KCS III-5

Engineered biosynthesis of antifungal antibiotics for plant disease control

Beom Seok Kim (Korea University, Seoul, Korea)

Laboratory of Plant Pharmacology, College of Life Sciences and Biotechnology, Korea University,Seoul, Korea

(E-mail: biskim@korea.ac.kr)

Exploitation of antimicrobial agents from microbial metabolites has been considered to be an approach to develop novel fungicides which meet the modern environmental requirements. Continuing efforts to find novel antifungal metabolites heavily depends on the screening of biological activity from pure cultured microorganisms, which caused increased rate of rediscovery of well-known antifungal families and necessitates the development of novel strategies to generate novel antifungal agents. These constraints lead us to manipulate biosynthetic pathway of microorganisms, so called metabolic engineering, which is expected to be an efficient way to generate novel analogues or intermediates of antimicrobial metabolite or to improve their productivity. Metabolic engineering requires a thorough knowledge of the biosynthetic pathways involved in the production of the antimicrobial metabolites. In this presentation, recent studies in metabolic engineering to generate novel antifungal antibiotics and to increase production rate of a desired compound will be discussed.

The anthracycline aromatic polyketide pradimicins produced by *Actinomadura hibisca*, have potent antifungal activity against several plant pathogenic fungi. We cloned the biosynthetic gene clusters of pradimicin and verified their deduced function. The genomic DNA fosmid clone harboring the part of pradimicin biosynthetic gene cluster (39 kb) was identified by using the probe designed from ketosynthase genes. Sequence analysis of the 39 kb locus revealed 28 open reading frames, which were assigned to the pradimicin biosynthetic gene cluster. The overall GC content of the sequenced region is 73.2%. All proteins were shown to share considerable homology with rubromycin, griseorhodin, and fredericamycin biosynthetic gene clusters. Based on homology searches, 28 open reading frames that consist of 15 genes for polyketide biosynthesis, 7 genes for sugar biosynthesis, and 2 genes for resistance were proposed. To confirm the gene cluster was responsible for the production of pradimicin, a mutated strain was generated by homologous recombination, in which the gene encoding the ketosynthase (*prmA* and *prmB*) was substituted by the apramycin resistance gene. Fermentation of the mutated strain clearly demonstrated the disappearance of pradimicin production, thereby proving that the gene cluster described

here is responsible for pradimicin biosynthesis. And we set out to generate a novel pradimicin analogue through mutagenesis of the methyltransferase gene. LC-MS and NMR analysis of the mutant strain revealed the generation of a novel 11-*O*-demethyl pradimicin.

Another talk will be protein engineering of the nonribosomal peptide synthetases (NRPSs) producing fusaricidin. The fusaricidins are a family of lipopeptide antibiotics containing a β-hydroxy fatty acid linked to cyclic hexapeptide and have potent antifungal activity against *Phytophthora capsici* and *Fusarium oxysporum* f. sp. *lycopersici*. The fusaricidin synthetase gene (*fusA*) comprising of 6 modules was identified in the producing strain DBB1709. The A domain substrate binding pocket of third module of FusA seems to have broad substrate specificity integrating any one of L-Tyr, L-Val, L-Ile, L-allo-Ile, and L-Phe into the cyclic peptide structure of fusaricidin, which result in the generation of various frusaricidin derivatives. Since the fusaricidin derivative containing L-Phe has been known to exhibit the most potent antifungal activity among the fusaricidin derivatives, we modified the A domain of third module to integrate mainly L-Phe into the cyclic peptide structure of fusaricidin through site-directed mutagenesis based on *in silico* analysis to decipher the selectivity-conferring amino acid residues in the binding pocket.