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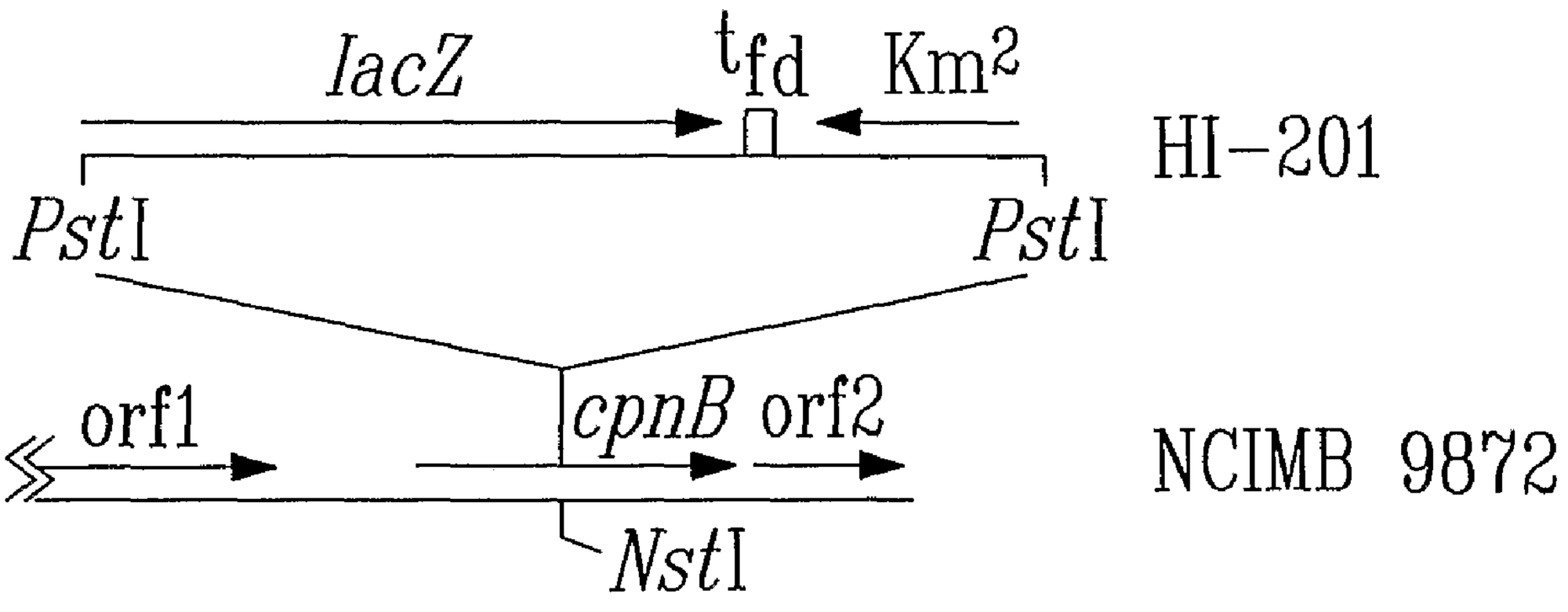
(54) Titre : CLONAGE, SEQUENCAGE ET EXPRESSION DU GENE CODANT POUR LE CYCLOPENTANONE 1,2-MONOOXYGENASE DU COMAMONAS DANS L'ESCHERICHIA COLI

(54) Title: CLONING, SEQUENCING AND EXPRESSION OF A COMAMONAS CYCLOPENTANONE 1,2-MONOOXYGENASE-ENCODING GENE IN ESCHERICHIA COLI



(57) Abrégé/Abstract:
Cyclopentanone 1,2-monooxygenase (CPMO) from Comamonas (previously Pseudomonas) sp. strain NCIMB 9872 carries out the second step of a degradation pathway that allows the bacterium to use cyclopentanol as a sole carbon source for growth. In the present invention there is reported the localization of the CPMO-encoding gene (cpnB) on a 4.3-kb SphI fragment, the determination of its sequence. The 550-amino acid CPMO polypeptide (M_r, 62,111) encoded by the gene was found to have 36.5% identity with the sequence of cyclohexanone 1,2-monooxygenase (CHMO) of Acinetobacter sp. strain NCIMB 9871. The 62-kDa CPMO was expressed in E. coli as an IPTG-inducible protein.

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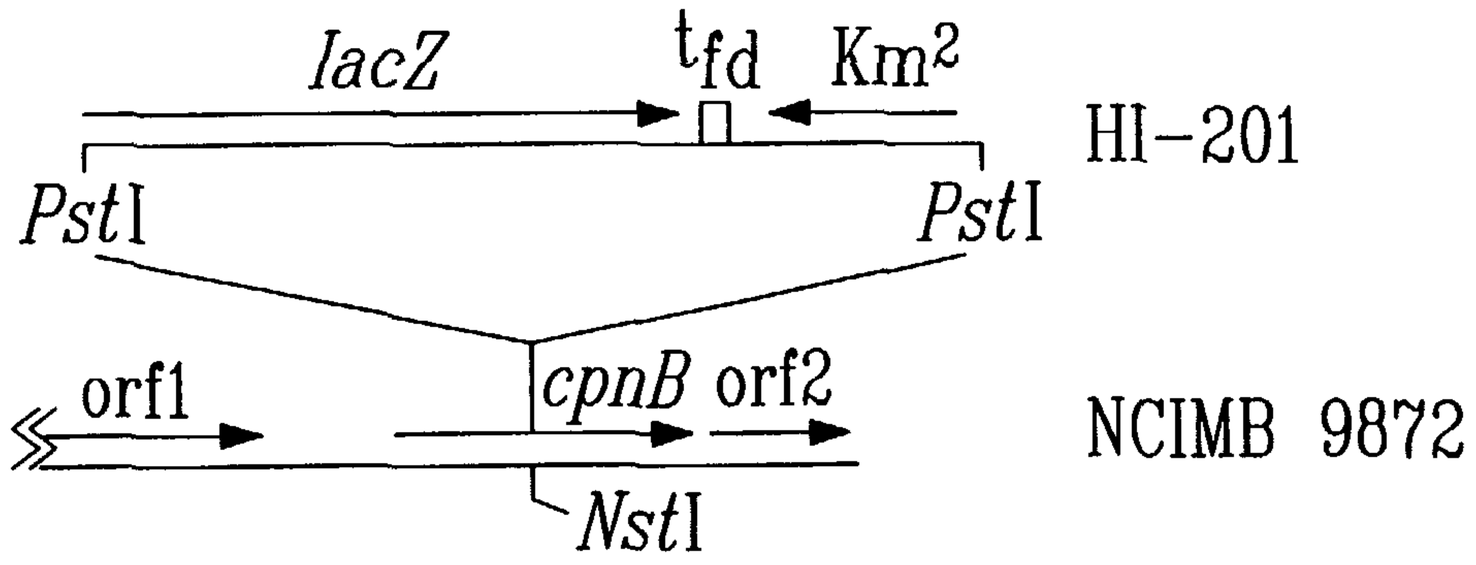
(54) Title: CLONING, SEQUENCING AND EXPRESSION OF A COMAMONAS CYCLOPENTANONE 1,2-MONOOXYGENASE-ENCODING GENE IN *ESCHERICHIA COLI*



(57) Abstract: Cyclopentanone 1,2-monooxygenase (CPMO) from *Comamonas* (previously *Pseudomonas*) sp. strain NCIMB 9872 carries out the second step of a degradation pathway that allows the bacterium to use cyclopentanol as a sole carbon source for growth. In the present invention there is reported the localization of the CPMO-encoding gene (*cpnB*) on a 4.3-kb *Sph*I fragment, the determination of its sequence. The 550-amino acid CPMO polypeptide (M_r, 62,111) encoded by the gene was found to have 36.5% identity with the sequence of cyclohexanone 1,2-monooxygenase (CHMO) of *Acinetobacter* sp. strain NCIMB 9871. The 62-kDa CPMO was expressed in *E. coli* as an IPTG-inducible protein.

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CLONING, SEQUENCING AND EXPRESSION OF A COMAMONAS CYCLOPENTANONE 1,2-MONOOXYGENASE-ENCODING GENE IN *ESCHERICHIA COLI*

TECHNICAL FIELD

The present invention relates to an isolated DNA encoding a cyclopentanone monooxygenase (CPMO), or an enzymatically active portion thereof, and expression vector and a transformed cell containing the isolates DNA.

BACKGROUND OF THE INVENTION

Comamonas (previously *Pseudomonas*) sp. NCIMB 9872 was one of the few microorganisms that have been characterized to produce a Baeyer-Villiger monooxygenase (BVMO; Griffin, M., et al., *Biochem. J.* **129:**595-603, 1972; Griffin, M., et al., *Eur. J. Biochem.* **63:**199-209, 1976; and Willetts, A., *Trends in Biotech.* **15:**55-62, 1997; for a recent review). BVMOs are flavoproteins that mimic the classical Baeyer-Villiger organic chemical reaction which is a peracid-catalyzed oxidation of a ketone to an ester or lactone. The use of enzyme substitutes for the production of lactones in high yield and optical purity is an attractive feature in current trends of research and development toward replacing chemical methods with biological alternatives (Stinson, S.C., *Chem. Eng. News*, 83-104, 1998). To date, the best characterized BVMO enzyme is that of cyclohexanone monooxygenase (CHMO) produced by *Acinetobacter* sp. NCIMB 9871 (Stewart, J.D., *Curr. Org. Chem.* **2:**195-216, 1998; Willetts, A., *Trends in Biotech.* **15:**55-62, 1997). This is also the only BVMO whose gene has been cloned and sequenced (Chen, et al., *J. Bacteriol.* **170:**781-789, 1988). Recently, this valuable resource was used to engineer a "designer yeast" in a whole-cell approach to effect a variety of asymmetric Baeyer-Villiger oxidations (Stewart, J.D., et al., *J. Am. Chem. Soc.* **120:**3541-3548, 1998).

It would be highly desirable to be provided with a new CPMO having an increased enzymatic activity for growing cells in a medium containing cyclopentanol or cyclopentanone as sole carbon source.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide a new CPMO having an increased enzymatic activity for growing cells in a medium containing cyclopentanol or cyclopentanone as sole carbon source.

In accordance with the present invention there is provided an isolated DNA encoding a cyclopentanone monooxygenase (CPMO), or an enzymatically active portion thereof, the isolated DNA being characterized by the ability to hybridize specifically with the complement of the DNA represented in SEQ ID NO:8 under stringent hybridization conditions.

Also in accordance with the present invention, there is provided an isolated DNA, wherein it codes for a cyclopentanone monooxygenase (CPMO), and contains:

(1) the nucleic acid sequence of SEQ ID NO:8;
(2) a sequence corresponding to said nucleic acid sequence in the scope of the degeneration of the genetic code; or
(3) a sequence hybridizing under stringent conditions with the sequence from (1) or (2), and still coding for cyclopentanone monooxygenase (CPMO).

Still in accordance with the present invention, there is provided an isolated DNA encoding a cyclopentanone monooxygenase (CPMO), or an enzymatically active portion thereof, said isolated DNA having SEQ ID NO:8.

The present invention further provides an isolated DNA expression vector encoding an enzymatically active cyclopentanone monooxygenase (CPMO) comprising a DNA characterized by a sequence as set forth in SEQ ID NO:8, or a portion thereof, said portion encoding said CPMO, in expressible form.

In accordance with the present invention, there is also provided a recombinant vector comprising the isolated DNA as decribed above, wherein the isolated DNA encodes cyclopentanone monooxygenase.

In a preferred embodiment of the present invention, the isolated DNA has a nucleic acid sequence of SEQ ID NO:8 or which, due to the degeneracy of the genetic code, is a functional equivalent thereof.

Also in accordance with the present invention, there is provided a recombinant vector containing one or more copies of a recombinant DNA described above.

The recombinant vector may be a prokaryotic vector. The recombinant vector may also be a plasmid.

Therefore, in accordance with the present invention, there is also provided a biologically functional plasmid or viral DNA vector, which contains a DNA as described above.

The present invention also provide a host cell comprising a recombinant vector as described above.

Accordingly, there is also provided a cell transformed with a heterologous DNA expression construct encoding an enzymatically active cyclopentanone monooxygenase (CPMO) comprising a DNA characterized by a sequence as set forth in SEQ ID NO:8, or a portion thereof, said portion encoding said CPMO, in expressible form.

The cell may be a prokaryotic cell or it may be *E. coli.*

Still in accordance with the present invention, there is also provided a purified cyclopentanone monooxygenase (CPMO) having :

a) an amino acid sequence as set forth in SEQ ID NO:5;

b) an amino acid sequence encoded by a nucleic acid sequence as set forth in SEQ ID NO:8; or

c) an amino acid sequence encoded by a nucleic acid sequence hybridizing to a nucleic acid sequence complementary to the nucleic acid sequence of step b) above under stringent conditions, said amino acid sequence encoded in step c) having a same activity as the amino acid sequence in a).

