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The role of putative -35 sequence in the *vir*E gene expression of *Agrobacterium tumefaciens.*

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The promoter of region of *vir*E gene of *Agrobacterium tumefaciens* has a *vir* box, an inverted repeated sequence, a putative -35 sequence, and a conserved -10 sequence. To understand the role of putative -35 sequence in the *vir*E promoter, a mutant in which the putative -35 sequence in *vir*E promoter was changed from CCGAGT to TTGACA, which is the consensus -35 sequence of *Escherichia coli* promoter, expressed 174% of the wild type *vir*E promoter activity. A mutant containing conserved -35 sequence but missing the region between 5'-end of the promoter and the middle of IR sequence showed 110% of the native *vir*E promoter activity. Another mutant containing conserved -35 sequence but lacking *vir* box and the left half of IR sequence expressed 130% of the native *vir*E promoter activity. These results demonstrated that the *vir*E promoter activity with the putative -35 sequence switched to the consensus -35 sequence of *E. coli* promoter is stronger than that of the native *vir*E promoter.

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Constructon of hybrid *phn* and *pah* genes for functonal analysis of aromatic ring dioxygenases

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We have constructed a hybrid multicomponent dioxygenase gene cluster in which the *pahAcAd* genes, coding for ISP subunits of aromatic dioxygenase from *Pseudomonas putida* OUS82, has been replaced by the *phnXY* and *phnAB* gene encoding the corresponding subunit of phenanthren dioxygenase from *Pseudomonas* sp. strain DJ77 and analyzed the function of a novel hybrid aromatic ring dioxygenase. *Escherichia coli* cells containing the chimeric dioxygenase acquired the novel capability to produce indigo from indole and to convert various aromatic compounds to the dihydrodiols, indicating that the hybrid terminal dioxygenase composed of *phnXY* and *phnAB* forms a functionally active multicomponent dioxygenase associated with ferredoxin(*pahAb*) and Fer reductase(*phaAa*). The results suggest that(*i*) the two subunits of terminal dioxygenase(ISP) are critically involved in the substrate specificity and (*ii*) the electron transport proteins, ferredoxin and Fer reductase, are exchangeable with one another between the *phn* and *pah* complex.