[S5-2] [10/11/2003(Sat) 10:10-10:50/ Grand Ballroom 102]

Combinatorial Biosynthesis of Polyketide Antibiotics Doxorubicin and Rubradirin

Young-Soo Hong[1], Jung-Joon Lee[1], Jae-Kyung Sohng[2], Jin-Chul Yoo[3], Chun-Gyu Kim[4]*

[1]KRIBB, [2]Sunmoon University, [3]Chosun University, [4]Inje University

A lot of polyketide antibiotics have been isolated from natural sources like microorganism, fungi and plant. The polyketide natural products have biologically and medically important activities, including antibacterial, anticancer, antiparasitic, and immunosuppressant properties. The diversified activities of polyketides are originated from their structural variety of which have been took advantage by several research groups for development of new drugs.

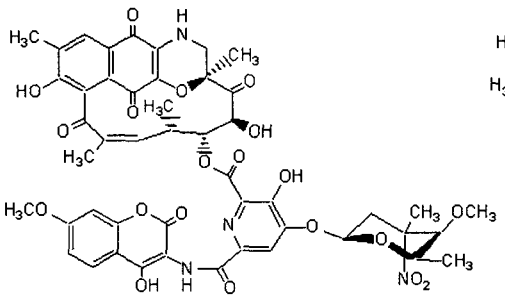
The polyketide antibiotics are biosynthesized by polyketide synthase (PKS) functioining as multienzyme system. The PKSs are divided into two types depending on operating as multifunctional enzyme complex (typeI) or monofuntional enzyme complex (typeII). For example, the aromatic antibiotic doxorubicin and the macrolactam antibiotic rubradirin are synthesized by typeII PKS and typeI PKS respectively.

Over the last decade, lots of PKS genes were cloned, sequenced, and analyzed and the knowledges thereafter were applied for structural modification of polyketide compounds. The domain and module swapping techniques were used for the typeI PKS modulation and the combination of alternative enzymes from different sources for the typeII PKS complex modulation. These techniques are now expanded into the solid combinatorial genetic manipulation system for diversified modification of polyketides compounds. In this seminar, we show the combinatorial genetic manipulation systems using the biosynthetic gene clusters of doxorubicin and ansamycins including rubradirin, geldanamycin and rifamycin (Fig.1).

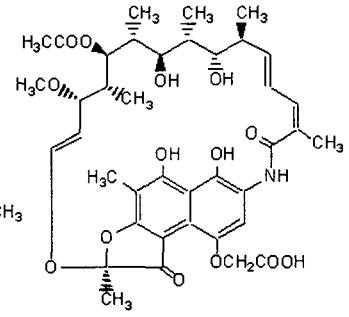
The 80kb biosynthetic gene cluster of doxorubicin isolated from *S. peucetius caesius* var. ATCC 27952 was sequenced and analyzed. The 13 genes known to be involved in the biosynthesis of ε-rhodomycinone, one of the doxorubicin intermediates, and two genes involved in nogalamycin and mithramycin biosynthesis were reorganized into two vectors, pDXR1 and pDR3, each of them containing apramycin and thiostrepton resistance marker gene respectively. The reorganized genes in each vector are all under the control of actIII-I promoter. The expression vector pDXR1 contains dpsABCDGEFY genes which would produce deoxyaklanonic acid. The expression vector pDXR3 contains dnrGCDEF, snoL2 and

mtmMII genes which would produce modificed ε-rhodomycinones when expressed properly together with the pDXR1 vector. The produced ε-rhodomycinone analogues could be utilized as a substrate for glycosylation of various deoxysugars. The thereafter modified anthracyclines are to be used to select the anticancer agents with improved activity.

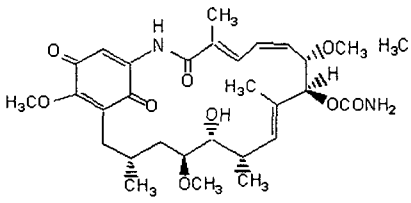
The biosynthetic gene clusters of three ansamycins including rubradirin, geldanamycin and a compound of unknown structure were sequenced and analyzed. The three gene clusters were compared all together with the reported gene clusters for the ansamycin antibiotics rifamycin and ansamitocin. Functions of the biosynthetic genes in the clusters were allocated based on the comparative analysis of the five gene clusters. The structure of the unknown ansamycin was also presumed from the domain analysis of the PKS. Several knockout mutants from the ansamycin producers were constructed and the metabolites from the mutants were identified resulting in confirmation of gene functions. Several strategies for modification of ansamycins are proposed.



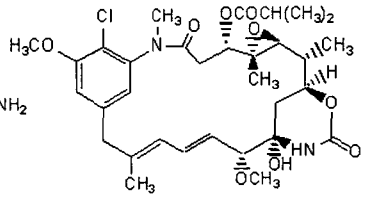
Rubradirin



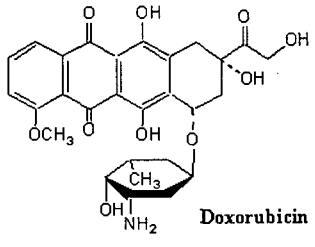
RifamycinB



Geldanamycin



Ansamitocin



Doxorubicin