

Organization and Transcriptional Characterization of the *cat₁* Gene Cluster in *Acinetobacter lwoffii* K24

Seung Il Kim,^{*,1} Sun-Hee Leem,[†] Jong-Soon Choi,^{*} and Kwon-Soo Ha^{*}

^{*}*Biomolecule Research Group, Korea Basic Science Institute, Taejeon 305-333, Korea; and*

[†]*Department of Biology, Dong-A University, Pusan 604-714, Korea*

Received November 12, 1997

Previously, we have reported that two clustered *cat* genes from *Acinetobacter lwoffii* K24 had different arrangements, *catB₁C₁A₁* and *catB₂A₂C₂* (Kim, S. I., S.-H. Leem, J.-S. Choi, Y. H. Chung, S. Kim, Y.-M. Park, Y. K. Park, Y. N. Lee, and K.-S. Ha. 1997, *J. Bacteriol.* **179**, 5226–5231). By further analysis of the organization of the *cat₁* gene cluster, we obtained a complete sequence of the *catB₁* gene, which encoded 40.8-kDa polypeptide containing 379 amino acids, and found a open reading frame (ORF) coding a putative regulatory protein in upstream region of *catB₁* on plasmid pCD1-1. This ORF encoded 34.2-kDa polypeptide containing 379 amino acids and had more than 40% identity with *catR*, *LysR* family regulatory protein of *Pseudomonas putida*. RT-PCR, Northern blot analysis and primer extension assay for transcriptional analysis of the *cat₁* gene cluster revealed that the *catB₁C₁* genes were cotranscribed and the *catA₁* gene was independently transcribed.

© 1998 Academic Press

The β -ketoadiphate pathway is widely used as a central reaction in microbial dissimilation of aromatic compounds [1]. In this pathway, catechol is converted into succinate and the *catABC* genes are involved in the first three steps. The *cat* genes are extensively studied in *Pseudomonas putida* and *Acinetobacter calcoaceticus* [2-7]. Two bacteria have different gene organization but similar transcriptional regulation. In *P. putida*, three *cat* genes are compactly clustered with the transcriptional activator *catR* in order of *catR*, *catBCA*. *CatR* can activate *catBC* and *catA* in the presence of intermediate metabolite *cis,cis*-muconate, which functions as a inducer [3, 8]. In *A. calcoaceticus*, the *catA* gene is separated 3.8 kbp away from *catBCEFD* and

the regulator gene *catM* is present in upstream of *catB* [2, 9, 10]. All of the genes of catechol degradation in *A. calcoaceticus* are activated by *catM*, which previously was thought to act as negative regulator [2], in concert with *cis,cis*-muconate [7]. Both *catR* and *catM* are *LysR*-type transcriptional regulators. The diversity in gene organization and regulation mechanism of the *cat* genes suggests the presence of different gene organization and transcriptional control in other bacteria. Recently *Rhodococcus erythropolis* 1CP was reported to have the *cat* genes in the order of *catA catB catC* [11].

Previously we have cloned two *catA* genes (*catA₁* and *catA₂*) from chromosomal DNA of *Acinetobacter lwoffii* K24 [12]. Two *catA* genes had different gene organization with other *cat* genes; *catB₁C₁A₁* and *catB₂A₂C₂*. The *catB₂A₂C₂* gene cluster has not been reported yet in other bacteria. In this study, we completely sequenced the *cat₁* gene cluster containing putative regulatory gene and analyzed transcriptional characterization of the *cat₁* gene cluster.

MATERIALS AND METHODS

Bacterial strains and plasmid. *Acinetobacter lwoffii* K24 is a wild type strain capable of growing on media containing aniline as a sole carbon and nitrogen source [12]. *A. lwoffii* K24 was cultured in aniline medium at 27°C as previously described by Kim et al [12]. *E. coli* DH5 α (pCD1-1) and *E. coli* DH5 α (pCD1) were selected in colony hybridization for having the *catA₁* gene of *A. lwoffii* K24. Plasmid pCD11, pCD12 and pCD13 were subcloned from pCD1 and pCD1-1 (Fig. 1).

Induction and enzyme activity assay. *E. coli* DH5 α containing the *cat* genes were grown to an optical density of 0.3-0.4 at 600 nm in LB containing Ap at a concentration of 100 μ g/ml and then incubated for overnight at 30°C in the final concentration of 1 mM isopropyl- β -D-thiogalactopyranoside (IPTG). Cells were harvested, resuspended in 2 ml of 50 mM Tris-HCl (pH 8.0) and disrupted by sonication (VCX 400 Sonics & Materials, Danbury, Conn.) and were centrifuged at 15,000 \times g for 10 min. The supernatant was used for enzyme assays. Catechol 1,2-dioxygenase (*catA*) activity was assayed as previously described [13]. Muconate lactonizing enzyme (*catB*) activity was assayed by the method of Ornston [14]. The substrate of muconate lactonizing enzyme, *cis,cis*-muconate was produced enzymati-

¹ To whom correspondence should be addressed. Fax: 82-42-865-3419. E-mail: ksi@comp.kbsi.re.kr.

cally from catechol by purified catechol 1,2-dioxygenase and purified by proRPC column (5 by 200 mm, Pharmacia).