The present invention also provides a recombinant cyclopentanone monooxygenase (CPMO) having an enzymatic activity superior to the one from a native *Pseudomonas*, and more preferably twice superior.

The recombinant cyclopentanone monooxygenase (CPMO) may be prepared from Comamonas sp. NCIMB 9872. The recombinant cyclopentanone monooxygenase (CPMO) has preferably a sequence as set forth in SEQ ID NO:5.

A method for growing cells *in vitro* in presence of cyclopentanol or cyclopentanone as sole source of carbon, said method comprising the steps of:

a) transforming a cell with the expression construct described above; and
b) growing the cell of step a) under suitable conditions in a medium containing cyclopentanol or cyclopentanone as a sole source of carbon.

To increase this gene potential, according to the invention it is reported herein the cloning of a cyclopentanone monooxygenase (CPMO)-encoding gene (*cpnB*) from Comamonas (*Pseudomonas*) sp. NCIMB 9872, the determination of its DNA and surrounding sequence and expression of CPMO activity and protein in *E. coli*.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the first two steps of cyclopentanol degradation by *Pseudomonas* sp. NCIMB 9872;

Fig. 2 illustrates the genetic organization in *Comamonas* sp. NCIMB 9872 in the *Sph*I fragment containing cyclopentanone monooxygenase-encoding gene (*cpnB*) and additional open reading frames;

Fig. 3 illustrates an alignment of the amino acid sequence of the CPMO of *Comamonas* sp. NCIMB 9872 with that of CHMO from *Acinetobacter* sp.

NCIMB 9871 and a steroid monooxygenase (STMO) from *Rhodococcus rhodochrous*;

Fig. 4 illustrates a SDS-PAGE of crude extracts from *E. coli* (pCMP201); and

Fig. 5 illustrates CPMO-encoding gene, designated cpnB.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

Cloning of the *Comamonas* sp. NCIMB 9872 CPMO-encoding gene

Pseudomonas sp. NCIMB 9872 (henceforth strain 9872) identified as a *Comamonas* by 16S rDNA sequencing in this study, was purchased from the National Collections of Industrial and Marine Bacteria Ltd (NCIMB, Aberdeen, Scotland) and grown at 30°C in Luria-Bertani (LB) broth (Sambrook, J., et al., Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y, 1989), or mineral salt medium (MSM), pH 7.0, containing 2 ml of cyclopentanone. The MSM recipe contains per liter: 1.0 g of NH_4NO_3, 1.5 g of KH_2PO_4, 1.5 g of Na_2HPO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g of $CaCl_2 \cdot 2H_2O$, 0.005 of $FeSO \cdot 7H_2O$, 0.002 g of $MnSO_4 \cdot 4H_2O$ and 0.1 g of yeast extract. Agar was added to 1.5% for plates. Genomic DNA of strain 9872 was prepared by the Marmur method (Marmur, J., *J. Mol. Biol.* **3**: 208-218, 1961). At first, a Southern hybridization of DNA digested with *Bam*HI was carried out using the *Acinetobacter* NCIMB 9871 CHMO-containing gene as probe. Since there was no positive result (hybridization conditions carried out at 65°C) the CPMO protein was purified in order to obtain an N-terminal amino acid sequence. The purification of CPMO protein from cyclopentanone-grown cells was according to Griffin and Turgill (Griffin, M., et al., *Eur. J. Biochem.* **63:**199-209, 1976). Using an automated protein sequencer (Perkin-Elmer model 477) a 40-residue amino-terminal sequence of the purified CPMO was obtained (Fig. 2). This sequence, longer by 11 amino acids, is in perfect agreement with that reported previously from the same organism (Willetts, A., *Trends in Biotech.* **15:**55-62, 1997). Two degenerate oligodeoxynucleotide primers (5'-ACIACIATGA CIACNATGAC-3' (SEQ ID NO:1) and 5'-ARRTGRTAIA

RYTGRTA-3' (SEQ ID NO:2), corresponding to amino acids 2-8 and 35-40, respectively) were synthesized to amplify a 116-bp product from total DNA prepared from strain 9872. The PCR amplification was performed in a Perkin Elmer-Model 2400 Thermal Cycler™ and the amplification conditions were 94°C for 1 min, 50°C for 1 min and 72°C for 1 min for 30 cycles. The amplified product was cloned directly in the pXcmkn12 vector (Cha, J., et al., *Gene* **136**, 369-370, 1993), transformed in *E. coli* JM109 and the resulting plasmid was designated pCMP10. Before using the amplified product as a gene probe its nucleotide sequence was confirmed. Nucleotide sequencing was determined by the Taq DyeDeoxy terminator cycle sequencing kit and the ABI Prism 310 Genetic Analyzer (Perkin Elmer). Plasmid isolation was performed by the method of Birnboim and Doly (Birnboim, H. C., and J. Doly, *DNA. Nucleic Acids Res.* **7:**1513-1523, 1979).

In Fig. 2, Orf1 is most likely a transcriptional activator of the NtrC-type (Morett, E., L. Segovia, *J. Bacteriol.* **175**:6067-6074, 1993). The amino acid sequence of ORF1 (C-terminal 391-amino acids) showed 38-40% identity to equivalent regions of proteins such as NTRC_ECOLI (Nitrogen regulation protein NR(I) from *E. coli*; Miranda, et al., *The complete nucleotide sequence of the glnALG operon of Eschericha coli K12* **15**:2757-2770, 1987), ACOR_ALCEU (Acetoin catabolism regulatory protein from *Ralstonia eutropha*; Kruger, N., et al., *J. Bacteriol.* **179**:4391-4400, 1992). The amino acid sequence of ORF2, showing similarity to enzymes of the short-chain alcohol dehydrogenase family (Jornvall, H., et al., *Biochemistry* **34**: 6003-6013, 1995), is most homologous (45-46% identity) to a putative oxidoreductase CY39.16C of *Mycobacterium tuberculosis* (Swiss Prot sp:Q10855) and fadG3 of *M. tuberculosis* (GenBank accession number Z74025). For *Pseudomonas* sp. strain HI-201 the *lacZ*-Km^r cassette from pKOK6.1 (Kokotek, W., et al., *Gene* **84**: 467-471, 1989) was inserted into *cpnB* at the *Nsi*I site.

In Fig. 2, the following terms are defined as follows: t_{fd}, transcriptional termination sequence of phage fd; Km^r, kanamycin resistance gene, *lacZ*, gene encoding b-galactosidase. Genes and markers are indicated with arrows.

To clone the CPMO-containing gene, the DNA insert from pCMP10 was amplified, labeled by the digoxigenin-11-UTP system according to manufacturer's instructions (Boehringer Mannheim GmbH) and used to probe a Southern hybridization of strain 9872 genomic DNA digested with various restriction enzymes (*Bam*HI, *Eco*RI, *Hin*dIII, *Kpn*I, *Nhe*I, *Pst*I, *Sal*I, *Sph*I and *Xba*I). As a result, a single hybridizing band of *ca* 4.3-kb *Sph*I fragment was obtained. Conditions of hybridization were as before. Subsequently, a purified 4.0- to 4.5-kb size fraction of *Sph*I-cut total DNA separated on a 0.8 % agarose gel was ligated to *E. coli* plasmid pUC18, which had been linearized and dephosphorylated. A clone containing the 4.3-kb insert was screened by colony hybridization using the PCR product as a probe; this recombinant plasmid was designated pCMP200.

DNA sequence of the CPMO-encoding gene (*cpnB*) and the flanking region

Nucleotide sequencing of the CPMO-encoding gene was initiated by using a primer designed from the sequence of the PCR product cloned in pCMP10 and further extended using oligonucleotides derived from the new sequence. Both DNA strands of the *Sph*I fragment were sequenced and found to consist of 4281 base pairs (bp). The sequence was analyzed by GENETYX-Mac (Software Development Co., Ltd. Chiba, Japan) and the BLAST program (Altschul, S. F., et al., *Nucleic Acids Res.* **25:**3389-3402, 1997). As a result three open reading frames (ORFs) arranged in the same direction were predicted (Fig. 2). The nucleotide sequence of the 1650-bp ORF encoding CPMO is preceded by a partial ORF1 (1173-bp) coding for the C-terminus of an NtrC-type transcriptional activator (Miranda, et al., *The complete nucleotide sequence of the glnALG operon of Eschericha coli K12* **15**:2757-2770, 1987) and by a complete ORF2 (750-bp) coding for a homolog of the short-chain dehydrogenases/reductases (Jornvall, H., et al., *Biochemistry* **34**: 6003-6013, 1995). The two intergenic regions are 244-bp and 32-bp, respectively. The CPMO-encoding gene is referred to *cpnB* (cyclopentanone and *B* designates the second step of the degradation pathway, see Fig. 1) hereafter. In Fig. 5, the CPMO-encoding gene starts at nucleotide position 1822 and ends 3471 that does not include the stop codon. Accordingly, the boundary of cpnA is 3507-

4256. The partial open reading frame preceding cpnB is from 1-1174.

Fig. 1 has been adapted from Griffin, M., et al. (Griffin, M., et al., *Biochem. J.* **129:**595-603, 1972). The designated genes are: *cpnA* encoding cyclopentanol dehydrogenase; *cpnB* encoding cyclopentanone 1,2-monooxygenase (CPMO). An alternative name for 5-valerolactone is 5-pentanolide. Subsequent reaction steps are the formation of 5-hydroxyvalerate, 5-oxovalerate, glutarate and finally acetyl CoA.

The amino acid sequence of the CPMO enzyme consists of 550 residues (Fig. 3). This sequence shows 36.5% identity and an additional 13.6% amino acid similarity with the 543-residue CHMO of *Acinetobacter* sp. strain NCIMB 9871. An equally related protein (549 amino acids; 37.3% identity and 12.4% similarity) is the putative steroid monooxygenase (STMO) of *Rhodococcus rhodochrous* (Morii, S., et al., GenBank accession number AB010439, 1998). The latter enzyme carries out the oxidation of progesterone to produce testosterone acetate. A CLUSTAL alignment of these three sequences gave 24.6% positional identity (Fig. 3).

In Fig. 3, asterisks indicate identical amino acids, dots indicated similar amino acids and dashes indicate gaps introduced to maximize the alignment. The amino-terminal peptide sequence confirmed by Edman degradation is underlined. The locations of the consensus FAD fingerprint sequences as described by Eppink et al. (Eppink, et al., *Prot. Sci.* **6:**2454-2458, 1997) are as indicated. The conserved GD motif found in flavoprotein hydroxylases as a second FAD fingerprint is also indicated. Not shown is the DG motif of flavoprotein hydroxylases which has the sequence of chhhssDGxcSxhR. Lower case letters identify certain residues types: h, hydrophobic residues, s, small residues, c, charged residues, and x, any residues. Note that a DG doublet is present in CPMO and STMO sequence.

A notable sequence motif present in CPMO and related proteins is the FAD-binding fingerprint (GXGXXG) that is similar to those found in flavoprotein hydroxylases (Eppink, et al., *Prot. Sci.* **6:**2454-2458, 1997). Flavoprotein hydroxylases (eg. phenol hydroxylase, the structure is now known; Enroth, C.,

et al., *Structure* **6:**605-617, 1998) are monooxygenases that catalyze the insertion of one atom of molecular oxygen into the substrate using NAD(P)H as electron donor. These proteins possess a conserved "Asp-Gly (DG)" motif for both FAD and NAD(P)H binding in between two fingerprint motifs for the FAD binding (fingerprint 1: GXGXXG; fingerprint 2: Gly-Asp [GD] motif). Sequence motifs in CPMO, STMO and CHMO differ from those in flavoprotein hydroxylases by having a repeated GXGXXG motif (amino acids 24 to 33 and 193 to 202 in CPMO numbering). The possibility that the second FAD fingerprint in CPMO and related proteins fulfils a dual role of FAD and NADPH binding awaits structural determination of a representative member of this family of proteins. It is reasonable to assume that a different mechanism in catalysis is reflected in the motifs seen in the two classes of proteins.

Expression of *cpnB* gene in *E. coli*

Two primers of the following sequence were synthesized to amplify the *cpnB* gene and the resultant 1.7-kb DNA fragment was cloned in the pSD80 plasmid to yield pCMP201. Plasmid pSD80 is a third generation derivative of the commercially available pKK223-3 vector (Pharmacia) that contains a *tac* promoter upstream of the multiple cloning site (MCS), an *unc* terminator sequence downstream of the MCS, and $lacI^q$ elsewhere on the plasmid (Smith, S.P., et al., *Biochemistry* **35:**8805-8814, 1996). The primers were: 5'-AAAAGGCCTG AACTTCAATT ATTTAGGAGA C-3' (SEQ ID NO:3) and 5'-AAAACTGCAG GAGTTGCACA ACAGAGTCTT AG-3' with built-in *Stu*I and *Pst*I restriction sites (underlined), respectively, to facilitate cloning at the compatible sites (*Sma*I and *Pst*I) of the pSD80 vector. Vent DNA polymerase (New England BioLabs, Beverly, MA) was used and the amplification conditions were 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, for 30 cycles. The amplified DNA fragment was purified from an agarose gel and digested with *Stu*I and *Pst*I. One of the resulting recombinant plasmids was designated pCMP201. By DNA sequencing it was established that no mutation had been introduced in the *cpnB* gene during PCR amplification.

