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Cloning and Sequencing of the Gene of Tryptophan-7-Halogenase from *Pseudomonas fluorescens* Strain CHA0

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Abstract—The gene of tryptophan-7-halogenase from the *Pseudomonas fluorescens* strain CHA0, a producer of the halogenated antibiotic pyrrolnitrin, has been cloned and sequenced.

Key words: halogenase, pyrrolnitrin, tryptophan, *Pseudomonas*

Halogenated substances quite often occur among natural biologically active compounds. Especially frequently they occur in marine organisms and microorganisms [1, 2]. It has been long believed that haloperoxidases, the only group of enzymes that are able to catalyze the formation of halogen–carbon bonds, are involved in the biosynthesis of halometabolites [2-4]. A cluster containing the genes of four enzymes (*prnABCD*) responsible for the conversion of tryptophan into pyrrolnitrin was isolated in 1997 from the *Pseudomonas fluorescens* strain BL915 [5] (Fig. 1). The product of the *prnA* gene catalyzes the chlorination of L-tryptophan to form 7-chloro-L-tryptophan. The product of the *prnB* gene catalyzes the regrouping of the cycle with subsequent decarboxylation, converting 7-chloro-L-tryptophan into monodechloro-aminopyrrolnitrin. The latter is chlorinated by the prnC enzyme at position 3 in the pyrrole cycle to form aminopyrrolnitrin. At the fourth (last) stage of pyrrolnitrin biosynthesis, the product of the *prnD* gene catalyzes the oxidation of the amino group to a nitro group [6]. Two of the listed enzymes, prnA and prnC, are halogenases. These are NADH- and $FADH_2$-dependent enzymes significantly differing in properties from haloperoxidases and, therefore, representing a new class of halogenating enzymes [7].

Earlier, we showed that a whole group of microorganisms producing halogen-containing antibiotics contain the genes of halogenating enzymes related to tryptophan-7- and monodechloroaminopyrrolnitrin halogenases from *P. fluorescens* BL 915 [8].

In this work, we cloned and sequenced the gene of tryptophan-7-halogenase from the pyrrolnitrin-producing *Pseudomonas fluorescens* strain CHA0.

METHODS OF INVESTIGATION

Culturing and selection of microorganisms. In this study, we used the *P. fluorescens* strain CHA0 (a producer of the antibiotic pyrrolnitrin) [9] and the *Escherichia coli* strain TG1.

Pseudomonas fluorescens CHA0 and *E. coli* TG1 cells were cultured at 30 and 37°C, respectively, in an aqueous medium containing bactotryptone (10 g/liter), yeast extract (5 g/liter), and NaCl (5 g/liter) or in Petri dishes in the same medium supplemented with 1% agar. *Escherichia coli* transformants were selected in solid media containing ampicillin (100 μg/ml), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal, 40 μg/ml), and 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG).

Hybridization, cloning, and sequencing. Chromosomal DNA from *P. fluorescens* and plasmids from *E. coli* were isolated using standard techniques [10].

All procedures with DNA were performed as described in [10, 11]. The search for the gene encoding tryptophan-7-halogenase was performed using the dioxygenin-marked *prnA* gene from the *P. fluorescens* strain BL915. DNA was sequenced on an A.L.F.-Sequenzer automated laser fluorescent sequencer (Pharmacia Biotechnologie, Sweden) using a kit from Pharmacia (Sweden).

