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Non-target effects of the microbial control agents *Pseudomonas fluorescens* DR54 and *Clonostachys rosea* IK726 in soils cropped with barley followed by sugar beet: a greenhouse assessment

Anders Johansen[a,*], Inge M.B. Knudsen[b], Svend J. Binnerup[a], Anne Winding[a], Jens E. Johansen[a], Linda E. Jensen[c], Karen S. Andersen[a], Mette M. Svenning[d], Torben A. Bonde[a]

[a]*Department of Environmental Chemistry and Microbiology, The National Environmental Research Institute, Frederiksborgvej 399, 4000 Roskilde, Postbox 358, Denmark*

[b]*Department of Plant Biology, Plant Pathology Section, The Royal Veterinary and Agricultural University; Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark*

[c]*Section of Genetics and Microbiology, Department of Ecology, The Royal Veterinary and Agricultural University, Thorvaldsensvej 40, DK-1871 Frederiksberg C, Denmark*

[d]*Faculty of Science, Department of Biology, University of Tromsø, N-9037 Tromsø, Norway*

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Abstract

Non-target effects of a bacterial (*Pseudomonas fluorescens* DR54) and a fungal (*Clonostachys rosea* IK726) microbial control agent (MCA), on the indigenous microbiota in bulk soil and rhizosphere of barley, and subsequent a sugar beet crop, were studied in a greenhouse experiment. MCAs were introduced by seed and soil inoculation. Bulk and rhizosphere soils were sampled regularly during the growth of barley and sugar beet. The soils were assayed for the fate of MCAs and various features of the indigenous soil microbiota. At the end of the experiment (193 d), DR54 and IK726 had declined by a factor of 10^6 and 20, respectively, and DR54 showed a short-lasting growth increase in the sugar beet rhizosphere. In general, the non-target effects were small and transient. IK726 seemed to have general stimulating effects on soil enzyme activity and the soil microbiota, and resulted in a significant increase in plant dry weight. The plant growth-promoting effect of DR54 was less pronounced and the DR54 displaced indigenous pseudomonads. DR54 stimulated growth of protozoans with a tolerance for the anti-fungal compound viscosinamide produced by DR54. Treatment with the fungicide Fungazil had no effects on plant growth or soil microorganisms. Phospholipid fatty acid (PLFA) analysis detected the perturbations of the soil microbial community structure in the MCA treatments as well as the return to non- perturbed conditions reflecting the decline of inoculant populations. The PLFA technique appears to be suitable for in situ monitoring of MCA non-target effects on the soil microbiota, but should be combined with assays for MCA survival and soil enzyme activity.

Keywords: Risk assessment; Microbial control agent; Soil microbiota; Non-target effect

1. Introduction

Many microorganisms have antagonistic properties against plant pathogenic fungi. Microbial control agents (MCAs) are utilized for plant protection in the field and to relieve the toxic effects of fungicides. However, there is concern that introduction of MCAs into the environment may cause adverse perturbations of the native soil microbiota and the nutrient turnover processes they are involved in.

Numbers of bacterial MCAs introduced into soil decline quickly. Non-target effects are frequently observed, but they are often small and transient and do not persist after the MCA has disappeared from the soil or is reduced substantially in numbers. The fate of MCAs and their non-target effects on the soil microbiota is discussed in detail in a review by Winding et al. (2004). Compared to bacterial MCAs, studies of fungal MCA non-target effects are few

* Corresponding author. Tel.: 46301316; fax: 46301114.
E-mail address: ajo@dmu.dk (A. Johansen).

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(Migheli et al., 1996; Brimner and Boland, 2003) and focus mainly on specific eco-physiological groups, especially arbuscular mycorrhizal fungi (McAllister et al., 1994; Burla et al., 1996; Green et al., 1999).

