bakersfield	50	0.94	0.02	0.97	0.49
Barley-Visalia	53	0.26	<0.01	0.07	>0.15
Radish-Moss Landing	52	0.27	<0.01	0.09	>0.15
Inoculated strains^c									
Potato-Bakersfield	50	0.92	<0.01	0.93	0.02
Potato-Ventura	60	0.21	<0.01	0.06	>0.15
Sugar beet-Davis	40	0.57	<0.01	0.95	0.15
Sugar beet-Greenhouse	49	0.76	<0.01	0.98	0.55
Seed or seed piece population^c									
Potato set 1	50	0.95	0.10	0.96	0.29
Potato set 2	50	0.85	<0.01	0.97	0.33
Sugar beet set 1	50	0.82	<0.01	0.97	0.35
Sugar beet set 2	50	0.83	<0.01	0.97	0.41

^aThe Shapiro-Wilk test for normality was performed on untransformed and transformed bacterial populations to obtain a *W* value of data sets containing ≤ 51 samples. A *W* value approaching 1.0 supports a normal distribution. *P* values (ie, quantiles) were obtained by using this *W* value to interpolate within a range of critical values provided by Shapiro and Wilk (28).

^bThe Kolmogorov test for goodness of fit was performed on untransformed and transformed bacterial populations to obtain a *D* value of data sets > 51 samples. A *D* value approaching zero supports a normal distribution. *P* values were calculated by using this *D* statistic to interpolate within a range of critical values provided by Stephens (30).

^cInoculum of rhizobacterial strains A1 and SH5 was applied to potato seed pieces or sugar beet seeds, respectively, before they were planted in the field. Root systems arising from treated seed were later sampled for rhizobacterial populations as described in Materials and Methods.

Davis. The variance in populations of strain A1 is larger than that of total fluorescent pseudomonad populations on potato root systems harvested near Bakersfield. Populations of inoculated rhizobacteria on seed or seed pieces were generally more uniform than those on root systems. Bacterial populations determined from 50 individual seeds or seed pieces in each of four experiments varied from a fourfold difference (3.7×10^8 to 1.5×10^9 cfu per seed piece) to a 34-fold difference (1.0×10^7 to 3.7×10^8 cfu per seed).

The variation in rhizosphere bacterial populations among a set of root systems could not be explained by variation in the size of individual root systems. No linear relationship was observed between \log_{10} (cfu per root system) and the mass of that root system (Fig. 5), nor was the variance expressed on a per-root-mass basis generally less than that expressed on a per-sample basis in any of the 10 data sets. Larger root systems did not generally support larger bacterial populations. Populations of fluorescent pseudomonads and of total aerobic bacteria estimated from potato roots of the flooded field in Bakersfield presented an exception to this generalization. Among root systems harvested from this field, there was a significant correlation between these bacterial populations and root system size. However, variance of these bacterial populations did not differ when expressed on a per-gram rather than a per-sample basis.

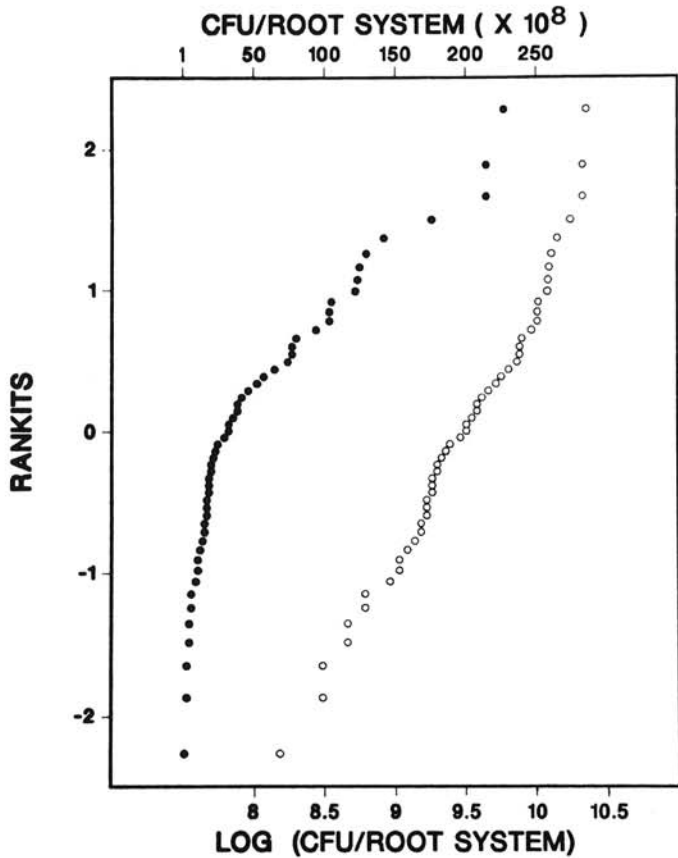


Fig. 2. Rankit diagram of total aerobic rhizosphere bacteria populations shown in Fig. 3. Symbols: ●, cfu per root system; and ○, \log_{10} (cfu per root system).