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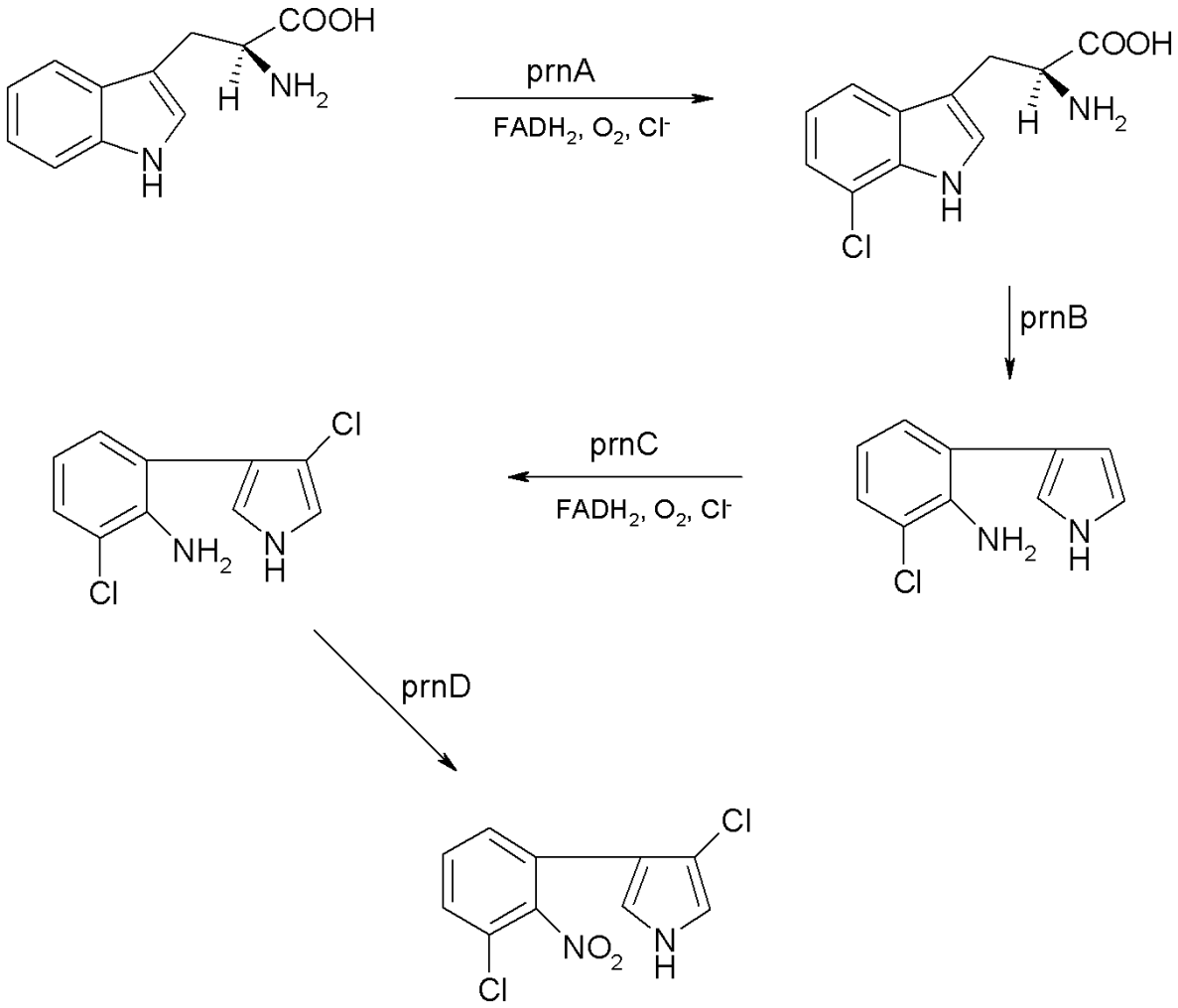

Fig. 1. Scheme of biosynthesis of pyrrolnitrin.

RESULTS AND DISCUSSION

To detect the gene of tryptophan-7-halogenase (*prnA*), chromosomal DNA from *P. fluorescens* CHA0 cells was hydrolyzed with a number of restriction endonucleases in individual samples. The fragments obtained were separated by electrophoresis and immobilized on a nylon membrane. Further search for the gene of interest was performed by hybridizing the samples with the gene of tryptophan-7-halogenase used as a probe. The result of this experiment is shown in Fig. 2. In further analysis, we used DNA fragments obtained by hydrolyzing chromosomal DNA with the restriction endonuclease *Sac*I. The length of the restriction fragment that gave a positive signal with the *prnA* probe was approximately 5 kb (Fig. 2).

DNA fragments (4-7 kb long), obtained by treating chromosomal DNA from *P. fluorescens* CHA0 cells with

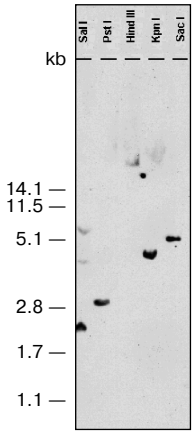

Fig. 2. Hybridization of chromosomal DNA of *P. fluorescens* CHA0 cells with the probe based on the gene of tryptophan-7-halogenase (*prnA*).

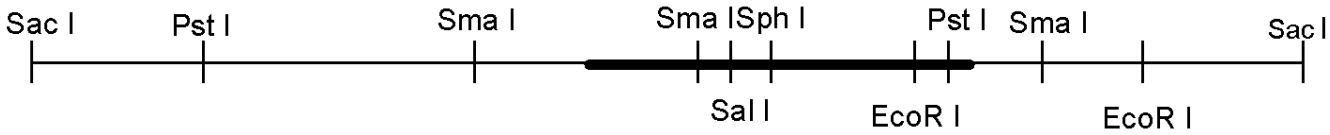

Fig. 3. Restriction map of the insertion of the recombinant clone pWB F1814 with the localized gene of tryptophan-7-halogenase from the *P. fluorescens* strain CHA0. Bold line indicates the gene of tryptophan-7-halogenase.

