

Molecular Ecology of Rhizosphere Microorganisms

Biotechnology and the
release of GMOs

Edited by

F. O'Gara, D.N. Dowling, B. Boesten



Weinheim · New York
Basel · Cambridge · Tokyo

This Page Intentionally Left Blank

Molecular Ecology of Rhizosphere Microorganisms

Edited by

F. O'Gara, D. N. Dowling, B. Boesten



© VCH Verlagsgesellschaft mbH, D-69451 Weinheim (Federal Republic of Germany), 1994

Distribution:

VCH, P. O. Box 101161, D-69451 Weinheim, Federal Republic of Germany

Switzerland: VCH, P. O. Box, CH-4020 Basel, Switzerland

United Kingdom and Ireland: VCH, 8 Wellington Court, Cambridge CB1 1HZ, United Kingdom

USA and Canada: VCH, 220 East 23rd Street, New York, NY 10010-4606, USA

Japan: VCH, Eikow Building, 10-9 Hongo 1-chome, Bunkyo-ku, Tokyo 113, Japan

ISBN 3-527-30052-X

Molecular Ecology of Rhizosphere Microorganisms

Biotechnology and the
release of GMOs

Edited by

F. O'Gara, D.N. Dowling, B. Boesten



Weinheim · New York
Basel · Cambridge · Tokyo

Editors:
Prof. Fergal O'Gara
Dr. David Dowling
Ir. Bert Boesten
BioMerit
Microbiology Department
University College
Cork
Ireland

This book was carefully produced. Nevertheless, authors, editors and publisher do not warrant the information contained therein to be free of errors. Readers are advised to keep in mind that statements, data, illustrations, procedural details or other items may inadvertently be inaccurate.

Published jointly by
VCH Verlagsgesellschaft mbH, Weinheim (Federal Republic of Germany)
VCH Publishers Inc., New York, NY (USA)

Editorial Director: Dr. Hans-Joachim Kraus
Production Manager: Dipl.-Wirt.-Ing. (FH) H.-J. Schmitt

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data:
A catalogue record for this book
is available from the British Library

Die Deutsche Bibliothek – CIP-Einheitsaufnahme:
Molecular ecology of rhizosphere microorganisms :
biotechnology and the release of GMOs / ed. by F. O'Gara ... –
Weinheim ; New York ; Basel ; Cambridge ; Tokyo : VCH, 1994
ISBN 3-527-30052-X
NE: O'Gara, Fergal [Hrsg.]

© VCH Verlagsgesellschaft mbH, D-69451 Weinheim (Federal Republic of Germany), 1994

Printed on acid-free and chlorine-free (TCF) paper

All rights reserved (including those of translation into other languages). No part of this book may be reproduced in any form – by photoprinting, microfilm, or any other means – nor transmitted or translated into a machine language without written permission from the publishers. Registered names, trademarks, etc. used in this book, even when not specifically marked as such are not to be considered unprotected by law.

Composition: Filmsatz Unger & Sommer, D-69469 Weinheim
Printing: Strauss Offsetdruck, D-69509 Mörlenbach
Bookbinding: Großbuchbinderei J. Schäffer, D-67269 Grünstadt
Printed in the Federal Republic of Germany.

Preface

Techniques in biotechnology and information science are revolutionising our ability to understand how microbes interact with their environment. The emerging field of molecular microbial ecology will allow technologists, researchers and regulators to monitor, model and predict with increased accuracy the outcome of a range of microbial applications of key biotechnological importance including; health-care (clinical microbiology), agribusiness (biocontrol and food microbiology) and environmental microbiology (bioremediation). This book is a collection of papers presented by the speakers and tutors at an EC sponsored BRIDGE Advanced Workshop in Biotechnology on "The Molecular Ecology of Rhizosphere Bacteria" held in Cork (22nd March – 2nd April 1993). The workshop consisted of an international research forum, co-organised by BIOMERIT (EC Comett II programme) and a practical component directed towards young researchers.

The genetic and molecular techniques that can be applied to the study of the ecology of rhizosphere microorganisms are as numerous and diverse as the microbes themselves. Experimental methods developed for unraveling the molecular complexity of the cell are being directed to the study of rhizosphere ecosystems and the integration of molecular methods with classical methods is expanding our understanding of rhizosphere microbial ecology. Biotechnology has been a major impetus in applying new methods in rhizosphere ecology. The availability for release of Genetically Modified Microorganisms (GMOs) has stimulated research programmes to evaluate their potential impact in the rhizosphere.

