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メタデータ	言語: English 出版者: 公開日: 2017-10-05 キーワード (Ja): キーワード (En): 作成者: Suzuki, Kenji, Sato, Hiroyuki, Kudo, Sayumi, Goto, Eiji, Matsuzawa, Chikara, Ohnishi, Kuniharu メールアドレス: 所属:
URL	http://hdl.handle.net/2297/6411

Proceedings: International Symposium of the Kanazawa University 21st-Century COE Program Vol. 1 (2003)
Edited by Naoto KAMATA
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Degradation of Aromatic Compounds by *Pseudomonas putida*

KENZI SUZUKI, HIROYUKI SATO, SAYUMI KUDO, EIJI GOTO, and CHIKARA MATSUZAWA
Department of Chemistry, Faculty of Science, Kanazawa University, Kanazawa, 920-1192, JAPAN
KUNIHARU OHNISHI
Department of Microbiology, Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, 920-1148, JAPAN

Abstract - From chromosomal DNA of *Pseudomonas putida* S-1, 4.2kbp-fragment was previously isolated and sequenced which contains *sal* and *salR* genes divergently oriented each other, encoding salicylate hydroxylase and its LysR-type regulater protein, respectively. In the intergenic region, promoters were found, separated from each other by 78 nucleotides. SalR protein was expressed and purified from *Esherichia coli* transformed by a plasmid containing *salR* gene. Molecular mass of SalR protein was determined to be 33kDa. The role of SalR was elucidated and discussed in term of the transcription of sal gene.

I. Introduction

Aromatic compounds are metabolized to inorganic compounds via TCA cycle by soil bacteria (Fig. 1). In typical aerobic pathways, the ring is first activated by hydroxylation on adjacent carbons to form a catechol-like compound. Ring cleavage, catalyzed by a dioxygenase, is then effected between the hydroxylated carbon atoms

