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FUNCTIONAL DIVERSITY INVESTIGATION OF BACTERIAL COMMUNITIES IN DISTINCT SOIL TYPES WITH RISA AFTER PRECULTURING (RISA-APC) METHOD.

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ABSTRACT – Functional diversity investigation of bacterial communities in distinct soil types with RISA after preculturing (RISA-APC) method.
Microorganisms play a leading role in soil development and preservation; moreover, they could indicate the soil health and conditions. In this study, we analyzed the bacterial composition of three different soil types with a newly developed RISA-APC method. A novelty of this method is the pre-culturing step: this preculturing of the bacterial communities were performed on solid media supplemented with different carbon sources (e.g.: carboxy-methyl cellulose, xylan, chitin, starch, tributyrin, casein and protocatechuic acid). For the investigation of heavy metal tolerant bacteria, a preculturing on YEG media containing $CuSO_4$ or $CdCl_2$ were used. The mini-colonies developed after a short incubation time was investigated. This RISA-APC method proved to be a useful tool for the comparison of different soil types, and for the examination of changes in the soil bacterial community structure. It was clearly shown that the most diverse functional diversity values occurred in the forest soil and the less diverse bacterial community was detected in sandy soil samples.

Keywords: bacterial community analysis, soil quality, soil type, preculturing, RISA-APC

INTRODUCTION

Soil is a very complex and dynamic biological system; microorganisms adapt to microhabitats and live there together in consortia. The extent of the diversity of microorganisms in soil seems to be critical to the maintenance of soil health and quality (RANJARD ET AL., 2001). Despite the relatively small biomass of the bacteria and fungi present in the soil, they play a key role in the carbon, nitrogen, sulphur and phosphor cycle (PANKHURST ET AL., 1997). Microbes affect the physical properties of the soil, for example the water holding capacity and soil structure (ELLIOTT ET AL., 1996). The chemicals, pesticides and heavy metals are significantly affecting the microbiological activities and trough this way, the soil health. The microbes respond very quickly to the external influences, so the investigation of the communities could refer to the different changes of the environmental factors (PANKHURST ET AL., 1995). For the rapid comparing studies of bacterial communities the most useful are the 16S rDNA genes. A large database of 16S rDNA sequences exists in the gene bank (www.ncbi.nlm.nih.gov) (NIELSEN AND WINDING, 2002). The region of the rRNA gene cluster between the small (16S) and large (23S) ribosomal RNA genes in bacteria is called the intergenic spacer region (ITS), which may encode tRNAs depending on the bacterial species, and displays significant heterogeneity in both length and nucleotide sequence. Both types of variation have been extensively used to distinguish bacterial strains and closely related

species. The ITS length polymorphism could be visualized with gelelectophoresis, and the resulted fingerprint of fragments is characteristic, such as a barcode and indicates the composition of the investigated bacterial community. In this study, we combined the RISA method with pre-culturing of the soil bacteria, and get a simple and reliable method for analyse and for compare different soil types.

MATERIAL AND METHOD

Sampling, culture conditions: Soil samples (top soils in all cases) were collected in Hungary from different areas, one from a wheat field, one from forest and one from sandy soil. The pre-culturing was performed on solid media containing (l^{-1}): agar 20 g, KH_2PO_4 5 g, $MgSO_4$ 1 g, $(NH_4)_2SO_4$ 1 g, supplemented with different carbon sources (2 g; carboxy-methyl cellulose, xylan, chitin, starch, tributyrin, casein and protocatechuic acid). As a control, a complex medium was used (l^{-1}): glucose 2 g, yeast extract 2 g, and agar 20 g. For the investigation of the heavy metal tolerance of bacteria, $CuSO_4$ or $CdCl_2$ were added to the complex medium. Fifty grams of the soil sample were diluted and homogenised in 50 ml isotonic NaCl solution (0.9%), and 50 µl aliquots were spreaded onto the agar plates. After desiccation, they were incubated at 20 °C for 20 hours. Isotonic NaCl solution (2 ml) was used to washed-down mini-colonies from the plates. The suspensions were centrifuged and the pellets were resuspended in 0.5 ml isotonic NaCl solution.

DNA isolation: The DNA isolation was carried out with Aqua Genomic Solution™ kit, according to the manufacturer's instructions.

PCR reaction, RISA: For the amplification of the bacterial ITS region, the Eub_ITSF as forward and Eub_ITSR as reverse primer were used. PCR was carried out in a final volume of 50 µl containing 5 µl of *Taq* polymerase 10x puffer, 1.6 mM $MgCl_2$, 200 µM for each of the dNTPs, 10 pM primers, 5 µl of template DNA (app. 100 ng) in distilled water and 1 U *Taq* DNA polymerase (Fermentas). The PCR product was visualized with gelelectophoresis, and the DNA fragments in the gels were stained with SYBR Green and analyzed under UV light.

RESULTS

RISA-APC profiles revealed different complexity resulted in by the different number and different relative intensity of their bands. From bacteriological point of view, the less complex habitat was the sandy soil (marked H). In many cases we did not get any bands after the PCR reaction, while culturing on complex medium resulted 9 bands. These sandy soil samples did not contain bacteria which could utilize the specific carbon sources tested. Forest soil sample (marked E) showed high diversity values on several carbon sources. The soil of wheat field (marked B) showed some similarity to soil sample of forest. However, substantial differences were observed in the number of the bands on carboxy-methyl cellulose, xylan and chitin (*Figure 1*.). This suggested the presence of bacteria with cellulose, xylan and chitin degrading potential. The estimated number of heavy metal resistant bacteria was nearly similar in forest and in wheat field samples (*Figure 2*.). A potential explanation is that both habitats can be exposed to these pollutants.

