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Detection of the prevalence, diversity and spread of antibiotic production genes in soils

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To shed light on the pathogen-suppressive power of soils, the prevalence and diversities of selected antibiotic production genes in soil under different management regimes (permanent grassland, arable land either under rotation or under monoculture of maize) as well as in the potato rhizosphere were assessed. Specifically, the production loci of pyrrolnitrin (PRN), 2,4-diacetylphloroglucinol (DAPG), phenazine-carboxylic acid (PCA) and selected polyketides were detected by PCR applied directly to soil-derived DNA, as well as cultured isolates. PCR was followed by hybridisation with specific probes to enhance the sensitivity and confirm the identity of products, as well as by cloning and sequencing to infer identity and diversity. Differences in the prevalence of antibiotic production loci were observed between treatments, with a tendency for stronger signals in plant-affected habitats. Quantitative real-time PCR detection was developed on the basis of the *prnD* gene to determine the prevalence of the PRN production locus in different treatments; this confirmed that the prevalence was greatly enhanced under the influence of plant (grass) roots.

Sequence analysis on the basis of fragments from soil as well as from cultured isolates revealed that the *prnD* gene was diverse and fell into several distinct taxonomic groups, under which *Pseudomonas* and *Burkholderia* species. This indicated the likelihood of horizontal gene transfer of these genes.

Type II ketosynthase (KS) genes of the aromatic polyketide antibiotic biosynthetic pathway were prevalent in the potato rhizosphere. Specific KS amplicons obtained both directly from soil and from isolates were sequenced. Phylogenetic analyses showed that the sequences from the isolates clustered separately, in two distinct clusters. The isolates were identified as (1) various *Streptomyces* species and (2) *Serratia*

ficaria and *S. proteomaculans*. The comparative sequence analysis revealed incongruity between the KS and 16S rDNA phylogenetic trees, as, for instance, the *Streptomyces caviscabies* KS gene sequence was more closely related to that of *Serratia* sp. than to those of the other *Streptomyces* sp. This suggested horizontal gene transfer of aromatic polyketide biosynthesis genes between these taxons. It remains open to what extent the current data and methods on gene prevalence and spread can serve to actually address antibiotic production activities in soil, but this is certainly a key issue in future work.