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# Comparison of the Chemotaxis Potential of Bacteria Isolated from Spinach Roots and Nonrhizosphere Soil

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**Abstract** In order to investigate the role of bacterial chemotaxis in root colonization, the chemotaxis potential of bacteria isolated from spinach roots was compared with that of bacteria from nonrhizosphere soil, with reference to the plant age (1,000 isolates), soil moisture conditions (1,400 isolates), and part of the root (200 isolates). The % CT (% occurrence of chemotaxis (+) isolates among total bacterial isolates) of the root isolates significantly fluctuated during the plant growth period, reaching a maximum after 10–15 days of growth. At this time period, the maximum % CT for the root isolates was around 70–80% CT under a soil moisture of 50% WFP (% volume of water-filled pores in total soil pores), and then gradually reduced with an increasing % WFP. The results of the chemotaxis potential of each of the 100 isolates from the spinach roots and nonrhizosphere soil under various % WFP demonstrated that the % CT of the root isolates were significantly higher than those of isolates from the nonrhizosphere soil under a wide range of soil moisture content (35–80% WFP). Furthermore, the % CT value (80%) from the upper root was significantly higher than that (55%) from the lower root. Compared with the % CT values of the roots, the values from the nonrhizosphere soil did not significantly vary relative to the plant age or % WFP. These results indicate that chemotaxis would appear to be a major factor in bacterial root colonization.

**Key words:** Bacterial chemotaxis, root isolates, nonrhizosphere soil isolates

Microbial communities in soil and plant rhizospheres have been studied for their structure and contributions to the nutrient cycle and plant growth. Among these contributions, studies on the parts played by rhizobacteria have been increasing [9, 13, 16, 18, 19].

A variety of rhizosphere bacteria are known to be mutualistic or commensal with plant roots rather than amensal or parasitic. The beneficial participation of these bacteria seems to operate through processes such as the decomposition of root exudates, the supply of nutrients and plant growth regulators [10], and antagonism against pathogens [18], although they may also exert some adverse effects through phytotoxins [12].

Considerable interest has recently been focused on the biocontrol functions of PGPR (plant-growth promoting rhizobacteria). Nevertheless, many papers have demonstrated that PGPR only make up a minor part of bacterial populations in a plant rhizosphere. Accordingly, these results infer that the implications of various rhizobacteria besides PGPR should not be overlooked in the study of root-microbe interactions.

The bacterial community structure in a plant rhizosphere depends not only on the plant species and age but also on the soil conditions by which the root exudation is significantly affected [7].

Rhizosphere populations must have advantageous traits for competitive root colonization, such as chemotaxis, higher growth rates, and survival potential in the rhizosphere [29]. The implications of chemotactic movement toward root-released substances still remains controversial [5, 6, 11, 17, 27]. This is due to limited information about the chemotactic behavior of bacteria in a plant rhizosphere, despite the fact that chemotaxis is a common feature of bacteria [14, 21].

In this paper, we describe the role of bacterial chemotaxis on root colonization, by comparing the chemotaxis potentials of bacteria isolated from spinach roots with those of isolates from nonrhizosphere soil.

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**Bacteria Isolated from Spinach Roots and Nonrhizosphere Soil with Plant Growth Stages and Various Soil Moisture Contents**

The soil samples (0- 10 cm depth, Brown Andosol) were taken in September 1995 at fallow sites in a vegetable-cropping green house, and preserved at 4°C in a cold room. Prior to use, the samples were mixed and passed through a 2-mm sieve. The content of exchangeable cations (cmol(+)$kg^{-1}$) was Ca 5.77, Mg 56, Na 0.127, and K 2.17, and the content of $NO_3$-N and Troug-$P_2O_5$ was 201 mg · $kg^{-1}$ and 583 mg · $kg^{-1}$, respectively.

i) During the 3-weeks plant growth period, spinach root samples were taken seven times in succession. Nonrhizosphere soil samples were taken from pots containing the same treated soil yet unplanted [20].

ii) The soil moisture content, the percent of the water-filled pore volume in the total volume of soil pore (% WFP, % volume of water-filled pore in the total volume of soil pore), was adjusted to 35, 40, 50, 65, and 80% by the method developed by Tate (1995). The resulting soil samples were placed in 100-ml plastic pots, 15 cm high and 3.3 cm in diameter.

The spinach seeds (*Spinacea oleracea* cv. "Atlas") were surface-disinfected by dipping in a 1% benzalkonium chloride solution and 1% hypochlorite solution each for 10 min and rinsing six times with sterile water for 3 min. Then, the seeds were planted in pots and put in a phytotron under a day-night regime at 20°C and 70% relative humidity, with a 12 h daytime under 7,000 micromoles/$cm^2$/s.