The chapters cover different areas of rhizosphere microbiology. They provide an overview of the current concepts and bottlenecks in our understanding of the molecular basis of rhizosphere microbial ecology and the impact that GMOs may have on this ecosystem. The contents of this book represent the synthesis of the authors contributions to the workshop which we hope will go some way towards defining a molecular basis to understanding rhizosphere microbial ecosystems.

We would like to thank the authors for their interest and commitment to the workshop and the members of the plant-microbiology group (UCC) who contributed to its success. The encouragement and support of Dr A. Leonard and Dr I. Economidis of the European Commission (DG12) were appreciated. Finally, we would like to thank Sheila Kelleher, Joan Buckley and Mary Cotter for their secretarial assistance.

Fergal O'Gara
David N. Dowling
Bert Boesten
Editors

This Page Intentionally Left Blank

List of Contributors

David M. Weller and Linda S. Thomashow
USDA-Agricultural Research Service,
Root Disease and Biological Control
Research Unit,
Pullman, Washington 99164-6430,
U.S.A.

John A. McInroy and Joseph W. Klopper
Department of Plant Pathology,
Biological Control Institute,
Alabama Agricultural Experiment
Station,
Auburn University,
Auburn, Alabama 36849, USA.

Maarten H. Ryder^{1,2}, Clive E. Pankhurst^{1,2}, Albert D. Rovira¹, Raymond L. Correll³ and Kathy M. Ophel Keller

¹ Cooperative Research Centre for Soil and Land Management,

² CSIRO Division of Soils and

³ CSIRO Biometrics Unit
Glen Osmond SA 5064
Australia

J.M. Lynch, F. A.A. M de Leij,¹
J.M. Whipps¹ and M.J. Bailey²
School of Biological Sciences,
University of Surrey,
Guildford, Surrey, GU2 5XH, UK

¹ Horticulture Research International,
Littlehampton, West Sussex,
BN17 6LP, UK

² NERC Institute of Virology and
Environmental Microbiology,
Mansfield Road, Oxford, OX1 3SR,
UK

David N. Dowling, Bert Boesten,
Paul R. Gill, Jr. and Fergal O'Gara
Department of Microbiology,
University College,
Cork,
Ireland

Christophe Voisard^{1,2,+}, Carolee T. Bull^{2,+}, Christoph Keel¹, Jacques Laville², Monika Maurhofer¹, Ursula Schnider^{2,+}, Geneviève Défago¹ and Dieter Haas^{2*}

¹ Department of Plant Sciences/
Phytomedicine and

² Department of Microbiology,
Eidgenössische Technische Hoch-
schule,
CH-8092 Zürich,
Switzerland.

⁺ Present address: Swiss Meteorologi-
cal Institute, CH-8044 Zürich,
Switzerland.

^{*} Laboratoire de Biologie Microbienne,
Université de Lausanne,
CH-1015 Lausanne,
Switzerland.

Victor de Lorenzo
Centro de Investigaciones Biológicas-
CSIC;
Velázquez 144, Madrid 28006, Spain.

Hans-Volker Tichy and Reinhard Simon
TÜV Südwest,
Fachgruppe Biologische Sicherheit,
Robert-Bunsen-Straße 1,
D-79108 Freiburg, Germany.

VIII *List of Contributors*

J.E. Cooper and A.J. Bjourson
Plant Pathology Research Division
Department of Agriculture for
Northern Ireland
Newforge Lane, Belfast BT9 5PX,
Northern Ireland

Pascal Simonet, Sylvie Nazaret and
Philippe Normand.
Laboratoire d'Ecologie Microbienne du
Sol,
URA CNRS 1450,
Université Claude Bernard Lyon I,
43 Bd du 11 Novembre 1918,
69622 Villeurbanne, France.

Peter Marsh and Elizabeth M. H.
Wellington
Department of Biological Sciences,
University of Warwick, Coventry, UK.

J.D. van Elsas and E. Smit
Institute for Soil Fertility Research,
P.O. Box 7060, 6700 GW Wageningen,
the Netherlands.