Fig. 4 shows the production of a 60-kDa protein in a Coomassie blue-stained SDS-polyacrylamide gel of the crude protein extract prepared from *E. coli* JM109 (pCMP201) cells that were induced by 0.1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG). The cells were induced at an absorbance (A600 nm) of 0.4 to 0.5 and the induction period was up to 4 hr. The observed molecular mass was in agreement with the predicted size of the 62-kDa CPMO. In the absence of IPTG, this protein band was not produced. Also, the CPMO enzyme activity was observed only in those cells grown in the presence of IPTG. CPMO activity was assayed at 25°C by measuring a decrease in absorbance at 340 nm in 50 mM phosphate buffer (pH 7.8) containing 1 µmol of cyclopentanone, 0.2 µmol of NADPH, and the crude enzyme extract prepared from *E. coli* JM109 (pCMP201). These cells were cultivated in 100 ml of LB medium containing 100 µg/ml of ampicillin at 25°C. The IPTG-induced cells were harvested by centrifugation, washed in 50 mM phosphate buffer (pH 7.2), resuspended in 1/20 volume of same buffer, and sonicated by four-20 sec bursts with a Braun-Sonifier™ 250 apparatus. After centrifugation for 30 min at 18,000 x g and at 4°C, the supernatant was used for determination of enzyme activity. One unit (U) of activity is defined as the amount of enzyme required to convert 1 µmol of substrate in 1 min. Protein concentration was determined by the method of Bradford (Bradford, M. M., *Anal. Biochem.* **72**: 248-254, 1976). As a result the specific activity of the CPMO enzyme was found to be 0.28 U/mg. The specific activity of CPMO in the native *Pseudomonas* was reported to be 0.11 U/mg (Griffin, M., et al., *Biochem. J.* **129:**595-603, 1972).

In Fig. 4, lane 1 has been loaded with extracts of IPTG-induced *E. coli* and lane 2 has been loaded with extracts of *E. coli* in absence of IPTG. M means molecular weight markers as indicated in kilo daltons. The arrow indicates the production of the desired 60-kDa protein.

Inactivation of *cpnB* gene

Pseudomonas sp. strain HI-201 was constructed by chromosomal inactivation of the *cpnB* gene using a *lacZ*-Km^r cassette from the mobilizable pKOK6.1 vector (Kokotek, W., et al., *Gene* **84**: 467-471, 1989). In pKOK6.1 the

lacZ gene is promoterless and in addition to Km^r it is ampicillin resistant (Ap^r). The *lacZ*-Km^r cassette was excised as a *Pst*I-fragment and inserted into the *Nsi*I site within the *cpnB* gene in pCMP200, yielding pCMP202. Electroporation of this plasmid into 9872 cells was carried out in the Gene Pulser™ (BioRads) and the parameters of electroporation were 2.5 kV, 25 uF and 200 ohm. The cells were initially washed with 1mM HEPES buffer and resuspended in 1mM HEPES containing 10% glycerol. Km^r colonies were selected on LB plates containing Km (250 µg/ml). To select for double crossover mutants, a second screening on LB plates containing Ap (300 µg /ml) was carried out. The inactivation of *cpnB* (Fig. 2), was confirmed by PCR. The resulting mutant HI-201 was found not to be able to grow on cyclopentanol or cyclopentanone as a sole carbon and energy source. This result indicated that *cpnB* is essential for the degradaton of cyclopentanol and it appeared that there was only one copy of the *cpnB* gene in strain 9872.

As expected of a flavoprotein the amino acid sequence of CPMO contains motifs of FAD fingerprints similar to those found in flavoprotein hydroxylases.

Nucleotide sequence accession number

The DNA sequence of the 4,281-bp *Sph*I fragment has been submitted to DDBJ and assigned accession number AB022102. The release of this data awaits the inventors' authorization.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

SEQUENCE LISTING

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HASEGAWA, Yoshie
LAU, Peter C.K.

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<223> Primers for Comamonas sp.

<221> modified_base
<222> (3)...(3)
<223> I

<221> modified_base
<222> (6)...(6)
<223> I

<221> modified_base
<222> (12)...(12)
<223> I

<221> unsure
<222> (15)...(15)
<223> N = any base

<400> 1
acnacnatga cnacnatgac 20

<210> 2
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Primers for Comamonas sp.

<221> modified_base
<222> (9)...(9)
<223> I

<400> 2
arrtgrtana rytgrta 17

<210> 3
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Primers for Comamonas sp.

<400> 3
aaaaggcctg aacttcaatt atttaggaga c 31

<210> 4
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Primers for Comamonas sp.

<400> 4
aaaactgcag gagttgcaca acagagtctt ag 32

<210> 5
<211> 550
<212> PRT
<213> Comamonas sp

<400> 5

Met	Thr	Thr	Met	Thr	Thr	Met	Thr	Thr	Glu	Gln	Leu	Gly	Met	Asn	Asn
1				5					10					15	
Ser	Val	Asn	Asp	Lys	Leu	Asp	Val	Leu	Leu	Ile	Gly	Ala	Gly	Phe	Thr
			20					25					30		
Gly	Leu	Tyr	Gln	Leu	Tyr	His	Leu	Arg	Lys	Leu	Gly	Tyr	Lys	Val	His
		35					40					45			
Leu	Val	Asp	Ala	Gly	Ala	Asp	Ile	Gly	Gly	Ile	Trp	His	Trp	Asn	Cys
	50					55					60				
Tyr	Pro	Gly	Ala	Arg	Val	Asp	Thr	His	Cys	Gln	Ile	Tyr	Gln	Tyr	Ser
65					70					75					80
Ile	Pro	Glu	Leu	Trp	Gln	Glu	Phe	Asn	Trp	Lys	Glu	Leu	Phe	Pro	Asn
				85					90					95	
Trp	Ala	Gln	Met	Arg	Glu	Tyr	Phe	His	Phe	Ala	Asp	Lys	Lys	Leu	Asp
			100					105					110		
Leu	Ser	Lys	Asp	Ile	Ser	Phe	Asn	Thr	Arg	Val	Gln	Ser	Ala	Val	Phe
		115					120					125			
Asp	Glu	Gly	Thr	Arg	Glu	Trp	Thr	Val	Arg	Ser	Ile	Gly	His	Gln	Pro
	130					135					140				
Ile	Gln	Ala	Arg	Phe	Val	Ile	Ala	Asn	Leu	Gly	Phe	Gly	Ala	Ser	Pro
145					150					155					160
Ser	Thr	Pro	Asn	Val	Asp	Gly	Ile	Glu	Thr	Phe	Lys	Gly	Gln	Trp	Tyr
				165					170					175	
His	Thr	Ala	Leu	Trp	Pro	Gln	Glu	Gly	Val	Asn	Met	Ala	Gly	Lys	Arg
			180					185					190		
Val	Ala	Ile	Ile	Gly	Thr	Gly	Ser	Ser	Gly	Val	Gln	Val	Ala	Gln	Glu
		195					200					205			
Ala	Ala	Leu	Asp	Ala	Lys	Gln	Val	Thr	Val	Tyr	Gln	Arg	Thr	Pro	Asn
	210					215					220				

```
Leu Ala Leu Pro Met His Gln Lys Gln Leu Ser Ala Glu Asp Asn Leu
225                 230                 235                 240
Arg Met Lys Pro Glu Leu Pro Ala Ala Phe Glu Arg Arg Gly Lys Cys
                245                 250                 255
Phe Ala Gly Phe Asp Phe Asp Phe Ile Ala Lys Asn Ala Thr Glu Leu
            260                 265                 270
Ser Ala Ala Glu Arg Thr Glu Ile Leu Glu Glu Leu Trp Asn Ala Gly
        275                 280                 285
Gly Phe Arg Tyr Trp Leu Ala Asn Phe Gln Asp Tyr Leu Phe Asp Asp
    290                 295                 300
Lys Ala Asn Asp Tyr Val Tyr Glu Phe Trp Arg Asp Lys Val Arg Ala
305                 310                 315                 320
Arg Ile Lys Asp Pro Lys Val Ala Glu Lys Leu Ala Pro Met Lys Lys
                325                 330                 335
Pro His Pro Tyr Gly Ala Lys Arg Pro Ser Leu Glu Gln Trp Tyr Tyr
            340                 345                 350
Glu Ile Phe Asn Gln Asn Asn Val Thr Leu Val Asp Val Asn Glu Thr
        355                 360                 365
Pro Val Leu Arg Ile Thr Glu Lys Gly Ile Val Thr Ala Glu Gly Glu
    370                 375                 380
Ala Glu Phe Asp Leu Ile Val Phe Ala Thr Gly Phe Asp Ala Val Thr
385                 390                 395                 400
Gly Gly Leu Thr Ser Ile Asp Phe Arg Asn Asn Gln Gly Gln Ser Phe
                405                 410                 415
Lys Asp Val Trp Ser Asp Gly Ile Arg Thr Gln Leu Gly Val Ala Thr
            420                 425                 430
Ala Gly Phe Pro Asn Leu Leu Phe Gly Tyr Gly Pro Gln Ser Pro Ala
        435                 440                 445
Gly Phe Cys Asn Gly Pro Ser Ser Ala Glu Tyr Gln Gly Asp Leu Leu
    450                 455                 460
Ile Gln Leu Met Asn Tyr Leu Arg Asp Asn Asn Ile Ser Arg Ile Glu
465                 470                 475                 480
Ala Gln Ser Glu Ala Gln Glu Glu Trp Ser Lys Leu Ile Ala Asp Phe
                485                 490                 495
Trp Asp Ser Ser Leu Phe Pro Arg Ala Lys Ser Trp Tyr Gln Gly Ser
            500                 505                 510
Asn Ile Pro Gly Lys Lys Val Glu Ser Leu Asn Phe Pro Leu Gly Leu
        515                 520                 525
Pro Thr Tyr Ile Ser Lys Phe Asn Glu Ser Ala Glu Lys Gly Tyr Ala
    530                 535                 540
Gly Phe Ser Leu Ala Ser
545                 550
```

<210> 6
<211> 549
<212> PRT
<213> Rhodococcus rhodochrous

<400> 6

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Met Asn Gly Gln His Pro Arg Ser Val Val Thr Ala Pro Asp Ala Thr
 1               5                  10                  15
Thr Gly Thr Thr Ser Tyr Asp Val Val Val Val Gly Ala Gly Ile Ala
            20                  25                  30
Gly Leu Tyr Ala Ile His Arg Phe Arg Ser Gln Gly Leu Thr Val Arg
        35                  40                  45
Ala Phe Glu Ala Ala Ser Gly Val Gly Gly Val Trp Tyr Trp Asn Arg
    50                  55                  60
Tyr Pro Gly Ala Arg Cys Asp Val Glu Ser Ile Asp Tyr Ser Tyr Ser
65                  70                  75                  80
```