The bacteria were isolated from the macerated spinach root and nonrhizosphere soil samples, using a plate method with 10% TSA(tryptic soy agar) containing 1.7 g/l bacto-tryptone, 0.3 g/l bacto-soytone, 0.25 g/l bacto-dextrose, 0.5 g/l NaCl, 0.25 g/l $K_2HPO_4$, and a 1.5% agar, and adjusted to pH 7.4.

iii) Bacterial isolates were also taken from different parts of the roots, including the upper root (basal 5 cm) and the lower root (top 5 cm). The % CT for the bacterial isolates from the upper roots, lower roots, and nonrhizosphere soil were assayed 14 days after spinach growth under a 50% WFP.

The isolates from the roots and the nonrhizosphere soil were precultured on a 10% TSA medium. One-hundred bacterial isolates were assayed in each treatment. The Gram-negative bacteria in the upper-root and the lower-root sections were also counted on a 10% TSA medium containing crystal violet (5 mg/l) [20].

**Chemotaxis Assay**

The chemotaxis potentials of the isolates from the roots and nonrhizosphere soil were assayed using a swarm plate method [4] with soft agar medium containing 1% polypeptone, 0.8% NaCl, and a 0.3% agar. After being inoculated with each isolate, the plates were incubated at 28°C for 24 h. The isolates forming a ring at the margin of the colony or those with flagellar movement under a microscope were identified as chemotaxis (+), whereas those without a ring and lacking flagellar movement under a microscope were identified as chemotaxis (-). % CT is defined as the sum of chemotaxis (+) isolates in each 100 isolates.

**Chemotaxis of the Isolates from Spinach Root and Nonrhizosphere Soil During Plant Growth Stages**

To assess the spinach growth stage when the soil bacteria actively migrate toward the roots, the occurrence of chemotaxis (+) isolates among the root isolates and nonrhizosphere soil isolates was investigated during the initial 3 weeks of spinach growth under a soil moisture condition of 50% WFP. The % CT for the root isolates substantially fluctuated during the growth period, reaching as high as 70 to 80% after 10- 14 days (Fig. 1). These values were remarkably higher than those for the soil isolates and more dependent on plant age.

**Comparison of % CT for Roots with That for Nonrhizosphere Soil Under Various Soil Moisture Conditions**

The responses of the % CT for the root and soil isolates relative to the soil moisture content are shown in Fig. 2. The % CT for the root isolates was higher regardless of the soil moisture conditions. Moreover, the % CT for the root isolates was significantly increased by optimizing the % WFP, reaching as high as 80% CT at a 50% WFP. In contrast, the % CT for the soil isolates was lower than 40%, and did not fluctuate much with the % WFP. Accordingly, the results in Fig. 1 and Fig. 2 suggest that the chemotaxis potential was a critical trait for the soil bacteria to colonize the spinach roots.

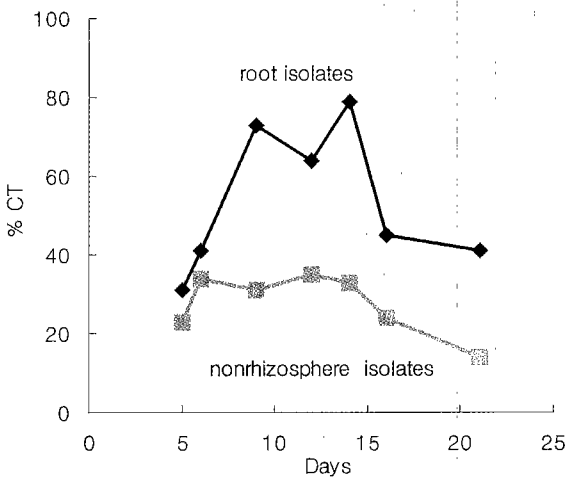


**Fig. 1.** Time-courses of % CT[1] in spinach roots and nonrhizosphere soil during plant growth under soil moisture of 50% WFP[2].
1) % occurrence of chemotaxis (+) isolates in total isolates. 2) % water-filled pore volume in the total pore volume in soil.

