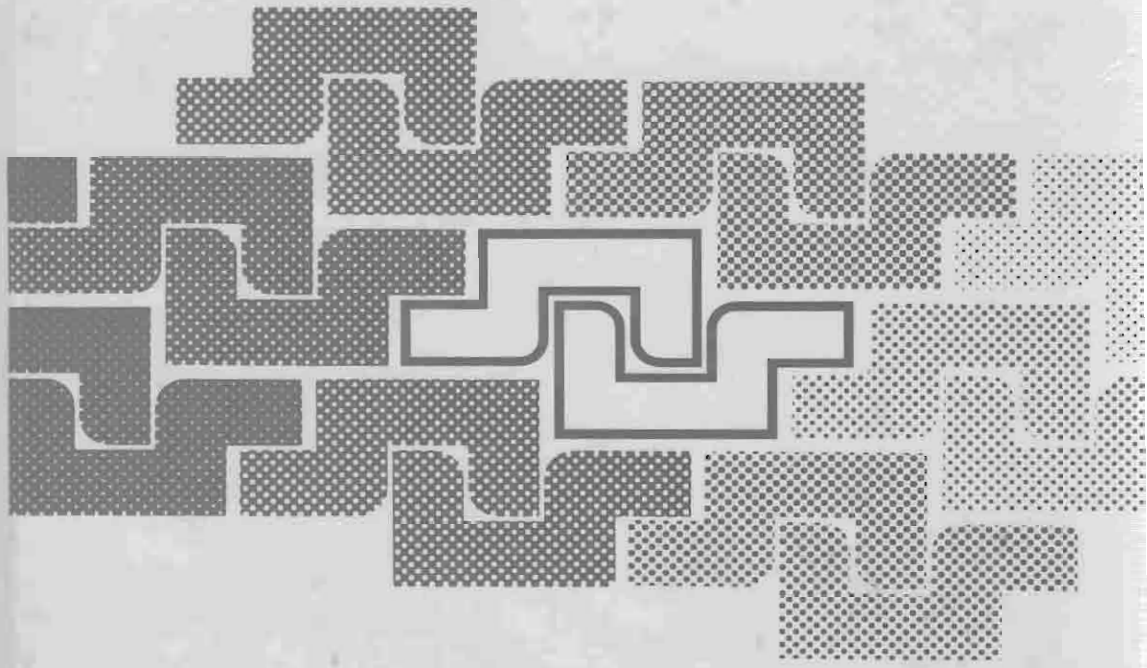

Azospirillum VI and Related Microorganisms

Genetics – Physiology – Ecology

Edited by
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NATO ASI Series

Series G: Ecological Sciences, Vol. 37

Adhesion of Rhizosphere Bacteria to Roots of Maize and Wheat

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Abstract

Several bacterial strains were isolated from the rhizosphere of important agronomical crops and classified in the genera *Enterobacter*, *Pseudomonas*, *Azospirillum*, *Azotobacter* and *Bacillus* on the basis of their morphological and biochemical characters. Investigations on the efficiency of colonization and adhesion of these bacteria to roots of maize and wheat by scanning electron microscopy have been carried out.

Keywords: rhizosphere, adhesion, root surface, SEM.

Introduction

The ability of rhizobacteria to colonize roots is an essential requisite for the success of the competition of the bacterium in the rhizosphere. The rhizobacteria dispose of various properties that are important to promote competitiveness, one of these is the capacity to adhere to root surface. Living firmly anchored to root, bacteria can benefit more quickly from root exudates (Michiels *et al.*, 1989).

In this work we have investigated the capacity to adhere to maize and wheat roots of several bacterial strains isolated from the rhizosphere of important agronomical crops.

NATO ASI Series, Vol. G 37
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Materials and Methods

Bacterial strains used are reported in Table 1, the species assignation was performed following Holt (1984). Plant inoculation was carried out as described by Bazzicalupo *et al.* (1985). Plants were maintained in controlled environment growth cabinet (16^h light, 21°C; 8^h dark, 17°C) for three days.

For scanning electron microscopy, samples of washed inoculated-roots were fixed for 18^h at 4°C with 2.5% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.0), washed three times, postfixed for 2^h at 4°C with 1% osmium tetroxide and dehydrated in a grade series of ethanol. The specimens were dried to critical point (Critical Point Dryer 020 Balzers) mounted on stubs, coated with gold and examined with a Philips 505 scanning electron microscope (Haahtela *et al.*, 1988, de Freitas and Germida, 1990).

Table 1. Bacterial strains used.

Strain	Source	Species
EnunAp2	barley	*Azospirillum lipoferum*
EnunAp3	barley	*Azospirillum lipoferum*
EnunEt5	wheat	*Enterobacter cloacae*
EnunEt7	wheat	*Enterobacter sakazakii*
EnunPs17	wheat	*Pseudomonas* sp.
EnunAz2	wheat	*Azotobacter chroococcum*
EnunB12	wheat	*Bacillus subtilis*
EnunB20	wheat	*Bacillus megatherium*

Results

SEM observations showed that all the bacterial strains tested were able to colonize and adher to root surface of maize and wheat, although the colonization level varied along each roo Figure 1A shows how most of the adhering bacteria were associated on the surface o epidermal cells in the root-hair regions, while the strains EnunB12 and EnunPs17 seemed to b connected to root surface and each other by fibrillar material (Fig. 1B). No bacteria colonization was observed near root meristem. Strains EnunEt5 and EnunPs17 were observe to be attached to the cell surface of maize and wheat roots by a single pole of the bacterial ce while the other strains along their full extent (Fig. 1C).