```
Phe Ser Pro Glu Leu Glu Gln Glu Trp Asn Trp Ser Glu Lys Tyr Ala
                85                  90                  95
Thr Gln Pro Glu Ile Leu Ala Tyr Leu Glu His Val Ala Asp Arg Phe
            100                 105                 110
Asp Leu Arg Arg Asp Ile Arg Phe Asp Thr Arg Val Thr Ser Ala Val
        115                 120                 125
Leu Asp Glu Glu Gly Leu Arg Trp Thr Val Arg Thr Asp Arg Gly Asp
    130                 135                 140
Glu Val Ser Ala Arg Phe Leu Val Val Ala Ala Gly Pro Leu Ser Asn
145                 150                 155                 160
Ala Asn Thr Pro Ala Phe Asp Gly Leu Asp Arg Phe Thr Gly Asp Ile
                165                 170                 175
Val His Thr Ala Arg Trp Pro His Asp Gly Val Asp Phe Thr Gly Lys
            180                 185                 190
Arg Val Gly Val Ile Gly Thr Gly Ser Ser Gly Ile Gln Ser Ile Pro
        195                 200                 205
Ile Ile Ala Glu Gln Ala Glu Gln Leu Phe Val Phe Gln Arg Ser Ala
    210                 215                 220
Asn Tyr Ser Ile Pro Ala Gly Asn Val Pro Leu Asp Asp Ala Thr Arg
225                 230                 235                 240
Ala Glu Gln Lys Ala Asn Tyr Ala Glu Arg Arg Arg Leu Ser Arg Glu
                245                 250                 255
Ser Gly Gly Gly Ser Pro His Arg Pro His Pro Lys Ser Ala Leu Glu
            260                 265                 270
Val Ser Glu Glu Glu Arg Arg Ala Val Tyr Glu Glu Arg Trp Lys Leu
        275                 280                 285
Gly Gly Val Leu Phe Ser Lys Ala Phe Pro Asp Gln Leu Thr Asp Pro
    290                 295                 300
Ala Ala Asn Asp Thr Ala Arg Ala Phe Trp Glu Glu Lys Ile Arg Ala
305                 310                 315                 320
Val Val Asp Asp Pro Ala Val Ala Glu Leu Leu Thr Pro Lys Asp His
                325                 330                 335
Ala Ile Gly Ala Lys Arg Ile Val Thr Asp Ser Gly Tyr Tyr Glu Thr
            340                 345                 350
Tyr Asn Arg Asp Asn Val Glu Leu Val Asp Leu Arg Ser Thr Pro Ile
        355                 360                 365
Val Gly Met Asp Glu Thr Gly Ile Val Thr Thr Gly Ala His Tyr Asp
    370                 375                 380
Leu Asp Met Ile Val Leu Ala Thr Gly Phe Asp Ala Met Thr Gly Ser
385                 390                 395                 400
Leu Asp Lys Leu Glu Ile Val Gly Arg Gly Gly Arg Thr Leu Lys Glu
                405                 410                 415
Thr Trp Ala Ala Gly Pro Arg Thr Tyr Leu Gly Leu Gly Ile Asp Gly
            420                 425                 430
Phe Pro Asn Phe Phe Asn Leu Thr Gly Pro Gly Ser Pro Ser Val Leu
        435                 440                 445
Ala Asn Met Val Leu His Ser Glu Leu His Val Asp Trp Val Ala Asp
    450                 455                 460
Ala Ile Ala Tyr Leu Asp Ala Arg Gly Ala Ala Gly Ile Glu Gly Thr
465                 470                 475                 480
Pro Glu Ala Val Ala Asp Trp Val Glu Glu Cys Arg Asn Arg Ala Glu
                485                 490                 495
Ala Ser Leu Leu Asn Ser Ala Asn Ser Trp Tyr Leu Gly Ala Asn Ile
            500                 505                 510
Pro Gly Arg Pro Arg Val Phe Met Pro Phe Leu Gly Gly Phe Gly Val
        515                 520                 525
Tyr Arg Glu Ile Ile Thr Glu Val Ala Glu Ser Gly Tyr Lys Gly Phe
    530                 535                 540
Ala Ile Leu Glu Gly
545
```

<210> 7
<211> 543
<212> PRT
<213> Acinetobacter sp

<400> 7

Met	Ser	Gln	Lys	Met	Asp	Phe	Asp	Ala	Ile	Val	Ile	Gly	Gly	Gly	Phe
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Gly	Gly	Leu	Tyr	Ala	Val	Lys	Lys	Leu	Arg	Asp	Glu	Leu	Glu	Leu	Lys
			20					25				30			
Val	Gln	Ala	Phe	Asp	Lys	Ala	Thr	Asp	Val	Ala	Gly	Thr	Trp	Tyr	Trp
		35					40					45			
Asn	Arg	Tyr	Pro	Gly	Ala	Leu	Thr	Asp	Thr	Glu	Thr	His	Leu	Tyr	Cys
	50					55					60				
Tyr	Ser	Trp	Asp	Lys	Glu	Leu	Leu	Gln	Ser	Leu	Glu	Ile	Lys	Lys	Lys
65					70					75					80
Tyr	Val	Gln	Gly	Pro	Asp	Val	Arg	Lys	Tyr	Leu	Gln	Gln	Val	Ala	Glu
				85					90					95	
Lys	His	Asp	Leu	Lys	Lys	Ser	Tyr	Gln	Phe	Asn	Thr	Ala	Val	Gln	Ser
			100					105					110		
Ala	His	Tyr	Asn	Glu	Ala	Asp	Ala	Leu	Trp	Glu	Val	Thr	Thr	Glu	Tyr
		115					120					125			
Gly	Asp	Lys	Tyr	Thr	Ala	Arg	Phe	Leu	Ile	Thr	Ala	Leu	Gly	Leu	Leu
	130					135					140				
Ser	Ala	Pro	Asn	Leu	Pro	Asn	Ile	Lys	Gly	Ile	Asn	Gln	Phe	Lys	Gly
145					150					155					160
Glu	Leu	His	His	Thr	Ser	Arg	Trp	Pro	Asp	Asp	Val	Ser	Phe	Glu	Gly
				165					170					175	
Lys	Arg	Val	Gly	Val	Ile	Gly	Thr	Gly	Ser	Thr	Gly	Val	Gln	Val	Ile
			180					185					190		
Thr	Ala	Val	Ala	Pro	Leu	Ala	Lys	His	Leu	Thr	Val	Phe	Gln	Arg	Ser
		195					200					205			
Ala	Gln	Tyr	Ser	Val	Pro	Ile	Gly	Asn	Asp	Pro	Leu	Ser	Glu	Glu	Asp
	210					215					220				
Val	Lys	Lys	Ile	Lys	Asp	Asn	Tyr	Asp	Lys	Ser	Leu	Gly	Trp	Cys	Met
225					230					235					240
Asn	Ser	Ala	Leu	Ala	Phe	Ala	Leu	Asn	Glu	Ser	Thr	Val	Pro	Ala	Met
				245					250					255	
Ser	Val	Ser	Ala	Glu	Glu	Arg	Lys	Ala	Val	Phe	Glu	Lys	Ala	Trp	Gln
			260					265					270		
Thr	Gly	Gly	Gly	Phe	Arg	Phe	Met	Phe	Glu	Thr	Phe	Gly	Asp	Ile	Ala
		275					280					285			
Thr	Asn	Met	Glu	Ala	Asn	Ile	Glu	Ala	Gln	Asn	Phe	Ile	Lys	Gly	Lys
	290					295					300				
Ile	Ala	Glu	Ile	Val	Lys	Asp	Pro	Ala	Ile	Ala	Gln	Lys	Leu	Met	Pro
305					310					315					320
Gln	Asp	Leu	Tyr	Ala	Lys	Arg	Pro	Leu	Cys	Asp	Ser	Gly	Tyr	Tyr	Asn
				325					330					335	
Thr	Phe	Asn	Arg	Asp	Asn	Val	Arg	Leu	Glu	Asp	Val	Lys	Ala	Asn	Pro
			340					345					350		
Ile	Val	Glu	Ile	Thr	Glu	Asn	Gly	Val	Lys	Leu	Glu	Asn	Gly	Asp	Phe
		355					360					365			
Val	Glu	Leu	Asp	Met	Leu	Ile	Cys	Ala	Thr	Gly	Phe	Asp	Ala	Val	Asp
	370					375					380				
Gly	Asn	Tyr	Val	Arg	Met	Asp	Ile	Gln	Gly	Lys	Asn	Gly	Leu	Ala	Met
385					390					395					400
Lys	Asp	Tyr	Trp	Lys	Glu	Gly	Pro	Ser	Ser	Tyr	Met	Gly	Val	Thr	Val
				405					410					415	

```
Asn Asn Tyr Pro Asn Met Phe Met Val Leu Gly Pro Asn Gly Pro Phe
            420                 425                 430
Thr Asn Leu Pro Pro Ser Ile Glu Ser Gln Val Glu Trp Ile Ser Asp
        435                 440                 445
Thr Ile Gln Tyr Thr Val Glu Asn Asn Val Glu Ser Ile Glu Ala Thr
    450                 455                 460
Lys Glu Ala Glu Glu Gln Trp Thr Gln Thr Cys Ala Asn Ile Ala Glu
465                 470                 475                 480
Met Thr Leu Phe Pro Lys Ala Gln Ser Trp Ile Phe Gly Ala Asn Ile
                485                 490                 495
Pro Gly Lys Lys Asn Thr Val Tyr Phe Tyr Leu Gly Gly Leu Lys Glu
            500                 505                 510
Tyr Arg Thr Cys Ala Ser Asn Cys Lys Asn His Ala Tyr Glu Gly Phe
        515                 520                 525
Asp Ile Gln Leu Gln Arg Ser Asp Ile Lys Gln Pro Ala Asn Ala
    530                 535                 540
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<210> 8
<211> 4281
<212> DNA
<213> Comamonas sp

<220>
<221> CDS
<222> (1822)...(3471)

<400> 8

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caccgacatc acatcgccgg tcaccggcga gaacattggc aagatcctgc gcttttcgcc    120
gctgaagccg cagggctacg agagaatttt tcagtccggc ctggcggact cgacccagca    180
caccccccagg atggtgcgtt tcggacagtt cctgtcctgt gatccagaga ccatcaacac    240
cctggagaca ttggagcgga tcgcgcgcgc cgacgtcaat gtcttgctgc atggagagac    300
gggcacgggc aaagagctga tagctcgcca cattcatgtc gcgagccgcc ggcgggatgc    360
gccctacctc gccatcaact gcggggcaat cagctcggag ttgctggaga gtacttttt     420
tggctatgtg cgcggagcat tctccggggc agatcccaag ggccgcgccg gctactttga    480
atcggtgggc gaaggcacct tgtttctgga cgagatcggc gagctgccct tggccatgca    540
ggcggcattg ttgcgcgtgc tggaagacgg aagctttctg cgtgtgggct catcgacccc    600
gcagcgcgcc tcgtgccgca ttatcgcggc cacgcaccgc aatctggagg aactcatcgc    660
acagggcctg tttcgccagg atctctacta ccgtctcaag atcgttcaga aaaggctcaa    720
gccgatacgg gaacgcacct gtgacatcgc cttgctggca gagcaattca aatctgcact    780
ggcgcaaaag caccagatcc cgaacgtgca gatccacccc gaggccatgg cagtcatgga    840
gcgctaccag tggccgggca atgcgcgcga aatccgcaat gtgatggaag ccgccctgat    900
ctgctccgat ggtgaaatca cgctcgccag cctgcccccg gaagtatctg agaactccac    960
ctatcctctg caaagccggg tggccgaagc gaatagcgag atgcctcccg tctccggcaa   1020
cgactatgaa cggcaactca tcgtgggttt gctgcgcaag taccgaaagg tcaatcatgt   1080
ggcgagggcc ctgggcctcg cgcgctccac gctctatcgg aaattcgcag acttgggcat   1140
tgaccaacgc gagtatgtca atgatctgtc cgattaacac tgctgcgccc tgcgcttgcg   1200
gcaacgcagt atcgtcggaa cgcgggcaac gctaaaggca gccgccgtcg gcatcggttg   1260
ccgcccggtg ttgtttgtcg gctgagaccg cctgcagcac cagcccctcc attcctcgtg   1320
tctagatctc cttgcaacgc tgtcacagcg tgctttttc gtggcgcggg acttctcgcg   1380
atgagggcct gcaagggcgg cgcatcaaaa caagcgccct gtcctcagga cgtttgcgcc   1440
gtcgctggct atgggcagtc tgcatgatcc gcttgggctt gcccaccatg cgcgcgactg   1500
cgctctttcc tgttgggcgc atcgtcatcc cgaccttcct gaaatccatt tccacagggc   1560
cggcctgagc cagacagcga tcggcaggct ctcgcatctc ggacagtggt gcgctacacc   1620
acaggacaca agtgtccggc gcgtggcacg cagcggcctt tgctgcgcac gatgcgtgct   1680
tttggcacat ggaaaccctc ggtttttccg aaccagagac ctggcacaag tcttgataac   1740
tcctgagtga ctgatgcaac gcatctcagt ccgggagcgt ggacgacacg cccctgaact   1800
tcaattattt aggagaccca t atg acc acc atg acc acc atg acc acc gaa      1851
                        Met Thr Thr Met Thr Thr Met Thr Thr Glu
                         1               5                  10
```