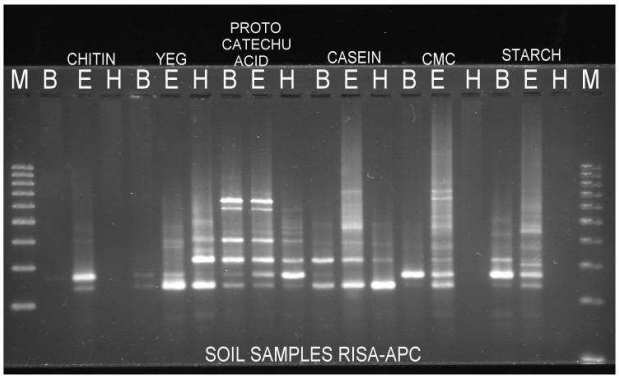

Figure 1. RISA-APC profiles from different soils: B-wheat field soil, E-forest soil, H-sandy soil. The preculturing was performed on different carbon sources.

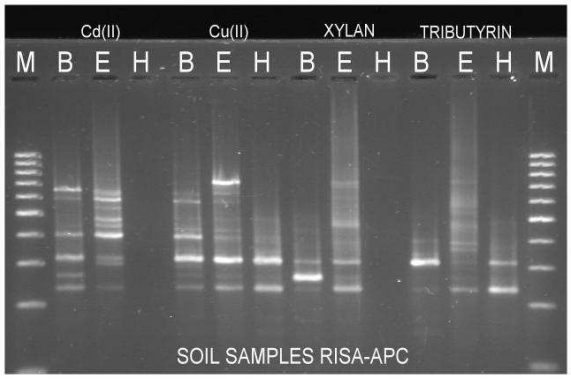

Figure 2. RISA-APC profiles from different soils. B-wheat field, E-forest soil, H-sandy soil. Cd(II) and Cu(II): the lines of metal resistant bacterial communities.

Fingerprint experiments showed high reproducibility, no difference were detected between replicates of RISA profiles obtained from amplifications of triplicate DNA extracts. The RISA-APC profiles were manually compared. The number of bands observed is summarized in *Table 1*.

Table 1. Comparison of RISA profiles (number of bands after PCR reaction).

Carbon source/heavy metal	H (sandy soil)	E (forest)	B (wheat field)
carboxy-methyl cellulose	0	13	3
xylan	0	9	6
chitin	0	4	1
starch	0	12	8
tributyrin	3	10	6
casein	4	8	5
protocatechuic acid	8	9	9
glucose	9	7	2
Cu (II)	6	10	9
Cd (II)	0	9	6

CONCLUSIONS

The results of RISA-APC clearly correlated, as regards the complexity of RISA-fingerprints, with the expected basic taxonomical complexity of various soil types. These results showed that the highest functional diversity values occurred in the forest soil, while the lowest were present in the sandy soil.

The distribution of the band number is similar in the case of preculturing on protocatechuic acid, a product of lignin biodegradation. The greatest differences were detected on carboxy-methyl cellulose, xylan and chitin carbon sources (Figure 3). The background of this result could be the high microbial diversity of forest surface soils. Dead plant material mostly consists of cellulose and xylan, which explains the presence of these types of degraders in high numbers. Similarly, the high incidence of chitin degrading bacteria in the forest soil could be explained with the presence of insects and fungi in this habitat.

The RISA-APC method developed in our study proved to be a useful tool for the comparison of different soil types, and for the examination of changes in the soil bacterial community structure. Furthermore, this approach could be combined with various statistical methods to analyse these correlations in detail. The main disadvantage of this approach is that the results do not provide bases for a precise species or genus identification (FISHER AND TRIPLETT 1999; JENSEN ET AL., 1993).

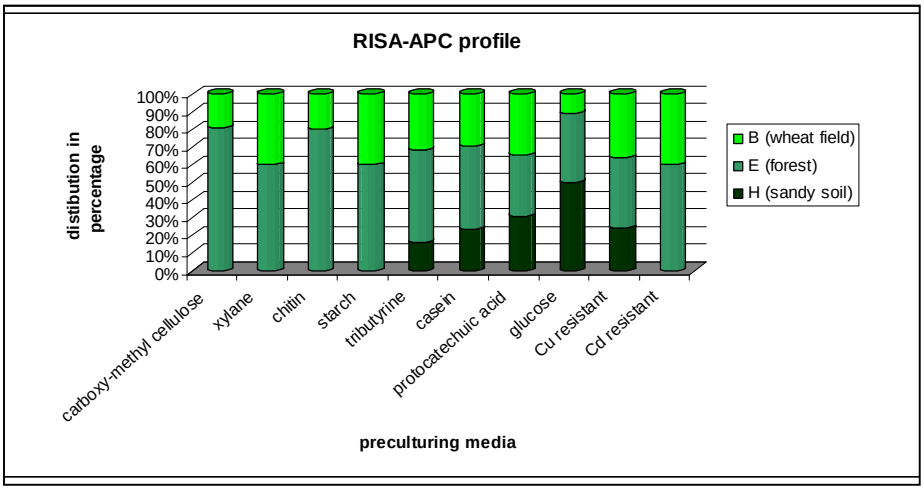

Figure 3. The diagram shows the number of the resulted bands with RISA-APC after preculturing on the different carbon sources or in the presence of heavy metals.

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