The majority of the bacterial cells occurred, on root surface, mainly in single units or smal aggregations, with the exception of the strain EnunB12 which formed extensive aggregations o the surface of roots (Fig. 1D).

Azospirillum and *Bacillus* strains were also able to adhere to root-hair surface (Fig. 1E). SEM observations provided evidence for internal colonization of *Azospirillum* (Fig. 1F).

Conclusions

Bacteria tested prefer to colonize root-hair regions in which there is more release of roo exudates; the modality of adhesion to root surface depends on bacterial strain and for some seems to be supported by fibrillar material anchoring.

Internal root colonization has been observed only for *Azospirillum* strains. It is known tha *Azospirillum* strains are able to external and internal colonization of roots. The way o *Azospirillum* penetration into intercellular spaces is still controversial matter and not fully understood (Bashan and Levanony, 1990), but in our case SEM observations suggested the penetration of bacteria in root injuries.

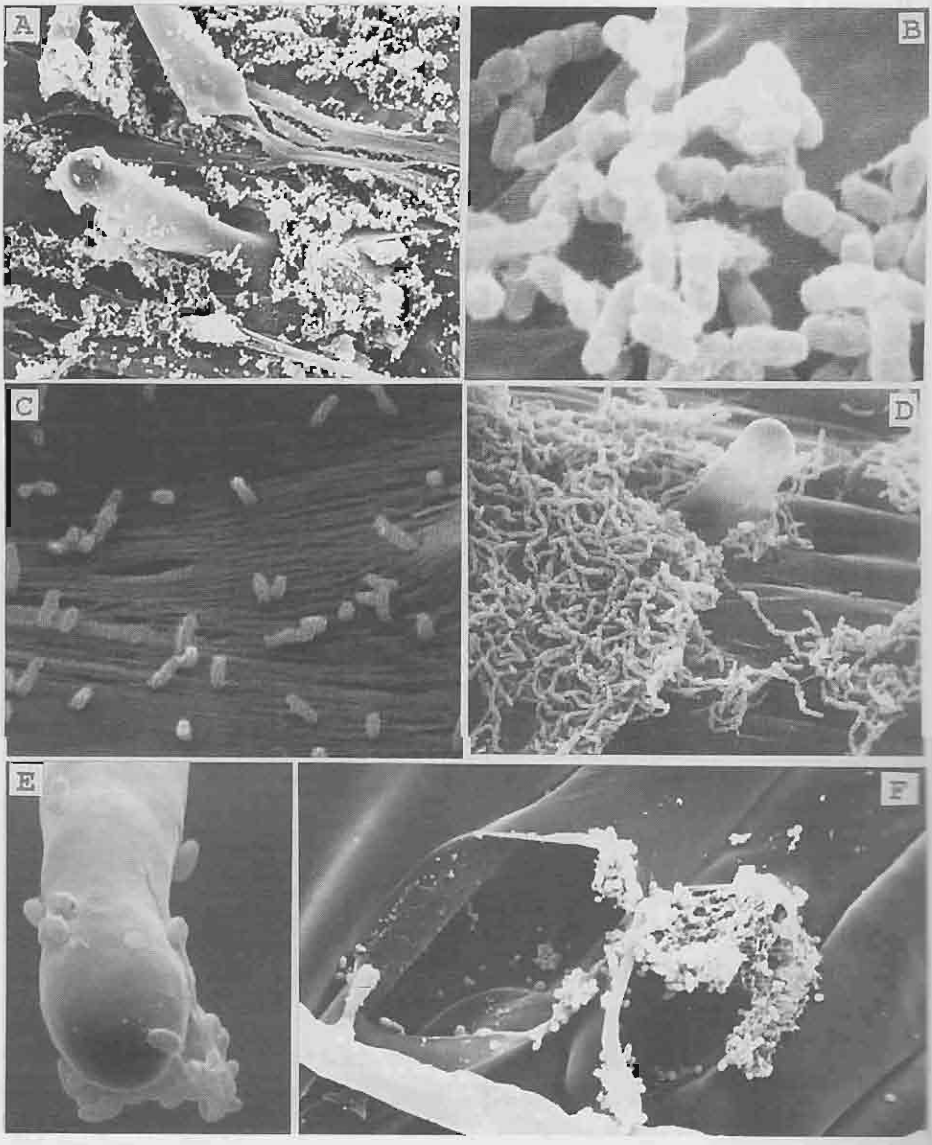

Figure 1. Scanning electron micrographs: (A) EnunAp3 adhered to maize root, X655; (B) EnunB12 anchored to root surface by fibrillar materal, X5000; (C) EnunEt5 anchored to maize root by a single pole of the bacterial cell, X2500, (D) EnunB12 formed aggregations on maize root surface, X600; (E) EnunAp2 adhered to root hair surface, X2620; (F) internal colonization of maize root by EnunAp3, X1250.

Acknowledgement

Work carried out according to a contract of Ministry for Universities and Scientific and Technological Research to Enichem Agricoltura S.p.A., referring to the National Research Program in Advanced Biotechnologies.

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