DNA manipulations. Plasmid DNA was isolated using QIAGEN plasmid kit (Chatsworth, Calif.). DNA fragments were isolated from 1% agarose gels by the prep-A clean kit (Bio-Rad, Hercules, Calif.) according to the recommendations of the supplier. DNA digestion with restriction enzymes, ligation and transformation were performed by the procedures of Sambrook et al [15].

Nucleotide sequence analysis. The nucleotide sequences of the upstream region of the *catB₁* gene in pCD1-1 were determined by a modification of the dideoxy-chain termination methods [16], using Prism DyeDeoxy Terminator Cycles Sequencing Kit (Perkin-Elmer, Norwalk, Conn.). Sequencing reactions were prepared according to the supplier's instructions and analyzed by electrophoresis using Applied Biosystem Model 373A DNA sequencer on 6% polyacrylamide gel. The oligonucleotides used as primers in sequencing reaction were supplied by Bioneer Corp (Taejon, Korea). Interpretation of the DNA sequence and the deduced amino acid sequence was done by MacDNASIS DNA and Protein Sequence Analysis System of Hitachi (San Bruno, Calif.). The nucleotide sequence reported here has been submitted to the GenBank database under accession number U77658.

RT-PCR. RT-PCR was performed with Promega Access RT-PCR system (Promega, Madison, Wisc). The used primers are listed in the Fig. 5C. PCR with 10 ng pCD1 as templates was carried out as a positive control in the same condition of RT-PCR except for reverse transcription. RNA was extracted from a log phase culture of *A. lwoffii* K24 in aniline media by Qiagen Rneasy kit (Chatsworth, Calif.) and was treated with Rnase-free Dnase I (Takara, Japan) to remove DNA contamination according to the supplier's protocol. To ensure prepared RNA has no DNA contaminant, PCR was performed with 1 µg prepared RNA as a template and founded to have no PCR products.

Northern blotting. RNA was extracted as described by Simpson et al. [17]. RNA (10 µg) was separated in 1% agarose-formaldehyde gel and transferred to a Zeta-probe blotting membrane in 10× SSC (1.5M NaCl plus 0.15 M sodium citrate) and immobilized on the membrane by UV irradiation (UV cross linker, Bio-Rad). DNA probes were prepared from the PCR product of 450-bp for *catA₁* [12] and radiolabelled by [³²P]dCTP (NEN, Beverly, Mass.) using Exo-free Klenow fragment (Takara). The filters were hybridized, washed at 65°C and then exposed to X-ray film overnight at 70°C.

Primer extension. A oligonucleotide primer for primer extension was 5'-end labeled with [γ-³²P]ATP (NEN) by using T4 polynucleotide kinase (promega). The labeled oligonucleotide was hybridized

with total RNA (10 µg) and used as primer by avian myeloblastosis virus reverse transcriptase (promega) for reverse transcription. The cDNA product was analyzed on 8% denaturing polyacrylamide gel with dideoxy sequencing standards prepared by using the same primer and T7 DNA polymerase (USB, Cleveland, Ohio). The primer was 5'CCCTTGCCTTCGAGATTGGTCGCCGCTTC3' (30mer) at position 2827 to 2798.

RESULTS

Cloning of the *cat₁* gene cluster. We have selected pCD1 containing the *catA₁* gene in colony hybridization and found the *cat₁* gene order as *catB₁*–*catC₁*–*catA₁* in subcloned vector pCD11. In this study, other positive clone, which have the upstream region of *catB₁* and shows *catA* and *catB* activity by IPTG, was selected and named as pCD1-1 (Fig. 1). *E. coli* containing subcloned vectors pCD12 and pCD13 also showed *catB* activity under IPTG induction.

DNA sequence analysis of *catB₁* and *ORF_{R1}* in the *cat₁* gene cluster. Nucleotide sequence analysis of the upstream of the *catB₁* gene in plasmid pCD1-1 was performed and 2009 bp nucleotides were sequenced (Fig. 2). The open reading frame (ORF) of the *catB₁* gene initiates at ATG (bp 1196) and terminates at TAA (bp 2335). *CatB₁* have 379 amino acids and deduced molecular weight is 40788 dalton. Multi-alignment analysis of the deduced amino acid sequence of the *catB₁* gene with other homologous genes showed that the gene has more than 50% identity with *A. calcoaceticus* (M76991) and *P. putida* PRS2000 [8]. The *catB₁* gene also have more than 40% identity with *clcB* [18], *tcbD* [19] and *tfdD* [20] (Data not shown). These results suggest that the *catB* and *catC* genes are more conserved than the *catA* genes in soil bacteria [11, 12]. Several amino acid residues (Asp, Glu and Lys) of the *catB* genes, which are assumed to be involved in magnesium coordination and the enzyme mechanism, are conserved in the *catB₁* gene [11]. By computer analysis,