Quantitative relationship of population means and variances.

The quantitative relationship between means and variances calculated for each of the 32 greenhouse data sets is described in Fig. 6. Means and variances of untransformed population values exhibited a nonlinear relationship. Means and standard deviations of these values were linearly related (Fig. 6B) although means and variances of the logtransformed values were not (Fig. 6C). The logtransformation of population values from these 32 sets of greenhouse-grown plants allows compliance with two assumptions of analysis of variance procedures, the normality of experimental error terms and the independence of means and variances.

DISCUSSION

The lognormal probability distribution applies theoretically to those situations in nature where the process underlying change or growth is multiplicative rather than additive. That is, the lognormal distribution arises from a theory of elementary errors combined by multiplicative processes just as the normal distribution arises from a theory of elementary errors combined by addition (1). For biological populations, increases or decreases in numbers are often proportional to the numbers already present, giving rise to variations which are proportional to the mean on an arithmetic

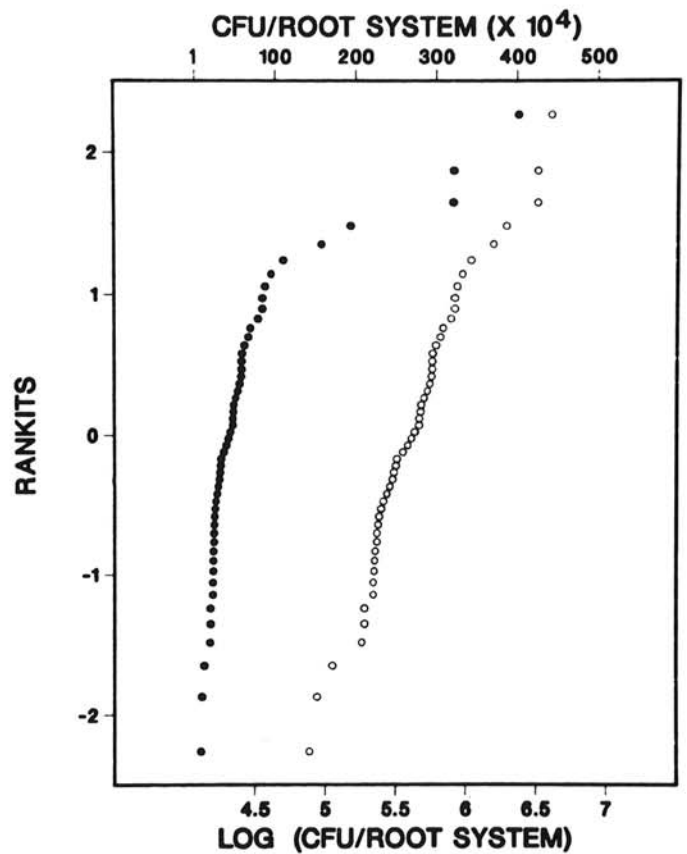


Fig. 3. Rankit diagram of total fluorescent bacterial populations determined from a set of 52 radish root systems. Symbols: ●, cfu per root system; and ○, \log_{10} (cfu per root system).

TABLE 2. Normality of each of 32 data sets of populations of inoculated bacteria as determined by the Shapiro-Wilk test

Data transformation	Number of data sets ^a with <i>P</i> value ^b									
	<0.01	0.01-0.02	0.02-0.05	0.05-0.10	0.10-0.50	0.50-0.90	0.90-0.95	0.95-0.98	0.98-0.99	>0.99
Cfu per root system	13	4	8	3	4	13	1	1	1	0
\log_{10} (cfu per root system)			2	4	10	13	1	1	1	0

^a Each data set composed of 10 root systems (sugar beet, potato, or tomato) grown in ceramic pots under greenhouse conditions. Inoculum of rhizobacterial strains A1 and SH5 was applied to seed or seed pieces before planting. Root systems arising from treated seed were later sampled for rhizobacterial populations as described in Materials and Methods.

