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# Sequence analysis of the upstream region of *dhlB*, the gene encoding haloalkanoic acid dehalogenase of *Xanthobacter autotrophicus* GJ10

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## Abstract

The DNA sequence upstream of the *dhlB* gene encoding the haloalkanoic acid dehalogenase of *Xanthobacter autotrophicus* GJ10 was determined and contained an open reading frame, designated *dhlC*, which encoded a protein with a significant similarity with the family of $Na^+$-dependent symport proteins. The *dhlC* gene was subcloned under control of a T7 promoter, and found to encode a polypeptide of 45 kDa on SDS-PAGE. Upstream of *dhlC*, a −24/−12 promoter sequence was found. Further upstream, in the opposite direction of transcription, another open reading frame, designated *dhlR* ,with homology with the family of $\sigma^{54}$-dependent transcriptional activator proteins was detected. The *dhlR* gene was cloned and expressed under the control of a T7 promoter and encoded a polypeptide of 51 kDa on SDS-PAGE. The genetic organization of the *dhlB* region suggested that the expression of *dhlC* and *dhlB* was controlled by the product of *dhlR* and $\sigma^{54}$ which may explain the observed overexpression of the haloalkanoic acid dehalogenase under starvation conditions.

*Abbreviations:* bp – base pair(s), *E. coli* – *Escherichia coli*, kb – kilobase(s) or 1000 bp, MCA – monochloroacetate, ORF – open reading frame, SDS-PAGE – sodiumdodecylsulfate-polyacrylamide gel electrophoresis, *X. autotrophicus* – *Xanthobacter autotrophicus*

## Introduction

The 1,2-dichloroethane-degrading bacterium *X. autotrophicus* GJ10 produces two different enzymes that hydrolytically remove a halogen from halogenated substrates (Janssen et al. 1985). The haloalkane dehalogenase is specific for halogenated aliphatic hydrocarbons, whereas the haloalkanoic acid dehalogenase is active with halogenated aliphatic carboxylic acids. Both enzymes have been purified and characterized (Keuning et al. 1985; Van der Ploeg et al. 1991). On basis of the stereoselectivity and gene sequence, the haloalkanoic acid dehalogenase can be classified as a member of the L-2-chloropropionic acid dehalogenases that invert the configuration of the substrate during the reaction (Van der Ploeg et al. 1991). This group of enzymes share 40–60% sequence identity and thus may have evolved from a common ancestor and have similar structures and reaction mechanisms (Janssen et al. 1994).

The gene encoding the haloalkanoic acid dehalogenase (*dhlB*) is located on the chromosome of *X. autotrophicus* GJ10 (Tardif et al. 1991) and has been cloned and sequenced (Van der Ploeg et al. 1991). The haloalkanoic acid dehalogenase appeared to be expressed constitutively (Janssen et al. 1989), but much higher levels of the enzyme were found in the starvation phase than in the exponential phase (A. Mars & D.B. Janssen, unpublished results). No consensus *E. coli* promoter sequence could be identified closely upstream of *dhlB* (Van der Ploeg et al. 1991) and the expression of this haloalkanoic acid dehalogenase in *E. coli* was very low (Janssen et al. 1989). Thus, the regulation of haloalkanoic acid dehalogenase activity is not well understood. In addition, it is not known whether

there is an uptake system for halogenated carboxylic acids in the organism.

Here, we describe the analysis of the DNA sequence upstream of *dhlB*. The results show that two open reading frames are present which can be expressed in *E. coli* and which, deducing from sequence similarity, may have a function in transport of acids and regulation of expression of *dhlB*.

## Materials and methods

### *Strains and plasmids*

*E. coli* strain JM101 (Yanisch-Perron et al. 1985) was cultivated in LB medium (Sambroek et al. 1989) at 37 °C. Antibiotics for maintenance of plasmids were used at the following concentrations: ampicillin, 100 μg/ml; tetracyclin, 12.5 μg/ml; kanamycin, 50 micro/ml. Plasmids pGEM5-Zf(−) and pGEM7-Zf(−) (Promega, Madison, WI) were used as cloning vectors.