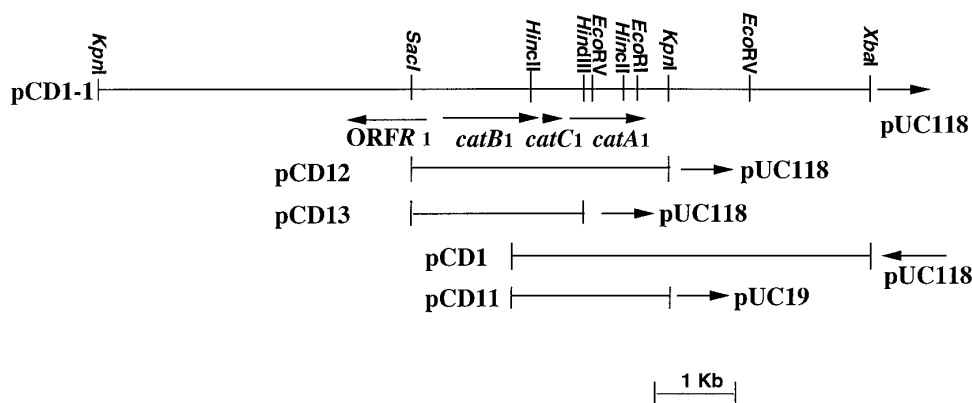


FIG. 1. Restriction endonuclease map of the 10-kbp *KpnI*-*XbaI* fragment of pCD1-1 containing the *cat₁* genes. Arrows indicate the direction of transcription.