^b *P* values were obtained from *W* values which were calculated as described by Shapiro and Wilk (28).

scale, but independent of the mean on a logarithmic scale (2). The logarithmically transformed values of the rhizosphere bacterial populations presented here are both normally distributed and have a variance independent of the mean, as expected for a lognormally distributed population. The lognormal probability distribution has been used to describe many biological populations in nature including the numbers of scale insects (29), or local lesions (17) on individual plant leaves, and more recently, leaf surface populations of epiphytic bacteria on individual leaves (12). Multiplicative processes, generally described by the lognormal probability distribution, are theoretically applicable to these biological systems, as well as to the dynamics of rhizosphere bacterial populations in nature. The spatial variability of several physical factors associated with soils of agricultural fields are also approximated by a lognormal distribution (3). The nonrandom distribution of these and other unidentified biological determinants may play a role in the population dynamics of rhizosphere bacteria.

An accurate determination of the mean rhizosphere bacterial populations of a set of root systems must consider the lognormal

probability distribution of those populations. The lognormal distribution is positively skewed as exemplified in Fig. 3A. A disproportionate number of bacterial cells are contributed to the arithmetic mean of this bacterial population by the root systems with the largest populations, resulting in a mean population (5.7×10^9 cfu per root system) which is 2.0 times larger than the median (2.9×10^9 cfu per root system). In contrast, transformed values given by the logarithm (base 10) of bacterial populations approximate a more symmetric normal distribution (Fig. 3B), resulting in a mean population (2.5×10^9 cfu per root system) which more closely approximates the median. The accuracy with which the arithmetic mean can be used to estimate the geometric mean is dependent upon the variance of the population. As the variance increases, the difference between these mean values also increases (10). The consequence of bulking root segments collected from more than one plant is the arithmetic averaging of bacterial populations that are lognormally distributed. This bulking of root samples will result in the overestimation of the mean bacterial population as described in the case of epiphytic bacterial populations (12), since the arithmetic rather than the geometric mean will be determined.

The perceived probability distribution of a given variable is dependent upon the size of the individual experimental unit chosen for study. The nonrandom distribution of bacteria along an individual plant root has been described by electron microscopy (24-26). This study, defining the experimental unit as an individual root system, excluded the nonrandom distribution of bacteria along an individual root but detected the distribution of bacterial populations among a set of plants in the field. The conditions of this

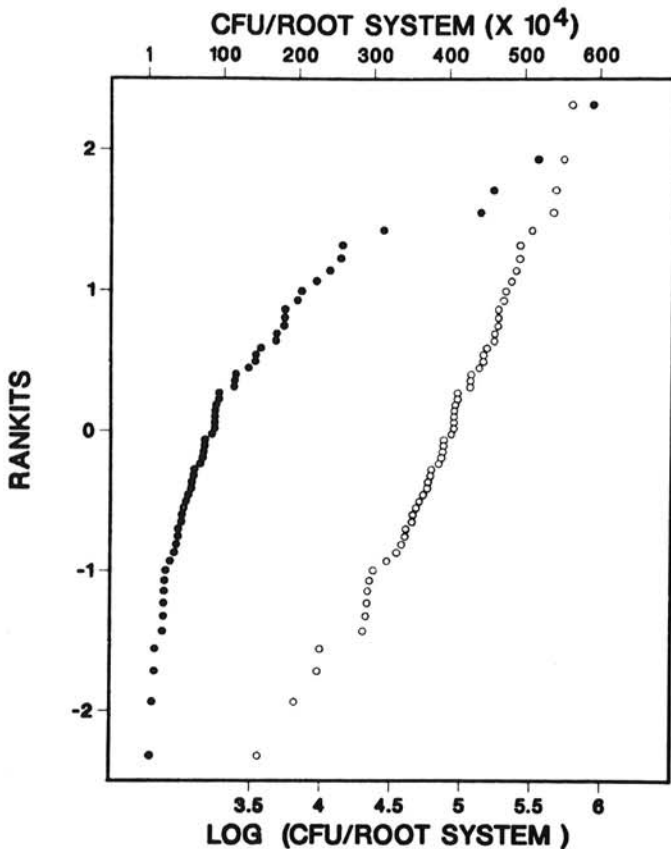


Fig. 4. Rankit diagram of populations of seed-inoculated *Pseudomonas putida* strain A1 as determined from a set of 60 potato root systems. Symbols: ●, cfu per root system; and ○, \log_{10} (cfu per root system).

