Form 3

Abstract of Thesis

Name （Yohanes Novi Kurniawan）	
Title	Differential roles of three regulatory proteins in IM-2/FarA signaling cascade governing secondary metabolism in *Streptomyces lavendulae* FRI-5 放線菌 *Streptomyces lavendulae* FRI-5 の IM-2/FarA 二次代謝制御系における転写調節因子群の機能解析

Abstract of Thesis

Members of the Gram-positive, soil-dwelling filamentous bacterial genus *Streptomyces* have been extensively studied due to their complex life cycle of morphological differentiation and their ability to synthesize secondary metabolites of structural and biological diversity possessing medical and industrial significance. These secondary metabolites production are tightly regulated in a hierarchical manner at several layers involving higher-level regulators regarded as global regulators that mediates stimuli from environment, controlled the activity of the pathway specific regulators which directly control the activation of biosynthetic genes for secondary metabolites. In streptomyces, the most well known hierarchical regulation is the γ-butyrolactone (GBL) signaling cascade consisting of a GBL molecule and a cognate GBL receptor protein which situated at the highest level hierarchy in the regulatory cascade. In the absence of GBL, the GBL receptor protein binds to the promoter region of target genes and represses its transcription. GBL molecule will bind and dissociates the receptor from the promoter of target genes and thereby triggering transcription of target genes and allowing the onset of secondary metabolism and/or morphological development.

The GBL signaling cascade in *Streptomyces lavendulae* FRI 5 composed of a GBL molecule IM-2 and cognate GBL receptor FarA. Unlike other GBL molecules which play positive roles in the regulation of secondary metabolite production, IM-2 exerts dual positive negative effects on the regulation of secondary metabolism; namely, switches on the production of blue pigment and nucleoside antibiotics and switches off the production of D-cycloserine. We previously found that the *farA*-flanking region has seven regulatory genes, including *farX*, an IM-2 biosynthetic gene, and comprises a *far* regulatory island. Two putative regulatory genes (*farR3* and *farR4*) encoding the *Streptomyces* Antibiotic Regulatory Protein (SARP)-family protein are present in the *far* regulatory island together with two more putative transcriptional regulatory genes (*farR1* and *farR2*) all of which are considered to be the direct transcriptional targets of FarA. This study is aimed to clarify the regulatory function of *farR2*, *farR3*, and *far4* in the IM-2/FarA signaling cascade.

In chapter 2, two regulatory genes, *farR3* and *farR4*, were characterized. The SARP family regulators are DNA-binding proteins transcriptional regulator and in general acts as activators for the production of secondary metabolites. *farR3* is transcribed both as a monocistronic RNA and as a bicistronic *farR4-farR3* mRNA, and the expression profile is tightly controlled by the IM-2/FarA system. Loss of *farR3* delayed and decreased the production of blue pigment indigoidine without any changes in the transcriptional profile of other *far* regulatory genes, indicating that FarR3 positively controls the biosynthesis of blue pigment indigoidine, and is positioned in the downstream region of the IM-2/FarA signaling system. Meanwhile, loss of *farR4* induced the early production of IM-2 by increasing transcription of an IM-2 biosynthetic gene, *farX*, indicating that FarR4 negatively controls the biosynthesis of IM-2. Thus, this study suggested differential contributions of the SARP-family regulators to the regulation of secondary metabolism in *S. lavendulae* FRI-5. This is the first report to show that a SARP-family regulator is involved in the biosynthesis of a signaling molecule functioning at the most upstream region of the regulatory cascade for *Streptomyces* secondary metabolism.

In chapter 3, a regulatory gene of the GBL receptor homologue family, *farR2*, which is located in the

downstream of *farR3* is characterized. Due to the high p*I* value, FarR2 falls to the class of pseudo-GBL receptor. In general, pseudo-GBL receptor negatively controls the production of secondary metabolites. Similar to *farR3* and *farR4*, transcription of *farR2* is tightly controlled by the IM-2/FarA system. Loss of *farR2* delayed the production of blue pigment indigoidine, indicating similar function with *farR3* to positively control blue pigment indigoidine production. In clear contrast of the delayed effect to blue pigment indigoidine production, loss of *farR2* caused transcriptional upregulation of *far*-regulatory genes at the late stage of secondary metabolism activated by the IM-2 signaling cascade, suggesting that FarR2 acts as a transcriptional repressor. In vitro analysis demonstrated that FarR2 binds to the upstream region of *farR1*, *farR2*, *farR4*, *farA*, and *farX*, suggesting a negative autoregulatory mechanism for *farR2* and a negative transcriptional regulation for IM-2 biosynthesis. Taken together, I suggested that FarR2 positively controls the initiation timing of blue pigment indigoidine production in response to the presence of IM-2 and is involved in the transcriptional repression of the *far* regulatory genes at the late stage of secondary metabolism, implying the functional diversity of the pseudo-GBL receptor regulators in streptomycetes.