Marco P. Nuti^{1,2}, Andrea Squartini¹
and Alessio Giacomini¹

¹ Dipartimento di Biotecnologie
agrarie,
Università di Padova,
via Gradenigo 6, 35121 Padova
(Italy)

² CRIBI Biotechnology Centre,
Università di Padova, Complesso
"A. Vallisneri",
via Trieste 75, 35121 Padova, (Italy)

Content

Preface V

List of Contributors VII

- 1 Current Challenges in Introducing Beneficial Microorganisms into the Rhizosphere** 1
 - 1.1 Introduction and Definitions 1
 - 1.2 Relationship of Root Colonization to Biocontrol and Growth Promotion 2
 - 1.3 The Process of Colonization 3
 - 1.4 Effect of Biotic and Abiotic Factors 4
 - 1.5 Bacterial Traits Contributing to Rhizosphere Competence 5
 - 1.6 Population Dynamics of PGPR in the Field 7
 - 1.7 Release of Genetically Engineered Rhizobacteria 8
 - 1.8 Mechanisms of Biological Control by PGPR 9
 - 1.9 Inconsistent Performance of PGPR 11
 - 1.10 Improving Root Colonizing and Biological Control 12
 - 1.11 Conclusion 13
 - 1.12 References 13

- 2 Studies on Indigenous Endophytic Bacteria of Sweet Corn and Cotton** 19
 - 2.1 Introduction 19
 - 2.2 Materials and Methods 20
 - 2.2.1 Media 20
 - 2.2.2 Field Experiments 20
 - 2.2.3 Sample Preparation and Surface Sterilization 20
 - 2.2.4 Growth Conditions, Bacterial Counts and Data Analysis 21
 - 2.2.5 Isolation and Preservation of Endophytes 21
 - 2.2.6 Strain Identification 21
 - 2.3 Results 22
 - 2.3.1 Population Dynamics 22
 - 2.3.2 Bacterial Identification 23
 - 2.4 Discussion 24
 - 2.5 References 27

- 3 Detection of Introduced Bacteria in the Rhizosphere Using Marker Genes and DNA Probes** 29
 - 3.1 Introduction 29
 - 3.2 Methods 30

3.2.1	Spontaneous Antibiotic Resistance	30
3.2.2	Marker Genes	31
3.2.2.1	New metabolic capability	32
3.2.2.2	Heavy metal resistance	33
3.2.2.3	Bioluminescence	33
3.2.2.4	Herbicide resistance	33
3.2.2.5	Transposons carrying antibiotic resistance	33
3.2.3	DNA Probes	35
3.2.4	Detection Limits, Amplification and Enrichment	37
3.2.4.1	Increased Sensitivity by PCR Amplification	37
3.2.4.2	Enrichment	37
3.3	Case Study: Tracking <i>LacZY</i> -labelled <i>Pseudomonas corrugata</i> in the Field	38
3.3.1	Pre-release Testing	39
3.3.2	Field Release	40
3.4	The Ecological Fitness of Genetically-Engineered Bacteria	41
3.4.1	Metabolic Load	41
3.4.2	Reduced Fitness	41
3.5	Conclusions	42
3.6	References	44
4	Impact of GEMMOs on Rhizosphere Population Dynamics	49
4.1	Introduction	49
4.2	A Most Probable Number (MPN) Recovery Technique	50
4.3	The Need for an Eco-Physiological Index (EPI)	51
4.4	Conclusions	53
4.5	References	54
5	Developing Concepts in Biological Control: A Molecular Ecology Approach	57
5.1	Introduction	57
5.2	Siderophore-Mediated Competitive Exclusion of Phytopathogens	58
5.3	Exploiting Antifungal Metabolites to Enhance Biological Control	60
5.4	Stability of Introduced Genes and Biological Containment Systems for GMO's	61
5.5	Conclusion	63
5.6	References	64
6	Biocontrol of Root Diseases by <i>Pseudomonas fluorescens</i> CHA0: Current Concepts and Experimental Approaches	67
6.1	Introduction	67
6.2	Mechanistic Studies on Biocontrol Traits of <i>Pseudomonas Fluorescens</i> CHA0	68
6.2.1	Chemical Identification of Extracellular Metabolites	68
6.2.2	Genetic Manipulation of Strain CHA0	70
6.2.3	Gnotobiotic System	73
6.2.4	Mutations Affecting Biocontrol Efficacy, Regulation of Secondary Metabolism, and some Caveats	75