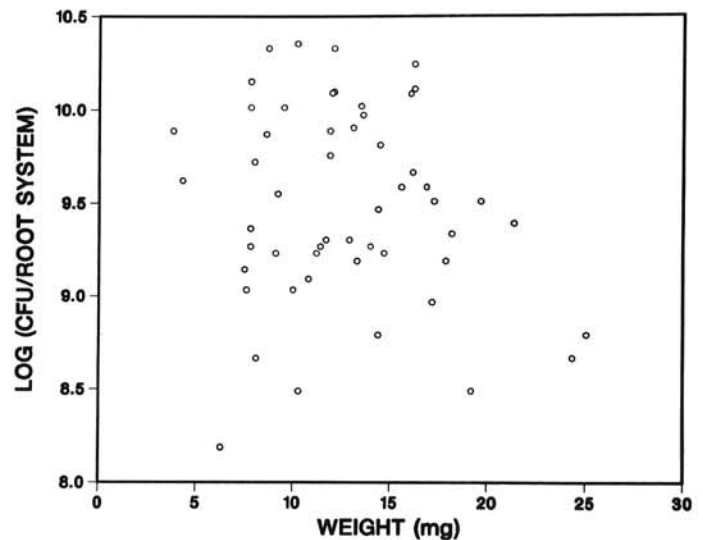


Fig. 5. Populations of total aerobic bacteria estimated from individual barley root systems and expressed as \log_{10} (cfu per root system) demonstrate no obvious quantitative relationship to the size of the corresponding individual root systems.

TABLE 3. Rhizosphere bacterial populations determined from field-grown seedlings and expressed as \log_{10} (cfu per root system)

Data set ^a (crop and location)	Root systems sampled (n)	Inoculated rhizobacteria ^a		Fluorescent pseudomonads		Total aerobic bacteria	
		Mean	Variance	Mean	Variance	Mean	Variance
Potato							
Bakersfield	50	4.51	0.18	6.15	0.03	8.84	0.12
Ventura	60	4.49	0.21
Sugar beet							
Davis	40	5.18	0.54
Barley							
Visalia	53	7.18	0.18	9.49	0.28
Radish							
Moss Landing	52	5.64	0.13	7.71	0.10

^aData sets and rhizobacterial strains are given in Table 1.

study facilitated the extraction of entire intact root systems. In studies of rhizosphere bacterial populations of mature plants or in clay soils, a portion of a root system must be the experimental unit. In such studies, care must be taken to collect root segments which have a similar position within a root system. Potato rhizosphere populations of an applied bacterium on a 4-cm root segment collected from the root tip often vary 1,000-fold from those on a 4-cm root segment collected near the seed piece of the same root. Preliminary experiments indicate that bacterial populations on such root segments may also be lognormally distributed (J. E. Loper, unpublished). Large variation in bacterial populations encountered within a given root system must be considered in studies addressing the quantitative variability and probability distribution of rhizosphere bacterial populations among a set of plants.

The quantitative variability of rhizosphere bacterial populations observed among plants in a single field could not be explained by the relatively small variation in the size of individual root systems. Other factors, including quantity of adhering soil, quantity and/or composition of root exudates, genetic variability among plants, variable numbers of soil microflora encountered by an elongating root, and differences in chemical or physical characteristics of the soil may have determined the bacterial population density at the

soil-root interface. Rhizosphere populations of strains A1 and SH5 on potato and sugar beet, respectively, after application to seed or seed pieces were extremely variable on field-grown plants. This variability was generally greater than that of totally fluorescent populations of *Pseudomonas*. Since the latter measurement describes an unknown number of individual bacterial strains or species resident within the set of root systems sampled, it is difficult to compare the variability of an inoculated strain to that of a strain naturally encountered by an elongating root penetrating through the soil. The moderate variability in seed populations of inoculated rhizobacterial strains may have contributed to, but cannot completely explain the large variability in rhizosphere populations of these strains.

The linear relationship of rhizosphere bacterial population means and standard deviations observed here is characteristic of a lognormal distribution (1,14). The logarithmic transformation of these populations allows conformance to two assumptions of analysis of variance procedures, normality and independence of the means and variances. Due to the large variability associated with rhizosphere bacterial populations, a highly replicated experiment may be required to separate treatment means of transformed population values. In the data sets presented here, an average of 14 and as many as 36 replications were needed to detect (with 80% confidence, $\alpha = 0.05$) a mean difference of 0.5 log units. An average of five and as many as 11 individual root systems were needed to detect a mean difference of 1.0 log units at the same level of confidence. One alternative to log transformation of data is the use of nonparametric statistical tests which do not assume a normal distribution. For example, the Wilcoxon's two-sample test has been used in other studies of rhizosphere bacterial populations (32,33).

