

Nucleotide sequence of the metapyrocatechase II (catechol 2,3-oxygenase II) gene *mpcII* from *Alcaligenes eutrophus* JMP 222

Michael Kabisch and Peter Fortnagel*

Universität Hamburg, Institut für Allgemeine Botanik, Abteilung für Mikrobiologie, Ohnhorstraße 18, D-2000 Hamburg 52, FRG

Submitted August 20, 1990

EMBL accession no. X52415

Many degradative pathways for aromatic compounds in bacteria include catechol as a central intermediary metabolite. Ring cleavage is achieved by its oxygenation in the 1,2-(*ortho*) or in the 2,3-(*meta*) position. During our investigation of the degradative capacity of aromatics in *Alcaligenes eutrophus* we could isolate and sequence the gene for metapyrocatechase I (*mpcI*) (1). Here we report that the strain used contains a second gene coding for an extradiol dioxygenase which we named metapyrocatechase II (*mpcII*). We have identified the gene on a 2 kb *EcoRV-SmaI* fragment in a gene bank prepared by inserting partially *Sau3A* digested total DNA of *Alcaligenes eutrophus* JMP 222 into the *Bam*HI site of pBR322. From the DNA sequence of this fragment we predict the *mpcII* gene product as a protein of 320 amino acids with a molecular weight of about 35 kdal which was confirmed in the maxi cell system (2). Since we find enzymic activity if the gene is cloned in the multi cloning site of the pIBI30 vector in either orientation we predict a ribosomal binding site, a transcriptional start and a promoter which function in *E. coli* upstream of the structural gene (Fig. 1). In addition an inverted repeat is present between the predicted start of transcription and the ribosomal binding site. The gene products of the *mpcI* (1) and the *mpcII* genes not only differ significantly in their amino acid composition but both enzymes have different substrate specificities (Table I).

Whereas the *mpcI* gene product has no similarity to other known extradiol dioxygenases (homology comparison <10%) the *mpcII* gene product has between 16 and 20% homology to other members of this class of enzymes such as *XYLE* from the

Table 1. Comparison of the cloned metapyrocatechase I and metapyrocatechase II of *Alcaligenes eutrophus* JMP 222

Substrate	Catechol		3-Methylcatechol		4-Methylcatechol	
	K _m ¹	V _{max} ²	K _m	V _{max}	K _m	V _{max}
MPCI	3.05	3.3	0.12	4.0	0.90	4.1
MPCII	1.28	1.0	0.33	266	<0.01	0.01

¹K_m = mol/l × 10⁻³
²V_{max} = μM/min × 10⁻²

TOL degradative plasmid pWW0 of *Pseudomonas putida* (16.3%) (3-4), *NAAH* from the NAH7 plasmid of *Pseudomonas putida* (17.6%) (5), *TODE* from the chromosome of *Pseudomonas putida* F1 (18.6%) (6), or *BPHCI* from the chromosome of *Pseudomonas pseudoalcaligenes* (19.5%) (7). Especially conserved are to 15 amino acid residues which seem to be essential in extradiol dioxygenases (5, 8).

