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Factors affecting transfer of degradative plasmids between bacteria in soils

Yujing Wang, Shumeng Kou, Qiuyue Jiang, Bin Xu, Xiaoxiang Liu, Jing Xiao, Yuhan Tian, Chenhao Zhou, Chuan Zhang, Ming Xiao *

Development Center of Plant Germplasm, College of Life and Environment Sciences, Shanghai Normal University, Shanghai 200234, China

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ABSTRACT

Horizontal gene transfer is useful for enhancing bioremediation through gene bioaugmentation. However, factors affecting transfer of degradative plasmids have not been systematically addressed. To this end, plasmid transfer experiments were performed using a TOL-like plasmid carrying the gene encoding for catechol 2,3-dioxygenase (C23O) between two soil bacteria under different conditions. Transfer frequency increased with air temperature in the range of 10–35 °C and reached 6×10^{-4} transconjugants per donor cell at 35 °C. The transfer frequency detected at soil depth 5–10 cm was significantly higher ($p < 0.05$) compared with other depths. Addition of 5–75% LB in the microbial inoculum promoted plasmid transfer frequencies. Addition of phenol to the experimental system resulted in significantly higher transfer frequency ($p < 0.05$) compared with no addition. Transfer frequency heat-moisture in loam was significantly higher ($p < 0.05$) than in other soils. The highest transfer frequency was found in the experiment containing tomato seedlings, with up to about 1.3×10^{-3} transconjugants per donor cell. Corn and wheat seedlings also led to significantly higher transfer frequencies ($p < 0.05$) compared with no plants. Furthermore, C23O activities of transconjugants formed under different conditions were measured, as a surrogate measure of the activity of transconjugant. Transfer temperature, soil and plant types had a minor influence on activities of transconjugants. Topsoil (0–5 cm) transconjugants expressed C23O more efficiently under normal incubation condition, but less efficiently when soils incubated with excessive LB medium concentrations, and in the absence of phenol in soil. These results suggested that transfer temperature, soil depth, dilutions of LB broth, phenol content, and soil and plant types had important effects on transfer of the TOL-like plasmid in soil, and some factors also affected activities of transconjugants.

1. Introduction

The anthropogenic pollution of soil has become an object of intensive scientific research since the end of the 20th century. Bioaugmentation has recently attracted interest as an in situ bioremediation method of cleaning up contaminated environments. Bioaugmentation includes both cell and gene bioaugmentation. Cell bioaugmentation is an in situ bioremediation method in which the expected enhanced degradation is due to survival of the introduced organism(s), while gene bioaugmentation is the in situ bioremediation method in which the expected enhanced degradation is due to plasmid transfer to indigenous microbial populations (Ikuma et al., 2012; Pepper et al., 2002). Horizontal gene transfer (HGT) is a successful mechanism of spreading plasmids harboring genes encoding degradative enzymes in different environments (Mars et al., 1999). Gene bioaugmentation depends on efficient HGT.

HGT has been found to play an important role in environmental bioremediation through gene bioaugmentation. Natural HGT of a naphthalene dioxygenase gene promoted naphthalene degradation in a contaminated hillside (Wilson et al., 2003). A psychrotrophic bacterium acquired the capacity to degrade and utilize toluate at temperatures as low as 0 °C by horizontal transfer of a TOL plasmid loaded with biodegradative genes from a mesophilic microorganism (Kolenc et al., 1988). A toluene-degrading endophytic bacterium was constructed by horizontal transfer of a plasmid loaded with the genetic determinants for this degradation, and improved phytoremediation of water-soluble,

* Corresponding author at: Biology Department, College of Life and Environment Sciences, Shanghai Normal University, Shanghai 200234, China.
Tel.: +86 21 64321022; fax: +86 21 65642468.
E-mail address: xiaom88@shnu.edu.cn (M. Xiao).

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volatile organic pollutants (Barac et al., 2004; Taghavi et al., 2005). Horizontal transfer of catabolic plasmids among endophytic bacteria led to toluene degradation and plant growth promotion (Wang et al., 2010). The reports presented above show that successful gene bioaugmentation relies on high transfer frequencies of catabolic plasmids. However, there are selective barriers that must be overcome for successful HGT. Factors affecting transfer of catabolic plasmids have not been elucidated. To this end, plasmid transfer experiments were performed using a mobile TOL-like plasmid carrying the gene encoding for catechol 2,3-dioxygenase (C23O), an important enzyme in the aerobic degradation pathway of aromatic compounds. *Pseudomonas aeruginosa* strain SZH16 has been shown to contain the TOL-like plasmid and has phenol-degrading activity, and *Pseudomonas fluorescens* strain P13 is a plant growth-promoting bacterium without phenol-degrading activity (Yang et al., 2011). The TOL-like plasmid could be transferred from strain SZH16 to strain P13, and the newly formed transconjugants were able to degrade phenol. Factors affecting transfer of the catabolic plasmid were systematically investigated using the TOL-like plasmid and the two strains.

