Root Prints: A Technique for the Determination of the In Situ Spatial Distribution of Bacteria on the Rhizoplane of Field-Grown Plants

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ABSTRACT

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Visualization of the in situ spatial distribution of bacteria on the rhizoplane of sugar-beet roots was accomplished by imprinting excised portions of roots on agar medium. Up to 20 consecutive prints could be made from a single 1-cm^2 section of rhizoplane. The number of colonies, which visually exhibited a random pattern of distribution, ranged from 48 to 113 (mean of 60) per square centimeter of rhizoplane. Total bacterial population size, estimated from dilution plating of washings from roots, ranged from 1.12 to 10.3×10^7 (mean of 5.1×10^7) colony-forming units/cm^2 of rhizoplane. *Bacillus* spp. were identified as the dominant bacteria (greater than 57% of the colonies) on both root prints and dilution plates. Fluorescent pseudomonads, when present, accounted for less than 3% of the colonies on prints and less than 20% of the colonies on dilution plates. The root-print technique was not restricted to large taprooted plants. Visualization of the spatial distribution of bacteria on the rhizoplane of small cylindrical roots (at least 0.3 mm in diameter) also was accomplished by rolling the root on the agar surface.

Manipulation of the rhizoplane-rhizosphere microflora, whether directed specifically at enhancing the competency of native or introduced beneficial microorganisms or decreasing the competency of deleterious root-infecting microorganisms, is dependent upon knowledge of their rhizoecology. Despite a wealth of information on the composition and frequency of microbial populations in the rhizosphere-rhizoplane (4,5,10), little information is available concerning microbial population dynamics or interactions, particularly as they exist under field conditions. The paucity of such knowledge can be attributed, in part, to the lack of technology for determining their in situ spatial distribution (1,13).

Presumably, the outcome of microbial interactions is dependent upon the proximity of various populations to each other. Current techniques employed for rhizoplane studies, that is, dilution plating of washings from roots (1,2,4–6,16), are totally disruptive to the natural spatial distribution of microbial populations. Only a few reports exist regarding the in situ spatial distribution of bacteria on the rhizoplane. Direct microscopic evidence of the distribution of bacterial colonies on the rhizoplane of greenhouse-grown plants has been obtained. (3,7–9,11,14). These studies suggested that bacterial colonies on the rhizoplane are aggregated and occupy 7–15% of the surface area. Whether this is the actual distribution, however, is not known with certainty because roots were washed before observation. Washing of roots before observation is reported to redistribute bacteria on the root surface (3). Microscopy, as a result of specimen preparation, also generally precludes identification of the observed bacteria or determination of their viability.

We have developed a simple technique that facilitates determination of the identity and the in situ spatial distribution of bacterial populations on the rhizoplane of field-grown plants—namely, root prints.

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MATERIALS AND METHODS

Root-print technique. The general procedure for making root prints (Fig. 1) was as follows. Roots of test plants were carefully extracted by spading from the field and transported to the laboratory. Sections of roots, bearing a rhizoplane surface area of 1 cm^2, were excised from the root and either pressed or rolled on the surface of agar media in petri dishes. Printed plates then were developed by incubation at 27 C for varied durations. The exact position and number of developing colonies per print were permanently recorded by photocopying or photographing the petri plate (Fig. 2). Once the permanent record had been made, the frequency of the dominant colony types on the print was recorded, and representatives of each colony type were isolated and identified to genus by the following tests: gram stain reaction and reaction profiles on API 20E test strips (Analytab Products, Plainview, NY).

Media employed in making root prints were King's medium B (KB) (1) and cornmeal agar (CMA) (Difco Laboratories, Detroit, MI). Media were amended with additional agar (final concentration was 4%), which prevented expression of water and the resultant smearing of bacterial colonies during the printing procedure but had no apparent effect on the number or growth of colonies. However, KB facilitated enumeration of fluorescent pseudomonads and CMA prevented the growth of spreading bacteria such as *Bacillus mycoides* Flugge. One or two colonies of the latter bacterium per print would completely overgrow an individual print within a 24-hr incubation period.

Dilution plating of washings from roots. In addition to root prints, quantitative estimates of bacterial population sizes per unit area of the rhizoplane also were assessed. Individual root segments, bearing 1 cm^2 of surface area, were placed in 100 ml of sterile distilled water in a 250-ml flask containing glass beads and vortexed for 15 min. Serial 10-fold dilutions were made, and 0.1-ml aliquots of appropriate dilutions were plated in triplicate on KB and CMA. Colony counts were made after 48 and 72 hr of incubation at 27 C. Fluorescent bacterial colonies

were enumerated under ultraviolet light (360 nm). The frequency of dominant colony types was recorded, and representatives of each type were isolated and identified.