CTGCTATCCCATCCATTCGAATGAACTCGAAATGGGTACGGTTCAACAGCCATATTTTCACGTGACCTCAGTGATAATCGTTTCAAGGGGCGACGCGTCTA	TCA	ATT	105
		*	
GTG AGT ATC CAA CCA TGC TGG ATG TTC GGC ATA CAT TTC GCG GAT CAA TTG TTT GGT CAG GCT AAT ATA CTT GGA GCT ATC			186
H T D L W A P H E A Y M E R I L Q K T T A S I Y K S S D			
GTT AGC CCG GTG ACT GAC GAT TAC CCG AGA GGT CGC GCG GTC GCC GTC TAT CAG CCG ATA ATG TAC ATC GTG GCG CAT CTG			267
N A R H S V I V P S T A R D G D I L R Y H V D H R M Q			
CCG CGC AGA CCG AGG TAT CAC GCA CAC CCC GAA TTC AGC TGC CAG CAG TCC CAA GGC GGT CTG GAT TTG CCG TAC CTC CAT			348
R A S A P I V C V G F E A A V L G L A T Q I E R V E M			
CAC TTG GCC TGG TTG TAC ATC GTG CCT ATC CAA GAT GTT CAG CAC TTG ATC GGC GAA GCC CGG GCG CGC CTC CTT TGG ATA			429
V Q G P Q V D H R D L I N L V Q A F G C P R P E K P Y			
AAC AAT CAA CTT TTC TCC GCG TAG CTG GTG AAC GGT TAG AGG TGT TGA CTC CCG AGC CAT GGG CGA CTC CAC CCA CGC			510
V I L K E G A L Q H V P L P T S E R A M P G S E M P L A			
AAC GGC AAG CCT TTC TTC GTG GAG AAC GAT GCT AGA CAC GTT CGG ATC GCT GTG ATG TAC CGC GGC GAA GCC GAT GTC GAT			591
V A L R E E H L V I S S V N P D S H H V R G F G I D I			
GCG GCC TTC GTT TAT CCG CTG GAT TTG CTG AAC CCA CAT GAG CTC GAC CAT TTG AAT GTC CAG CTC CCG CGC GTT GTG CGC			672
R G E N I A Q I Q V S M L E V M Q I D L E P A H Q R			
GAG TTT GCG CAT CAG TGT CCG CAA TAC GCC GTA GAG AGT AGA TGC GAA AAA GCC CAT CGA CAA AAC GCT ACG CTG ATG TAA			753
L K R M L T P L V G Y L T S A V F G I S L V S R Q H L			
TCC CAC TCG CCG CGT GGC CGC TTG CAT CTG TTC GAC GCG CCC CAG CAC CTG GAT CGC CTG CTC AAA GAA CAG ACG ACC CGC			834
G V R R T A A Q M Q E V R R G L V Q I A Q E F F L R G A			
GTC GGT TAG CTG CAG CCG CCG GCT GTT GCG AAT CAG AAG AGG AAC CCC CAG CTC TTC CTC GAG CAA CTG TAT CTG ACG GCT			915
D T L Q V P R S N R I L L P V V E E E L L Q I Q R R S			
CAA TGG TGG CTG AGC GAT GTT GAG CTG TCT GGC TGC CGT GAA GTT GCG CTC GCG GGC GAG GGC GAA GTA GCG AAA			996
L P P Q A I N L Q R A A R T F N R E R A V A V F Y R F			
CTG GCG CAG ATC CAT AGTCTGATTGGAGTCGAGTGTGGTTTCGCGCGGACTTGAAGCATTCGAACGCTAAATTCGCGTTGGGCATAAAGCGCTCTCTA			1095
Q R L D M	← ORF _{r1}		
TACTGTAAAGGTATCAAAATCCTATAAAAAAGGTGTGGACGTCGAGGTGGCCCGCAGCGTATAAAACCTCATTTACAGACAATACGGATGCCACCTCGCAG		ATG	1198
	catB₁	M	
TCC AGT GTA ACG ATT GAA CCG ATC GAA ACT TGC CTC CTC GAT TTG CCA ACG ATT CGG CAC AAG TTG TCT GTC GCC ACG			1279
S S V V T I E R I E T C L V D L P P T I R P P H K L S V A T			
ATG TAC GGA CAG ACT TTG ATG CTG GTG AAG GTG TAT TGC ACT GAC GGC GCG GTC GGC ATC GGT GAG GGC ACC ACG ATC GCC			1360
M Y G Q T L M L V K V Y C T D G A V G I G E G T T I A			
GGG ATG GCG TAC GGC CCG GAA AGT CCG GAA GCG ATG AAT TTG GCG ATC GAC GCG TAC TTC GCG CGC GCG CTG GTC GCG AAG			1441
G M A Y G P E S P E A M K L A I D A Y F A P A L V G K			
GAC GCG ACA CCG ATC CAG ACG TTG ATG GCA CAC CTT GGC AAG CTG GTG AAA ATC AAC CAC TTC CCG AAG AGC GCA CTC GAA			1522
D A T R I Q T L M A H L G K L V K I N H F A K S A L E			
ACC GCG CTG CTT GAG GCA CAT GGT AAG CCA CTT GCG GTA GCT GTC AGT GAA CTC CTT GGT GGG GCG CGT CGT GAA GCG CTA			1603
T A L L D A H G K R L G V A V S E L L G G R R R E R L			
CCG GTT GCC TGG ACG CTG GCG TCC GGC GAT ACC TCG GCG GAC ATC GCC GAA GCA GAG CAA ATG ATT GAG GTT CCG GCG CAC			1684
P V A W T L A S G D T S R D I A E A E Q M I E V R R H			
AAC GTA TTC AAT CTG AAG ATC GGT GCG AAG GAA GAT ATG AAG ACC GAT ATT AAA CAC GTA GCC GAG ATC AAG AGG GTG GTT GGG			1765
N V F K K L K I G A K E L K T D I K H V A E I K R V V G			
GAG CAT GCG GCG GTG CCG GTC GAC GTG AAC ATG CCA TGG AGC GAG ACG CAG GCA GCG TGG GCG ATT CCA GCA CTC GCC GAT			1846
E H A A V R V D V N M A W S E T Q C A A W A I P A L A D			
GCC GGT TGT GAA TTG GTT GAG CAG CCG GTC GCG TCG GCG GCG CTC GCG CGG TTG ATG CCG CGC TTT CCG GTT GCT TTG			1927
A G C E L V E Q P V A S A A A L A R L M R R P P V A L			
ATG GCC GAC GAA ATC CTG CAA GGC CCG GAC AAT CCG TTG TAT GAG ATT CCG CGA GTA AAC GGG GCT GAG GTT TTT GCG ATC AAG			2008
M A D E I L Q G C P D N A F E I A R V N G A D V F A I K			
ATC GAA CAG AGT GGT GGG CTA TTC GCC GCG CAG CCG GTG GCG CCG ATC GCC GAC GCG GCC GGC ATC GAG CTG TAC GGT GGC			2089
I E Q S G G L F A A Q R V A A I A D A A G I E L Y G G			
ACC ATG CTG GAA GGC CCG TTC AGC ACG GTC GCG TCA GCG CAT CTA TTC GCG AGC TTC GCG AAC CTG CAA TGG GCG ACC GAA			2170
T M L E G A F S T V A S A H L F A S F A N L Q W G T E			
CTG TTT GCG CCG CTG TTG ATC ACC GAA GAA ATT CTG AAC AAG CCG CTA GAT TAC AGT GAT TAC CAG TTG ACC GTG CCA GAC			2251
L F G P L L I T E E I L T K P L D Y S D Y Q L T V P D			
GGT CCT GCG CTT GCG ATC GAA CTC GAG GAA GAG AAG GTC CCG CGT TTC ACG CCG CAG GGG CTG ATC AAG GTC ACG AAG GCG			2332
G P G L G I E L D E E K V R R F R D G L I K V T K A			
TAA CCCCGGACGAAAAAAGAGAGGATCAGC ATG CTT TTC CAT GTA CCG ATG GAT GTG AAT ATT CCG GAC GAT ATG CCG GTC GAG			2413
	catC₁	M	
GTG GCA GAC GAA ATC AAG GCA CCG GAG AAG GCG TAT TCG CAG GCG TTG CAA AAG AGC GGC AAA TGG CCT CAC ATC TGG CCG			2494
V A D E I K A R R E K A Y S Q A L Q K S G C K W P H I W R			
CTC GTT GGT GAT TAT GCG AAC TAC AGC ATC TTC GAT GTT GAG AGC AAT GCG GAA CTC GAC GGC ATC CTG ACC GGA CTG CCG			2575
L V G E Y A N Y S I F D V E S N A E L H G I L T G G L P			
CTG TTT TCA TAT ATG AAG ATG GAG GTG ACG CCG CTG TGC CGT CAC CCG TCG TCG AIT CCG CAG GAG TCG TGA TCGGAT			2656
L F S Y M K I E V T P L G R H P C S I R D D E S *			
GAT TCAACAGAGGGCGCGCTTCGAGGGCGAGCGCGCTTCCACAGGGTTCGTTACCCGATATGTGCGCCCAAACTACTCGATGGAGACTGGCATC ATG AGC			2759
+1 →	catA₁	M	S
ATC AAA GTG TTC GGT ACG AAG GAA GTG CAA GAC CTG CTG AAG GCG GCG ACG AAT ATC CTA GAA GGC AAG GGC ACG GGC ACG			2840
I K V F G G T K E V Q D L L K A A T N L E G K G G N A R			