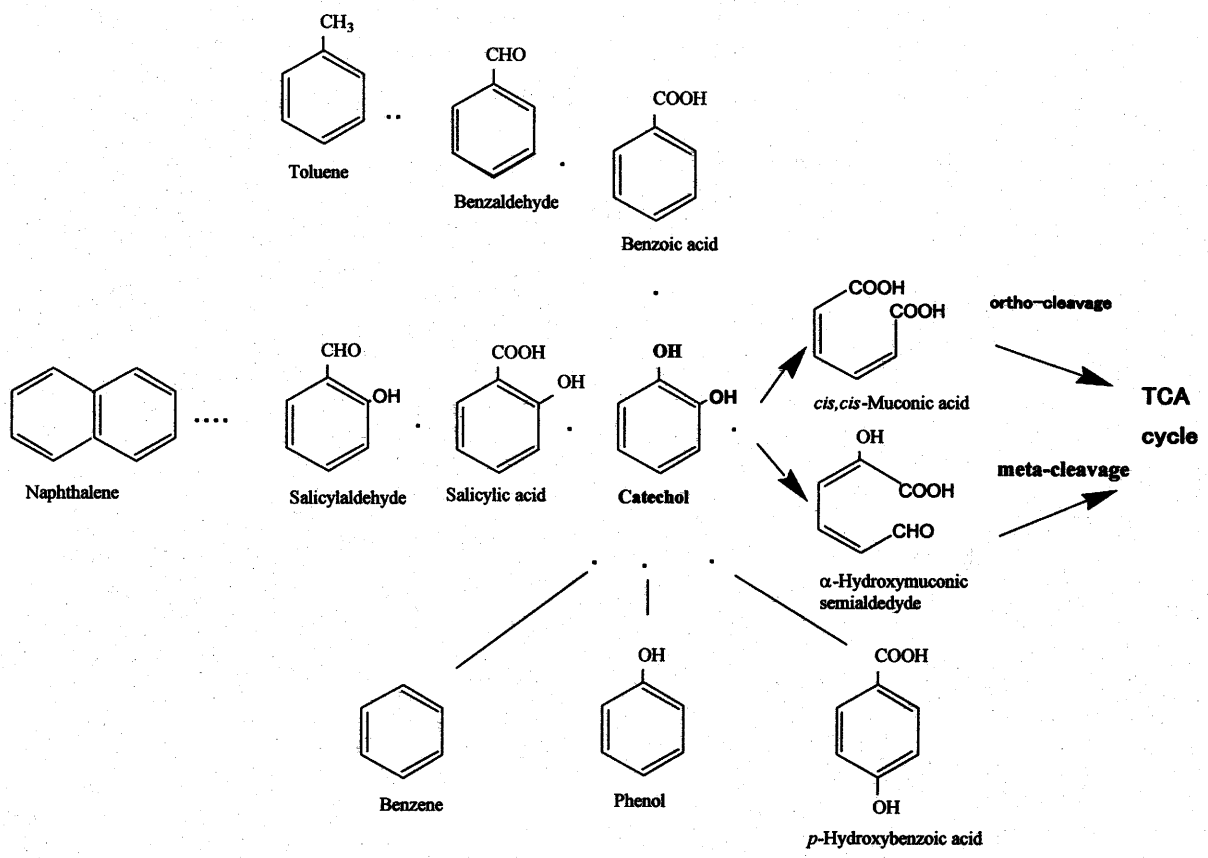

Fig. 1. Metabolic pathways of aromatic compounds.

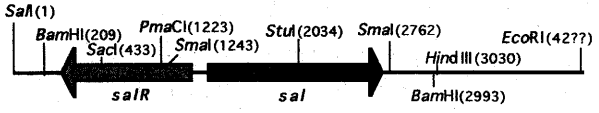

Fig. 2 The gene orientation of 4.2-kb fragment

(intradiol or ortho-cleavage) or adjacent to one of these carbon atoms (extradiol or meta-cleavage). *P. putida* S-1 was previously isolated from soil by a carbon enrichment method with salicylate as a single carbon source. This bacteria metabolizes salicylate by ortho-cleavage pathway. We cloned a chromosomal gene of 4.2 kbp-DNA fragments and sequenced. In this fragment, *salR* gene was locatd divergently oriented from *sal* gene which encodes salicylate hydroxylase. The salR gene consists of 930 bp starting from a GTG codon and encodes a protein of 309 amno acids with a molecular mass of 34542Da. The amino-acid sequence is homologous to LysR family regulatory proteins such as CatR of *P. putida* RB1 and has helix-turn helix DNA binding motif near its N-terminal.

II. Materials and methos

P. putida S-1 was used as a source of DNA and protein. *E. coli* JM109 and *E. coli* BL21(DE3) cells were purchased from Toyobo and Novagens, respectively. The plasmids, pUC18 and pET28a(+) were purchased from Toyobo and Novagens, respectively. The plasmid pSAH1 was constructed by ligation of a 4.2-kb DNA fragment of *P. putida* S-1 chormosome to the multi-cloning site of pUC18 plasmid vector, as reported previously. The *sal-lacZ* protein fusion gene, pLACZ12SH was constructed by ligation of lacZ gene and pLACZ12a which was constructed from *salR* gene fragment and pSAH1.

DNA foot printing. PCR was performed with 355 bp-DNA fragment containing promoter region as the template. The product was digested with CfrI31 and 240 bp-DNA fragment was purified and labeled with ^{32}P which was used as a probe of the coding strand of *sal* (244 bp). Nucleotide fragments with DNase1 were detected by electrophoresis, followed by autoradiography.

III Results and discussions

Subcloning of the *sal* and *salR* genes

The 4.2 kb-plasmid containing *sal* and *salR* gene, pSAH1, was sequenced. These genes were oriented divergently as shown in Fig. 2. The plasmid was transformed into *E. coli* JM109 and the transformants were grown in Luria-Bertani medium containing ampicillin in the presence of 0.1% salicylate as inducer. Cell extracts of *E. coli* harboring pSAH1 grown in the presence of salicylate exhibited a significant increase in the activity of salicylate hydroxylase (2.9 U/g of cells)