```
caa ctc ggc atg aac aac tct gtc aat gac aag ctt gac gtt ttg ctc      1899
Gln Leu Gly Met Asn Asn Ser Val Asn Asp Lys Leu Asp Val Leu Leu
                15                  20                  25

atc ggc gcc ggc ttc acc ggt ctc tac cag ctc tat cac ctg cgc aag      1947
Ile Gly Ala Gly Phe Thr Gly Leu Tyr Gln Leu Tyr His Leu Arg Lys
            30                  35                  40

ctg ggc tac aag gtt cat ctc gtc gac gcc ggt gcc gat att ggc ggg      1995
Leu Gly Tyr Lys Val His Leu Val Asp Ala Gly Ala Asp Ile Gly Gly
        45                  50                  55

atc tgg cat tgg aac tgc tac ccc gga gcg cgt gtg gat acc cac tgc      2043
Ile Trp His Trp Asn Cys Tyr Pro Gly Ala Arg Val Asp Thr His Cys
    60                  65                  70

cag atc tac cag tac tcc att cca gag ttg tgg cag gag ttc aac tgg      2091
Gln Ile Tyr Gln Tyr Ser Ile Pro Glu Leu Trp Gln Glu Phe Asn Trp
75                  80                  85                  90

aaa gag ctg ttc cct aac tgg gcg caa atg cgc gag tat ttc cat ttt      2139
Lys Glu Leu Phe Pro Asn Trp Ala Gln Met Arg Glu Tyr Phe His Phe
                95                  100                 105

gcc gac aag aag ctc gac ctg agc aag gac atc agc ttc aac acc cgt      2187
Ala Asp Lys Lys Leu Asp Leu Ser Lys Asp Ile Ser Phe Asn Thr Arg
            110                 115                 120

gtg cag tcg gcc gtc ttt gac gaa ggc aca cgc gaa tgg acg gta cgc      2235
Val Gln Ser Ala Val Phe Asp Glu Gly Thr Arg Glu Trp Thr Val Arg
        125                 130                 135

tcg atc gga cac cag ccg atc cag gcc agg ttc gtc atc gcc aac ctt      2283
Ser Ile Gly His Gln Pro Ile Gln Ala Arg Phe Val Ile Ala Asn Leu
    140                 145                 150

ggc ttc ggt gcc agc ccc agc acg ccc aat gtc gat ggc atc gag aca      2331
Gly Phe Gly Ala Ser Pro Ser Thr Pro Asn Val Asp Gly Ile Glu Thr
155                 160                 165                 170

ttc aaa ggc cag tgg tat cac act gct ctg tgg ccc cag gaa ggc gtg      2379
Phe Lys Gly Gln Trp Tyr His Thr Ala Leu Trp Pro Gln Glu Gly Val
                175                 180                 185

aac atg gcc ggc aag cgc gtg gcc atc att ggc acc ggc tcc agc ggg      2427
Asn Met Ala Gly Lys Arg Val Ala Ile Ile Gly Thr Gly Ser Ser Gly
            190                 195                 200

gtc cag gtc gcc cag gag gct gcc ctt gat gcg aaa cag gtg acg gtg      2475
Val Gln Val Ala Gln Glu Ala Ala Leu Asp Ala Lys Gln Val Thr Val
        205                 210                 215

tac cag cgc acc ccc aac ctg gcc ttg ccc atg cat cag aag cag ctc      2523
Tyr Gln Arg Thr Pro Asn Leu Ala Leu Pro Met His Gln Lys Gln Leu
    220                 225                 230

agc gcc gag gac aat ctg cgc atg aag ccc gag ctt ccc gca gcg ttc      2571
Ser Ala Glu Asp Asn Leu Arg Met Lys Pro Glu Leu Pro Ala Ala Phe
235                 240                 245                 250
```

```
gag aga cgc ggc aag tgc ttc gcc ggc ttc gac ttc gac ttc atc gcc      2619
Glu Arg Arg Gly Lys Cys Phe Ala Gly Phe Asp Phe Asp Phe Ile Ala
                255                 260                 265

aag aac gcg acc gag ctg tcc gct gcg gag cgc aca gag atc ttg gaa      2667
Lys Asn Ala Thr Glu Leu Ser Ala Ala Glu Arg Thr Glu Ile Leu Glu
            270                 275                 280

gag ctg tgg aac gcc ggc ggc ttc cgc tac tgg ctg gcc aat ttc caa      2715
Glu Leu Trp Asn Ala Gly Gly Phe Arg Tyr Trp Leu Ala Asn Phe Gln
        285                 290                 295

gac tat ctg ttc gat gac aag gcc aac gat tac gtc tac gag ttc tgg      2763
Asp Tyr Leu Phe Asp Asp Lys Ala Asn Asp Tyr Val Tyr Glu Phe Trp
    300                 305                 310

cgc gac aag gtc cgc gcc cgc atc aag gat ccg aaa gtt gcc gag aag      2811
Arg Asp Lys Val Arg Ala Arg Ile Lys Asp Pro Lys Val Ala Glu Lys
315                 320                 325                 330

ctc gcc ccc atg aag aag ccg cat cct tac gga gcc aag cgc cct tcg      2859
Leu Ala Pro Met Lys Lys Pro His Pro Tyr Gly Ala Lys Arg Pro Ser
                335                 340                 345

ctg gag cag tgg tac tac gag atc ttc aat cag aac aac gtc acg ctg      2907
Leu Glu Gln Trp Tyr Tyr Glu Ile Phe Asn Gln Asn Asn Val Thr Leu
            350                 355                 360

gtg gat gtc aac gaa aca ccg gtg ctt cgc atc acc gag aaa ggc atc      2955
Val Asp Val Asn Glu Thr Pro Val Leu Arg Ile Thr Glu Lys Gly Ile
        365                 370                 375

gtg acc gct gag ggt gaa gcc gaa ttc gac ctg atc gtg ttc gcg acc      3003
Val Thr Ala Glu Gly Glu Ala Glu Phe Asp Leu Ile Val Phe Ala Thr
    380                 385                 390

ggc ttc gac gca gtg acc ggg gga ctc acc agc atc gac ttc cgc aac      3051
Gly Phe Asp Ala Val Thr Gly Gly Leu Thr Ser Ile Asp Phe Arg Asn
395                 400                 405                 410

aac cag ggc cag agc ttc aag gat gtg tgg tct gac gga atc cgc acc      3099
Asn Gln Gly Gln Ser Phe Lys Asp Val Trp Ser Asp Gly Ile Arg Thr
                415                 420                 425

cag ctg gga gtg gcc acg gca ggt ttt ccc aac ttg ctc ttt ggc tac      3147
Gln Leu Gly Val Ala Thr Ala Gly Phe Pro Asn Leu Leu Phe Gly Tyr
            430                 435                 440

gga cct caa tcg cct gcg ggc ttc tgc aac ggt ccg tcg agc gcc gaa      3195
Gly Pro Gln Ser Pro Ala Gly Phe Cys Asn Gly Pro Ser Ser Ala Glu
        445                 450                 455

tac cag ggc gat ctg ctg atc cag ctg atg aac tac cta cgc gac aac      3243
Tyr Gln Gly Asp Leu Leu Ile Gln Leu Met Asn Tyr Leu Arg Asp Asn
    460                 465                 470

aac atc tcg cgc atc gaa gcc cag tcc gag gca cag gaa gaa tgg agc      3291
Asn Ile Ser Arg Ile Glu Ala Gln Ser Glu Ala Gln Glu Glu Trp Ser
475                 480                 485                 490
```

```
aag ctg atc gca gac ttc tgg gac agc tcg ctg ttc ccc cgc gca aag      3339
Lys Leu Ile Ala Asp Phe Trp Asp Ser Ser Leu Phe Pro Arg Ala Lys
                495                 500                 505

tcc tgg tac caa gga tcc aac atc ccg ggc aag aaa gtc gag agc ctg      3387
Ser Trp Tyr Gln Gly Ser Asn Ile Pro Gly Lys Lys Val Glu Ser Leu
            510                 515                 520

aac ttc ccg ctg ggg ctg cca acc tat ata tcc aaa ttc aat gaa agc      3435
Asn Phe Pro Leu Gly Leu Pro Thr Tyr Ile Ser Lys Phe Asn Glu Ser
        525                 530                 535

gct gaa aaa gga tat gca ggc ttc tcg ctg gcc agc taagactctg          3481
Ala Glu Lys Gly Tyr Ala Gly Phe Ser Leu Ala Ser
    540                 545                 550

