Research Note

BEAN ROOT COLONIZATION BY PSEUDOMONAS CEPACIA *UPR 5C*[1,2]

Numerous microorganisms beneficially affect plant development when applied to seeds or incorporated into the soil. The rhizobacteria that colonize plant roots and stimulate plant growth are called plant growth-promoting rhizobacteria (PGPR).[3]

A major constraint to the successful agronomic use of bacterial inocula is the need to establish high population densities of the introduced bacterium. The root environment is characterized by the intense microbial competition for nutrients.[4] Rhizobacteria are members of the soil microbial population that are able to colonize roots. Root colonization is defined as the bacterial capacity to multiply and keep pace with the growing root in field soil.[5] Bacterial inocula on seeds planted into field soils multiply in the seed zone in response to seed exudation before germination. Root-colonizers transfer from seed zone to the developing root where they multiply and persist through the growing season. To demonstrate that plant growth response is due to an introduced strain, the introduced bacteria should successfully colonize the root environment. One method for monitoring bacterial root colonization involves the use of antibiotic-resistant strains.[6] This methodology has allowed detailed ecological analysis of bacterial root colonization under field conditions.

There are several possible mechanisms to explain the effect of PGPR. One such potential mechanism is the protection of roots from pathogenic fungi. Several authors report the isolation of strains of *Pseudomonas cepacia* with antagonistic effect towards plant pathogenic fungi.[7,8,9] In petri dish bioassay we have found a strain of *P. cepacia* capable of strongly inhibiting the growth of *Macrophomina phaseolina*.[10] The objective of the study herein reported was to determine the ability of *P. cepacia* strain UPR 5C to colonize the roots of *Phaseolus vulgaris*.

Experiments were carried out at the Biotechnology Nitrogen Fixation Laboratory (BNF) of the Agronomy and Soils Department. *P. cepacia* (strain UPR 5C, naturally resistant to 200 µg/ml kanamycin and 1000 µg/ml streptomycin) from the BNF collection and seed of common bean cv. PC-50 (Pompadour type) were used. *P. cepacia* UPR 5C was grown on tryptone yeast (TY) broth. Before planting, bean seeds were surface-sterilized with acidified mercuric chloride solution and washed five times with sterile distilled water.[11]

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Seeds were dipped in a bacterial suspension (approximately 10^9 cells per milliliter). Control seeds were treated with sterile distilled water. Seeds were planted in Leonard jars containing a 1:1 mixture of vermiculite and sand. Plants were grown in a controlled environment at 26/20° C (day/night) with a 14-h photoperiod. A qualitative replica-plating procedure[12] was used to assess the degree of colonization of bean seedling roots by *P. cepacia*. The seedling root was transferred to a sterile 150 X 15 mm Petri dish and pressed onto plates of King's B medium containing kanamycin (50 µg/ml) and streptomycin (500 µg/ml). The plates were incubated at 28° C for 5 days. The quantitative assay involved cutting 1-cm root segments along the root at the upper, middle and lower portions. Root segments were shaken in 10 ml of sterile water on a reciprocating shaker for 15 min, serially diluted and plated on King's B medium containing the antibiotics. Colonies were counted ten and 15 days after planting.

The number of colony forming units (CFU) was determined before and after seed inoculation. All treatments were replicated four times in a completely randomized design. The experimental units were the Petri dishes containing King's B medium.

To evaluate root colonization by a rhizobacteria, it is necessary to determine the bacterial seed population. The number of *P. cepacia* bacteria per seed averaged 9.3 X 10^5 after inoculation and before planting. Hebbar et al.[7] obtained a population of 7 X 10^5 in corn seeds. Suslow and Schroth[13] indicate that to achieve uniform colonization and growth promotion the inoculum needs to contain at least 10^5 CFU per seed or 10^7 CFU/g of dry inoculum.

The quantitative evaluation indicated that *P. cepacia* (UPR 5C) migrated along with the growing root after planting (table 1). However, colonization was highest for the upper root section 10 and 15 days after planting. Up to 366 colonies per centimeter were obtained at the lower section of the bean root system. Juhnke et al.[14] reported similar results. They observed that bacterial colonization was highest on the upper section of wheat roots.

The qualitative studies showed that when *P. cepacia* was inoculated on the seed, it colonized all of the surfaces. Ten days after planting the root system was completely colonized. Roots from plants not inoculated with *P. cepacia* (control) showed no colonies on King's B medium with antibiotics.

TABLE 1.— *Number of viable colonies of* Pseudomonas cepacia *(UPR 5C) on different segments of bean roots*

Days after planting	Root Segment Portion		
	Upper	Middle	Lower
10	4,700[1]	2,133	366
15	9,600	6,800	696

[1]Mean of four replications.

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Many bacteria have biological control potential in vitro. Nevertheless, to be useful in the field, rhizobacteria should be able to grow fast and colonize roots.[4] However, many bacteria may persist in the root zone after inoculation without necessarily colonizing developing roots. The bean root colonization of *P. cepacia* (UPR 5C) agrees with reports of effective colonization of roots of other crops by strains of *P. putida* and *P. fluorescens*.[6,12,15] It has been shown that other strains of *P. cepacia* colonized and multiplied in the maize rhizosphere.[7] Taken together, these results agree with those of Hozore and Alexander[16] suggest that mobility along the root is important to successful rhizosphere competition.

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