### *DNA manipulation and analysis*

For plasmid isolation, restriction enzyme digestion, ligation and transformation, standard procedures were used (Sambrook et al. 1989).

The generation of nested deletions in plasmids was as described by Henikoff (1984). Double-stranded DNA from these deletions was sequenced with the dideoxynucleotide chain termination method of Sanger et al. (1977) with $^{35}$S-dATP. When necessary, appropriate DNA fragments were cloned and the resulting plasmids sequenced to fill remaining gaps. Primers used for sequencing were the T7 and SP6 promoter primer (Promega). Nucleotide and protein sequence analysis was done with the University of Wisconsin Genetics Computer Group package release 7.3 (Devereux et al. 1984) or with PC/GENE (Genofit, Geneva, Switzerland).

The nucleotide sequence data reported in this paper will appeaar in the EMBL, Genbank and DDBJ Nucleotide Sequence Databases under the accession number X86084.

### *Expression of plasmid encoded proteins*

Plasmid encoded proteins were expressed according to Ausubel et al. (1990). Cells of *E. coli* strain K38(pGp1–2) (Tabor & Richardson 1985) were grown in 1 ml LB medium supplemented with ampicillin (100 μg/l) and kanamycin (50 μg/l) to an optical density of 0.5 and washed 4 times in 1 ml of M9 medium. Cells were resuspended in M9 medium (Ausubel et al. 1990) with all amino acids (0.005% w/v) except methionine and cysteine added, and grown for another 30 min at 30 °C. Cells were then transferred to 42 °C for 30 min. Rifampicin was added to a concentration of 200 μg/ml and incubation was continued for 30 min at 42 °C. Cells were transferred to 30 °C for 30 min. Subsequently, radiolabelled methionine was added (10 μCi (specific activity > $1.10^6$ mCi/nmol)) and incubation was continued at 30 °C for 5 min. The cells were centrifuged, resuspended and lysed in electrophoresis buffer (100 μl) and proteins were subjected to SDS-PAGE. Labelled proteins were visualized by fluorography on Kodak XAR5 film.

## Results and discussion

### *Sequence analysis of the upstream region of dhlB*

The gene encoding haloalkanoic acid dehalogenase of *X. autotrophicus* GJ10 was previously found to be located on a 10.2 kb *Eco*RI fragment (with one internal *Eco*RI site) that was cloned in the broad host range vector pLAFR1. From this clone (pPJ66), plasmids pPS7 and pPS8 were constructed by cloning a 5.7 kb *Eco*RV fragment in opposite orientations in the *Sma*I site of the vector pGEM7-Zf(−) (Van der Ploeg et al. 1991). In Fig. 1, the restriction site map of this *Eco*RV fragment is shown. The nucleotide sequence of the 4.2 kb fragment was determined from the *Eco*RV site to the *Pst*I site in *dhlB*. This was done with both strands using a set of unidirectional nested deletions, constructed with exonuclease III (Fig. 2). The sequence contains a part (bp 3583 and further) of the already reported sequence of the *dhlB* (Van der Ploeg et al. 1991).

The G+C content of the complete sequence of 4152 bp was 66.4%, which is similar to that reported for the genus *Xanthobacter* (Wiegel & Schlegel 1984). Inspection of the sequence revealed two large open reading frames which had codon usages similar to that of the protein encoded by *dhlB*.

A long ORF which ends 30 bp upstream of *dhlB* was designated *dhlC*. There are two potential translation initiation sites present that are both preceded by good ribosome binding sites. The smaller ORF (bp 2206–3756) can encode a protein of 516 amino acids with a calculated molecular mass of 53,308 Da, while the