In this thesis, the regulatory function of three regulatory genes, two of which belongs to the SARP-family, *farR3* and *farR4*, and a pseudo-GBL receptor homologue, *farR2* was characterized and demonstrated the exquisite regulation of blue pigment indigoidine and IM-2 biosynthesis in the IM-2/FarA signaling cascade. FarR3 positively controls the production of blue pigment indigoidine while FarR4 is an important determinant for controlling the initiation time of IM-2 production. FarR2 (a pseudoreceptor regulator) is under the tight and direct transcriptional control of the IM-2/FarA system, and revealed that FarR2 acts as a pathway-specific activator of the onset of blue pigment indigoidine production as well as a repressor of the *far* regulatory genes, including *farR2* itself, at the late stage of secondary metabolism, suggesting that FarR2 makes distinct contributions to two physiological processes in different stages of secondary metabolism.

様式 7

論文審査の結果の要旨及び担当者

氏　名	（　Yohanes Novi Kurniawan　）		
		（職）	氏　名
論文審査担当者	主 査	教授	仁平　卓也
	副 査	教授	福崎　英一郎
	副 査	教授	渡邉　肇
	副 査	教授	紀ノ岡　正博
	副 査	教授	福井　希一
	副 査	教授	村中　俊哉
	副 査	教授	大政　健史
	副 査	教授	藤山　和仁
	副 査	教授	永井　健治

論文審査の結果の要旨

土壌微生物として知られる放線菌は、多くの有用生理活性物質を二次代謝産物として生産する微生物である。この放線菌の二次代謝制御系を遺伝子レベルで理解することは、有用化合物の効率的生産や新規天然物の発見などに繋がることが期待されている。しかし、放線菌の二次代謝制御系では、複数の制御因子が有用化合物の生産を緻密に制御していることが推測されているが、二次代謝制御系間の共通性や相違性についての知見は、未だ集積段階にある。本論文は、代表的放線菌である *Streptomyces* 属放線菌の二次代謝制御系について、遺伝子レベルで解析し、二次代謝誘導低分子信号伝達系の支配下にある転写制御因子の機能を解明すると同時に、放線菌二次代謝の新たな制御系モデルを提唱するものである。得られた知見を要約すると、以下の通りである。

1）　青色色素を二次代謝産物として生産する放線菌 *Streptomyces lavendulae* FRI-5 において、二次代謝誘導因子 IM-2 受容体遺伝子の近傍にある 2 つの SARP 型制御因子の機能を遺伝子破壊法により解析している。青色色素がインジゴイジンであることを質量分析により示すと共に、この SARP 型制御因子が全く異なる制御経路を介して、インジゴイジン生産を調節することを明らかにしている。また、二次代謝制御系の下層に位置すると知られる SARP 型制御因子が、上位にある二次代謝誘導因子の生合成を支配する結論は、SARP 型制御因子の普遍的機能解明に一石を投ずる知見である。

2）　同菌株の二次代謝における IM-2 受容体相同遺伝子の制御機能を、遺伝子破壊解析により解明している。この制御因子は IM-2 制御系により厳密に転写調節されており、またインジゴイジン生産の開始時期を正に調節することを明らかにしている。また、1）で示した SARP 型制御因子を含む IM-2 受容体遺伝子近傍の複数の制御因子を転写調節することを示している。この解明に基づき、今までに示されてない複雑な二次代謝の制御系モデルを呈示している。

以上のように、本論文では、青色色素インジゴイジン生産放線菌を対象にし、二次代謝誘導因子の支配下にある制御因子の機能を、遺伝子破壊解析などにより、詳細に解明している。また、放線菌の二次代謝制御機構に関わる様々なレベルの遺伝的情報が示されると同時に、新しい概念の二次代謝制御モデルも提唱している。本研究で得られた成果は、生理活性物質などの有用化合物の効率的生産において遺伝子操作の有効性を示唆するとともに、放線菌の二次代謝制御に関する研究を大きく進展させるものと期待される。

よって、本論文は、博士論文として価値あるものと認める。