Plants assayed and field conditions. Roots of the following plants, ranging in age from 3 to 8 mo, were assayed: *Beta vulgaris* L., *Gossypium hirsutum* L., and *Sorghum vulgare* Pers. Taproots of mature sugar beets, however, were the principal root assayed. The rhizoplane of a mature sugar beet commonly exceeds 500 cm^2. Thus, several individual 1-cm^2 sections from the rhizoplane of a single root were used for root printing and dilution plating. All plants were collected from furrow-irrigated field plots located at the University of Arizona Campbell Avenue Farm in Tucson, AZ. The soil type at this location was a loam, pH 7.25 (51.9% sand, 33.0% silt, and 15.1% clay). Roots were collected when soil-moisture levels were between −0.03 and −1.0 MPa.

RESULTS AND DISCUSSSON

The in situ spatial distribution of bacterial colonies on the rhizoplane of mature sugar-beet taproots are presented in Figure 2. Bacterial colonies, which visually exhibited a random pattern of distribution, developed within 16 hr, and the maximum number of colonies was recorded within 20 hr of incubation at 27 C after printing of the rhizoplane on agar medium. Up to 20 consecutive prints could be made from a single 1-cm^2 rhizoplane section (Fig. 3), indicating that a single rhizoplane section can be used as a template for replica plating on various selective media. Thus, the technique can be employed for estimating the location of different bacteria on the same rhizoplane. The occasional occurrence of high numbers of colonies on the first one to three consecutive prints of some rhizoplane sections most likely represented bacterial colonies associated with residual soil particles that closely adhered to the rhizoplane. This hypothesis was supported by the observation that preprinting of the rhizoplane section on the sticky surface of masking tape before printing on agar medium eliminated the origin of such colonies (Fig. 4). Assuming that actual rhizoplane colonies were represented on the fourth consecutive print, the mean number of colonies from eight 1-cm^2 rhizoplane sections (two sections from each of four sugar beets) was 60, range = 48–113 (Fig. 3). Assuming that the circumference of bacterial colonies on the rhizoplane ranged from 0.25 to 0.5 mm in diameter (which was 25 to 50% of their mean diameter on root prints after 20 hr), we estimated that 60 bacterial colonies would occupy between 2.9 and 11.8%, respectively, of the total surface area of a 1-cm^2 rhizoplane section. These data are in agreement with previous estimates of percent bacterial cover of the root surface (9,11).

The mean total bacterial population size from 12 1-cm^2 rhizoplane sections (three sections from each of four sugar-beet roots) was 5.1×10^7 (range $1.12–10.32 \times 10^7$) (Table 1). Such populations are in accord with those commonly reported on roots (1,4,5). The large bacterial populations estimated from dilution plate counts appeared, at first, to be at variance with the relatively low number of bacterial colonies that developed on root prints. However, we estimated that 60 bacterial colonies, each measuring 0.25 mm in diameter and composed of a monolayer of bacterial cells each measuring 1×3 μm, would contain about 1×10^6 bacterial cells. Sixty colonies of the same dimensions with bacteria stacked 20 cells deep would contain about 2.0×10^7 bacteria. Thus, relatively few bacterial colonies on the rhizoplane, depending upon their size, can account for large bacterial populations estimated by dilution plate counts of washings from roots.

Between 57 and 100% of the colonies on individual rhizoplane prints and dilution plates were identified as *Bacillus* spp. Fluorescent pseudomonads, when present, accounted for less than 3% of the total number of colonies per print and less than 20% of the colonies on dilution plates. Obviously, many factors, including the specific agar medium and temperature employed, will influence the growth of specific bacteria. In our study, we were interested in the distribution and identity of rapidly growing bacteria that may compete with *Pythium aphanidermatum*