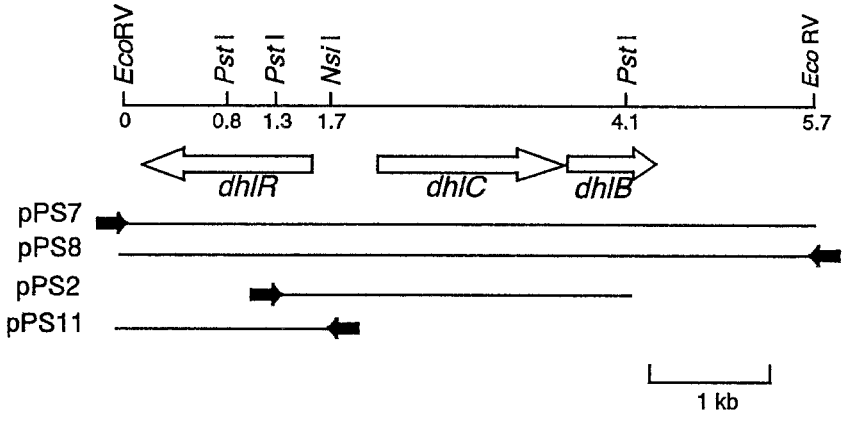


*Fig. 1.* Restriction site map of the 5.7 kb*Eco*RV fragment containing *dhlB*. Only relevant restriction sites are shown. The location of putative genes *dhlC* and *dhlR* are shown, as well as that of *dhlB*. The orientation of inserts in plasmids relative to the T7 promoter (⇒) ( of pGEM-7Zf(−) (pPS7 and pPS8) and pGEM-5Zf(−) (pPS2 and pPS11) is shown.

larger ORF (bp 1831–3756) can encode a protein of 641 amino acids with a molecular mass of 66,457 Da. The smaller ORF was used in sequence comparisons. The restriction map shown in Fig. 1 is derived from the sequence.

Upstream of *dhlC*, in the opposite direction of transcription, an open reading frame bp 1657–263, designated *dhlR*, could encode a protein of 464 amino acids with a calculated molecular mass of 50,511 Da. This ORF was preceded by a reasonable ribosome binding site (bp 1667–1663).

*Sequence comparison with dhlC*

Using the programs FASTA and TFASTA (Pearson & Lipman 1988), the protein encoded by *dhlC* was compared with the SWISS-PROT protein database release 29 and the EMBL nucleotide database release 39 respectively. A low similarity was found with some of the members of the $Na^+$-dependent family of symporter systems. There was similarity with the human $Na^+$-dependent glucose carrier, 15.5% identity (Hediger et al. 1989); the proline carrier PutP from *E. coli* 17.3% identity (Nakao et al. 1987); and the panthotenate transporter PanF from *E. coli* 20% identity (Jackowski & Alix 1990). The alignment of DhlC with PutP and PanF is shown in Fig. 3. The positions of membrane-spanning segments were calculated by the method of Klein et al. (1985) with a window of 15 amino acids and were predicted to be in similar regions (Fig. 3). This window was chosen since it represents the minimum number of amino acids needed for a membrane spanning region. Kyte and Doolittle hydrophobicity plots (Kyte & Doolittle 1982) also showed very similar patterns of hydrophobic and hydrophilic regions of DhlC and PutP (results not shown), indicating that the three proteins have a similar transmembrane structure.

A high similarity of DhlC (62.2% identity in 123 amino acids) was also found with an unidentified ORF located immediately upstream of the *hadD* and *hadL* genes, encoding haloalkanoic acid dehalogenases specific for D- and L-chloropropionic acid respectively, from *P. putida* strain AJ1. This ORF was supposed to be in one operon with the two haloalkanoic acid dehalogenase genes (Barth et al. 1992). There was also a high similarity with two ORFs of unknown function of *E. coli* (ORF f549, 38.8% identity) (Blattner et al. 1993) and *B. subtilis* (ORF ipa-31r, 44.8% identity) (Glaser et al. 1993).