ttgtgcaact cctggagaca agcatatggg acgtgtaaat gacaaagtgg ttctcgtcac   3541
tggcggtgcc atgggaatgg gcctgaccca ttgcaccttg ctggcccgcg agggggccac   3601
cgtttacctc agcgacatga atgaggaact gggccaccaa gccgtagcgg agatccgtcg   3661
gcaaggtggc aaggcccact tcctgcacct ggacgtgacc aacgagaatc actggacagg   3721
tgccgtggac accatcctcg ccgagtccga ccggctggac gcgctggtca acaacgccgg   3781
cattttgacc ctcaaacccg ttcaggacac cagcaacgaa gaatgggatc gcatctttga   3841
aatcaatgtc cgcagtgtgt tcttgggcac gcgagcagtg atcgagccca tgcgtaaagc   3901
ccataaaggc tgcatcgtca atgtgtcgtc catctacggt ctggtgggtg cgccgggagc   3961
tgcggcctac gaggcctcca aaggtgccgt gcgcttgttt accaaggctt gcgcggtgga   4021
cctggcaccg ttcaatattc gggtcaactc ggtccaccca ggggtgatcg caacacccat   4081
gacgcagcag atcctggacg ccccacagag cgcacgtgcg cttctgggcc ccacgctgct   4141
gggtcgtgcg gcccagccga tggaggtctc ccaggcggtg cttttcctgg tctcggacga   4201
ggcctcgttc gtgcatggct ctgaactcgt cgtcgacgga gggtatacgg cgaactgaga   4261
aaggaacatc ctcggcatgc                                              4281
```

WHAT IS CLAIMED IS:

1. An isolated DNA encoding a cyclopentanone monooxygenase (CPMO), or an enzymatically active portion thereof, the isolated DNA being characterized by the ability to hybridize specifically with the complement of the DNA represented in SEQ ID NO:8 under stringent hybridization conditions.

2. An isolated DNA, wherein it codes for a cyclopentanone monooxygenase (CPMO), and contains:

(a) the nucleic acid sequence of SEQ ID NO:8;

(b) a sequence corresponding to said nucleic acid sequence in the scope of the degeneration of the genetic code; or

(c) a sequence hybridizing under stringent conditions with the sequence from (1) or (2), and still coding for cyclopentanone monooxygenase (CPMO).

3. An isolated DNA encoding a cyclopentanone monooxygenase (CPMO), or an enzymatically active portion thereof, said isolated DNA having SEQ ID NO:8.

4. An isolated DNA expression vector encoding an enzymatically active cyclopentanone monooxygenase (CPMO) comprising a DNA characterized by a sequence as set forth in SEQ ID NO:8, or a portion thereof, said portion encoding said CPMO, in expressible form.

5. A recombinant vector comprising the isolated DNA of any one of claims 1 to 3, wherein the isolated DNA encodes cyclopentanone monooxygenase.

6. The recombinant vector of claim 5, wherein the isolated DNA has a nucleic acid sequence of SEQ ID NO:8 or which, due to the degeneracy of the genetic code, is a functional equivalent thereof.

7. A recombinant vector, wherein it contains one or more copies of a recombinant DNA according to claim 1, 2 or 3.

8. A recombinant vector according to claim 4, 5, 6 or 7, wherein it is a prokaryotic vector.

9. A recombinant vector according to claim 4, 5, 6 or 7, wherein it is a plasmid.

10. Biologically functional plasmid or viral DNA vector, which contains a DNA as defined in claim 1, 2 or 3.

11. A host cell comprising a recombinant vector of any one of claims 4 to 9.

12. A cell transformed with a heterologous DNA expression construct encoding an enzymatically active cyclopentanone monooxygenase (CPMO) comprising a DNA characterized by a sequence as set forth in SEQ ID NO:8, or a portion thereof, said portion encoding said CPMO, in expressible form.

13. A cell as defined in claim 12, which is a prokaryotic cell.

14. A cell as defined in claim 12, which is E. coli.

15. A purified cyclopentanone monooxygenase (CPMO) having :

(a) an amino acid sequence as set forth in SEQ ID NO:5;
(b) an amino acid sequence encoded by a nucleic acid sequence as set forth in SEQ ID NO:8; or
(c) an amino acid sequence encoded by a nucleic acid sequence hybridizing to a nucleic acid sequence complementary to the nucleic acid sequence of step b) above under stringent conditions, said amino acid sequence encoded in step c) having a same activity as the amino acid sequence in a).

16. A recombinant cyclopentanone monooxygenase (CPMO) having an enzymatic activity superior to the one naturally occurring.

17. The recombinant cyclopentanone monooxygenase (CPMO) of claim 16, wherein said CPMO is prepared from Comamonas sp. NCIMB 9872.

18. The recombinant cyclopentanone monooxygenase (CPMO) of claim 16, wherein said CPMO has a sequence as set forth in SEQ ID NO:5.

19. The recombinant cyclopentanone monooxygenase (CPMO) of claim 16, wherein said CPMO has an enzymatic activity twice superior to that of a CPMO from a native *Pseudomonas*.

20. A method for growing cells in vitro in presence of cyclopentanol or cyclopentanone as sole source of carbon, said method comprising the steps of:

(a) transforming a cell with the expression construct of claim 4, 5, 6, 7, 8 or 9; and

(b) growing the cell of step a) under suitable conditions in a medium containing cyclopentanol or cyclopentanone as a sole source of carbon.

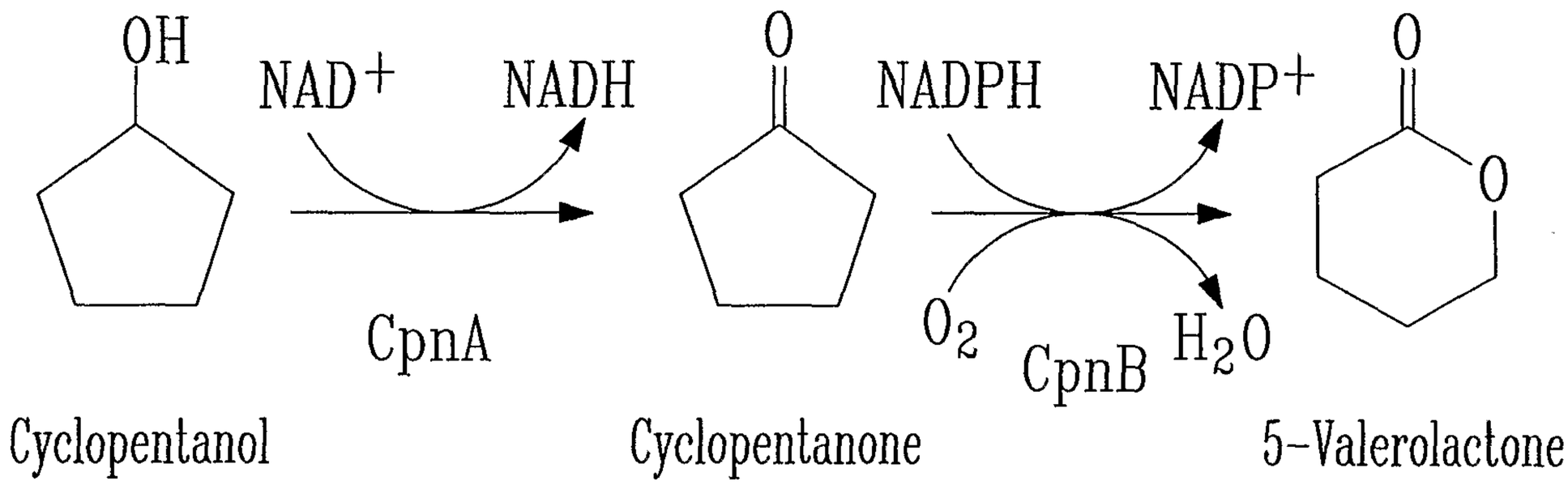
OH
NAD+
NADH
O
NADPH
NADP+
O
O
CpnA
O2
CpnB
H2O
Cyclopentanol
Cyclopentanone
5-Valerolactone

Fig. 1

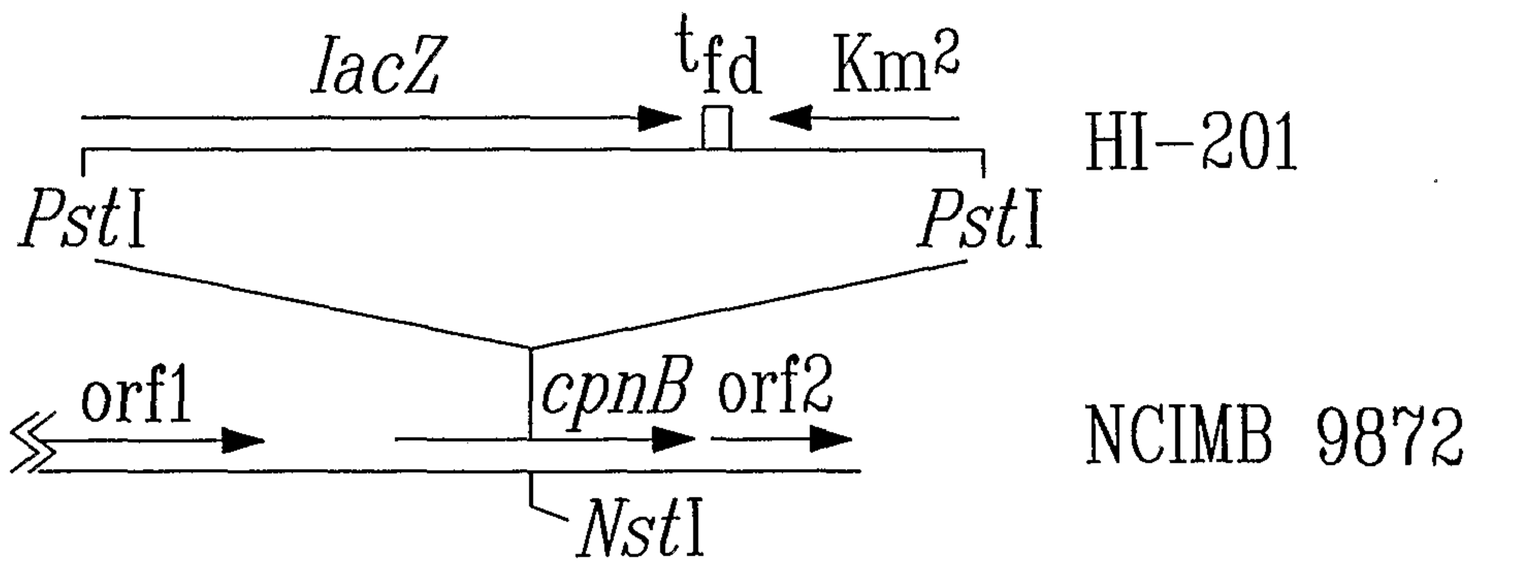
lacZ
tfd
Km2
HI-201
PstI
PstI
orf1
cpnB
orf2
NstI
NCIMB 9872

Fig. 2

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GxGxxG

```
CHMO    1:---------------MSQKMDFDAIVIGGGFGGLYAVKKLRDELELKVQAFDKATDVAGT 45
STMO    1:MNGQHPRSVVTAPDATTGTTSYDVVVVGAGIAGLYAIHRFRSQ-GLTVRAFEAASGVGGV 59
CPMO    1:MTTMFTMTTEQLGMNNSVNDKLDVLLIGAGFTGLYQLYHLRKL-GYKVHLVDAGADIGGI 59
                          .     * ...* *. *** . ..*      *.  .  . . *

CHMO   46:WYWNRYPGALTDTETHLYCYSWDKELLQSLEIKKKYVQGPDVRKYLQQVAEKHDLKKSYQ 105
STMO   60:WYWNRYPGARCDVESIDYSYSFSPELEQEWNWSEKYATQPEILAYLEHVADRFDLRRDIR 119
CPMO   60:WHWNCYPGARVDTHCQIYQYSI-PELWQEFNWKELFPNWAQMREYFHFADKKLDLSKDIS 118
             *.** ****  *     * **   ** * .  . .    ..  *.     . ** .

CHMO  106:FNTAVQSAHYNEADALWEVTTEYGDKYTARFLITALGLLSAPNLPNIKGINQFKGELHHT 165
STMO  120:FDTRVTSAVLDEEGLRWTVRTDRGDEVSARFLVVAAGPLSNANTPAFDGLDRFTGDIVHT 179
CPMO  119:FNTRVQSAVFDEGTREWTVRSIGHQPIQARFVIANLGFGASPSTPNVDGIETFKGQWYHT 178
             *.* * **  .*    * * .  .   ***..  *  .   *   *.. * *.  **

                          GxGxxG

CHMO  166:SRWPDD-VSFEGKRVGVIGTGSTGVQVITAVAPLAKHLTVFQRSAQYSVPIGNDPLSEED 224
STMO  180:ARWPHDGVDFTGKRVGVIGTGSSGIQSIPIIAEQAEQLFVFQRSANYSIPAGNVPLDDAT 239
CPMO  179:ALWPQEGVNMAGKRVAIIGTGSSGVQVAQEAALDAKQVTVYQRTPNLALPMHQKQLSAED 238
             . **  * . **** .*****.*.*     *  *... *.**. . ..*  .  *

CHMO  225:VKKIKDNYDKSLGWCMNSALAFALNESTVPAMSVSAEERKAVFEKAWQTGGGFRFMFETF 284
STMO  240:RAEQKANYAERRRLSRESGGGSPHRPHPKSALEVSEEERRAVYEERWKLGG--VLFSKAF 297
CPMO  239:NLRMKPELPAAFERRGKCFAGFDFDFIAKNATELSAAERTEILEELWNAGG-FRYWLANF 297
             *  .        .              *  .*  **  . *. *  **      *
```

FIG 3A