- 6.2.5 Induced Systemic Resistance in Plants 77
- 6.2.6 Genetic Instability of Strain CHA0: Effects on Secondary Metabolism and Biological Control 78
- 6.3 Environmental Impact of Bacterial Inoculants 79
 - 6.3.1 Environmental Monitoring 80
 - 6.3.2 Microcosms 80
- 6.4 Potential Applications 81
- 6.5 Conclusion 82
- 6.6 References 83

- 7 Genetic Strategies to Engineer Expression Systems Responsive to Relevant Environmental Signals 91**
 - 7.1 Introduction 91
 - 7.2 Mini-Transposons as Genetic Tools 91
 - 7.2.1 Rationale for the Utilization of Mini-Tn5 Transposons 92
 - 7.2.2 A Universal Suicide Delivery System 92
 - 7.2.3 Alternative Selection Markers 94
 - 7.3 Engineering Gene Expression within Mini-Transposons 95
 - 7.3.1 Selecting an Adequate Level of Transcription 95
 - 7.3.2 Post-Transcriptional Bottlenecks 96
 - 7.4 Engineering Alkyl- and Halo-aromatic Responsive Phenotypes 97
 - 7.5 Outlook 99
 - 7.6 References 100

- 8 Genetic Typing of Microorganisms: Current Concepts and Future Prospects 103**
 - 8.1 Introduction 103
 - 8.2 Techniques for the Analysis of DNA Sequence Polymorphisms 104
 - 8.2.1 Southern Blot and Hybridization 104
 - 8.2.2 PCR-Amplification of Polymorphic DNA 105
 - 8.2.3 Ribotyping of Bacterial Strains 105
 - 8.2.4 Fingerprinting by Arbitrarily Primed PCR 106
 - 8.2.5 Fingerprinting by tRNA Consensus Primed PCR 107
 - 8.2.6 Automated Analysis of Fingerprints 109
 - 8.3 Outlook 109
 - 8.4 References 111

- 9 Development of Subtraction Hybridization Procedures for Generating Strain-Specific *Rhizobium* DNA Probes 113**
 - 9.1 Introduction 113
 - 9.2 System with Biotinylated and Mercurated Subtractor DNA 113
 - 9.3 Combined Subtraction Hybridization and PCR Amplification Procedure 114
 - 9.3.1 Technical details 115
 - 9.3.1.1 Isolation of DNA 116
 - 9.3.1.2 Synthesis of oligonucleotides and preparation of linkers 116

XII *Content*

- 9.3.1.3 Preparation of probe strain DNA 117
- 9.3.1.4 Preparation of subtracter DNA 117
- 9.3.1.5 Subtraction hybridization 117
- 9.3.1.6 Isolation of probe strain DNA sequences from the subtraction mixture 117
- 9.4 Results 118
- 9.5 Conclusions 118
- 9.6 References 119

10 Molecular Characterization and Detection of the Actinomycete *Frankia* in the Environment 121

- 10.1 Introduction 121
- 10.2 Taxonomy 122
 - 10.2.1 DNA-DNA hybridization data 122
 - 10.2.2 Sequencing of 16S rDNA genes 123
- 10.3 Characterization of *Frankia* 125
 - 10.3.1 Conventional Techniques 125
 - 10.3.2 Sequence Based Characterization 126
 - 10.3.2.1 Intergenic Spacers 126
 - 10.3.2.2 PCR/RFLP 127
- 10.4 Detection and Enumeration 128
 - 10.4.1 Detection of *Frankia* in Actinorhizae 128
 - 10.4.2 Direct Detection of *Frankia* Present in the Soil 129
- 10.5 Conclusion 130
- 10.6 References 130

11 Molecular Ecology of Filamentous Actinomycetes in Soil 133

- 11.1 Introduction 133
- 11.2 Life-cycle of Streptomycetes in Soil 134
 - 11.2.1 Spore Germination and Mycelial Development in Soil 135
 - 11.2.2 Molecular Monitoring of Differentiation in Soil 136
- 11.3 Potential for Genetic Interactions between Actinomycetes in Soil 138
 - 11.3.1 Conjugative Interactions between Streptomycetes in Soil 139
 - 11.3.2 Gene Exchange between Actinomycetes and Other Bacteria 140
 - 11.3.3 Interactions between Streptomycetes and Actinophages in Soil 141
- 11.4 Detection and Expression of Specific Genes in Soil 142
 - 11.4.1 Antibiotic Resistance Genes and Expression of Antibiotic Production Genes in Soil 143
 - 11.4.2 Detection of Amplified Genes in Soil 144
- 11.5 Conclusions 145
- 11.7 References 146