The similarity to $Na^+$-dependent transport proteins suggests that *dhlC* encodes a protein that has an uptake function. Haloalkanoic acids are negatively charged at physiological pH values, and it is likely that these substrates need to be transported through the membrane by a carrier protein. Southern blot analysis has shown that a region homologous to *dhlC* is not present in *X. autotrophicus* XD, a strain which can not grow with haloalkanoic acids and which does not possess haloalkanoic acid dehalogenase activity (J.R. van der Ploeg, unpublished results). This suggests that *dhlC*

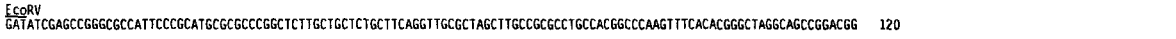


*Fig. 2.* Nucleotide sequence of the region upstream of *dhlB* (Genbank accession number [Submission after acceptance]). Deduced amino acid sequences are shown in the one letter code. The amino acid sequence of *dhlR* is from the reverse complement. Some of the relevant restriction sites are shown, stop codons are indicated by a –, potential ribosome binding sites are shown in asterisks under the sequence, and the putative –24/–12 promoter sequence is underlined. The possible second initiation codon of *dhlC* is double underlined.

is specifically involved in growth with haloalkanoic acids.

The active uptake of halogenated carboxylic acids was observed in *Pseudomonas putida* PP3 (Slater et al. 1985). In mutants of strain PP3 that were resistant to these compounds, the rate of uptake of monochloroacetate decreased. It was proposed that the genes encoding the protein that mediates transport of halogenated carboxylic acids and the dehalogenase were closely associated (Slater et al. 1985). It is interesting to note that the growth rate of strain GJ10 with monochloroacetate as substrate was poor compared to that with 2-monochloropropionate (Janssen et al. 1985). The haloalkanoic acid dehalogenase has enough activity to permit growth with MCA and there is no indication that MCA is toxic (J.R. van der Ploeg & D.B. Janssen, in preparation). The difference in growth rate may therefore be caused by a difference in affinity of the transport protein for these substrates.

Recently, we have isolated and characterized mutants of strain GJ10 that show increased resistance to and growth on bromoacetic acid. In some of these mutants, *dhlC* is corrupted by an insertion element that is copied to a position in the structural *dhlC* gene presumably yielding no or defective DhlC protein (Van der Ploeg et al. 1995). Thus, completely intact DhlC is not essential for growth on haloalkanoic acids under all conditions, and the insertion element may uncouple the expression of the two proteins by the presence of transcription stop signals which could induce resistance.