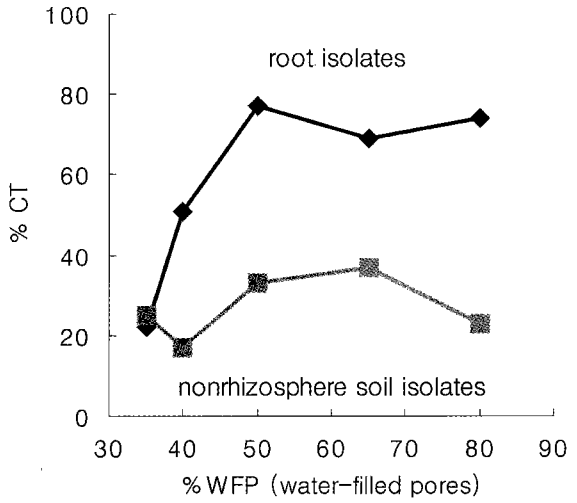


**Fig. 2.** Percent occurrences of chemotaxis (+) isolates in total isolates (% CT) in spinach roots and nonrhizosphere soil under various soil moisture conditions.

### Comparison of % CT for Upper Root Isolates with That for Lower Root Isolates During Initial 14-day Growth Period

As illustrated in Fig. 3, the % CT for the upper-root isolates was as high as 80%, while it was only 55% for the lower-root isolates. The % CT for the nonrhizosphere soil isolates was less than 35% (Fig. 1), which was substantially lower than the values for the roots. The counts of Gram-negative bacteria in the upper and lower roots were $22 \times 10^6$ and $5.8 \times 10^6$, respectively (Fig. 3). The former count was almost 4 times higher than the latter. These results would seem to suggest that the population of chemotactic bacteria in the upper root was larger than that in the lower root.

The results obtained in these experiments indicate that there was a remarkable difference in the % CT between the bacterial populations in the roots and those in the nonrhizosphere soil. In the roots, the % CT was as high as 80%, while it was only 35% or lower in the soil (Fig. 1 and Fig. 2). These results also indicate that the chemotaxis potential seemed to be an essential trait for the soil bacteria to competitively colonize the root. In addition, the higher population of chemotactic bacteria in the upper root than in the lower root may explain why it took time for the soil bacteria to chemotactically migrate toward and colonize the growing roots, thereby resulting in the characteristic upper-root populations being more responsive to the root exudates than the lower-root populations (Fig. 3).

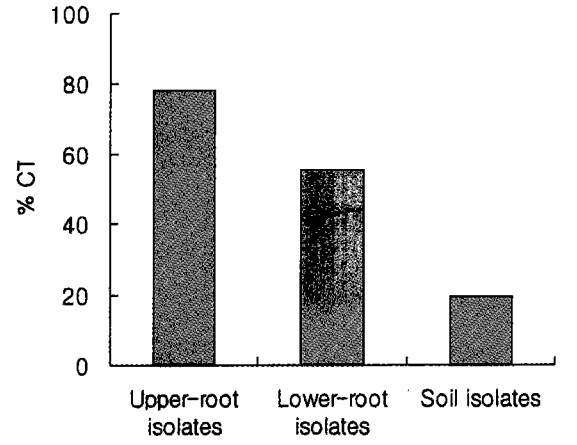


**Fig. 3.** Percent CT in basal and top 5-cm parts of spinach roots and rhizosphere soil after 14 days of growth under a soil moisture content of 50% WFP.

Adler [1] was the first to demonstrate the important role of bacterial chemotaxis as the function through which bacteria can move toward more favorable habitats *in situ*. Nevertheless, discussions about the ecological role of bacterial motility in the soil-root system are still inconsistent. This is because many of these discussions have been restricted to the flagellar movement of *Bacillus*, *Azotobacter*, *Azospirillum brasilense*, or fluorescent pseudomonads in unplanted soils [2, 3, 30]. The migration of *Rhizobium* or fluorescent pseudomonads towards roots and root exudates [8, 23, 24, 25], or the chemotaxis-motility mechanism in some PGPR strains, represented a competitive advantage for root colonization [8, 28]. In contrast, Kloepper [18] reported that chemotaxis towards root exudates is not essential for fluorescent pseudomonads to colonize plant roots, and Boelens *et al.* [4], Howie *et al.* [15], and Parke *et al.* [22] also obtained similar results.

Beneficial rhizobacteria, so-called PGPR, generally form a limited part of bacterial populations in a plant rhizosphere. Accordingly, a variety of rhizobacteria besides PGPR also need to be studied in regard to the role of chemotaxis potentials in root colonization.

In summary, we describe in this work the largest screening of a soil-root system for chemotaxis potential, the knowledge of which is important in respect to root colonization. This study tested a total of 2,600 bacterial isolates from spinach roots and nonrhizosphere soil with plant growth stages, various soil moisture conditions, and parts of the root. Almost 80% of the root isolates had chemotaxis capacities (Fig. 1 and Fig. 2), thus indicating the importance of chemotactic migration through soil in the process of root colonization.

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