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- 300  GGATTGGGTGTTTCATGGCGTCTTCTTACAGGGATTACCCCTCATGGGGTTTTCCCTGATGTGACAAAAGGAATGTGGCGTTGCAGCATTAAGTAAATG
- 200  TACGTATGCGAAAAGCAACCTCGGTTGGAAAAGGCAACTACCGCCGGCGCCCGCTGGCTGGCGAAGAACCGGCAACCGGCAACCGGCAACCGGCAAC
- 100  CCAGCCCGCGCAGTGTGACTCATGATGCTCTCCGTCACACACCGGCAAAATATCAAATCTTTTATATATATTTTTGGATATATAGCTTTT
+1   AACCAAGTTCGCGCAACGGCGAGTTCACTAGCCCGAAGAACCGGCCCGCGCTGGCGGCGGTTACAGAAAACAGAAAACCGGCAACCGGCAACCGGCAAC
100  ATGGAACACTCATGCGCGAGTTCGAGGCCAGCGTTCGCAAGCGCCCGCGCTGACCGCGCCATGCCCTGACTCGATGACCACTTACCGCGCTGGAAGTG
M D T H R A D A S Q R S Q A P A A R P R H A V H S I D H Y A L E V
199  CCTGACTGGCAGTGGCGAGCGCTTCTCGACGCGTTGGGCTGACCGTGGCACTACCGCGAATGCTCTGAGGCTATGCGCTGACGACGCGCTG
P D L A V R E R F L D A T F G L T V A R T F E C L E V Y A R D Q R E C
298  TGGCGTGGTTCTAAGAGCGAACCGAAGCGCTCGCTACCTGAGCTTCACTGCTCGAAGCGGATTTCCGGGCGCATCCCGGAGGCTTGGCGCC
N A R F Y E G E R K R L A Y L S F S C F E G D F A G I R Q Q L L A A
397  AGTGGCGGCACTCTGTGACGCAACCGCGCTATGGCGGAGCTGGGATCTGGTCTTCGATCCCGAAGGTAACTGGTCCAGGTGAAGATCGGCCCC
S G A T L V E D P R Y G D E S G V W F F P D
496  AAGACTTCCGACGACAGTCTCCGCGCGCTGGAGGCGCGCGCTGTCAGCGTGGCGGCTGCTGCTGACCAAGTCCAGCGCTGCTGCTGCA
K T S P S S K S P A R L E G A P G G Q R G A V V R S Q V Q R V L F
595  CGCCGCTGTGCAAGTCTGTGTTTCAACCGGAGCTGCGAGCTGCTGCACTTACCGCGAAGCGCTGGGAGCTGGCGCTTCCGACCGATCCGAT
R R L S H V L L F T P S V Q R A L D F Y R D A L C L R L S D R S D
694  GAGCTATTCGCTTACGCAATCCCGTTATGGCAGCGATCCACTCTCTGCTGGCTGCGTGAAGAGCTGGCGCAGGCTGGCCACCGCGCATGGAG
D V I A F T H A P Y G S D H L L D A L V E S A R G W H H A A N D
793  GTCGCGACGCTCAAGAGTGGCCGAGCGGCGCAGCGAGATGGCAAGGCGGCGTCAACGCAAGGCTGGGATCCGGGCGCATGCTGCTCGACTCGAAC
V A D V N E V G Q C A S Q H A K A G Y T Q G M G T G R H V L G S H
892  TACTTCTCTATGTAAGTCCGACCGGCTGGCGCTGCTGCGAGTACTGGCGGAGTGGAGTATCCCGCGCGGACAGGCTGGCGCGGCGGCGGCTTT
Y F F Y V L D P W G S F P C E Y S A D I D Y I P A G Q A M P A G D F
991  CGCGCAGGAGCTCCCTACTACAGTGGGGCGGAGCTGCCAGTACTCTGCTCGCAATCCGAGGCTGAGCGGCAACCGCGGCGGCGGCGGCGGCGGCA
A A E D S L Y Q W G P D V F E Y F V R H T E A 899
1090  CCGTACTTTCACAAATTCGCCCGCATGGCAATTCGCACTGAGCTGGAGATGAACTTGGAGCTTTCTGCTGATGGCCAGCAAGAGTGG
1190  GCCTGTATCCGACGAAAGGCTTCTGCTGCGCGCGGTTGGCGAAGGAGTGGCGGCGCTTATGACGCGCGGATTCGGCCCGCAGCGTGGCACAGCC
1290  TGGTGGCCAGGAGTGCATGCACTGCGCGCTGACTGCTGCGCGGCTGACTGCGCGGCGGCAAGATCTCTGCGTGGCGGCTGAACATGCGGAGCC
1390  ATACCAGGGGCTCTTACGACAGGAGTCCCGAGCGCTTTCTGCGCGTTCGCGCGGCGGCAAGTGGAGATGAGCTGGCGGATCTGCGCGGCTGGCAG
CGACAAGTGGCTACGAGGCTGACTGCGGCTGCTGATGGCAAGGTTGCGAAGCATATCCGACGGGAGACGCTCAGCCATGCTGCTCGCTACTCCG
1490  TCTTCAGACGCGCTCGATCCGAGTACCAGTTCAGGTCGCCGAAATGGAAGGCTGGCGAAGACTTCGACGCAACCGCGGCGCTTTTGGTCCCTATTCG
1590  TGACCGGGCGAAGTCCCGG
1690
    
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* To whom correspondence should be addressed