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Transfer and loss of naturally-occurring plasmids among isolates of *Rhizobium leguminosarum* bv. *viciae* in heavy metal contaminated soils

Amir Lakzian[a], Phillip Murphy[a], Ken E. Giller[a,b,*]

[a]*Department of Biological Sciences, Imperial College at Wye, University of London, Wye, Ashford, Kent TN25 5AH, UK*
[b]*Plant Production Systems, Department of Plant Sciences, Wageningen University, P.O. Box 430, Postbus 430, 6700 AK Wageningen, The Netherlands*

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Abstract

Plasmid transfer among isolates of *Rhizobium leguminosarum* bv. *viciae* in heavy metal contaminated soils from a long-term experiment in Braunschweig, Germany, was investigated under laboratory conditions. Three replicate samples each of four sterilized soils with total Zn contents of 54, 104, 208 and 340 mg kg^{-1} were inoculated with an equal number (1×10^5 cells g^{-1} soil) of seven different, well-characterized isolates of *R. leguminosarum* bv. *viciae*. Four of the isolates were from an uncontaminated control plot (total Zn 54 mg kg^{-1}) and three were from a metal-contaminated plot (total Zn 340 mg kg^{-1}).

After 1 year the population size was between 10^6 and 10^7 g^{-1} soil, and remained at this level in all but the most contaminated soil. In the soil from the most contaminated plot no initial increase in rhizobial numbers was seen, and the population declined after 1 year to <30 cells g^{-1} soil after 4 years. One isolate originally from uncontaminated soil that had five large plasmids (no. 2-8-27) was the most abundant type re-isolated from all of the soils. Isolates originally from the metal-contaminated soils were only recovered in the most contaminated soil. After 1 year, four isolates with plasmid profiles distinct from those inoculated into the soils were recovered. One isolate in the control soil appeared to have lost a plasmid. Three isolates from heavy metal contaminated soils (one isolate from the soil with total Zn 208 mg kg^{-1} and two isolates from the soil with total Zn 340 mg kg^{-1}) had all acquired one plasmid. Plasmid transfer was confirmed using the distinct ITS–RFLP types of the isolates and DNA hybridization using probes specific to the transferred plasmid. The transconjugant of 2-8-27 which had gained a plasmid was found in one replicate after 2 years of the most contaminated soil but comprised more than 50% of the isolates. A similar type appeared in a separate replicate of the most contaminated soil after 3 years and persisted in both of these soils until the final sampling after 4 years. After 2 years isolates were recovered from four of the soil replicates with the chromosomal type of 2-8-27 which appeared to have lost one plasmid, but these were not recovered subsequently.

Isolate 2-8-27 was among the isolates most sensitive to Zn in laboratory assays, whereas isolate 7-13-1 showed greater zinc tolerance. Acquisition of the plasmid conferred enhanced Zn tolerance to the recipients, but transconjugant isolates were not as metal tolerant as 7-13-1, the putative donor. Laboratory matings between 2-8-27 and 7-13-1 in the presence of Zn resulted in the conjugal transfer of the same small plasmid from 7-13-1 to isolate 2-8-27 and the transconjugant had enhanced metal tolerance. Our results show that transfer of naturally-occurring plasmids among rhizobial strains is stimulated by increased metal concentrations in soil. We further demonstrate that the transfer of naturally-occurring plasmids is important in conferring enhanced tolerance to elevated zinc concentrations in rhizobia.

Keywords: Conjugation; Zinc; Metal toxicity; Sewage sludge; Microbial ecology

1. Introduction

The sensitivity of *Rhizobium* to heavy metals in soil is well established (Giller et al., 1989, 1998). Toxic effects of heavy metals are a cause for concern in agricultural soils which become contaminated as heavy metals accumulate in soils which receive repeated additions of sewage sludges

*Corresponding author. Plant Production Systems, Department of Plant Sciences, Wageningen University, P.O. Box 430, 6700, Building No. 537, Haarweg 333, AK Wageningen, The Netherlands.
Tel.: +31 317 485818; fax: +31 317 484892.
E-mail address: Ken.Giller@wur.nl (K.E. Giller).

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