FIG. 2. Nucleotide sequences and deduced amino acid sequences of ORF_{R1} and the *cat1* genes in *Acinetobacter lwoffii* K24. The putative ribosome-binding sites are underlined. Asterisks indicate the translational stop codons. The arrow of ORF_{R1} indicated the direction of transcription. The transcription start sites of *catA1* was defined by +1.

another open reading frame was found in 224 bp upstream of *catB*₁. This ORF has reverse direction and significant identity with *catRs* (43.9% and 44.1%), *catM* (38.2%) and *clcR* (31.4%) (Fig. 3). *CatR*, *catM* and *clcR* are known as *LysR* family regulator proteins in *P. put*

ida and *A. calcoaceticus* [2, 5, 8, 18]. This ORF has also common characteristics of *LysR* family in molecular weight, highly homology in the N-terminal region containing helix-turn-helix motif and lower homology in the C-terminal region [21]. We designated this ORF as

		10	20	30	40	50
ORFR1	1	MDLRQFRYFV	AVARERNFTR	AARQLNIAQP	PLSRQIQLE	BEVGVPIITR
catR-PRS2000	1	MELRHLYRFK	VLAETLNFTTR	AAELLHIAQP	PLSRQISOLE	DELGT-LLIA
catR-RB1	1	MELRHLYRFK	VLAETLNFTTR	AAELLHIAQP	PLSRQISOLE	DOLGT-LLIV
catM	1	MELRHLYRFV	TVVEEQSISK	AAEKLCTAQP	PLSRQIQKLE	BELGIQLFER
clcR	1	MEFRQLRYFT	AVAEENIGTA	AARRLHISQP	BITRQIQALE	QDLGVVLFER
		60	70	80	90	100
ORFR1	51	NSRPVQLTDA	GRFFFEQATQ	VLGRVEQMA	ATRRVGLHOR	SVLSIGFVAS
catR-PRS2000	51	RERPLRLTEA	GRFFYEQTCT	VL-QLQNISD	NTRRIGQGOR	QWLIGIFAPS
catR-RB1	51	RERPLRLTEA	GRFFYEQSCCT	VL-QLQNISD	NTRRIGQGOR	QWLIGIFAPS
catM	51	GFRPAKVTEA	GMFFYOHAVQ	LLHTTAQAAS	MAKRIA-TVSS	QTLRIGYVSS
clcR	51	THRGVELTAA	GITFLEDARR	LLHVTEISRV	RSFAASRGEI	GELRVAYFGT
		110	120	130	140	150
ORFR1	101	TLYGVLPTLM	RKLROHAPEL	DIOMVVELMS	VQOIQAINEG	RIDIGFGRVH
catR-PRS2000	101	TLYNVLPETLI	RELRODS-EL	ELGLS-EMTT	LQOVEALKSG	RIDIAFGRIR
catR-RB1	101	TLYKVLPELI	RELRODS-EL	ELGLN-EMTT	LQOVEALKSG	RIDIAFGRIR
catM	101	LLYGLLPETLI	YLFROQNPET	HIEL-IECGT	KDQINALKQG	KIDLGFGRLE
clcR	101	VVLHTLPPLL	ROLLSVAPSA	TVSLT-QMSK	NRQIEALDAG	TIDIGFGRFY
		160	170	180	190	200
ORFR1	151	HSIDPNVSSIV	LHEERLAVAL	PMESPMAR-E	STPLFVHOLA	GEKLTIVPKE
catR-PRS2000	151	IEDAATAQOV	LREDPLVAVL	PKGHPLA---	GSPLSLAOLA	GEAFILYPAN
catR-RB1	151	IEDPAIHQOV	LCEDPLVAVL	PKOHPLA---	SSPLTLAOLA	GEAFILYPAN
catM	151	IEDPATRRIV	LHKEQLKLAT	HKHHHLNOFA	ATGVHLSQIT	DEPMILYPVS
clcR	151	PYQEGVVVRN	VINERLFLGA	QKSR--ARSF	GEQVHCALR	NEPFILFPRE
		210	220	230	240	250
ORFR1	201	PRPSFADQVL	NILDRHJVOP	GOVMEVREIQ	TALGLVAAEF	GVCVTPASAR
catR-PRS2000	201	PRPSYADHVL	ALFAQHGMST	RVSQWANELQ	TAIGLVAVGV	GVTLPVPSVQ
catR-RB1	201	PRPSYADHVL	ALFAQHGMST	HVSQWANELQ	TAIGLVAVGV	GVTLPVPSVQ
catM	201	QKPNFAFFIQ	SLFTELGLVP	SKLTETREIQ	LALGLVAAGE	GVCIVPASAM
clcR	201	GRPSFADEVI	GVFKNARVEP	KVVAIVEDVN	AAMALALAGV	GVTIVEPETA
		260	270	280	290	300
ORFR1	251	QM-RHDVHYR	LIDGDRATSP	VIVSHRANDS	SKYISLTKQL	IREMYAEHPA
catR-PRS2000	251	QQHRTDIEYV	GLLDSSAVSP	IILSRKGDV	SPIVORCLGL	IALQ-----
catR-RB1	251	QQHRTDIEYV	SLLDSSAVSP	IILSRKGDV	SPIVORCLTL	IAQQ-----
catM	251	DIGVKNLLYI	PILDD-AYSP	ISLAVANMDH	SNYIPKILAC	VQEVFATHHI
clcR	251	MISWPDGFT	ELVGSKATVP	VSCIYRHDI	APILKTFNL	LP-----
		301				
ORFR1	301	WLDTHN				
catR-PRS2000	301	----TV				
catR-RB1	301	----AE				
catM	301	R-PLTE				
clcR	301	-IRESQ				