12 Some Considerations on Gene Transfer between Bacteria in Soil and Rhizosphere 151

- 12.1 Introduction 151
- 12.2 Soil and Rhizosphere as Habitats for Bacteria 152

12.3	Gene Transfer in Soil and Rhizosphere	153
12.3.1	Transformation	153
12.3.2	Transduction	155
12.3.3	Conjugation	157
12.4	Concluding Remarks	159
12.5	References	161
13	European Community Regulation for the Use and Release of Genetically Modified Organisms (GMOs) in the Environment	165
13.1	Introduction	165
13.2	The International Regulatory Framework	167
13.3	The European Community Regulation	168
13.4	Biosafety Results of Field Tests of GMOs	169
13.5	Concluding Remarks	171
13.6	References	171
	Index	175

This Page Intentionally Left Blank

1 Current Challenges in Introducing Beneficial Microorganisms into the Rhizosphere

David M. Weller and Linda S. Thomashow

1.1 Introduction and Definitions

Since 1904, when Lorenz Hiltner introduced the term rhizosphere, much has been learned about its biology, microbiology, and ultrastructure. The rhizosphere is the narrow zone of soil surrounding the root that is subject to the influence of the root. Intense microbial activity and larger microbial populations occur in this zone as compared to the bulk soil, in response to the release from roots of large amounts of organic matter (50–100 mg/g of root), in the form of exudates, lysates, and mucilages. As much as 18% of carbon assimilated as photosynthate can be released from roots. Organic compounds lost from roots include sugars, amino acids, organic acids, fatty acids, nucleotides, vitamins and enzymes. Since the rhizosphere is rich in exudates, the microbial population can reach up to 1×10^9 cells per cm^3 , 10–100 times larger than the population in the bulk soil. Rhizosphere microorganisms include bacteria, viruses, fungi, arthropods, mites, amoebae, and flagellates. The rhizosphere extends away from the root for 1–2 mm, but some organisms may be stimulated up to 5 mm away.

The rhizoplane refers to the actual surface of the root; however, as a root ages, cortical cells undergo autolysis (a genetically controlled trait) and the boundary between the rhizoplane and the rhizosphere becomes blurred. The root cortex becomes colonized by microorganisms such that only the tissues of the stele remain alive. Thus, a part of the root becomes an extension of the rhizosphere known as the endorhizosphere. The use of the term endorhizosphere recently has been questioned (1). Interestingly, despite the intense microbial activity in the rhizosphere, only about 7–15% of the actual root surface (rhizoplane) is covered with microorganisms. They are clumped into microcolonies in sites where nutrients are most abundant. These include grooves between epidermal cells, root hairs, lesions and sites where lateral roots break through cortical cells. The rhizosphere is a dynamic environment, and rhizosphere interactions have substantial impact on plant growth and development (2,3).

Rhizobacteria are plant-associated bacteria that are able to colonize and persist on roots (4). Rhizobacteria are subdivided into beneficial, deleterious and neutral groups on the basis of their effects on plant growth. Studies during the late 1970s

and early 1980s at the University of California, Berkeley demonstrated that certain fluorescent *Pseudomonas* strains, termed plant growth-promoting rhizobacteria (PGPR), could improve the growth of potato and sugar beet when applied to seeds or seed pieces (5,6). The results of these studies along with public concerns about the adverse affects of chemical pesticides helped to catalyze a resurgence of research worldwide on bacterial inoculants to control pathogens and improve plant growth. The term PGPR is now applied to a wide spectrum of strains that have, in common, the ability to promote the growth of plants following inoculation onto seeds or subterranean plant parts (4). Growth promotion can occur through direct stimulation of the plant either by increasing the supply of mineral nutrients, such as phosphorous and nitrogen, or by the production of phytohormones (7). PGPR also improve growth indirectly through the suppression of major and minor soilborne pathogens (8,9). Major pathogens produce the well-known root or vascular diseases with obvious symptoms. Minor pathogens are parasites or saprophytes that damage mainly juvenile tissue such as root hairs and tips and cortical cells, and produce symptoms that are not obvious (10). Schippers et al. (11) distinguished the parasitizing minor pathogens from the nonparasitizing deleterious rhizosphere microorganisms (DRMO). DRMO include deleterious rhizobacteria (12) and deleterious fungi. PGPR have been identified from many genera besides *Pseudomonas* these include *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Enterobacter*, *Erwinia*, *Flavobacteria*, *Hafnia*, *Klebsiella*, *Rhizobium*, *Serratia* and *Xanthomonas*.