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                        1                                                  50
P.fluorescens BL915     MNKPIKNIVI VGGGTAGWMA ASYLVRALQQ QANITLIESA AIPRIGVGEA
P.fluorescens CHA0      MNKPIKNIVI VGGGTAGWMA ASYLVRALQQ QVNITLIESA AIPRIGVGEA
                        51                                                 100
P.fluorescens BL915     TIPSLQKVFF DFLGIPEREW MPQVNGAFKA AIKFVNWRKS PDPSRDDHFY
P.fluorescens CHA0      TIPSLQKVFF DFLGIPEREW MPQVNGAFKA AIKFVNWRKS PDPSREDYFY

                        101                                                150
P.fluorescens BL915     HLFGNVPNCD GVPLTHYWLR KREQGFQQPM EYACYPQPGA LDGKLAPCLS
P.fluorescens CHA0      HLFGSVPNCD GVPLTHYWLR KREQGFQQPM AYACYPQPGA LDGKLAPCLA

                        151                                                200
P.fluorescens BL915     DGTRQMSHAW HFDAHLVADF LKRWAVERGV NRVVDEVVDV RLNNRGYISN
P.fluorescens CHA0      DGTRQMSHAW HFDAHLVADF LKRWAVERGV NRVVDEVVEV QLNDRGYIST

                        201                                                250
P.fluorescens BL915     LLTKEGRTLE ADLFIDCSGM RGLLINQALK EPFIDMSDYL LCDSAVASAV
P.fluorescens CHA0      LLTKEGRTLE ADLFIDCSGM RGLLINQALK EPFIDMSDYL LCDSAVASAV

                        251                                                300
P.fluorescens BL915     PNDDARDGVE PYTSSIAMNS GWTWKIPMLG RFGSGYVFSS HFTSRDQATA
P.fluorescens CHA0      PNDDAREGVE PYTSAIAMNS GWTWKIPMLG RFGSGYVFST KFTSRDQATA

                        301                                                350
P.fluorescens 915       DFLKLWGLSD NQPLNQIKFR VGRNKRAWVN NCVSIGLSSC FLEPLESTGI
P.fluorescens CHA0      DFLKLWGLSD NQQLNQINFR VGRNKRAWVN NCVSIGLSSC FLEPLESTGI

                        351                                                400
P.fluorescens BL915     YFIYAALYQL VKHFPDTSFD PRLSDAFNAE IVHMFDDCRD FVQAHYFTTS
P.fluorescens CHA0      YFIYAALYQL VKHFPDTSFD PRLRDAFNAE IVYMFDDCRD FVQAHYFTTS

                        401                                                450
P.fluorescens BL915     RDDTPFWLAN RHDLRLSDAI KEKVQRYKAG LPLTTTSFDD STYYETFDYE
P.fluorescens CHA0      REDTPFWLAN RHELRLSDAI QEKVERYKAG LPLTTTSFDD STYYETFDYE

                        451                                                500
P.fluorescens BL915     FKNFWLNGNY YCIFAGLGML PDRSLPLLQH RPESIEKAEA MFASIRREAE
P.fluorescens CHA0      FKNFWLNGNY YCIFAGLGML PDRSLPLLQH RPESIQKAEA MFASIRREAE

                        501                                        539
P.fluorescens BL915     RLRTSLPTNY DYLRSLRDGD AGLSRGQRGP KLAAQESL
P.fluorescens CHA0      RLRTSLPTNY DYLRSRRDGD AQLSRNQHGP TLAAQERQ
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Fig. 4. Amino acid sequence of tryptophan-7-halogenases from the *P. fluorescens* strain BL915 [13] and *P. fluorescens* strain CHA0. The non-coincident amino acid residues are shown in bold.

the restriction endonuclease *Sac*I, were separated by electrophoresis in agarose gel, eluted, and then ligated with the pUC18 vector. The recombinant plasmid was used for transfecting competent *E. coli* cells. After selecting colonies on media containing ampicillin, X-gal, and IPTG, a gene library with a size of 1296 clones was obtained. Further search for the clone of interest was performed using the hybridization procedure. As a result, we obtained the pWB F1814 clone containing the target *Sac*I insert (5.1 kb in length) with a localized tryptophan-7-halogenase gene. In accordance with the restriction map (Fig. 3), several DNA fragments containing the gene of interest were subcloned and sequenced. The results of DNA sequencing were deposited in the NCBI (National Center for Biotechnology Information) database [12]. The amino acid sequence of the enzyme is shown in Fig. 4.

The analysis and comparison of our data with the results of sequencing the known gene *prnA* [13] showed that the genes analyzed consist of equal number of nucleotides (1617 bp) and share a high (more than 90%) degree of homology. A similar conclusion was made based on the results of analysis of the amino acid sequences of the corresponding enzymes (Fig. 4). Both halogenases consist of 539 amino acid residues, differing at 27 positions.

Thus, in this study, we isolated and sequenced the gene of tryptophan-7-halogenase from the *P. fluorescens* strain CHA0, a producer of the chlorine-containing antibiotic pyrrolnitrin. The degree of homology between the isolated enzyme and the tryptophan-7-halogenase from the *P. fluorescens* strain BL915 is 94%.

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