FIG. 3. Multiple alignment of the deduced amino acids of ORF_{R1} of *Acinetobacter lwoffii* K24 with homologous proteins of various species. ORFR1, ORF_{R1} of *cat*₁ of *A. lwoffii* K24; catR-PRS2000, *catR* of *P. putida* PRS2000 [8]; catR-RB1, *catR* of *P. putida* RB1 [5]; catM, *catM* of *A. calcoaceticus* [2]; clcR, *clcR* of *P. putida* [27].

ORF_{R1}. ORF_{R1} has 303 amino acids and its deduced molecular weight is 34245. The ORF_{R1}-*catB*₁ intergenic region has highly homologous sequence with the sequence of repression binding site (RBS) and activation binding site (ABS) in the *catR*-*catB* intergenic regions of *P. putida* (Fig. 4). Interestingly, suggested consensus binding motif of *LysR* family regulatory protein, T-N₁₁-A is founded in this region (T₁₀₉₆-A₁₁₀₈ of *cat*₁ genes). Codon usage analysis showed that the *cat*₁ gene cluster has higher biased G or C (71.4% for *cat*₁ genes) in the wobble position than total genes of *E. coli* (55.9%) and have similar ratio to that of *P. putida* (72.4 %) [22].

Transcriptional analysis of the *cat*₁ gene cluster. To analyze the transcriptional characterization of the *cat*₁

*B*₁C₁A₁ gene cluster of *A. lwoffii* K24, RT-PCR was carried out using the oligonucleotides synthesized on the base of nucleotide sequence of the *cat*₁ genes. Five oligonucleotides used in PCR and RT-PCR were indicated in Fig. 5 C. By this method mRNA coding *catB*₁, *catB*₁C₁ and *catA*₁ were detected but not *catB*₁C₁A₁ (Fig. 5 A). This results show that *catB*₁ and *catC*₁ may be cotranscribed but *catA*₁ may be independently transcribed (Fig. 5 B). Northern blotting and primer extension analysis confirmed this proposal. Northern blotting with 450 bp PCR product of *catA*₁ [12] revealed that the transcript coding *catA*₁ was about 1.6 kb, which is enough size for covering the *catA*₁ gene but not the *catB*₁ gene (Fig. 6 A). To identify the 5' termini

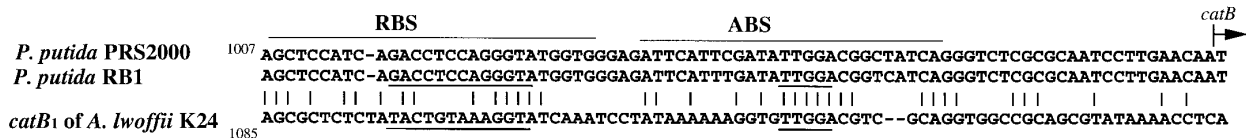


FIG. 4. Alignment of promoter regions of the *catB₁* gene and the *catB* genes of *P. putida* PRS2000 [8] and *P. putida* RB1 [3]. Homologous nucleotides are indicated by bars. The sequences of RBS and ABS in *P. putida* RB1 were indicated and were from the studies by Parsek et al.[3]. The -35 hexamer sequences, G-N₁₁-A sequence in *P. putida* and T-N₁₁-A sequence in *A. lwoffii* K24 were underlined.

of the *catA₁* gene, primer extension analysis was performed with RNA purified from *A. lwoffii* K24 cultured in aniline media. The extension product of the *catA₁* gene was ended at T₂₆₅₉ on the coding strand suggesting the transcription start of *catA₁* at T₂₆₅₉ (Fig. 6 B).

DISCUSSION

The *cat₁* gene cluster of *A. lwoffii* K24 was sequenced and the transcriptional pattern was characterized. The *cat₁* gene cluster has the gene order of ORF_{R1}-*catB₁*-*catC₁*-*catA₁*, which is similar to the *cat* gene cluster of *P. putida* PRS2000 [8]. The *cat₁* genes have other common properties with those of *P. putida* PRS2000. 1) Independent transcription of *catA₁* and cotranscription of *catB₁*C₁. 2) Significant homology in the promoter region of the *catB₁* gene and the *catB* gene of *P. putida* PRS2000 (Fig. 4). 3) Putative regulatory protein coding gene, ORF_{R1}, which has 43.9% identity with *catR* of *P. putida* PRS2000. But the *cat₁* genes have different characteristics from *cat* genes of *P. putida* PRS 2000. 1) Difference in the length and DNA sequence of in-