Previous quantitative studies of rhizosphere bacterial populations have focused on the mean population size. Although much insight into the role of specific components of the rhizosphere microflora on plant growth has been gained in these studies, other statistical parameters such as population variance and probability distribution can contribute to this knowledge. For example, consider the rhizosphere bacterial populations estimated from root systems of potato plants harvested from the flooded field near Bakersfield. These populations exhibited one or more of the following: low variance, low mean, a significant linear relationship between population size and root mass, and approximation by a normal distribution. These data sets are atypical in contrast to the other sets presented, perhaps due to the excessive moisture conditions in this field. Studies of environmental or treatment effects on rhizosphere bacterial populations may be more conclusive when considering several statistical parameters.

This study and that of Hirano et al (12) indicate that bacterial populations associated with plant surfaces are approximated by a lognormal distribution. Bacterial populations were extremely variable among plants in both studies. Populations of applied bacteria were more variable than those of total bacteria or of a specific bacterial component on leaf surfaces (12) and in the rhizosphere. The predictive models based on the lognormal distribution of bacterial populations on individual leaves (12), may also be applicable to rhizosphere populations. That is, the population of a plant growth-promoting rhizobacterium or biocontrol strain on an individual root system, rather than the mean population among a set of plants, may influence the growth of that individual plant. The frequency with which a threshold population of an applied beneficial bacterial strain is met or exceeded on individual root systems may be of predictive value in determining plant growth, health, or yield. The definition of this threshold bacterial population size may clarify the relationship between plant growth-promoting or biocontrol strains and their influence on plant growth.

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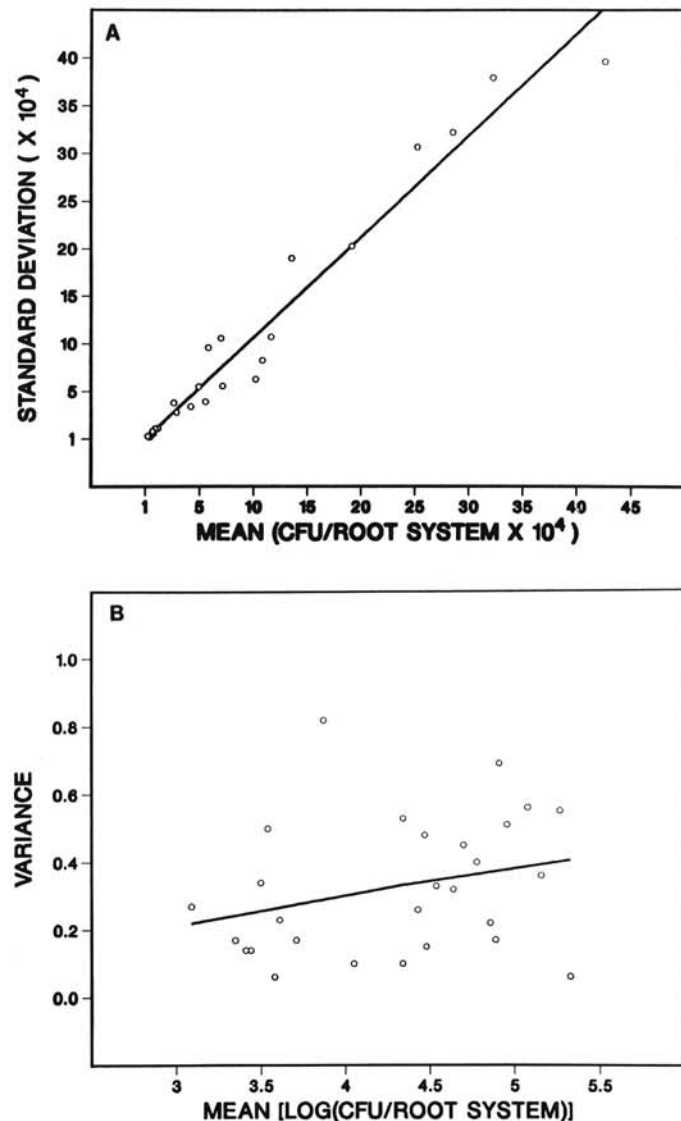


Fig. 6. Quantitative relationship between A, means and standard deviations of untransformed values (cfu per root system) or B, means and variances of transformed values (\log_{10} (cfu per root system)). Values represent rhizosphere populations of inoculated rhizobacterial strains estimated from 32 data sets, each composed of 10 greenhouse-grown plants.

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