

Plasmid pool in pesticide-contaminated soils



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INTRODUCTION

Plasmids are bacterial mobile genetic elements that facilitates rapid evolution and adaptation of their hosts to changing environmental conditions. Genes coded on plasmids has a big impact on their bacterial hosts, their importance for soil properties and fertility cannot be disregarded. This is especially important in agricultural soils, which are often treated with toxic chemical compounds, like pesticides. Soils contaminated with pesticides are often enriched in bacterial or fungi species capable to degrade deadly compounds. Moreover genes located on mobile elements are known to play important role in resistance of microorganisms to chemical pollution. In presented work plasmid diversity in pesticide (mainly organochlorine) contaminated soils was assessed using PCR replicon typing and deep metagenome sequencing.

SAMPLES

Soil samples (coming from 7 sites) were collected in 2010 and 2011 during elimination of underground infrastructure where obsolete pesticides were stored from 1960's.

DNA was isolated with modified method of Zhou and colleagues (1996). Standard Illumina paired-end libraries were prepared and sequenced on Illumina hardware.

Physicochemical properties of collected samples were assessed with standard methods. Pesticides were detected and quantified by GC-MS and HPLC-MS.

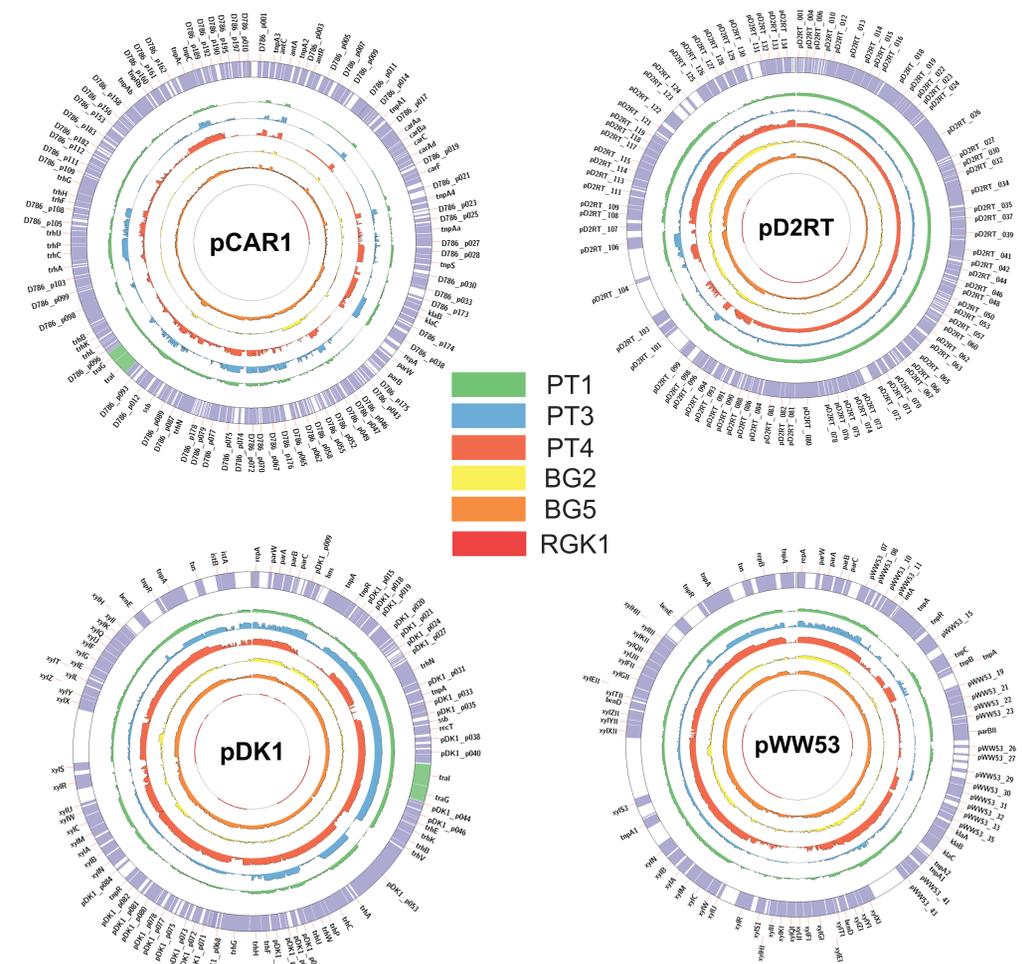
16S rRNA genes fragments (357-786) were amplified via PCR and sequenced on 454 GS FLX Titanium machine. Obtained reads were analyzed using functions contained in MOTHUR software. OTUs were constructed using average neighbor clustering method.