### *Sequence comparison with dhlR*

The protein encoded by *dhlR* was compared with the SWISS-PROT protein database (Figs. 2, 4). A high similarity was found with proteins from the family of transcriptional activators which activate expression from $-24/-12$ promoters together with the alternative sigma factor $\sigma^{54}$ (Morett & Segovia 1993). The highest similarity was found in the central domain of these proteins (Morett & Segovia 1993) whereas the N-terminal part of the protein were different (Fig. 4). The C-terminal domain showed some homology in the putative DNA-binding motif (Morett & Segovia 1993). Indeed, a helix-turn-helix DNA-binding motif (residues 438–459) was predicted for DhlR with the method of Dodd & Egan (1990) with a score of 1576 (significance ± 4.55 SD).

```
DhlC      MKL---LALAIF-VAILALTLAITWWAARRTRTASDFYTADGTLGPIQNGFALAGDWMSAA
PanF      MQLFVILPLVAYLVVVFGISV-YAMRKRSTGTFLNEYFLGSRSMGGIVLAMTLTATYISAS
PutP      MAISTPM-LVTFCVYIFGMIL-IGFIAWRSTKNFDDYILGGRSLGPFVTALSAGASDMSGW
          * .  . * . * .... .                .. . ..* . . . . .*.

AFLGFSGLAALYGMDGS--LYAAGALVAFLAILMLVAEPIRNTGRF-----TLGDVIAFRMQRPQARLAAVL
SFIGGPGAAYKYGLG--WVLLAMIQLPAVWLSLGILGKKFAILAR-RYNAVTLNDMLFARYQ---SRLLVWL
LLMGLPGAVFLSGISEWIAIGLTL--GAWINWKLVAGRLRVHTEYNNNALTLPDYFTGRFED-KSRILRII
 ..* .*    *.     .    .    ... .              ** * . * .  .*.   .

GTVVVSLAYLV---PQMAGGAALIKLLLGVPYAISIVVVGLGMLVYVVFGGMIATTWVQIIKAVMLLSAAAV
ASLSLLVAFVGAMTVQFIGGARLLETAAGIPYETGLLIFGISIALYTAFGGFAPAAERHHARACDA-----D
SALVILLFFTIYCASGIVAGARLFESTFGMSYETALWAGAAATILYTFIGGFLAVSWTDTVQASLM-----I
... . . .  .** *.   *..*  ..  . . .* .** . . . .*

LVALLLAHFGFDPFSVFAAAEDTYGAKILAPGNYLK--HPLDQLSLGLSFAFGTAGLPHVMTRFYTVPDAQT
WHRC-AAYWRSTCAGGLSNAVQTLQTIDPQLVTPQGADDILSPAFMTSFWVLVCFG---VIG--LAHTAVRC
FALILTPVIVIISVGGFGDSLEVIKQKSIENVDMLKGLNFVAIISLMG-WGLGYFGQPHILARFMAADSHHS
     .     . . . .                . .  .  .  * .. .  .

---ARRSAVWLMFLAGSFFLVTTLIGLGSAVLVGQDAIRAADKGGNLALPLLAQHLGGGPDSIGGQVFLAVV
ISYKDSKAVHRGIIIGTIVVAILMFGMHLAGALGRAVIPDLHVPDLVIPTLMVKVL----PPFAAGIFLAAP
IVHARRISMTWMILCLAGAVAVGFFGIAYFNDHPALAGAVNQNAERVFIELAQILF----NPWIAGILLSAI
     ..  .. . .  .*.      .  .  *  .  . . ..*.

VAVAFATILAVVAALTLSTSGAIAHDLYVNVLRGGHVSEQEQVKVARISTVIVSAFAILFGLLAQGINVAVL
MA----AIMSTINAQLLQSSATIIKDLYLNIRPDQMQNETRLKRMSAVITLVLGALLLLAAMKPPEM-IIWL
LA----AVMSTLSCQLLVCSSAITEDLYKAFLRKH-ASQKELVWVGRVMVLVVALVAIALAANPENR-VLGL
.*    .... .   * *..*  ***          ..    .. .  ....  .  .  .  . *

VILAISVAASANFPVIVLSLFWQRFNTAGVVTGMVAGLVSAVALALTGPAFMGADALFP-VVNPAIASVPIG
NLLAFGGLEAVFLWPLVLGLYWERANAKGALSAMIVGGVLYAVLATLNIQYLGFHPIVPSLLLS---LLAF-
VSYAWAGFGAAFGPVVLFSVMMSRMTRNGALAGMIIGAL--TVIVWKQFGWLGLYEIIPGFIFGSIGIVVFS
   * .  .    ..... * *   * ...*. * .   .      .*  . * .    . .

FLGAILGALLSPRDAVSEAQFDEVVFRANTGLRDDAQAGKSLH
LVGN--------RFGTSVPQ--------ATVLTTDK-------
LLGKAPSAAMQKRFAEADAHYH----SAPPSRLQES-------
..*       * . . .. .
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*Fig. 3.* CLUSTALV amino acid sequence comparison (Higgins & Sharp 1988) of DhlC with PutP (Nakao et al. 1987) and PanF (Jackowski & Alix 1990) from *E. coli*. Symbols: *, identical residue; ·, conserved residue. Putative membrane-spanning segments predicted by the method of Klein et al. (1985) are underlined.

### *Expression of dhlC and dhlR*

To identify the proteins encoded upstream of *dhlB*, fragments containing *dhlC* and *dhlR* were cloned in the vector pGEM5-Zf(−) downstream of the T7 promoter (Fig. 1). Expression of the plasmid encoded genes was induced by temperature shock as described by Ausubel et al. (1990). Protein patterns of whole cells were subsequently determined with SDS-PAGE (Fig. 5). Cells harboring plasmid pPS2, containing the complete *dhlC* gene, produced a protein with an apparent molecular mass of 45 kDa. This is lower than expected from calculation of the molecular mass of the smallest ORF (53.3 kDa). This low apparent molecular mass is not surprising, since an increased mobility of integral membrane proteins during SDS-PAGE is not uncommon.

Cells harboring plasmid pPS11, which contains *dhlR* downstream of the T7 promoter, produced a protein of 51 kDa, which is in agreement with the predicted size.