tergenic space between the *cat* genes. The 85-bp palindromic sequence, which cause genetic difference in *P. putida* PRS2000 and *P. putida* RB1, was not found in the *cat₁* genes in *A. lwoffii* K24. 2) In spite of different placement of an 85-bp palindromic sequence, the *cat* genes of *P. putida* PRS2000 and *P. putida* RB1 have about 90% homology showing their close relation, but the *cat₁* genes of *A. lwoffii* K24 have less than 60% homology with the *cat* genes of these bacteria. These results suggest that though the *cat* genes of two soil bacteria have the same gene arrangement, there is considerable gene diversity between the *cat₁* genes of *A. lwoffii* K24 and the *cat* genes of *P. putida* PRS2000. Primer extension analysis of *catA₁* suggests the possibility of regulatory protein binding on the *catC₁* region for transcription of *catA₁*. *CatR* of *P. putida* PRS 2000 was reported to bind to the *catC* region as well as the upstream region of the *catB* gene and *catM* of *A. calcoaceticus* also was known to bind to the upstream region of *catA* [7, 8]. However, we could not found common DNA sequences for binding of *LysR* family regulatory proteins in this region. It is necessary to study the

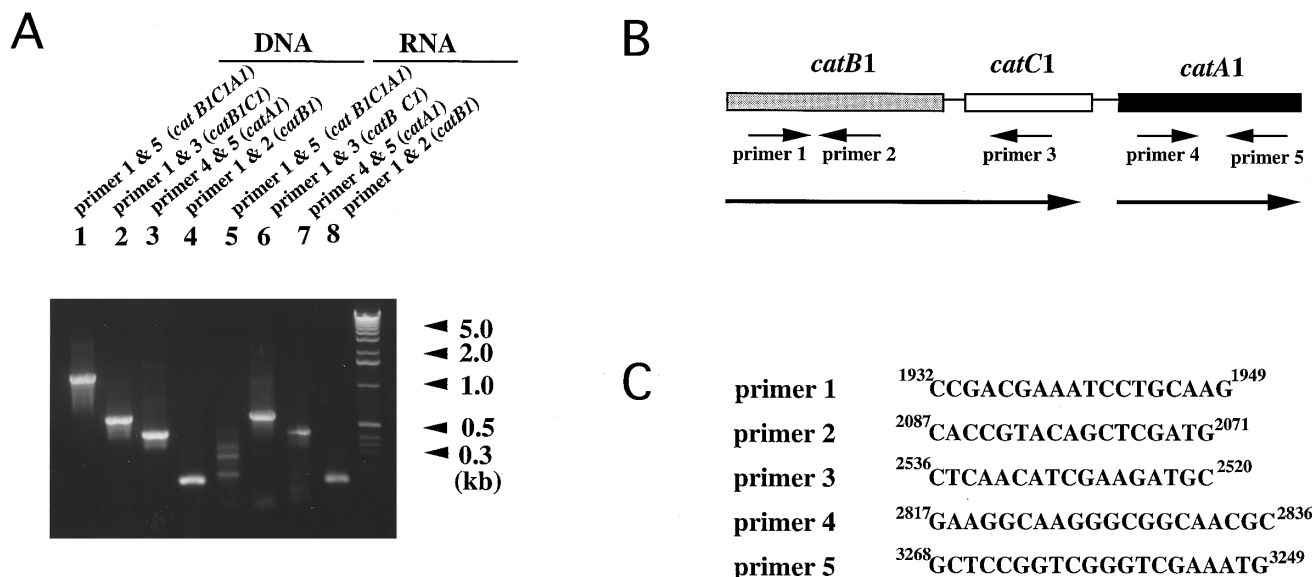


FIG. 5. Analysis of transcriptional pattern of the *catB₁C₁A₁* gene cluster by RT-PCR. (A) Lane 1-4; control PCR. pCD1-1 was used as a template. Lane 5-8; RT-PCR was performed according to the Material and Methods. (B) Transcription pattern of the *cat₁* genes was depicted as bold line. (C) The used oligonucleotides.

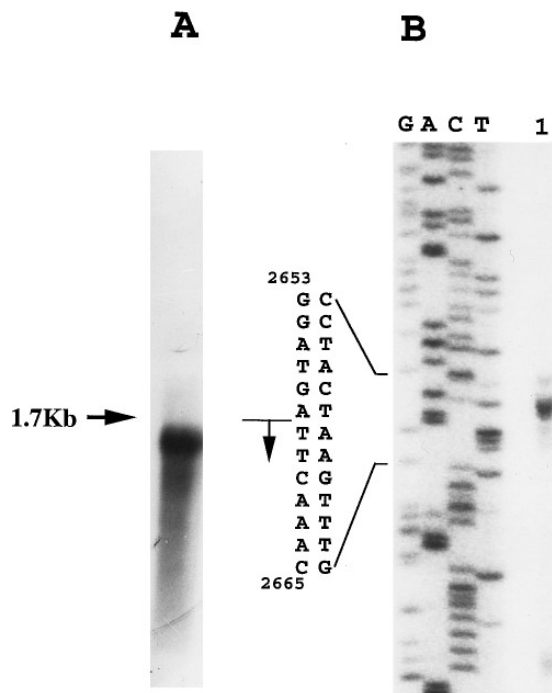


FIG. 6. (A) Northern blot analysis with 450bp PCR product of *catA₁* as probe. (B) Determination of *catA₁* transcription start site by primer extension. The sequence in the region of transcription initiation is shown at the left side, and the arrow indicates the sequence positions of the major extension products.

