Dissimilar Plasmids Isolated from *Pseudomonas diminuta* MG and a *Flavobacterium* sp. (ATCC 27551) Contain Identical *opd* Genes

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The *opd* (organophosphate-degrading) gene derived from a 43-kilobase-pair plasmid (pSM55) of a *Flavobacterium* sp. (ATCC 27551) has a sequence identical to that of the plasmid-borne gene of *Pseudomonas diminuta*. Hybridization studies with DNA fragments obtained by restriction endonuclease digestion of plasmid DNAs demonstrated that the identical *opd* sequences were encoded on dissimilar plasmids from the two sources.

*Pseudomonas diminuta* MG and a *Flavobacterium* sp. (ATCC 27551) have the ability to degrade a broad spectrum of organophosphorous triesters (2, 4) by virtue of a constitutively expressed organophosphorous acid anhydrase (11, 14). This enzyme stereospecifically hydrolyzes the triester bond common to organophosphorous pesticide molecules without a phosphorylated enzyme intermediate (7). Neither of the strains appears to utilize organophosphorous molecules as nutrients, nor do the substrates appear to be directly toxic to the bacterial cultures. These independent soil isolates encode organophosphorous detoxification genes (*opd*) on large plasmids (10, 13), and the plasmid-borne genes have been cloned (8, 10, 12; C. S. McDaniel, Ph.D. dissertation, Texas A&M University, College Station, 1985). The *Pseudomonas* gene has been sequenced (8).

Recently, it has been shown that crude whole-cell extracts from both *P. diminuta* MG and a *Flavobacterium* sp. (ATCC 27551) were able to degrade disopropyl fluorophosphate (1), an analog of the nerve gas Soman. Purified enzyme preparations of the cloned *Pseudomonas* gene product have been shown to specifically degrade disopropyl fluorophosphate (D. Dumas, J. Wild, and F. Raushel, submitted for publication). Furthermore, methyl parathion-degrading *Pseudomonas* isolates that possess DNA homologous to the *opd* gene have been obtained (3). The studies reported in this paper describe the sequencing of the *Pseudomonas* plasmid-encoded gene and the comparison of that genetic system with the *opd* gene and the plasmid of the *Pseudomonas* strain.

*Escherichia coli* required a host promoter immediately 5' of the *opd* sequence. For that reason, the subcloning of the 7.3-kb *opd*-containing fragment from the *Flavobacterium* sequence (pWWM44) was achieved by *PstI* digestion and insertion of the fragment into M13mp11 and by screening for whole-cell organophosphorous acid anhydrase activity (9) under lac control. Those clones possessing lactose-inducible organophosphorous acid anhydrase activity (as well as clones having reverse orientation, i.e., inactive isolates) were sequenced. Similarities in the restriction endonuclease digestion patterns between the two *opd* genetic regions prompted the sequencing of the *PstI* fragment from the *Flavobacterium* plasmid by using the techniques and DNA primers previously described for the *Pseudomonas* gene (8).

The two genes were shown to be identical, with the possible exception of a G-to-C transversion at base 295 (Fig. 1). This identity extended beyond the putative gene sequence in both the 5' (62-base-pair [bp]) and 3' (286-bp) directions and included the entire *PstI* fragment. By using the Align subroutine of the SEQ program (Intelligenetics, Palo Alto, Calif.), the 5' region common to both genes was compared with the *Pseudomonas* prototype promoters and the *nif* promoter (5) (see sequences below). The 5'-flanking region of the *opd* open reading frame contains the invariant dinucleotides with a critical spacing of 10 bp, which corresponds to the recognition sequence (*G* [30 bp-*G*) for the core of sigma-60 rather than the consensus sequences of *E. coli* (TTGACA-16 or 17 bp-TATAAT) recognized for the core of sigma-70 (5).

| *Pseudomonas* | NT - GGCNNNNTTNGCT
| *opd* | CTCGGCACCAGTCGCTGCAAGCAGAGTCGTAAGCAATCGCAAGGGGGCAGCATG
| *nif* | CT - GGCA - CNNNNNTTGCA