2. Materials and methods

2.1. Plasmid transfer in soil

Plasmid transfer in soil was carried out between *P. aeruginosa* strain SZH16 (accession no.: GU384267, as a donor) and *P. fluorescens* rifampin-resistant P13 strain (as a recipient). The rifampin-resistant P13 strain was isolated by isolating of antibiotic-resistant strains from *P. fluorescens* strain P13 (accession no.: EF487999) in a previous study (Yang et al., 2011). Donor and recipient strains were grown in Luria-Bertani (LB) medium. These strains at the late exponential growth phase were harvested, and resuspended in different dilutions of LB broth (0, 5, 15, 25, 50, 75 or 100%) at approximately 6×10^8 CFU/ml. Samples of four types of soil (loamy sand, sandy loam, sandy clay loam or loam) were collected from the botanical garden of Shanghai Normal University (Table 1), finely pulverized and sterilized by autoclaving three times for 30 min at 121 °C. Each sample was examined for sterility by plating method, and then spiked with different concentrations of phenol (0, 50, 100, 150, 200, 250 or 300 mg/kg soil), as described by Wang et al. (2010). The above four types of soil containing different concentrations of phenol were mixed with donor and recipient strains resuspended in different dilutions of LB broth, respectively, at a final density of 7.9×10^6 cells (5.9×10^6 donor cells + 2.0×10^6 recipient cells)/g soil, respectively. The ratio of donor:recipient cells in the soil was approximately 3:1. Autoclaved clay pots, 40 cm in height and 20 cm in diameter, were used in the experiments. The phenol-spiked and bacteria-inoculated soils were put into the pots, with three pots per treatment. The pots were covered with eight layers of gauze and placed in independently controlled compartments in a sterile greenhouse. The temperatures of these compartments were 10, 15, 20, 25, 30, 35 and 40 °C, respectively. Thermocouples were used to measure the temperatures in these compartments. Distributed air conditioners were used for individual compartment temperature control. The greenhouse and the compartments were sterilized by ultraviolet radiation. Soil water content was measured every day with a TRASE time domain reflectometer (TDR) system (SEC, Santa Barbara, USA), and maintained by watering with sterile water.

Table 1
Physical properties of soils.

Physical properties	Loamy sand soil	Sandy loam soil	Sandy clay loam soil	Loam soil
Sand	86%	73%	51%	43%
Silt	7%	15%	22%	34%
Clay	7%	12%	26%	23%
WHC[a]	22%	29%	34%	14%
pH	6.3	6.6	6.4	6.2

[a] WHC is water holding capacity.

Soil samples were collected at different depths (0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm, 20–25 cm or 25–30 cm) after 20 days of incubation. Selective medium for phenol-degrading bacteria was prepared as described previously (Wang et al., 2007). Transconjugants, donors and recipients were isolated from these soil samples as previously described with slight modification (Hohnstock et al., 2000). Transconjugants were spread onto the selective medium amended with rifampin. Donor cells only were enumerated on the selective medium, and recipient cells were enumerated on LB agar plates amended with rifampin. With transconjugants growing onto the selective medium amended with rifampin as controls, the numbers of donor and recipient cells were obtained by total number of bacterial colonies growing on corresponding media minus the transconjugant colonies, respectively. Both donor and recipient controls were also plated onto the selective medium amended with rifampin to account for spontaneous rifampin resistance. If required, 16S rRNA gene and the plasmids carrying the C23O gene were examined and southern and colony hybridization were performed. Transfer frequencies were measured by dividing the number of transconjugants (minus spontaneous rifampin resistance) by the viable counts of donor cells.

2.2. C23O activity assays

C23O activities of transconjugants were measured following the modified method of Ikuma and Gunsch (2010). In brief, 1 μl of bacterial suspension (10^8 CFU/ml) were inoculated into LB broth. Cell cultures (50 ml) at the late exponential growth phase were harvested by centrifuging for 10 min at $5000 \times g$, washed twice with 20 mM phosphate buffered saline (pH 7.5), then resuspended in 3 ml of the same buffer. Cells were lysed by sonication on ice (six cycles of alternating 30 s of sonication and 30 s of rest on ice). After centrifugation at $10{,}000 \times g$ for 30 min at 4 °C, the supernatants were used as cell-free extracts. C23O activity was estimated by measuring the generation of 2-hydroxymunoic semialdehyde at 25 °C. The amount of this product was determined spectrophotometrically at 375 nm following the addition of 4 mM catechol to the cell-free extract. The concentration of the product was calculated by using an extinction coefficient of 36/mM/cm. One unit of enzyme activity was defined as the amount of enzyme that forms 1 mmol of product per min. Protein concentration was determined by using a Bio-Rad protein assay kit (Bio-Rad USA).

2.3. Plasmid transfer in soil–plant system

A soil–plant system was constructed essentially as described previously (Yang et al., 2011). In brief, seeds of corn (*Zea mays* 'Shentian-1'), wheat (*Triticum durum* 'Huaimai-12') or tomato (*Solanum lycopersicum* 'Hezuo 903'), purchased from Shanghai Howard Seed Co., Ltd., were surface sterilized with 0.01% $HgCl_2$ for 10 min and then washed thoroughly with distilled water. Subsequently, these seeds were placed on Petri plates padded with wet cotton for germination at 24 °C. Donor and recipient strains resuspended in 15% LB broth were introduced to the above sterilized sandy loam soil with phenol (100 mg/kg soil) at a final density of 7.9×10^6 cells (5.9×10^6 donor cells + 2.0×10^6 recipient cells)/g soil, respectively. Healthy one-week-old seedlings, after washing with sterile distilled water, were transferred into the autoclaved pots containing the above sterilized sandy loam soil with phenol and bacteria. Four corn seedlings, four wheat