Several definitions of root colonization by rhizobacteria have been proposed (4,13,9). In this chapter it is defined as the process whereby bacteria introduced on seeds or vegetatively propagated plant parts become distributed along roots growing in raw soil, multiply, and survive for several weeks in the presence of indigenous soil microflora. Root colonization includes colonization of the root surface, the inside of the root and/or the rhizosphere. Rhizosphere competence describes the relative root-colonizing ability of a rhizobacterium. The rhizosphere competence of a strain can be quantified by determining the population size it attains on a root, the length or number of roots colonized and/or the length of time the bacteria survive (9).

In spite of the "biological buffering" that generally limits introducing microorganisms into soil (14), PGPR can be established successfully because they are highly adapted to the rhizosphere. Further, the application of large populations allows rapid occupation of preferred sites where exudates are greatest.

1.2 Relationship of Root Colonization to Biocontrol and Growth Promotion

It is generally accepted that PGPR must become positioned on or in the root or in the rhizosphere to promote growth (7,15,16,17). Rhizobacteria growing in or near infection courts on roots, as well as in channels in the rhizosphere that provide physical

access to the root, are ideally positioned to limit the establishment and spread of pathogens. Several studies have demonstrated that PGPR suppress populations of root pathogens (18,19,20). For example, Xu and Gross (21) applied *Pseudomonas putida* W4P63 to potatoes and monitored its population and that of its target *Erwinia carotovora* on roots in the field. The populations of W4P63 ranged between 10^4 and 10^5 colony forming units per g root, while the population of *E. carotovora* on the same root was only 10% of that on roots without W4P63. However, the threshold populations required to achieve pathogen suppression or growth promotion have not been well defined for most PGPR. Bull et al. (22) studied the relationship between the population size (ranging from 10^1 - 10^5 per cm root) of *P. fluorescens* 2-79 established on wheat roots via seed treatments, and the number of lesions caused by the take-all pathogen, *Gaeumannomyces graminis* var. *tritici*. Linear regression analysis demonstrated an inverse relationship between the population size of 2-79 and the number of lesions, thus indicating that as colonization increased, take-all control improved.

Another important question is the duration that populations of PGPR must be maintained in order to close the "window of vulnerability" to infection by pathogens. For some diseases, such as *Pythium* seed rot and damping-off, protection may be required for only hours to days, whereas, for diseases like take-all, protection will need to last weeks to months. In general, the smaller the "window of vulnerability" the greater are the chances of successful biocontrol.

1.3 The Process of Colonization

How are PGPR translocated from sources of inoculum on seeds or seedpieces to critical sites along the root and in the rhizosphere? Howie et al. (23) hypothesized a two phase process. In Phase I, bacteria on the seed attach to the emerging root tip and are passively transported into the soil. During root growth some cells remain associated with the tip while others are left behind on older portions of the root and in the rhizosphere. Bacteria may be dislodged as the root extends through the soil or become adsorbed to soil particles (24). In Phase II, bacteria deposited along the root multiply and form microcolonies in nutrient-rich microsites, compete with indigenous microflora and avoid displacement. Any bacterium applied to the seed can be transported into the soil with the roots but only those that are rhizosphere competent will maintain or increase their population (25). The concept of Phase I and II is meant to define two stages in the life history of introduced PGPR, rather than a strict temporal sequence of events because both phases occur simultaneously on different parts of the roots. Although percolating water is not required for root colonization (22,23,24), Liddell and Parke (15) demonstrated that it can make a major contribution to the long distance transport of introduced PGPR. They applied *P. fluorescens* PRA25 to pea seed and monitored root colonization in a Plainfield sand incubated at 24-26°C. In the absence of infiltrating water PRA25 was restricted to root segments 0-4 cm below the seed at matric potentials of -1, -6 and -10 kPa.