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Characterization of a collection of plasmid-containing bacteria isolated from an on-farm biopurification system used for pesticide removal

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ABSTRACT

Biopurification systems (BPS) are complex soil-related and artificially-generated environments usually designed for the removal of toxic compounds from contaminated wastewaters. The present study has been conducted to isolate and characterize a collection of cultivable plasmid-carrying bacterial isolates recovered from a BPS established for the decontamination of wastewater generated in a farmyard.

Out of 1400 isolates, a collection of 75 plasmid-containing bacteria was obtained, of which 35 representative isolates comprising in total at least 50 plasmids were chosen for further characterization. Bacterial hosts were taxonomically assigned by 16S ribosomal RNA gene sequencing and phenotypically characterized according to their ability to grow in presence of different antibiotics and heavy metals. The study demonstrated that a high proportion of the isolates was tolerant to antibiotics and/or heavy metals, highlighting the on-farm BPS enrichment in such genetic traits. Several plasmids conferring such resistances in the bacterial collection were detected to be either mobilizable or selftransmissible. Occurrence of broad host range plasmids of the incompatibility groups IncP, IncQ, IncN and IncW was examined with positive results only for the first group. Presence of the IS*1071* insertion sequence, frequently associated with xenobiotics degradation genes, was detected in DNA obtained from 24 of these isolates, strongly suggesting the presence of yet-hidden catabolic activities in the collection of isolates. The results showed a remarkable diversity in the plasmid mobilome of cultivable bacteria in the BPS with the presence of abundant resistance markers of different types, thus providing a suitable environment to investigate the genetic structure of the mobile genetic pool in a model on-farm biofilter for wastewater decontamination in intensive agricultural production.

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1. Introduction

In recent years, a great interest has arisen in the study of microbial communities in contaminated environments since their associated microbiota represents a reservoir for diverse types of resistance determinants as well as catabolic genes. Several polluted environments have been chosen as target sites for searching genes for organic-compounds degradation (Choi et al., 2009; Herrick et al., 1997; Zhang and Anderson, 2012), antibiotic resistance (Smalla et al., 2000) and heavy-metal resistance (Rajkumar et al., 2012; Smit et al., 1998) among others, and many of those loci have been demonstrated to be encoded on plasmids and other mobile genetic elements (MGE) (Anjum et al., 2012; Sharma and Thakur, 2009; Shin et al., 2012; Tabata et al., 2011).

In order to minimize agricultural contamination, on-farm biopurification systems (BPS) have been developed as a strategy for removing toxic compounds from polluted wastewater sources generated in the farmyard. BPS receive high loads of pesticides and in cases other toxic compounds at relatively rich concentrations during a substantial part of the year, thus imposing a strong, constant and long-term selective pressure on the evolution and growth of the associated microbiome (Sniegowski et al., 2011). For these reasons bacterial communities within BPS are research targets of practical and basic interest.

In the present study, we characterized a collection of plasmid-containing bacteria isolated from an on-farm BPS composed of a mixture of substrates such as cow manure, straw, willow chippings, soil, coconut chips, garden-waste compost and peat (De Wilde et al., 2010; Dunon et al., 2013).The list of all pesticides added to the BPS was reported in detail by Dealtry et al. (2014) and included at least fifteen different compounds.

The study of catabolic plasmids has been focused on the characterization of small and large plasmids that can be potentially used either in biotechnological assays or bioremediation strategies (Zhang et al., 2012). Particularly, we characterized community members from a BPS that was previously pointed as an environment with high abundance of IncP plasmids and other MGE (Dealtry et al., 2014; Gaze et al., 2013).

We present here the phenotypic and molecular characterization of a collection of plasmid-containing cultivable bacteria isolated from our model BPS. We characterized plasmid abundance and taxonomy of the bacterial hosts, plasmid size, their incompatibility groups, mobilization properties, and finally the presence of common traits frequently associated to plasmids and other MGE. Although the experimental strategy was limited to cultivable microorganisms, this *modus operandi* offered several advantages. First, the methodology allowed maintenance of plasmids in their natural hosts, enabling host-identification. Second, no plasmid-encoded selectable markers were required, which enabled the acquisition of a wider range of plasmid-containing bacteria, since many plasmids do not confer easily identifiable phenotypes on their host bacteria. The results that we present in this work show a remarkable diversity of cultivable plasmid-containing bacterial hosts in the biofilter, a significant proportion of transmissible plasmids, and several phenotypic characteristics that suggest the exposition of the BPS to other selective pressures (i.e. antibiotics, heavy metals) than the known high loads of pesticides.

2. Materials and methods

2.1. Bacterial strains and plasmids

Escherichia coli CV601 (Rf^s GFP) (Smalla et al., 2006) and *E. coli* CSH26 (Rf^r) (Miller, 1972) were used as recipient strains in bi- and triparental matings. *E. coli* HB101 carrying plasmid pRK600 (Finan et al., 1986) or pRK2013 (Figurski and Helinski, 1979) were used as helper strains in the triparental matings. *Ensifer* (*Sinorhizobium) meliloti* MVII-1 was used as plasmid molecular weight marker in *in situ* lysis gel electrophoresis (Kosier et al., 1993).

2.2. Biopurification system sampling

Samples were obtained from a pesticide-containing biofilter in Kortrijk, Belgium, containing a biomix composed of coco chips, straw, manure and field soil (Dunon et al., 2013). The BPS was used to treat water contaminated with different types of pesticides resulting from spillage and for residue water collected when cleaning the spraying equipment. Of the BPS mixture, 250 g were collected in March 2011 as was described by Dealtry et al. (2014) and stored for 2 weeks at 4 °C in the dark.

2.3. Recovery of bacteria from a BPS

Plasmid-containing bacteria were isolated as follows: 5 g of BPS sample was resuspended in 50 ml of sterile physiological-saline solution and the suspension was shaken at 180 rpm for 1 h at room temperature. The bacteria were harvested from the supernatant and aliquots plated in different cycloheximide-containing media (200 μg/ml) as well as in antibiotic-containing Luria-Bertani (LB) medium (Sambrook et al., 1989) and incubated at 28 °C for 48 h. Eight different types of agar plates were used for colony isolation: LB, tryptone-yeast (TY; Beringer, 1974), eosin–methylene-blue (EMB; Levine, 1918), MacConkey (Mck; Macconkey, 1905), dextrose-agar (Dex; Sigma-Aldrich), Mueller-Hinton (MH; Oxoid Ltd, UK), glutamate-sucrose minimal medium (GS) (Del Papa et al., 1999) and M9 minimal medium (M9) (Kempner and Miller, 1972). When required, the following antibiotics were added to LB medium: 120 μg/ml neomycin (Nm), 400 μg/ml streptomycin (Str), 200 μg/ml ampicillin (Ap), 10 μg/ml tetracycline (Tc), 25 μg/ml kanamycin (Km), 50 μg/ml gentamicin (Gm), 20 μg/ml trimethoprim (Tp), 200 μg/ml erythromycin (Er), 20 μg/ml chloramphenicol (Cm), 5 μg/ml nalidixic acid (Nx), 50 μg/ml carbenicillin (Cb) or 100 μg/ml rifampicin (Rf).

2.4. Plasmid screening

Single colonies obtained from plating on different media were grown in LB liquid medium overnight at 28 °C and