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INDUCTION CONDITION OF SECONDARY METABOLITES PRODUCTION IN SOIL ACTINOMYCETES, *RHODOCOCCUS JOSTII* RHA1

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SUMMARY: *Rhodococcus jostii* RHA1 is a potent polychlorinated biphenyl-degrading soil actinomycete that catabolizes a wide range of compounds and represents a genus of considerable chemical and industrial interest. Genome of RHA1 contains nonribosomal peptide synthase (NRPS) genes, and polyketide synthase (PKS) genes, providing evidence that RHA1 harbor an extensive secondary metabolism as same as other actinomycetes. To elucidate R. jostii RHA1 may produce any kinds of secondary metabolites, induction condition of these secondary metabolite production genes were investigated. Type I, type III, PKS-NRPS hybrid located on chromosome, and NRPSs located on plasmid were typically induced on solid medium with specific nutrient or environment condition. On the other hands, type II PKSs located on chromosome were mainly induced on liquid medium with specific condition. These results indicated that secondary metabolites induced on specific condition on solid or liquid medium by RHA1 might play a key role of surviving under severe environmental condition.

Keywords—*Actinomycete*, *Rhodococcus*, Secondary metabolites, gene regulation, environmental condition

INTRODUCTION

Actinomycetales are an order of nonmotile Gram-positive bacteria that live in a broad range of environments, including soil, water, and eukaryotic cells. This order includes some of the most important organisms known to humankind, including streptomycetes, which produce most of the antibiotics in use today. The most industrially important genus of actinomycetes not used for antibiotic production is arguably *Rhodococcus* (1). Applications of rhodococci include bioactive steroid production, and fossil fuel biodesulfurization, the most commercially successful application of a microbial biocatalyst (2). The biotechnological importance of rhodococci derives from their lifestyles; these heterotrophs commonly occur in soil where they degrade a wide range of organic compounds. Their assimilatory abilities have been attributed to their diversity of enzymatic activities as well as their mycolic acids, proposed to facilitate the uptake of hydrophobic compounds (3). In addition to their industrial importance, rhodococci offer advantages as experimental systems over more familiar actinomycetes. Despite their importance, rhodococci have not been well characterized.

Polyketides (PKs) and nonribosomal peptides (NRPs) are two large groups of natural products with remarkable structural diversity and biological activities, for example, the antibiotics erythromycin and penicillin, the antifungals amphotericin and echinocandin, the anticancer agents epothilone, the cholesterol-lowering lovastatin, the immunosuppressants FK506, and the veterinary antibiotics monensin and avermectin (4). Polyketide synthase (PKS) and nonribosomal peptides synthase (NRPS) are molecular assembly lines that direct product formation on a protein template. Both systems accomplish their task by maintaining reaction intermediates covalently bound as thioesters on the same phosphopantetheine prosthetic group. PKSs are generally classified into three types. PKSs generate polyketide chains through the oligomerization of small carboxylic acids. The NRPSs are arranged in a modular structure, in which each module is a relatively independent functional block that fulfills a cycle of peptide elongation (5).

Rhodococcus jostii RHA1 was isolated from a lindane-contaminated soil and is known for its ability to transform polychlorinated biphenyls (PCB), and to utilize a wide range of aromatic compounds. It contains approximately 9.7 Mbps arranged in one linear replicon and three additional linear plasmids (6). *R. jostii* RHA1 also contains 24 NRPS genes, and 7 PKS genes, providing evidence of an extensive and uncharacterized secondary metabolism.

In this study, we explored the induction condition of secondary metabolites genes to investigate secondary metabolites production from genus rhodococcus.

2. MATERIALS AND METHODS

2.1. Strains, plasmid, chemicals and growth conditions

R. jostii RHA1 and its derivertives was grown at 30°C in W minimal salt medium containing 20 mM each growth substrate or in Luria-Bertani (LB), 1/5LB medium for stock cultures and gene disruption procedures. Cultures were incubated at 30°C with shaking at 120 rpm. All of the aromatic compounds used in this sturdy were purchased from Sigma-Aldrich (St. Louis, MO) or TCI (Tokyo) and were at least 95% pure.

2.2. Analytical methods

Cells were grown on particular condition for 7 days. Total protein content of crude extract was determined in cells disrupted by sonication (10 cycles of 30 s) by using the Bradford protein assay (BioRad, Hercules, CA) and BSA as standard. Protein concentration was adjusted at 100 μg of each to compare wild type and each RFP insertion mutant. Fluorescence measurements were performed in a Spectrophotometer FL F-2500 fluorometer (Hitachi) at room temperature. The emission maximum of RFP at 591 nm was used for the measurement of fluorescent intensity derived from RFP translation.

