Molecular Ecology of Rhizosphere Microorganisms

Biotechnology and the release of GMOs

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

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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 committment 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.

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Content

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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 x 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.