The initial cloning of the *Flavobacterium* sp. *opd* gene region was performed as described previously (10) by using partial *EcoRI* digests of the 43-kilobase-pair (kb) plasmid from the *Flavobacterium* sp.; however, no enzymatic activity was observed for the subclones containing the large fragment. Earlier work on cloning the *Pseudomonas* *opd* gene had demonstrated that its heterologous expression in *Escherichia coli* required a host promoter immediately 5' of the *opd* sequence. For that reason, the subcloning of the 7.3-kb *opd*-containing fragment from the *Flavobacterium* sequence (pWWM44) was achieved by *PstI* digestion and insertion of the fragment into M13mp11 and by screening for whole-cell organophosphorous acid anhydrase activity (9) under lac control. Those clones possessing lactose-inducible organophosphorous acid anhydrase activity (as well as clones having reverse orientation, i.e., inactive isolates) were sequenced. Similarities in the restriction endonuclease digestion patterns between the two *opd* genetic regions prompted the sequencing of the *PstI* fragment from the *Flavobacterium* plasmid by using the techniques and DNA primers previously described for the *Pseudomonas* gene (8).

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In addition to a potential *Pseudomonas*-like promoter sequence in the 5'-flanking region of the *opd* gene, there is also a potential ribosome-binding site (AAGGGGG) just 5' of the presumed translational start codon (underlined above).

The nonidentical nature of the two separately isolated plasmids was demonstrated by using a pair of *PstI* fragments from the *P. diminuta* plasmid (pCMS1) as probes against the plasmid DNA of the *Flavobacterium* sp. (Fig. 2). The cloning of the two *PstI* fragments (C' and D) from pCMS1 which were used in this study has been described previously (8). The C' fragment from the *Pseudomonas* plasmid (1,326

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FIG. 1. Nucleotide sequence of Flavobacterium opd gene fragment. Base 295, which may represent a G-to-C transversion with respect to the Pseudomonas gene, is underlined. Bold type indicates the putative coding sequence for the gene. Base numbers are given on the left.

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FIG. 2. Derivation of plasmids used in this study. (A) P. diminuta plasmid pCMS1 (51 kb) is the native plasmid containing the opd gene. Two pBR322 clones containing either the opd gene (1.3 kb, C' fragment) or a fragment from the Pseudomonas plasmid lacking the opd gene (0.9 kb, D fragment) were constructed. (B) Flavobacterium sp. plasmid (43 kb) is the native plasmid containing the opd gene. pWWM44 is the pBR325 derivative containing an EcoRI fragment of the native plasmid which possessed the opd gene sequence. M13-067 is the M13mp11 derivative of pWWM44 which contains the 1.3-kb active fragment opd.
bp) containing the opd gene was shown by sequence analysis to be identical to the same-size fragment from the *Flavobacterium* plasmid. A second *PstI* fragment (D) of approximately 900 bp was chosen as a probe since it was separated from the region containing the known homology by approximately 22 kb, as estimated by a preliminary restriction digest map of the *Pseudomonas* plasmid (McDaniel, Ph.D. dissertation). For all of the hybridization studies, the methods of Southern (15) were used.

Figure 3 demonstrates the strong hybridization of both *Pseudomonas* and *Flavobacterium* plasmid DNAs with the 1.326-bp (C") fragment containing the opd gene sequence. The *PstI*-digested plasmids differed considerably in their restriction profiles (Fig. 3A). There appeared to be a single plasmid in the *Flavobacterium* strain, although it was present in several forms. Upon restriction, a single hybridizing band was observed for each of the two plasmid sources of the gene (Fig. 3B), and the overall restriction endonuclease pattern is similar to that observed for the isolated plasmid (unpublished observations).

When the 900-bp fragment (D) was used as a probe against both plasmid DNAs (Fig. 4A and B), it hybridized to DNA in the control (*PstI*-digested pCMS1) and the unrestricted *Pseudomonas* plasmid. However, the fragment failed to hybridize to either the native or the restricted plasmid DNA from *Flavobacterium* sp. These results are consistent with the restriction site data reported previously (10) and reiterate the dissimilarity of the two plasmids.

Other examples of genetic homology among *Pseudomonas* degradative plasmids, such as TOL, NAH, and SAL, indicate evolutionary interrelationships. Appreciable homology has been found between the chlorobenzoate degradative plasmid pAC25 and plasmids such as TOL, SAL, and a *Pseudomonas* antibiotic resistance plasmid, even when these plasmids differ in incompatibility and host range characteristics (6). This finding suggests that there may be more-extensive homology between the two parathion-degradative plasmids than just the opd gene itself. Studies are under way to determine the nature of the junction between the homologous and nonhomologous regions of the two plasmids containing the opd gene. Sequence analysis in the flanking regions may provide insight into a mechanism of genetic transfer for a degradative plasmid-borne gene in soil microorganisms.

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**LITERATURE CITED**


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