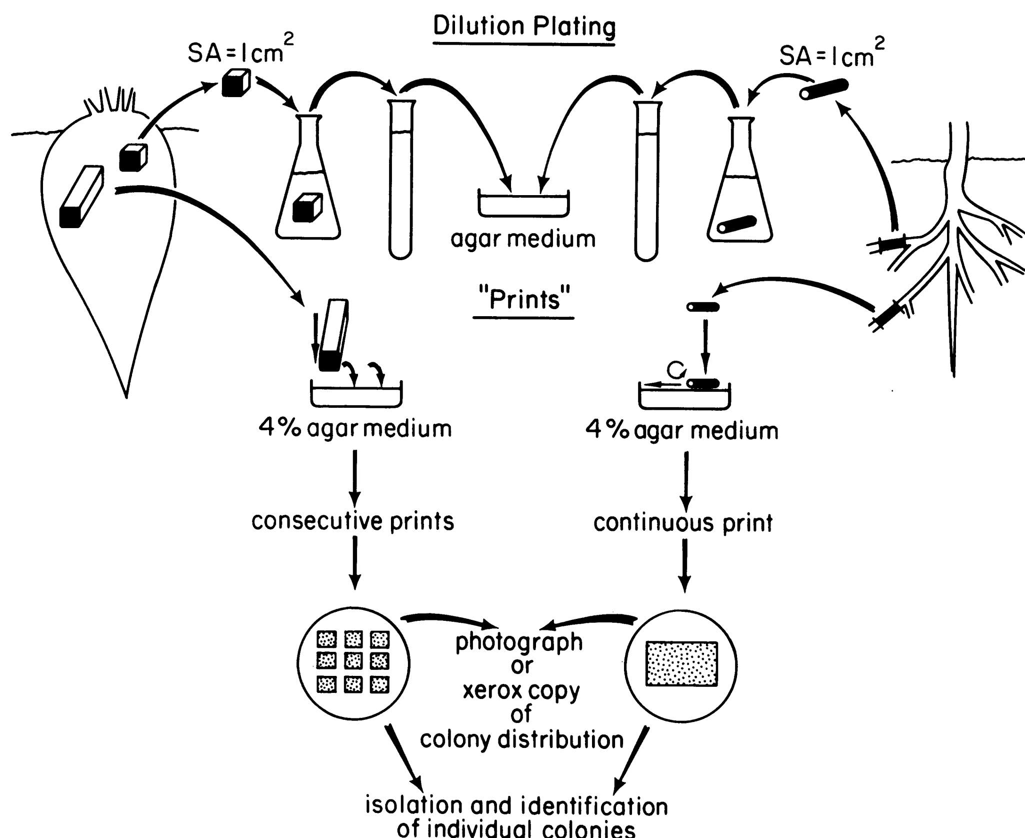
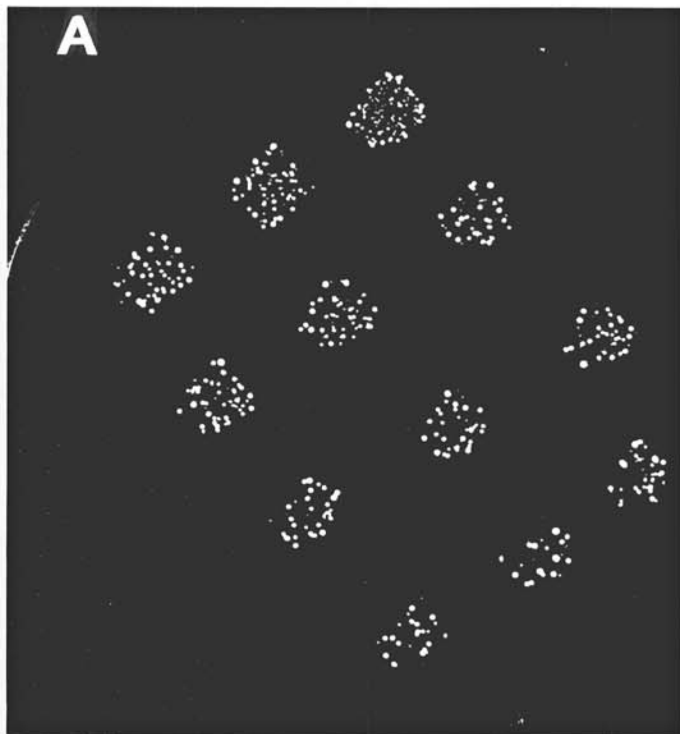


Fig. 1. Illustration of the root-print technique. SA = surface area.

(Edson) Fitzp. for colonization of the rhizoplane. The interaction(s) between *P. aphanidermatum*, a pathogen of mature sugar-beet taproots (12,15), and rhizoplane bacteria currently is being investigated.

Although sugar beets were the principal plant assayed in our study, the root-print technique was not restricted to plants with large taproots. Visualization of the distribution of bacterial colonies on small cylindrical roots (0.3 mm in diameter or greater) also was accomplished by rolling root sections of cotton and sorghum on the surface of agar medium (Fig. 1). This modification resulted in a continuous print of an individual root segment. The rhizoplane surface area of a cylindrical root measuring 1 cm in length and 0.31 cm in diameter is 1 cm^2. Thus, comparative studies of the spatial distribution of bacteria on the rhizoplane of different plants or underground plant parts can be compared on an area rather than a weight basis. As pointed out by several researchers (6,11), the use of root surface area is more realistic that root