```
DhlR    1       MGRTRATRDTPQPPPLSERDFGEIVRNSFDGIFVADGEGRTLLVNPGCER
         51     NYDIRAADVVGRPVSDLEADGIIRPVIAPRVIASGERVTAIQRTHKGKTI
        101     FATGIPLFDEAGRVRRVIINSRDTTELDQLQAELSRIRGDLARAQTEVAQ

DhlR  151       LREEGQGAGGPVVHGETTRRIADLLRRVAGSDATVLLTGESGVGKEVFAR
NtrC  139                 VGRSAAMQEIYRVLARLMQTDLTLMITGESGTGKELVAR
XylR  236                 IGHSPAYKRICETIDKAARGRVSVLLLGETGVGKEVIAR
DctD  146                 IGQTPVMENLRNILRHIADTDVDVLVAGETGSGKEVVAQ
NifA   13                 VGESAALKEVLEIAQIVARSNSPVLLRGESGTGKEFFAK
                          .       .             ... **.* *** *.

FVHRESARSKAPFIKINCGALPRDLIESELFGYEAGAFTGAQRGGKPGMIEMANTGTLFLDEIGEL
ALHDYGKRRNGPFVAINMAAIPRDLIESELFGHEKGAFTGAQTR-STGRFEQAEGGTLFLDEIGDM
SVHLRSERAEQPFVAVNCAAIPPDLIESELFGVDKGAYTGAVNA-RAGRFERANGGTIFLDEVIEL
ILHQWSHRRKGNFVALNCGALPETVIESELFGHERGAFTGAQKR-RTGRIEHASGGTLFLDEIESM
LIHDSSSRHEKPFVKLNCAALSAGVLESELFGHEKGAFTGATSQ-KEGRFELAHGGTLLLDEIGEI
 .*  . *    *. .* .*..  ..****** . **.***      * .* *  **..***.  .

PLDMQVKLLHVLQDRIIARLGATRSIPLDIRVVAATNRDLAKAVETGAFRGDLFYRLNVVPVVVPP
PMDAQTRLLRVLQQGEYTTVGGRTPIRSDVRIVAATNKDLKQSINQGLFREDLYYRLNVVPLRLPP
TPRAQATLLRVLQEGELERVGGDRTRKVDVRLITATNENLEEAVKMGRFRADLFFRLNVFPVHIPP
PAATQVKMLRVLEMREITPLGTNEVRPVNLRVVAAAKIDLGDPAVRGDFREDLYYRLNVVTISIPP
SAEFQAKLLRVLQEGELERVGGTRTLKVNVRLVCATNKDLETAVAAGEFRADLYYRINVVPITLPP
    *  .*.**.      .*       ..*.. *.. .*  .   * ** **..*.** . .**

LRERRDDILPLLRQALASFNAQYCT-AKQLSHAAARTLVAHDWPGNIRELRNMVERLVV-TVSHDV
LRDRAEDIPDLVRHFVQQA-EKEGLDVKRFDQEALELMKAHPWPGNVRELENLVRRLTALY-PQDV
LRERVEDIPLLVEHFLRRHHKEYGKKTLGLSDRAMEACLHYQWPGNIRELENALERGVILTESNES
LRERRDDIPLLFSHFAARAAERFRRDVPPLSPDVRRHLASHTWPGNVRELSHYAERV---------
LRQRDGDIPRLAQKFLQRF-NRENGRSLSFAPATLDILSKCEFPGNIRELQNCTQRTATLA-RSDV
**.*  **  *                .              ***.*** .   *

