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Dual labeling of *Pseudomonas Putida* ND6 with fluorescence proteins for exploring the conjugal transfer of pND6-1 and pND6-2 plasmid

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Dual labeling of *Pseudomonas putida* ND6 with fluorescence proteins for exploring conjugal transfer of pND6-1 and pND6-2 Plasmid: Gram-negative *Pseudomonas putida* ND6 possess two large plasmids pND6-1 and pND6-2. The former one which carries the genes encoded for naphthalene degradation in the catechol-meta-cleavage pathway belongs to the IncP-7 conjugative plasmid. Several genes involved in the Type IVB Secretion System are located in the later plasmid. In order to well-understand the characteristics of these two plasmids during conjugation, pND6-1 and pND6-2 were labeled with red fluorescent protein gene (dsred) and green fluorescent protein gene (gfp) respectively by homologous recombination via biparental mating. In view of the narrow host range of the IncP-7 plasmid, Poprl promoter (located before the oprl gene) from *Pseudomonas putida* ND6 was attached to dsred and gfp and inserted into the non-functional region of plasmid together to avoid affecting the expression of functional genes on the plasmid. Both red and green fluorescent proteins were co-expressed in the isolated conjugon GROND6 (pND6-1::dsred, pND6-2::gfp). Furthermore, the results suggested that Poprl promoter could better improve the red fluorescent expression when compared with the green fluorescent protein in *P. putida* ND6. The dual-labeled GROND6 with red and green fluorescent proteins was subsequently tested its conjugation transfer by mating experiment with *P. putida* KT2440 as the recipient. The screened transconjugant KT2440RG exhibited both red and green fluorescence under fluorescence microscopy, indicating that the constructed dual-fluorescent-labeled strain GROND6 (pND6-1::dsred, pND6-2::gfp) can be used to in situ detect the transfer of two mobile plasmids in ND6 in the various environment.

Biography

Shan Wang is pursuing her Doctor's degree in Power Engineering and Engineering Thermophysics at Xi'an Jiaotong University. Her study focuses on the functional mechanism of the conjugative transfer system in *Pseudomonas putida* ND6 and the monitoring of conjugation in distinct environments in situ.

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