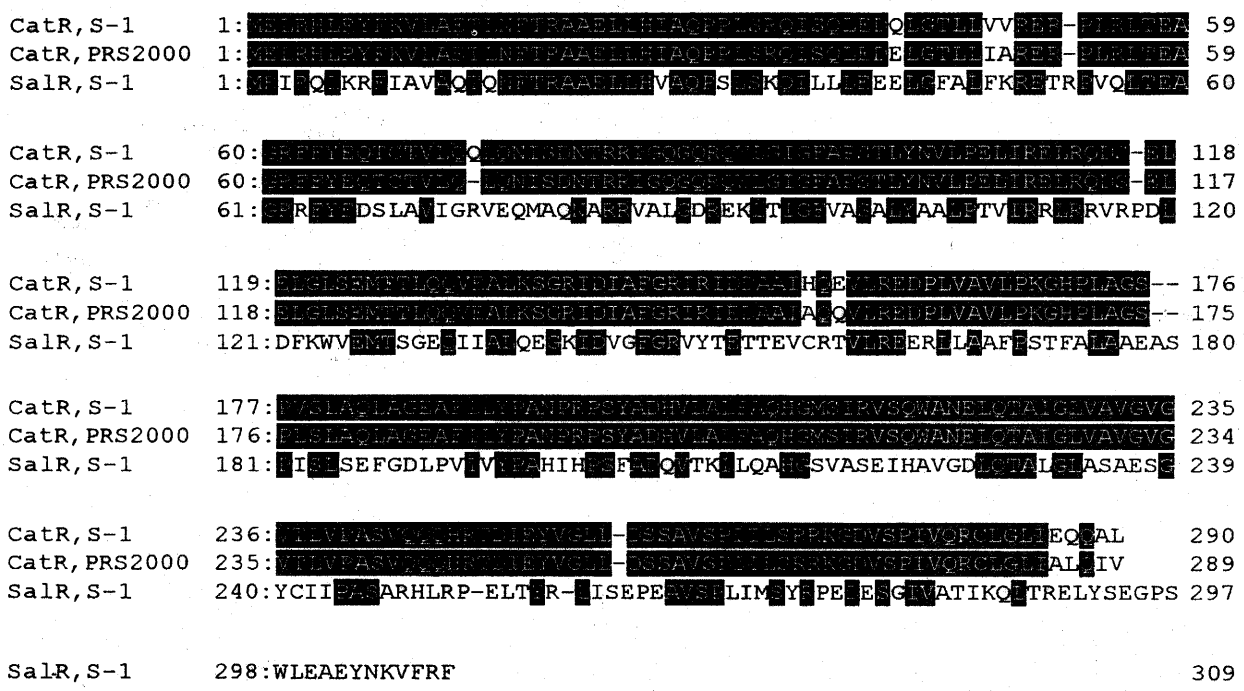

Fig. 3. The amino acid sequence of SalR

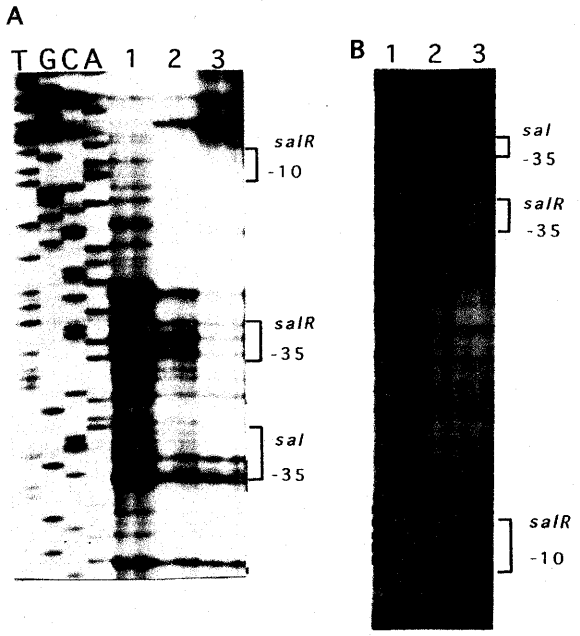

Fig. 4 The foot printing

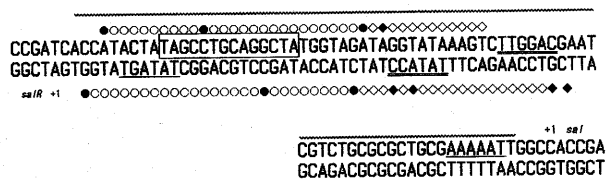

Fig. 5 The promoterregion of salR-Sal

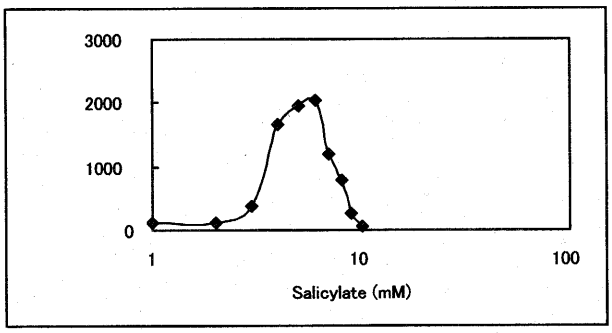

Fig. 6. The expression of sal gene by salicylateas the inducer

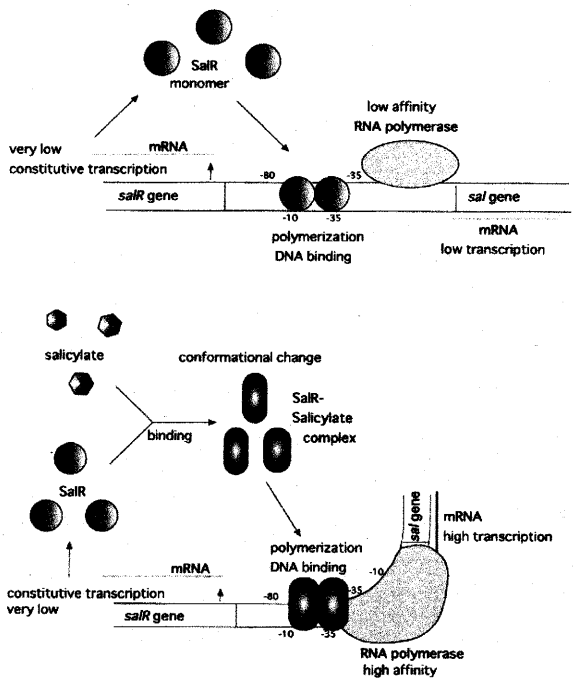

Fig. 7. The regulation mechanism by SalR

Nucleotide sequence of the 5'-flanking region of *sal* gene

The nucleotide sequence of the 5'-flanking region of sal gene was determined. Amino acid sequence of SalR was deduced from the sequence of the *salR* gene as shown in Fig.3. The amino acid sequence of SalR with the same LysR-type regulaters, CatR from *P. putida* S-1 and P. putida PRS2000 was compared in this figure. Helix-turn-helix motif which plays a role of binding with promoter was observed near N-terminal of the sequence.

Foot printing analysis

The electrophoretic pattern of the foot printing was shown in Fig. 4. The decolorized parts were suggested to be protected from DNase, indicating the binding region with SalR.. The results shows the binding region of the promoter with SalR and inducer, as shown in Fig 5.

Construction of pLACZ12SH

The sal-lacZ protein gene, pLACZ12SH was constructed to determine the regulation of SalR for the expression of *sal* gene. The maximam activity was observed by the addition of 1 mM salicylate as the inducer (Fig. 6). The salicylate analogs were also determined for the inducer, as shown in Table 1. 3-Metylsalicylate was effective.

Table 1.
Salicylate analogs as the inducer

Inducer	β-Galactosidase activity (relative)
None	1
Salicylate	17.8
Salicylaldehyde	1.1
o-Iodophenol	1.1
Catechol	1.1
Benzoate	1.1
o-Aminobenzoate	1.4
Acetylsalicylate	4.6
p-Aminosalicylate	1.1
3-Methylsalicylate	31.6
2,3-Dihydrobenzoate	1.1

The results showed the orientation of *salR* and *sal* genes and the replication mechanism of *sal* gene which was shown in Fig. 7.