Tab. 1. Basic characteristics of analyzed samples

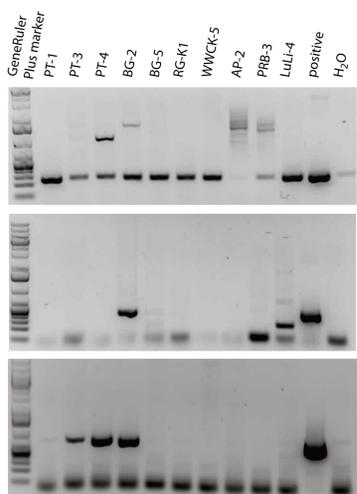
sample	species (16S based)	contaminants concentration [ng/ul]
PT-1	36	73382.33
PT-3	21	26848.33
PT-4	135	221637.72
BG-2	183	2781.32
BG-5	419	28288.39
RG-K1	84	426.37
WWCK-5	360	78.36
AP-2	794	46.00
PRB-3	280	2.0
LuLi-4	535	27216.00

PLASMID BACKBONES PRESENT IN METAGENOMIC SAMPLES

Selected metagenomes were sequenced on Illumina platform. Obtained sequencing reads were mapped to known plasmid sequences (NCBI) using bowtie2 to find possible plasmid backbones. Several IncP1, IncP7 and IncP9 backbones were found (Tab. 2., Fig. 2), including: pDK1, pCAR1, pWW53 (IncP7), pC1-1 (IncP1), as well as other plasmid backbones, like pD2RT. It is worth to note that most of identified sequences originate from plasmids described as catabolic, in many cases connected with organochlorines degradation. Almost no plasmid sequences were detected in one sample, RGK1, despite of high depth of sequencing.



IncP PLASMIDS DIVERSITY IN CONTAMINATED SOILS



Replicons of selected IncP groups were detected with standard PCR replicon typing method, using primers *trfa21/trfa22* (IncP1, targeting fragment of *trfA2* gene), *tolRepF/tolRepR* (IncP9, targeting fragment of *rep* gene) and *RepRmsF/RepRmsR* (IncP7, targeting fragment of *rep* gene).

Analysis revealed presence of IncP1 plasmids in all analysed samples. IncP9 and IncP7 was restricted to more polluted samples (Fig. 1).

Fig. 1 PCR replicon typing of metagenomic samples coming from pesticide-contaminated soils.

HIGHLIGHTS

- * PCR replicon typing can be used to determine plasmids presence in metagenomic samples
- * IncP1 is a most common group of plasmids in analyzed soils
- * IncP9 and IncP7 groups are present mainly in contaminated soils
- * Deep metagenomic sequencing can be used to reconstruct plasmid backbones

Fig. 2 Plasmid backbones identified in sequenced metagenomes. Outer circle shows plasmid annotation, on inner circles coverage of reads from metagenomic samples were plotted (logarithmic scale). Annotations were downloaded from Genbank.

plasmid	accession	length	sequence coverage					
			PT1	PT3	PT4	BG2	BG5	RGK1
pD2RT	NC_021250	129894	95,28%	89,94%	92,07%	91,36%	98,96%	0,68%
pDK1	NC_014124	128921	91,40%	89,32%	94,47%	83,38%	98,35%	1,92%
pWW53	NC_008275	107929	88,47%	75,95%	89,53%	80,56%	98,87%	1,04%
pCAR1	NC_004444	199035	58,87%	43,46%	58,02%	25,83%	93,65%	0,40%
reads sequenced [mln]:			13,86	211,36	160,02	63,1	32,06	219,71

Tab. 2. Most common plasmid sequences identified in sequenced metagenomes

ACKNOWLEDGEMENTS

This work was supported by the Polish National Science Centre grant DEC-2012/05/N/NZ9/01393

This investigation was cofinanced by the European Union from the resources of the European Regional Development Fund under the Innovative Economy Programme

This work has been supported with a scholarship from the European Social Fund, Human Capital Operational Programme for the execution of the Programme "Support for bio med tech scientists in technology transfer (UDA-POKL.08.02.01-14-041/09)

