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Halogenating Enzymes in the Biosynthesis of Antibiotics

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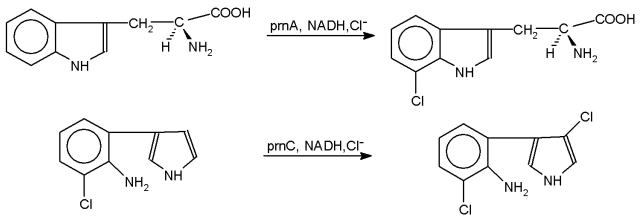
Abstract—Using blot hybridization, it has been shown that microorganisms producing halogen-containing antibiotics—*Pseudomonas pyrrocinia*, *P. aureofaciens* ACN, *P. aureofaciens* Pa1, *P. fluorescens* CHA0, *Actinoplanes* sp., *Kitasatasporia* sp., *Sacharothrix aerocolonigenes*, *Actinomadura melliaura*, and *Streptomyces albogriseolus*—contain the genes of the halogenating enzymes related to tryptophan-7-halogenase and monodechloroaminopyrrolnitrin halogenase from *P. fluorescens* BL 915.

Key words: halogenase, pyrrolnitrin, pentachloropseudilin, pyoluteorin, pyrroindomycin, rebeccamycin, thienodolin, *Pseudomonas*, *Actinoplanes*, *Kitasatasporia*, *Sacharothrix*, *Actinomadura*, *Streptomyces*

To date, more than 2000 halogen-containing compounds have been isolated from natural sources [1, 2]. Most of these compounds have been found in marine algae and microorganisms. The presence of halogen in a molecule often provides the compound with a high physiological activity, allowing a number of halometabolites to be used as medicaments [1, 3]. Until recently, only one group of the enzymes capable of catalyzing the formation of the halogen–carbon bond was known. Since the reaction of halogenation proceeds only in the presence of hydrogen peroxide, these enzymes were named haloperoxidases (EC 1.11.1.10). Investigation of biosynthesis of such antibiotics as chlortetracycline and pyrrolnitrin showed that the enzymes catalyzing their halogenation must exhibit high specificity [3]. Haloperoxidases, in contrast, exhibit wide substrate specificity and low regiospecificity. Genetic research of the producers of chloramphenicol and pyrrolnitrin [4, 5] revealed that the chloroperoxidases contained in these strains are not involved in biosynthesis of the indicated compounds.

Recently, a cluster containing genes of four enzymes catalyzing the transformation of tryptophan into pyrrolnitrin was isolated from the BL 915 strain of *Pseudomonas fluorescens* [6]. Two of these enzymes are halogenases: tryptophan-7-halogenase (prn A) and monodechloroaminopyrrolnitrin halogenase (prn C) [7].



Both enzymes exhibited high specificity and use NADH as the cofactor. Comparative studies of their molecular and catalytic properties, as well as corresponding genes indicated that these enzymes belong to a new group of halogenating enzymes.

In the present work, using the isolated genes as probes, we screened the chromosomal DNAs of a number of microorganisms producing halogen-containing compounds.

MATERIALS AND METHODS

In this work we used the following strains of microorganisms: *Pseudomonas pyrrocinia* ATCC 15958, *Pseudomonas aureofaciens* ACN [8], *Pseudomonas aureofaciens* Pa1, *Pseudomonas fluorescens* CHA0 [9], *Actinoplanes* sp. ATCC 33002, *Kitasatasporia* sp. LL42D005, *Sacharothrix aerocolonigenes* ATCC 39243, *Actinomadura melliaura* ATCC 39691, and *Streptomyces*