3. RESULTS AND DISCUSSION

3.1. Selection of secondary metabolite production genes to identify induction condition

To determine the induction condition of secondary metabolites production genes, we selected genes as follow, ro04065 (Type I PKS), ro04231 (Type I PKS), ro00739 (Type II PKS), ro01201 (Type II PKS), ro01257 (Type II PKS), ro05206 (Type III PKS), ro02209 (PKS-NRPS hybrid), ro08547 (NRPS on plasmid 1), ro08649 (NRPS on plasmid 1) (Fig. 1). To identify induction condition of each gene, a gene encoding red fluorescent protein (RFP) was inserted into the inside of gene.

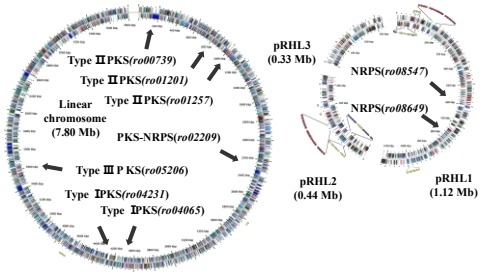

Fig. 1 Schematic representation of *R. jostii* RHA1 linear chromosome and 3 liner plasmid, pRHL1, pRHL2, and pRHL3. Arrows indicated that secondary metabolite production gene were selected for the determination of induction condition in this study.

3.2. Induction of secondary metabolite producing genes under various nutrient or osmotic stress.

For the determination of induction condition under the condition of nutrient and osmotic differentiation, each RFP insertion mutants were inoculated on LB and 1/5 LB including different concentration of NaCl (Table 1). We defined that fold change is more than 2 is significantly up regulated on specifies condition compared with wild type. In case of type I PKSs, ro04065 and ro04231, the up regulation of these genes are recognized grown on solid medium, but not on liquid medium. Incase of type II PKSs, ro00739, ro01201, and ro01257, most of up regulation conditions of these gene are recognized grown on liquid medium but not on solid medium. In case of PKS-NRPS hybrid, ro02209, and NRPS located on plasmid, ro08547, up regulation of these genes are recognized under a few conditions of growth of solid medium, but not on liquid medium. A NRPS located on plasmid, ro08649, does not show any up regulation on any condition that we have tested in this study.

By the differentiation of nutrient and osmotic stress, type II PKSs, ro00739, ro01201, and ro01257, were highly up regulated under liquid culture on low osmotic condition (0.17% NaCl). On solid medium, type III PKS, ro05206, and PKS-NRPS hybrid, ro02209, were highly up regulated on low osmotic stress. In case of high osmotic condition (1.5% NaCl), type I PKSs, ro04605 and ro04231, type II PKS, ro00739 and ro01257, were highly up regulated on solid culture, and type II PKSs, ro00739 and ro01257, were highly up regulated on liquid culture. The highest up regulation under these condition was type II PKS, ro00739, which showed 39.3 fold compared with wild type on liquid culture of LB medium and medium NaCl condition.

These results indicated that RHA1 produces proper secondary metabolite(s) recognizing specific environmental condition. Especially, type I PKSs, PKS- NRPS, and NRPS located on plasmid, showed up regulation on solid culture, but type II PKSs, ro00739, ro01201, and ro01257, mainly showed up regulation on liquid culture.

Table 1. Summary of induction condition

Gene	Growth condition	LB			1/5LB		
		0.17% NaCl	0.5% NaCl	1.5% NaCl	0.17% NaCl	0.5% NaCl	1.5% NaCl
ro00739	liquid	8.57	39.3	2.10	-	-	-
	solid	-	-	-	-	-	3.00
ro01201	liquid	2.49	-	-	-	-	-
	solid	-	-	-	-	-	-
ro01257	liquid	2.01	6.18	2.60	-	-	-
	solid	-	-	-	-	28.4	-
ro02209	liquid	-	-	-	-	-	-
	solid	-	2.28	-	7.54	-	-
ro04065	liquid	-	-	-	-	-	-
	solid	-	2.59	4.44	-	2.58	3.74
ro04231	liquid	-	-	-	-	-	-
	solid	-	-	2.09	4.80	3.77	6.89
ro05206	liquid	-	-	-	-	-	-
	solid	-	-	-	2.26	-	-
ro08547	liquid	-	-	-	-	-	-
	solid	-	-	-	2.53	-	-
ro08649	liquid	-	-	-	-	-	-
	solid	-	-	-	-	-	-

*Horizontal bar indicates that fold change of fluorescent intensity showed less than 2 compared with wild type.

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