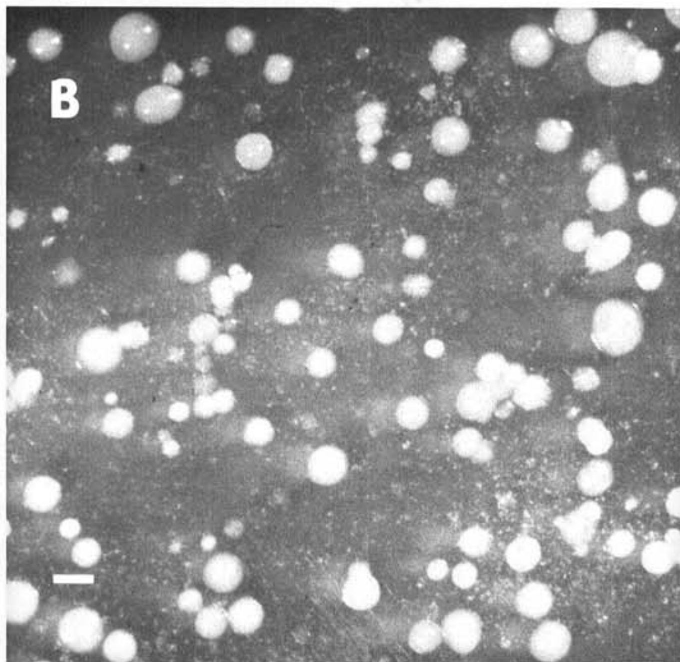


Fig. 2. The in situ spatial distribution of bacterial colonies on the rhizoplane of a sugar-beet taproot. **A,** Photocopy of a petri plate (cornmeal agar) with 12 consecutive prints of a single 1-cm^2 rhizoplane section excised from a taproot. **B,** Photograph of bacterial colonies on a print of a rhizoplane (bar = 1 mm).

TABLE 1. Bacterial populations per unit area of the rhizoplane of field-grown mature sugar-beet taproots

Rhizoplane sections	Total aerobic bacteria (colony-forming units $\times 10^7/cm^2$ of rhizoplane) on: Cormeal agar	King's medium B	Percent fluorescent pseudomonads
Sugar beet A			
Section 1	2.24	4.00	0
Section 2	1.80	2.48	1.0
Section 3	2.40	1.12	0
Sugar beet B			
Section 1	6.56	8.08	0.6
Section 2	6.88	9.06	1.2
Section 3	8.24	10.32	0.7
Sugar beet C			
Section 1	1.84	2.56	0
Section 2	1.76	2.36	0
Section 3	4.32	6.40	1.0
Sugar beet D			
Section 1	7.81	7.36	15.0
Section 2	4.08	4.13	14.6
Section 3	6.24	7.80	18.1

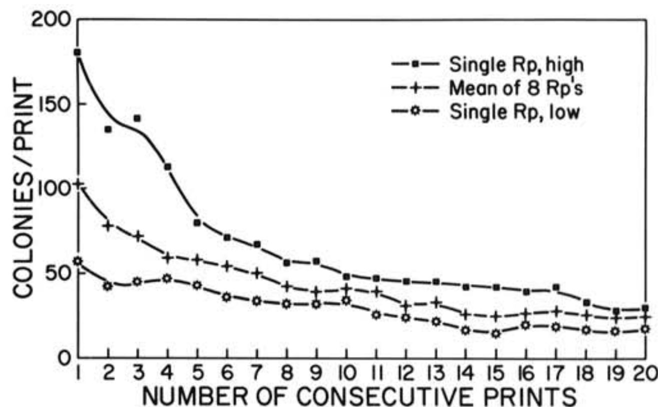


Fig. 3. Number of bacterial colonies on 20 consecutive prints from each of eight individual 1-cm^2 rhizoplane sections excised from a sugar-beet taproot. Rp = rhizoplane; high = high population; low = low population.

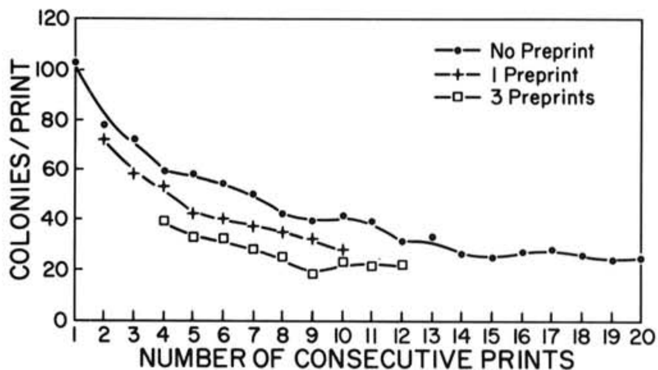


Fig. 4. Effect of preprinting a rhizoplane section on sticky tape before printing on an agar medium for determination of the in situ spatial distribution of bacteria on the rhizoplane.

weight as a basis in elucidating microbial population dynamics in the rhizoplane-rhizosphere.

Determination of the in situ spatial distribution of bacteria on the rhizoplane via the root-print technique, coupled with the ability to identify individuals, will enable rhizosphere microbiologists (particularly those interested in biological control or management of native or introduced microorganisms) to more accurately assess microbial interactions as they exist in nature.

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