```
CHMO 285:GDIATNMEANIEAQNFIKGKIAEIVKDPAIAQKLMPQD----LYAKRPLCDSGYYNTFNR 340
STMO 298.PDQLTDPAANDTARAFWEEKIRAVVDDPAVAELLTPKDH--AIGAKRIVTDSGYYETYNR 355
CPMO 298:QDYLFDDKANDYVYEFWRDKVRARIKDPKVAEKLAPMKKPHPYGAKRPSLEQWYYEIFNQ 357
          *   .  **     *   *.   . ** .*. * *         ***    .  **. .*.
CHMO 341.DNVRLEDVKANPIVEITENGVKLENGDFVELDMLICATGFDAVDGNYVRMDIQGKNGLAM 400
STMO 356:DNVELVDLRSTPIVGMDETGIVT-TGAHYDLDMIVLATGFDAMTGSLDKLEIVGRGGRTL 414
CPMO 358:NNVTLVDVNETPVLRITEKGIVT-AEGEAEFDLIVFATGFDAVTGGITSIDFRNNQGQSF 416
         .** * *.   *.. . * *.        ..*... ******. *    ...    * ..
CHMO 401:KDYWKEGPSSYMGVTVNNYPNMFMVLGPNGP--FTNLPPSIESQVEWISDTIQYTVENNV 458
STMO 415:KETWAAGPRTYLGLGIDGFPNFFNLTGPGSPSVLANMVLHSELHVDWVADAIAYLDARGA 474
CPMO 417:KDVWSDGIRTQLGVATAGFPNLLFGYGPQSPAGFCNGPSSAEYQGDLLIQLMNYLRDNNI 476
          *. *  *  . .*.    .**..  **  *  . *     * . . . . . *
CHMO 459:ESIEATKEAEEQWTQTCANIAEMTLFPKAQSWIFGANIPGKKNTVYFYLGGLKEYRTCAS 518
STMO 475:AGIEGTPEAVADWVEECRNRAEASLLNSANSWYLGANIPGRPRVFMPFLGGFGVYREIIT 534
CPMO 477:SRIEAQSEAQEEWSKLIADFWDSSLFPRAKSWYQGSNIPGKKVESLNFPLGLPTYISKFN 536
           **   **  .* .   .  . .*.  *.**  *.****.      .  *.  *
CHMO 519:NCKNHAYEGFDIQLQRSDIKQPANA 543 SEQ ID NO:7
STMO 535:EVAESGYKGFAILEG---------- 549 SEQ ID NO:6
CPMO 537:ESAEKGYAGFSLAS---------- 550 SEQ ID NO:5
          .  .  * ** .
```

Fig 3B

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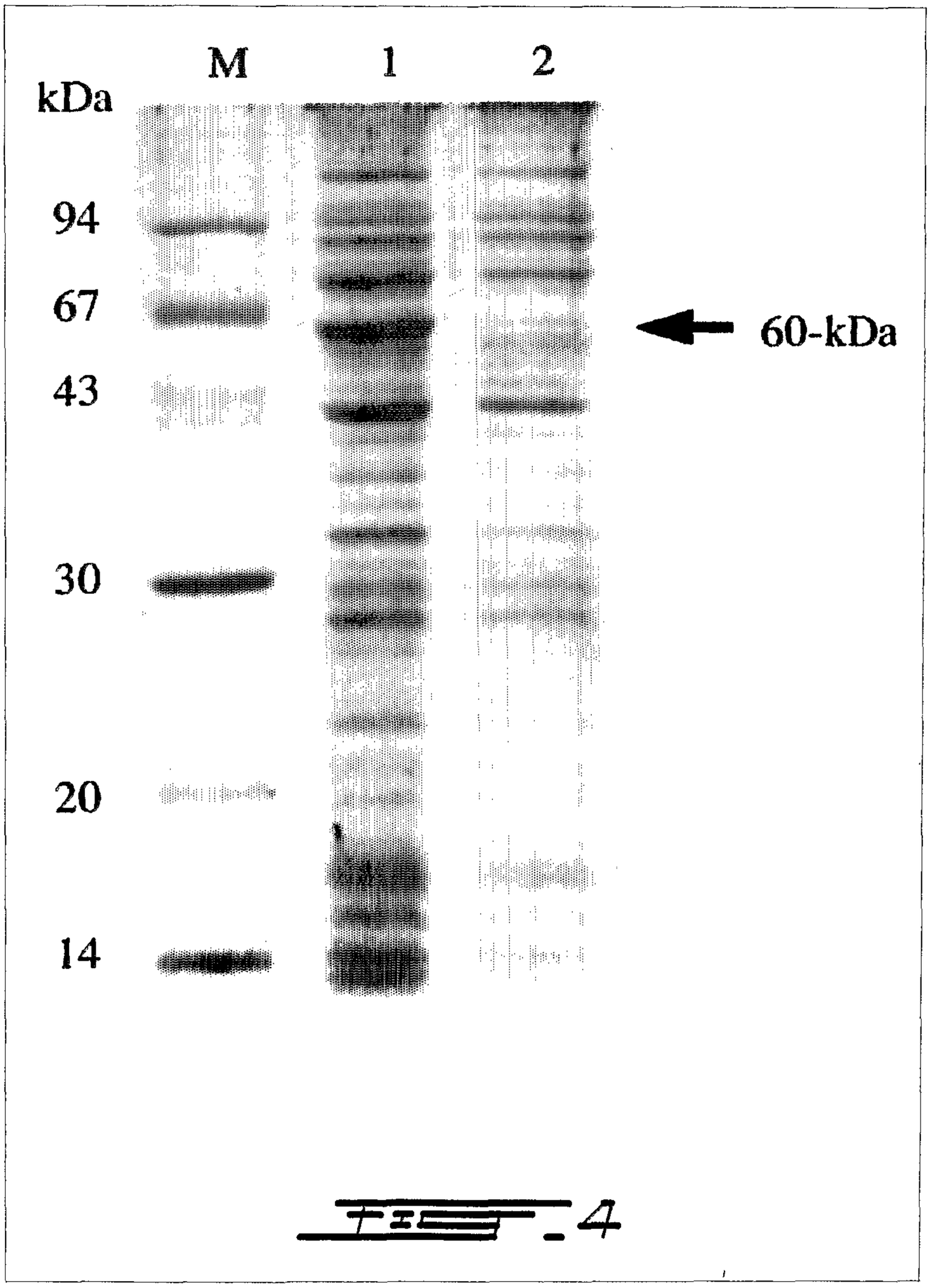

FIG. 4

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```
                10        20        30        40        50        60
                |         |         |         |         |         |
1     GCATGCGATAGCGGCACGTTGGTGGGCGGGCGAGCGCGGCCACGAGCGCGTCTCTCGCTC
      CGTACGCTATCGCCGTGCAACCACCCGCCCGCTCGCGCCGGTGCTCGCGCAGAGAGCGAG
61    CACCGACATCACATCGCCGGTCACCGGCGAGAACATTGGCAAGATCCTGCGCTTTTCGCC
      GTGGCTGTAGTGTAGCGGCCAGTGGCCGCTCTTGTAACCGTTCTAGGACGCGAAAAGCGG
121   GCTGAAGCCGCAGGGCTACGAGAGAATTTTTCAGTCCGGCCTGGCGGACTCGACCCAGCA
      CGACTTCGGCGTCCCGATGCTCTCTTAAAAAGTCAGGCCGGACCGCCTGAGCTGGGTCGT
181   CACCCCCAGGATGGTGCGTTTCGGACAGTTCCTGTCCTGTGATCCAGAGACCATCAACAC
      GTGGGGGTCCTACCACGCAAAGCCTGTCAAGGACAGGACACTAGGTCTCTGGTAGTTGTG
241   CCTGGAGACATTGGAGCGGATCGCGCGCGCCGACGTCAATGTCTTGCTGCATGGAGAGAC
      GGACCTCTGTAACCTCGCCTAGCGCGCGCGGCTGCAGTTACAGAACGACGTACCTCTCTG
301   GGGCACGGGCAAAGAGCTGATAGCTCGCCACATTCATGTCGCGAGCCGCCGGCGGGATGC
      CCCGTGCCCGTTTCTCGACTATCGAGCGGTGTAAGTACAGCGCTCGGCGGCCGCCCTACG
361   GCCCTACCTCGCCATCAACTGCGGGGCAATCAGCTCGGAGTTGCTGGAGAGTACTTTTTT
      CGGGATGGAGCGGTAGTTGACGCCCCGTTAGTCGAGCCTCAACGACCTCTCATGAAAAAA
421   TGGCTATGTGCGCGGAGCATTCTCCGGGGCAGATCCCAAGGGCCGCGCCGGCTACTTTGA
      ACCGATACACGCGCCTCGTAAGAGGCCCCGTCTAGGGTTCCCGGCGCGGCCGATGAAACT
481   ATCGGTGGGCGAAGGCACCTTGTTTCTGGACGAGATCGGCGAGCTGCCCTTGGCCATGCA
      TAGCCACCCGCTTCCGTGGAACAAAGACCTGCTCTAGCCGCTCGACGGGAACCGGTACGT
541   GGCGGCATTGTTGCGCGTGCTGGAAGACGGAAGCTTTCTGCGTGTGGGCTCATCGACCCC
      CCGCCGTAACAACGCGCACGACCTTCTGCCTTCGAAAGACGCACACCCGAGTAGCTGGGG
601   GCAGCGCGCCTCGTGCCGCATTATCGCGGCCACGCACCGCAATCTGGAGGAACTCATCGC
      CGTCGCGCGGAGCACGGCGTAATAGCGCCGGTGCGTGGCGTTAGACCTCCTTGAGTAGCG
661   ACAGGGCCTGTTTCGCCAGGATCTCTACTACCGTCTCAAGATCGTTCAGAAAAGGCTCAA
      TGTCCCGGACAAAGCGGTCCTAGAGATGATGGCAGAGTTCTAGCAAGTCTTTTCCGAGTT
```

Fig. 5A

```
721  GCCGATACGGGAACGCACCTGTGACATCGCCTTGCTGGCAGAGCAATTCAAATCTGCACT
     CGGCTATGCCCTTGCGTGGACACTGTAGCGGAACGACCGTCTCGTTAAGTTTAGACGTGA
781  GGCGCAAAAGCACCAGATCCCGAACGTGCAGATCCACCCCGAGGCCATGGCAGTCATGGA
     CCGCGTTTTCGTGGTCTAGGGCTTGCACGTCTAGGTGGGGCTCCGGTACCGTCAGTACCT
841  GCGCTACCAGTGGCCGGGCAATGCGCGCGAAATCCGCAATGTGATGGAAGCCGCCCTGAT
     CGCGATGGTCACCGGCCCGTTACGCGCGCTTTAGGCGTTACACTACCTTCGGCGGGACTA
901  CTGCTCCGATGGTGAAATCACGCTCGCCAGCCTGCCCCCGGAAGTATCTGAGAACTCCAC
     GACGAGGCTACCACTTTAGTGCGAGCGGTCGGACGGGGGCCTTCATAGACTCTTGAGGTG
961  CTATCCTCTGCAAAGCCGGGTGGCCGAAGCGAATAGCGAGATGCCTCCCGTCTCCGGCAA
     GATAGGAGACGTTTCGGCCCACCGGCTTCGCTTATCGCTCTACGGAGGGCAGAGGCCGTT
1021 CGACTATGAACGGCAACTCATCGTGGGTTTGCTGCGCAAGTACCGAAAGGTCAATCATGT
     GCTGATACTTGCCGTTGAGTAGCACCCAAACGACGCGTTCATGGCTTTCCAGTTAGTACA
1081 GGCGAGGGCCCTGGGCCTCGCGCGCTCCACGCTCTATCGGAAATTCGCAGACTTGGGCAT
     CCGCTCCCGGGACCCGGAGCGCGCGAGGTGCGAGATAGCCTTTAAGCGTCTGAACCCGTA
1141 TGACCAACGCGAGTATGTCAATGATCTGTCCGATTAACACTGCTGCGCCCTGCGCTTGCG
     ACTGGTTGCGCTCATACAGTTACTAGACAGGCTAATTGTGACGACGCGGGACGCGAACGC
1201 GCAACGCAGTATCGTCGGAACGCGGGCAACGCTAAAGGCAGCCGCCGTCGGCATCGGTTG
     CGTTGCGTCATAGCAGCCTTGCGCCCGTTGCGATTTCCGTCGGCGGCAGCCGTAGCCAAC
1261 CCGCCCGGTGTTGTTTGTCGGCTGAGACCGCCTGCAGCACCAGCCCCTCCATTCCTCGTG
     GGCGGGCCACAACAAACAGCCGACTCTGGCGGACGTCGTGGTCGGGGAGGTAAGGAGCAC
1321 TCTAGATCTCCTTGCAACGCTGTCACAGCGTGCTTTTTTCGTGGCGCGGGACTTCTCGCG
     AGATCTAGAGGAACGTTGCGACAGTGTCGCACGAAAAAAGCACCGCGCCCTGAAGAGCGC
1381 ATGAGGGCCTGCAAGGGCGGCGCATCAAAACAAGCGCCCTGTCCTCAGGACGTTTGCGCC
     TACTCCCGGACGTTCCCGCCGCGTAGTTTTGTTCGCGGGACAGGAGTCCTGCAAACGCGG
1441 GTCGCTGGCTATGGGCAGTCTGCATGATCCGCTTGGGCTTGCCCACCATGCGCGCGACTG
```

Fig - 5B

```
      CAGCGACCGATACCCGTCAGACGTACTAGGCGAACCCGAACGGGTGGTACGCGCGCTGAC
1501  CGCTCTTTCCTGTTGGGCGCATCGTCATCCCGACCTTCCTGAAATCCATTTCCACAGGGC
      GCGAGAAAGGACAACCCGCGTAGCAGTAGGGCTGGAAGGACTTTAGGTAAAGGTGTCCCG
1561  CGGCCTGAGCCAGACAGCGATCGGCAGGCTCTCGCATCTCGGACAGTGGTGCGCTACACC
      GCCGGACTCGGTCTGTCGCTAGCCGTCCGAGAGCGTAGAGCCTGTCACCACGCGATGTGG
1621  ACAGGACACAAGTGTCCGGCGCGTGGCACGCAGCGGCCTTTGCTGCGCACGATGCGTGCT
      TGTCCTGTGTTCACAGGCCGCGCACCGTGCGTCGCCGGAAACGACGCGTGCTACGCACGA
1681  TTTGGCACATGGAAACCCTCGGTTTTTCCGAACCAGAGACCTGGCACAAGTCTTGATAAC
      AAACCGTGTACCTTTGGGAGCCAAAAAGGCTTGGTCTCTGGACCGTGTTCAGAACTATTG
1741  TCCTGAGTGACTGATGCAACGCATCTCAGTCCGGGAGCGTGGACGACACGCCCCTGAACT
      AGGACTCACTGACTACGTTGCGTAGAGTCAGGCCCTCGCACCTGCTGTGCGGGGACTTGA
1801  TCAATTATTTAGGAGACCCATATGACCACCATGACCACCATGACCACCGAACAACTCGGC
      AGTTAATAAATCCTCTGGGTATACTGGTGGTACTGGTGGTACTGGTGGCTTGTTGAGCCG
1861  ATGAACAACTCTGTCAATGACAAGCTTGACGTTTTGCTCATCGGCGCCGGCTTCACCGGT
      TACTTGTTGAGACAGTTACTGTTCGAACTGCAAAACGAGTAGCCGCGGCCGAAGTGGCCA
1921  CTCTACCAGCTCTATCACCTGCGCAAGCTGGGCTACAAGGTTCATCTCGTCGACGCCGGT
      GAGATGGTCGAGATAGTGGACGCGTTCGACCCGATGTTCCAAGTAGAGCAGCTGCGGCCA
1981  GCCGATATTGGCGGGATCTGGCATTGGAACTGCTACCCCGGAGCGCGTGTGGATACCCAC
      CGGCTATAACCGCCCTAGACCGTAACCTTGACGATGGGGCCTCGCGCACACCTATGGGTG
2041  TGCCAGATCTACCAGTACTCCATTCCAGAGTTGTGGCAGGAGTTCAACTGGAAAGAGCTG
      ACGGTCTAGATGGTCATGAGGTAAGGTCTCAACACCGTCCTCAAGTTGACCTTTCTCGAC
2101  TTCCCTAACTGGGCGCAAATGCGCGAGTATTTCCATTTTGCCGACAAGAAGCTCGACCTG
      AAGGGATTGACCCGCGTTTACGCGCTCATAAAGGTAAAACGGCTGTTCTTCGAGCTGGAC
2161  AGCAAGGACATCAGCTTCAACACCCGTGTGCAGTCGGCCGTCTTTGACGAAGGCACACGC
      TCGTTCCTGTAGTCGAAGTTGTGGGCACACGTCAGCCGGCAGAAACTGCTTCCGTGTGCG
```