MCAs typically have repressive effects on the growth or reproduction of plant pathogens (Whipps, 2001; Raaijmakers et al., 2002). They may affect microbial communities by competing for nutrient resources (Wicklow, 1992), by predation, parasitism or by direct production of toxins (Knudsen et al., 1997). If the MCAs are effective in controlling plant pathogens, it is somewhat unexpected that in general the observed non-target effects are small and brief (Winding et al., 2004). However, due to the large variability in the physical and chemical conditions in soil as well as the variation in microbiota, risk assessment of soil microorganisms is complex and obviously our understanding of the in situ conditions is not complete. Poor MCA survival may also be part of the explanation or maybe perturbations are not revealed if they predominantly affect members of the soil microbiota that are not cultivatable. The last situation complicates detection of non-target effects as most soil microorganisms fail cultivation (Torsvik et al., 1990; Winding et al., 1994). It is likely that new MCAs will be isolated or engineered that are far more powerful in their antagonistic traits (production of antibiotics, competitive ability, etc.) and with an enhanced survival competence in soil. Consequently, it is necessary for public authorities to have the knowledge and the tools for proper environmental risk assessment of future MCAs.

Our main objective was to assess the risks and benefits to the indigenous microbiota associated with the introduction of MCAs into soil. Greenhouse conditions were designed to create controlled experimental environmental conditions close to a field situation and allow for sufficient sampling of bulk and rhizosphere soil throughout the entire life cycle of a barley crop, followed by replanting with sugar beet. In addition to assessing the effects on the indigenous microbiota, a second objective was to study the fate of the MCAs in the rhizosphere and bulk soil and their effects on plant growth. A range of methods was applied to monitor the indigenous soil microbiota at several biological levels: the microorganisms (numbers, taxonomic groups, diversity); their functional ability/diversity (enzyme activity profile); community structure/dynamics (changes in structure) and nutrient turnover processes driven by soil microorganisms. Based on the present and previous work, a risk-assessment procedure of MCAs is discussed.

2. Materials and methods

2.1. Soil origin and characteristics

The soil (a sandy loam) was collected in September 1999, at the Højbakkegård Experimental Station, the Royal Veterinary and Agricultural University, Copenhagen Denmark. Using a small bulldozer, 1.5 t of soil was removed from the upper 10 cm of an experimental stubble barley field. The soil, holding 5% water at sampling, was sieved (10-mm mesh) and stored at ambient temperature for 3 weeks before establishing the experiment. Important characteristics of the soil are presented in Table 1.

Table 1
Højbakkegård soil characteristics

Soil texture	
Coarse sand (0.2–2.0 mm)	67.9%
Fine sand (0.02–0.2 mm)	20.1%
Silt (0.002–0.02 mm)	3.5%
Clay (<0.002 mm)	4.6%
Humic material	3.9%
pH	6.2
Total organic C	8.3 g kg^{-1} dry soil
Total N	799 mg kg^{-1} dry soil
Ammonium-N	0.1 mg kg^{-1} dry soil
Nitrate-N	27 mg kg^{-1} dry soil
Calcium	790 mg kg^{-1} dry soil
Sodium	200 mg kg^{-1} dry soil
Cation exchange capacity	96 meq kg^{-1} dry soil
Inorganic P	260 mg kg^{-1} dry soil
Total P	12 g kg^{-1} dry soil

2.2. The experimental design

The greenhouse experiment was designed to assess risks and benefits associated with the use of one bacterial (*P. fluorescens* DR54) and one fungal (*C. rosea* IK726) anti-fungal MCA in a soil planted with barley (*Hordeum vulgare* L., Lamba) and subsequently sugar beet (*Beta vulgaris* L., Marathon). The MCA treatments were compared to a treatment where the barley seeds were coated with a conventional fungicide (Fungazil) and a non-treated control. As a wide range of experimental variables was assessed, only one inoculation rate was used for each MCA. It was decided to inoculate to a density above recommended practise, representing 'a worst case scenario' in order to identify microbial variables that can be perturbed by the MCAs. The soil was inoculated homogeneously to lower the spatial variation and the seeds were inoculated before sowing.

Thirty-six pots (with 2 kg d. wt. soil) and 48 growth containers (with 20 kg d. wt. soil) were arranged in a random block design in the greenhouse. Experimental units were planted with barley and harvested sequentially. Following final harvest of barley at maturity, the soil was sieved (10 mm) and transferred to new pots before seeded with sugar beet.

The schedule of experimental set-up and sampling was as follows: inoculation of soils and packing into growth units at d 0; inoculation and sowing of barley seeds at d 12; non-destructive soil samplings of containers at d 1, 6 and 12; destructive samplings of pots at d 18 and 25 and containers at d 39, 74, 103 and 130; sowing of sugar beet at d 136 and destructively sampling of pots at d 152, 165 and 193.