Microbiomics, metabolomics and mobilomics suggest a high level of genetic adaptation towards pesticide biodegradation in on farm biopurification systems

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Introduction

Biopurification systems (BPSs) are used at farms to treat pesticide-contaminated wastewater. BPSs act as biofilters in which the water is treated on a solid matrix composed of waste materials like straw, peat and soil and in which the pesticides are removed by sorption and biodegradation. On-farm BPSs receive pesticides at high concentrations during a substantial period of the year, leading to strong selective pressures for evolution and growth of pesticide degrading bacteria. This enables bacteria to develop novel enzymatic activities and metabolic pathways to use such organic xenobiotics as sole source of energy and carbon or as a nutrient source (van der Meer, 2007). Genes involved in the degradation of organic xenobiotics are often located on mobile genetic elements (MGEs), such as plasmids and transposons, collectively called the mobilome. These MGEs are considered as crucial agents in the evolution and adaptation of bacterial communities by controlling the intra- and interbacterial exchange of genetic material (Heuer & Smalla, 2012; Top & Springael, 2003). Among the best described MGEs that carry xenobiotic catabolic gene clusters are the broad host range IncP-1 plasmids and the composite IS\textsubscript{1071} transposons. The latter MGE is also often found to be present on IncP-1 plasmids. Interestingly two defined insertion hot spots are found on the IncP-1 plasmid backbone genes where the ‘accessory load’, not necessary for plasmid functioning, replication or maintenance, is inserted. It is hypothesized that due to the xenobiotic selective pressure, MGEs like IncP-1 plasmids and IS\textsubscript{1071} elements are often associated with xenobiotic degradation and will be enriched in the bacterial community of a BPS as carrier of genes for pesticide catabolism. The prevalence of IncP-1 plasmids and IS\textsubscript{1071} elements were therefore studied in microcosm setups to which different pesticide stress conditions were applied. Furthermore several operational BPS systems were investigated for their MGE content. To unravel the ecological role of IncP-1 plasmids and IS\textsubscript{1071} elements in bacterial communities we developed a novel method to access the accessory load on these elements. In our approach we designed primers targeting conserved genes on these MGEs to develop long range (LR) PCRs. For IS\textsubscript{1071} the transposase gene was targeted and for IncP-1 plasmids primers were designed on the backbone genes adjacent to the two insertion hotspots trf\textsubscript{A}-ori\textsubscript{V} and trb-operon-traC. With this novel methodology the accessory load of these MGEs were explored on two pesticide contaminated ecosystems, namely an operational on-farm BPS system and a pesticide treated BPS microcosm experiment.

Material and methods

BPS material was collected from different operational on-farm BPS systems. Also different BPS microcosm setups were investigated, to which either tap water or tap water with pesticides was applied. PCR and qPCR based detection of genetic markers for MGEs and catabolic genes were performed. Catabolic activities were examined by mineralization of $^{14}$C-
labelled pesticides and degradation of chloroaromatic compounds. Long range (LR) PCR methods directed toward amplification of accessory genes of IS1071 composite transposons or IncP-1 plasmids were developed. Efficiency of our LR PCR system was evaluated on metagenomic DNA of an operational on-farm BPS to which a dilution series of a reference strain containing the catabolic IncP-1 plasmid pNB8c was added. The presence of the desired amplicon was confirmed by Southern blot hybridizations targeting the catabolic gene dcaQ. LR amplicons were generated on metagenomic DNA from a microcosm BPS and BPS in operation and sequenced using Illumina HiSeq 2000. Rapid gene predictions and annotation were performed using RAST and predicted protein sequences were further analysed for conserved protein domains using HMMER3.

Results

High prevalence of IncP-1 plasmids and IS1071 elements were observed in pesticide treated microcosms simulating the BPS filter matrix and in BPS in operation at farms. This high MGE content was concomitant with an increase in the catabolic capacity for pesticide and haloaromatic degradation and in the abundance of catabolic genes compared to non-treated systems. The LR PCR approach generated amplicons up to 33kb, with detection limits of $10^5$ copies per gram of soil. The method was successfully applied on DNA extracts from a microcosm BPS and a BPS in operation. Interestingly in the IS1071 LR PCR only the pesticide treated microcosm BPS samples yielded large amplicons, while water treatment didn't show any amplification. While in the microcosm BPS a rather limited amount of IS1071 LR amplicons were observed, a larger abundance of amplicons were generated in the operational BPS, mostly between 7 and 20 kb in length. This suggests that pesticide stress conditions drive the increase of IS1071 elements and that the accessory load is important for the adaptation of the bacterial community. Next generation sequencing of the amplified LR amplicons revealed around 150 kb of unique DNA in the pesticide treated microcosm BPS and around 650 kb in the operational BPS. In both LR amplicon sequence data sets a high enrichment of hypothetical enzyme functions for organic xenobiotic catabolism was observed, including dioxygenases, dehalogenases and amidohydrolases. Moreover genes previously reported to be participating in the degradation of the pesticide linuron were retrieved in both the microcosm and operational BPS. Moreover the atzA gene responsible for the first step in the atrazine degradation was found in the operational BPS. This data is in accordance with the mineralization capacity of these pesticides in these ecosystems.

Conclusion

Our data show the extensive catabolic potential of microbiota in a BPS at the genetic level and suggest that the mobilome is an important mediator in shaping this genetic content. The results further remove uncertainties about the biological aspect of pesticide contaminated wastewater treatment in BPS.

References