Fig. 5C

```
2221 GAATGGACGGTACGCTCGATCGGACACCAGCCGATCCAGGCCAGGTTCGTCATCGCCAAC
     CTTACCTGCCATGCGAGCTAGCCTGTGGTCGGCTAGGTCCGGTCCAAGCAGTAGCGGTTG
2281 CTTGGCTTCGGTGCCAGCCCCAGCACGCCCAATGTCGATGGCATCGAGACATTCAAAGGC
     GAACCGAAGCCACGGTCGGGGTCGTGCGGGTTACAGCTACCGTAGCTCTGTAAGTTTCCG
2341 CAGTGGTATCACACTGCTCTGTGGCCCCAGGAAGGCGTGAACATGGCCGGCAAGCGCGTG
     GTCACCATAGTGTGACGAGACACCGGGGTCCTTCCGCACTTGTACCGGCCGTTCGCGCAC
2401 GCCATCATTGGCACCGGCTCCAGCGGGGTCCAGGTCGCCCAGGAGGCTGCCCTTGATGCG
     CGGTAGTAACCGTGGCCGAGGTCGCCCCAGGTCCAGCGGGTCCTCCGACGGGAACTACGC
2461 AAACAGGTGACGGTGTACCAGCGCACCCCCAACCTGGCCTTGCCCATGCATCAGAAGCAG
     TTTGTCCACTGCCACATGGTCGCGTGGGGGTTGGACCGGAACGGGTACGTAGTCTTCGTC
2521 CTCAGCGCCGAGGACAATCTGCGCATGAAGCCCGAGCTTCCCGCAGCGTTCGAGAGACGC
     GAGTCGCGGCTCCTGTTAGACGCGTACTTCGGGCTCGAAGGGCGTCGCAAGCTCTCTGCG
2581 GGCAAGTGCTTCGCCGGCTTCGACTTCGACTTCATCGCCAAGAACGCGACCGAGCTGTCC
     CCGTTCACGAAGCGGCCGAAGCTGAAGCTGAAGTAGCGGTTCTTGCGCTGGCTCGACAGG
2641 GCTGCGGAGCGCACAGAGATCTTGGAAGAGCTGTGGAACGCCGGCGGCTTCCGCTACTGG
     CGACGCCTCGCGTGTCTCTAGAACCTTCTCGACACCTTGCGGCCGCCGAAGGCGATGACC
2701 CTGGCCAATTTCCAAGACTATCTGTTCGATGACAAGGCCAACGATTACGTCTACGAGTTC
     GACCGGTTAAAGGTTCTGATAGACAAGCTACTGTTCCGGTTGCTAATGCAGATGCTCAAG
2761 TGGCGCGACAAGGTCCGCGCCCGCATCAAGGATCCGAAAGTTGCCGAGAAGCTCGCCCCC
     ACCGCGCTGTTCCAGGCGCGGGCGTAGTTCCTAGGCTTTCAACGGCTCTTCGAGCGGGGG
2821 ATGAAGAAGCCGCATCCTTACGGAGCCAAGCGCCCTTCGCTGGAGCAGTGGTACTACGAG
     TACTTCTTCGGCGTAGGAATGCCTCGGTTCGCGGGAAGCGACCTCGTCACCATGATGCTC
2881 ATCTTCAATCAGAACAACGTCACGCTGGTGGATGTCAACGAAACACCGGTGCTTCGCATC
     TAGAAGTTAGTCTTGTTGCAGTGCGACCACCTACAGTTGCTTTGTGGCCACGAAGCGTAG
2941 ACCGAGAAAGGCATCGTGACCGCTGAGGGTGAAGCCGAATTCGACCTGATCGTGTTCGCG
```

Fig. 50

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```
      TGGCTCTTTCCGTAGCACTGGCGACTCCCACTTCGGCTTAAGCTGGACTAGCACAAGCGC
3001  ACCGGCTTCGACGCAGTGACCGGGGGACTCACCAGCATCGACTTCCGCAACAACCAGGGC
      TGGCCGAAGCTGCGTCACTGGCCCCCTGAGTGGTCGTAGCTGAAGGCGTTGTTGGTCCCG
3061  CAGAGCTTCAAGGATGTGTGGTCTGACGGAATCCGCACCCAGCTGGGAGTGGCCACGGCA
      GTCTCGAAGTTCCTACACACCAGACTGCCTTAGGCGTGGGTCGACCCTCACCGGTGCCGT
3121  GGTTTTCCCAACTTGCTCTTTGGCTACGGACCTCAATCGCCTGCGGGCTTCTGCAACGGT
      CCAAAAGGGTTGAACGAGAAACCGATGCCTGGAGTTAGCGGACGCCCGAAGACGTTGCCA
3181  CCGTCGAGCGCCGAATACCAGGGCGATCTGCTGATCCAGCTGATGAACTACCTACGCGAC
      GGCAGCTCGCGGCTTATGGTCCCGCTAGACGACTAGGTCGACTACTTGATGGATGCGCTG
3241  AACAACATCTCGCGCATCGAAGCCCAGTCCGAGGCACAGGAAGAATGGAGCAAGCTGATC
      TTGTTGTAGAGCGCGTAGCTTCGGGTCAGGCTCCGTGTCCTTCTTACCTCGTTCGACTAG
3301  GCAGACTTCTGGGACAGCTCGCTGTTCCCCCGCGCAAAGTCCTGGTACCAAGGATCCAAC
      CGTCTGAAGACCCTGTCGAGCGACAAGGGGGCGCGTTTCAGGACCATGGTTCCTAGGTTG
3361  ATCCCGGGCAAGAAAGTCGAGAGCCTGAACTTCCCGCTGGGGCTGCCAACCTATATATCC
      TAGGGCCCGTTCTTTCAGCTCTCGGACTTGAAGGGCGACCCCGACGGTTGGATATATAGG
3421  AAATTCAATGAAAGCGCTGAAAAAGGATATGCAGGCTTCTCGCTGGCCAGCTAAGACTCT
      TTTAAGTTACTTTCGCGACTTTTTCCTATACGTCCGAAGAGCGACCGGTCGATTCTGAGA
3481  GTTGTGCAACTCCTGGAGACAAGCATATGGGACGTGTAAATGACAAAGTGGTTCTCGTCA
      CAACACGTTGAGGACCTCTGTTCGTATACCCTGCACATTTACTGTTTCACCAAGAGCAGT
3541  CTGGCGGTGCCATGGGAATGGGCCTGACCCATTGCACCTTGCTGGCCCGCGAGGGGCCA
      GACCGCCACGGTACCCTTACCCGGACTGGGTAACGTGGAACGACCGGGCGCTCCCCCGGT
3601  CCGTTTACCTCAGCGACATGAATGAGGAACTGGGCCACCAAGCCGTAGCGGAGATCCGTC
      GGCAAATGGAGTCGCTGTACTTACTCCTTGACCCGGTGGTTCGGCATCGCCTCTAGGCAG
3661  GGCAAGGTGGCAAGGCCCACTTCCTGCACCTGGACGTGACCAACGAGAATCACTGGACAG
      CCGTTCCACCGTTCCGGGTGAAGGACGTGGACCTGCACTGGTTGCTCTTAGTGACCTGTC
```

Fig. 5E

```
3721 GTGCCGTGGACACCATCCTCGCCGAGTCCGACCGGCTGGACGCGCTGGTCAACAACGCCG
     CACGGCACCTGTGGTAGGAGCGGCTCAGGCTGGCCGACCTGCGCGACCAGTTGTTGCGGC
3781 GCATTTTGACCCTCAAACCCGTTCAGGACACCAGCAACGAAGAATGGGATCGCATCTTTG
     CGTAAAACTGGGAGTTTGGGCAAGTCCTGTGGTCGTTGCTTCTTACCCTAGCGTAGAAAC
3841 AAATCAATGTCCGCAGTGTGTTCTTGGGCACGCGAGCAGTGATCGAGCCCATGCGTAAAG
     TTTAGTTACAGGCGTCACACAAGAACCCGTGCGCTCGTCACTAGCTCGGGTACGCATTTC
3901 CCCATAAAGGCTGCATCGTCAATGTGTCGTCCATCTACGGTCTGGTGGGTGCGCCGGGAG
     GGGTATTTCCGACGTAGCAGTTACACAGCAGGTAGATGCCAGACCACCCACGCGGCCCTC
3961 CTGCGGCCTACGAGGCCTCCAAAGGTGCCGTGCGCTTGTTTACCAAGGCTTGCGCGGTGG
     GACGCCGGATGCTCCGGAGGTTTCCACGGCACGCGAACAAATGGTTCCGAACGCGCCACC
4021 ACCTGGCACCGTTCAATATTCGGGTCAACTCGGTCCACCCAGGGGTGATCGCAACACCCA
     TGGACCGTGGCAAGTTATAAGCCCAGTTGAGCCAGGTGGGTCCCCACTAGCGTTGTGGGT
4081 TGACGCAGCAGATCCTGGACGCCCCACAGAGCGCACGTGCGCTTCTGGGCCCCACGCTGC
     ACTGCGTCGTCTAGGACCTGCGGGGTGTCTCGCGTGCACGCGAAGACCCGGGGTGCGACG
4141 TGGGTCGTGCGGCCCAGCCGATGGAGGTCTCCCAGGCGGTGCTTTTCCTGGTCTCGGACG
     ACCCAGCACGCCGGGTCGGCTACCTCCAGAGGGTCCGCCACGAAAAGGACCAGAGCCTGC
4201 AGGCCTCGTTCGTGCATGGCTCTGAACTCGTCGTCGACGGAGGGTATACGGCGAACTGAG
     TCCGGAGCAAGCACGTACCGAGACTTGAGCAGCAGCTGCCTCCCATATGCCGCTTGACTC
4261 AAAGGAACATCCTCGGCATGC
     TTTCCTTGTAGGAGCCGTACG
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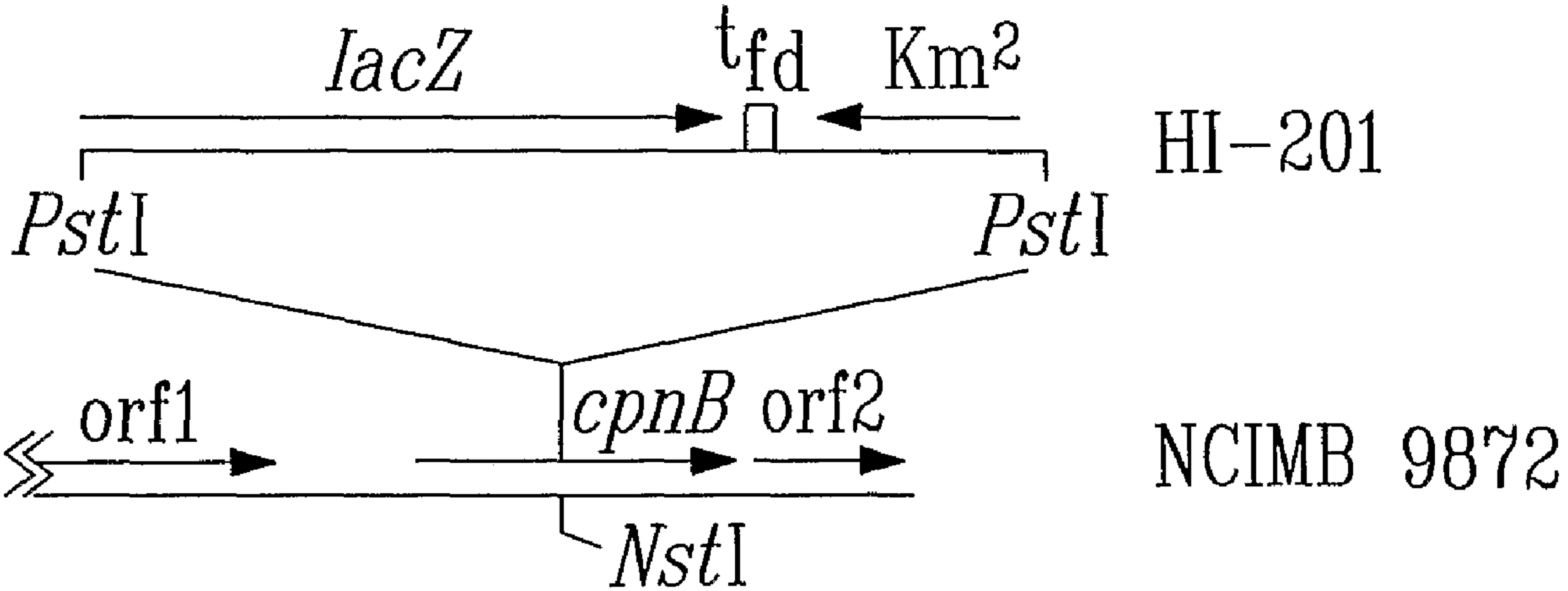
Fig 5F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