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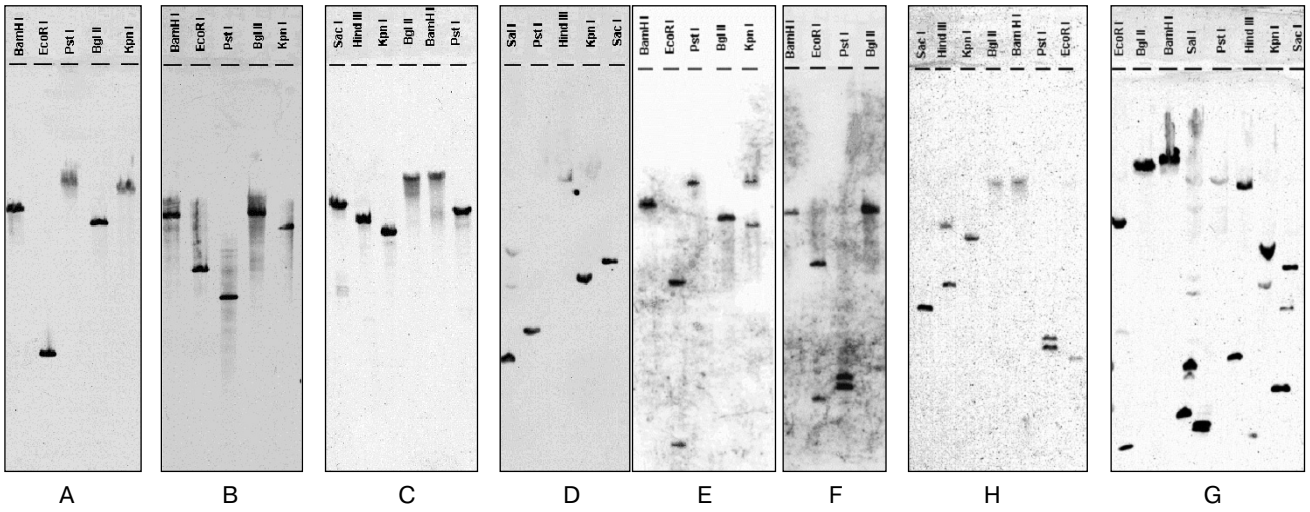


Fig. 1. Blot hybridization of chromosomal DNAs of *P. pyrrocinia* (A, E), *P. aureofaciens* ACN (B, F), *P. aureofaciens* Pa1 (C, H), and *P. fluorescens* CHA0 (D, G) with the *prn*A probe (A-D) and the *prn*C probe (E-G).

albogriseolus [10]. Cell biomass of *Pseudomonas* genus was grown at 30°C in medium containing meat extract (3 g/liter), yeast extract (5 g/liter), meat peptone (5 g/liter), and sodium chloride (5 g/liter). Cells of actinomycetes were grown at 30°C in medium containing glucose (4 g/liter), yeast extract (4 g/liter), malt extract (10 g/liter), and calcium carbonate (2 g/liter).

Chromosomal DNA was isolated after the treatment of the cells with lysozyme (for *Pseudomonas*) or proteinase K (for Actinomycetes) using known methods [11]. Methods for cleaving DNA with endonucleases, electrophoresis, blotting, and preparing of the genetic probe and hybridization are described in [12].

RESULTS AND DISCUSSION

Chromosomal DNA of the investigated strains was treated with endonucleases, and the resulting fragments were separated by electrophoresis in agarose gel and immobilized on a nylon membrane. The microorganisms were selected considering the structural analogy of their halometabolites to tryptophan or to monodechloropyrrolnitrin (table). Hybridization was performed under conditions providing the extent of homology within 65-100%.

The same metabolites are usually synthesized in microorganisms using similar pathways, analogous reactions being catalyzed by related enzymes. The same picture is observed in our case. All investigated producers of pyrrolnitrin exhibited a positive reaction with both probes, the extent of homology constituting more than 90% (Fig. 1).

The strain *P. fluorescens* CHA0 produces two halogen-containing antibiotics: pyrrolnitrin and pyoluteorin (table). The latter contains two chlorine atoms in the pyrrole cycle. Evidently, the number of genes homologous to the *prn*C gene can vary between 1 and 3. As seen from Fig. 1 (G), the chromosome of this strain contains at least two sites homologous to the *prn*C gene that are situated close to each other. The cluster of biosynthesis of pyoluteorin is presumably located close to that of pyrrolnitrin.

Figure 2 presents the results of the hybridization of DNAs of *S. aerocolonigenes* (A), *S. albogriseolus* (B), *A. melliaura* (C), and *S. kitasatasporia* sp. (D) with the *prn*A probe. The extent of homology constituted 65%. As seen,