IDVGDL-------------AIPAAAPRGAG-----------------------GASLEEQ-----
ITREIIENELR---SEIPDSPIEKAAARSGSLSISQAVEENMRQ-----YFASFGDALPPSGLYDR
INVESL-------FPGLATATEGDRLSSEGRLE-EESGDSWFRQIIDQGV----------------
------------------------------VLGVEGGGAAAVPPQ-------PTGATLPER-----
IVPQDLACEQGRCYSPILKKAVAEQVGKGAIHGLARGETESMGQPCDVGVFAA-ETVMGQSGLIGR

-VRRFEMALIEDALRRCITTRA-AARDLRVSQSTIVRKLKGGGFA-------A
VLAEMEYPLILAALTATRGNQIKAADLLGLNRNTLRKKIRELGVS--VYRSLA
SLEDLEAGLMRTAMDRCGQNISQAARLLGLTRPAMAYRLKKLDPSLSVKAMGR
-LERYEAEIIRDTLSANDGDVRRTIEALGIPRKTFYDKLQRHGINRGGYSSRK
--ERLEQAMATAGWV-----QAKAARLLGRTPRQVGYSLRRHGIE----RKVF
    *  .             .  *          ..  .

          ____________
          helix-turn-helix
```

*Fig. 4.* CLUSTALV amino acid sequence alignment of DhlR with NtrC from *Rhizobium meliloti* (Szeto et al. 1987), XylR from *P. putida* (Inouye et al. 1988), DctD from *Rhizobium* (Jiang et al. 1989) and NifA from *Rhizobium leguminosarum* biovar *trifolii* (Iismaa & Watson 1989). Only the alignment of the central and C-terminal domains (Morett & Segovia 1993) is shown.

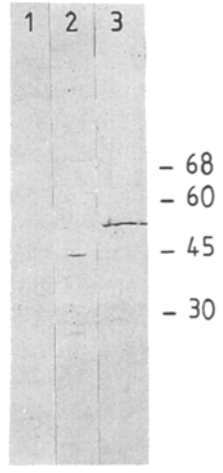


*Fig. 5.* Expression of *dhlC* and *dhlR*. Lane: 1, pGEM-7Z(−); 2, pPS2; 3, pPS11. The sizes of the molecular masses of the markers are shown to the right and are in kDa.

### *Putative transcriptional control of dhlB expression*

No *E. coli*-like promoters could be detected using the method of Staden (1984). However, upstream of *dhlC*, a sequence which closely resembled the −24/−12 or *ntr* promoter motif (Thöny & Hennecke 1989) was found (Fig. 2). Expression from these promoters requires the RNA polymerase factor $\sigma^{54}$ and a transcriptional activator. Thus, the expression of *dhlC* and possibly *dhlB* may be under positive regulatory control of *dhlR* and dependent on $\sigma^{54}$. In *P. putida* PP3, expression of *dehI*, the gene encoding haloalkanoic acid dehalogenase I, is also under control of a −24/−12 promoter (Thomas et al. 1992; Topping et al. 1995). In agreement with this, no expression of *dehI* was observed in an *rpoN* mutant of *P. putida* (Thomas et al. 1992). The 571 amino acids regulatory protein DehRI is also encoded before *dehI* in the opposite direction (Topping et al. 1995).

In general, activation of $\sigma^{54}$-dependent promoters occurs in response to a situation of environmental stress (Thöny & Hennecke 1989). In this respect it is interesting that *dhlB* is overexpressed in stationary phase grown cells. *X. autotrophicus* mutant GJ10M41 grows very poorly with ethanol and has a 4-fold higher expression compared to the wild type strain grown with the same substrate (Janssen et al. 1987). In fed-batch cultures of *X. autotrophicus* GJ10, a 50-fold higher expression than during the exponential growth phase was found under maintenance conditions with methanol as carbon source (A. Mars & D.B. Janssen, unpublished results). The genetic organization of the *dhlB* region appears to be such that high level expression of the haloalkanoic acid dehalogenase is achieved under starvation conditions. A high expression level of an initial catabolic enzyme increases the specific affinity of an organism for its substrate, allowing more efficient substrate utilization under such conditions.

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