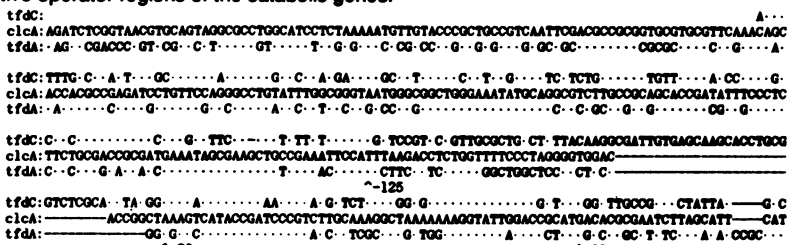
Volume 16 Number 14 1988 Nucleic Acids Research

Partial nucleotide sequence of the chlorocatechol degradative operon *tfdCDEF* of pJP4 and similarity to promoters of the chlorinated aromatic degradative operons *tfdA* and *clcABD*

Edward J.Perkins, George W.Bolton, Milton P.Gordon and Paul F.Lurquin[1]

Department of Biochemistry, SJ-70, University of Washington, Seattle, WA 98195 and [1]Program in Genetics and Cell Biology, Washington State University, Pullman, WA 99164-4350, USA
Submitted May 2, 1988 Accession no.X07754

We have sequenced the 1.6 kb *Hin*dIII G fragment of the *Alcaligenes eutrophus* plasmid pJP4 which contains the promoter region of *tfdCDEF* and the complete sequence of *tfdC*, encoding chlorocatechol 1,2-dioxygenase. This *Hin*dIII fragment has also been sequenced by Ghosal and You (1), although only the open reading frame (ORF) encoding TfdC has been published. We have compared the promoter region of this sequence using the computer program GENEPRO (Riverside Scientific, Seattle WA) to those published for the 2,4-dichlorophenoxyacetate monooxygenase gene, *tfdA*, of pJP4 and the chlorocatechol degradative operon, *clcABD*, of the *Pseudomonas putida* plasmid pAC27 (2,3; see figure). Two regions are highly similar between the three promoter sequences. Region one, from -33 to -86, suggests a possible operator region for regulatory protein(s). Regulatory proteins have been shown to act within the -60 to -80 region of several operons (4). Region two extends from -125 to the end of each of the three sequenced fragments. This region is composed of an ORF, without termination, of 230 bp in the *clcABD* promoter region, 542 bp in the *tfdA* promoter region, and 138 bp in the *tfdCDEF* promoter region. The predicted amino acid sequences of the ORFs are over 65% identical. In searching PROTEIN IDENTIFICATION RESOURCE version 15 using the program FASTP (5), we have found that these three putative proteins are similar in sequence to the regulatory proteins LysR of *Escherichia coli* and NodD of *Rhizobium meliloti*. Additionally, the 1.5 kb *Bam*HI-*Eco*RI fragment, which encodes both the putative protein and TfdA, positively regulates the expression of 2,4-dichlorophenol hydroxylase of pJP4 (E.P. et al, in preparation). In pAC27, the ORF upstream of *clcABD* continues into the adjacent *Bgl*I-*Eco*RI fragment, the presence of which is required for positive regulation of *clcABD* (6). These observations lead us to propose that these ORFs may encode positive regulatory proteins and which may act upon the putative operator regions of the catabolic genes.



Legend. Positions are numbered relative to the transcription initiation start of *clcABD* (3) and ending with the first codon of each catabolic protein. Dashes represent gaps introduced for optimal alignment, dots indicate bases identical to the promoter sequence of *clcABD*. The ribosome binding site is underlined.

References

1. Ghosal,D and I.-S.You (1988) Mol. Gen. Genet. 211,113-120.
2. Streber,W. et al. (1987) J. Bacteriol. 169,2950-2955.
3. Frantz,B. and A.Chakrabarty (1987) Proc. Natl. Acad. Sci. USA 84,4460-4464.
4. Raibaud,O. and M.Schwartz (1984) Ann. Rev. Genet. 18,173-206.
5. Lipman,D. and W.Pearson (1985) Science 227,1435-1441.
6. Ghosal,D. et al. (1985) Proc. Natl. Acad. Sci. USA 82,1638-1642.

E.P. was supported by NSF grant DMB-8410058. G.B. was supported by a grant from Occidental Chemical, Corp.