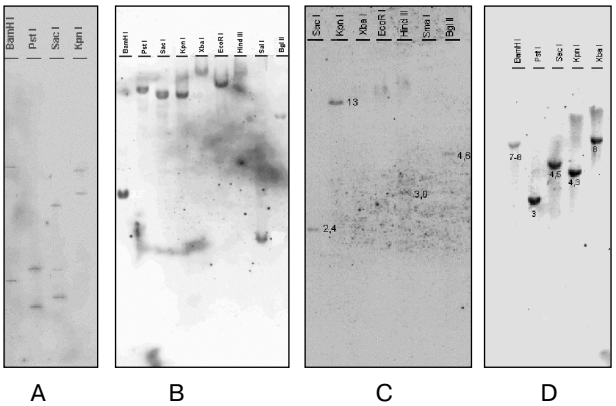
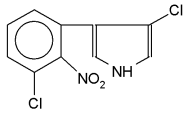
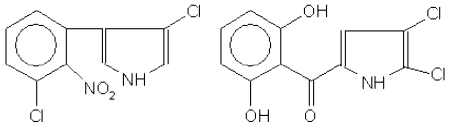
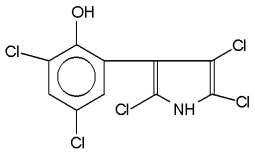
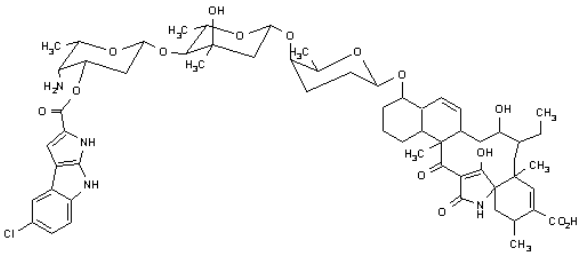
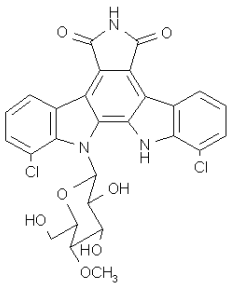
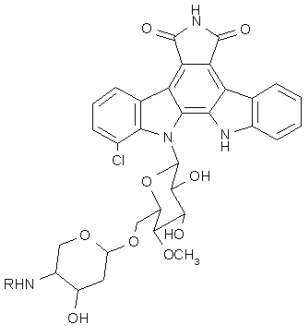
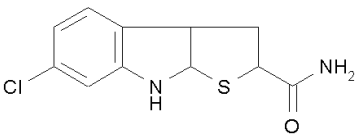


Fig. 2. Blot hybridization of the chromosomal DNA of *S. aerocolonigenes* (A), *S. albogriseolus* (B), *A. melliaura* (C), and *S. kitasatasporia* sp. (D) with the *prn*A probe.

Strains of microorganisms and antibiotics produced

Strain	Antibiotic	
	Name	Structural formula
Pseudomonas pyrrocinia *Pseudomonas aureofaciens ACN* *Pseudomonas aureofaciens Pa1*	Pyrrolnitrin	
Pseudomonas fluorescens CHA0	Pyrrolnitrin, pyoluteorin	
Actinoplanes species	Pentachloropseudilin	
Kitasatasporia species	Pyrroindomycin*	
Sacharothrix aerocolonigenes	Rebeccamycin*	
Actinomadura melliaura	Antitumor complex AT2433*	 R = H; CH_3.
Streptomyces albogriseolus	Thienodolin	

* R, radicals of complex structure not containing halogen atoms.

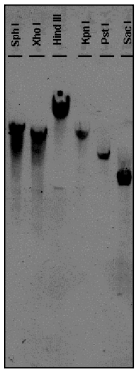


Fig. 3. Blot hybridization of the chromosomal DNA of *Actinoplanes* sp. with the *prn*C probe.

all DNAs indicated contain sites homologous to the *prn*A gene. It can be assumed that one of the steps of biosynthesis of the indicated antibiotics is chlorination of tryptophan or its derivative having similar structure.

It is interesting to note that tryptophan-7-halogenase exhibits high regiospecificity, catalyzing chlorination of the indole core of the tryptophan molecule at the 7th position. The antibiotics pyrroindomycin and thienodolin consequently contain the indole fragments chlorinated at positions 5 and 6. The halogenases revealed in these strains are also expected to exhibit high regiospecificity.

The antibiotic pentachloropseudilin (*Actinoplanes* sp.) contains five chlorine atoms, two of which are in the benzene ring, and three are in the pyrrole ring. The hybridization with the *prn*A probe did not give positive results, while the *prn*C probe revealed relative sites with the homology of 65% (Fig. 3).

Thus, the present study demonstrated that the halogenases of a new type related to tryptophan-7-halogenase and monodechloroaminopyrrolnitrin halogenase found in the strain of *P. fluorescens* are present in a number of other microorganisms and are probably involved in biosynthesis of the corresponding halogen-containing antibiotics.

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