function of the putative regulatory protein-coding gene, ORF_{R1} as regulator for the *cat₁* and *cat₂* cluster. *CatR*, the regulator of the *catBC* and *catA* gene of *P. putida* RB1, was known to be able to control the transcription of the *pheBA* operon in *P. putida* PaW85 as well as the *clcABD* gene clusters in *P. putida* PRS2000 [23, 24]. The cross regulation of *catR* is common characteristics found in other Lys-R family member such as *benR* and *TcbR* [25, 26]. High homology of ORF_{R1} with *catR* of *P. putida* (44.1%) shows the possibility of cross talking of ORF_{R1} and *catR* as regulators.

REFERENCES

1. Harwood, C. S., and Parales, R. E. (1996) *Annu. Rev. Microbiol.* **50**, 553–590.
2. Neidle, E. L., Hartnett, C., and Ornston, L. N. (1989) *J. Bacteriol.* **171**, 5410–5421.
3. Parsek, M. R., Shinabarger, D. L., Rothmel, R. K., and Chakrabarty, A. M. (1992) *J. Bacteriol.* **174**, 7798–7806.
4. Rothmel, R. K., Shinabarger, D. L., Parsek, M. R., Aldrich, T. L., and Chakrabarty, A. M. (1991) *J. Bacteriol.* **173**, 4717–4724.
5. Rothmel, R. K., Aldrich, T. L., Houghton, J. E., Coco, W. M., Ornston, L. N., and Chakrabarty, A. M. (1990) *J. Bacteriol.* **172**, 922–931.
6. Chugani, S. A., Parsek, M. R., Hershberger, C. D., Murakami, K., Ishihama, A., and Chakrabarty, A. M. (1997) *J. Bacteriol.* **179**, 2221–2227.
7. Romero-Arroyo, C. E., Schell, M. A., Gaines III, G. L., and Neidle, E. L. (1995) *J. Bacteriol.* **177**, 5891–5898.
8. Houghton, J. E., Brown, T. M., Appel, A. J., Hughes, E. J., and Ornston, L. N. (1995) *J. Bacteriol.* **177**, 401–412.
9. Neidle, E. L., and Ornston, L. N. (1986) *J. Bacteriol.* **168**, 815–820.
10. Shanley, M. S., Neidle, E. L., Parales, R. E., and Ornston, L. N. (1986) *J. Bacteriol.* **165**, 557–563.
11. Eulberg, D., Golovleva, L. A., and Schlömann, M. (1997) *J. Bacteriol.* **179**, 370–381.
12. Kim, S. I., Leem, S.-H., Choi, J.-S., Chung, Y. H., Kim, S., Park, Y.-M., Park, Y. K., Lee, Y. N., and Ha, K.-S. (1997) *J. Bacteriol.* **179**, 5226–5231.
13. Aoki, K., Konohana, T., Shinke, R., and Nishira, H. (1984) *Agric. Biol. Chem.* **48**, 2087–2095.
14. Ornston, L. N., and Stanier, R. Y. (1966) *J. Biol. Chem.* **241**, 3795–3799.
15. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, New York.
16. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
17. Simpson, D. A., Hammarton, T. C., and Roberts, I. S. (1996) *J. Bacteriol.* **178**, 6466–6474.
18. Franz, B., and Chakrabarty, A. M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4460–4464.
19. van der Meer, J. R., Eggen, R. I. L., Zehnder, A. J. B., and de Vos, W. M. (1991) *J. Bacteriol.* **173**, 2425–2434.
20. Perkins, E. J., Gordon, M. P., Caceres, O., and Lurquin, P. F. (1990) *J. Bacteriol.* **172**, 2351–2359.
21. Schell, M. A. (1993) *J. Annu. Rev. Microbiol.* **47**, 597–626.
22. Wada, K. -N., Aota, S.-I., Tsuchiya, R., Ischibach, F., Gojobori, T., and Ikemura, T. (1990) *Nucleic Acids Research* **18**, 2367–2370.
23. Kasak, L., Horak, R., Nurk, A., Talvik, K., and Kivisaar, M. (1991) *J. Bacteriol.* **175**, 8038–8042.
24. Parsek, M. R., McFall, S. M., Shinabarger, D. L., and Chakrabarty, A. M. (1994) *Proc. Natl. Acad. Sci.* **91**, 12393–12397.
25. Jeffrey, W. H., Cuskey, S. M., Chapman, P. J., Resnick, S., and Olsen, R. H. (1992) *J. Bacteriol.* **174**, 4986–4996.
26. Matrubutham, U., and Harker, A. R. (1994) *J. Bacteriol.* **176**, 2348–2353.
27. Coco, W. M., Rothmel, R. K., Henikoff, S., and Chakrabarty, A. M. (1993) *J. Bacteriol.* **175**, 417–427.