CLONING AND CONFIRMATION OF PLECOMACROLIDE BIOSYNTHETIC GENE CLUSTER OF STREPTOMYCES GRISEUS DSM 2608

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Introduction. *Streptomyces griseus*, DSM2608 (TÜ 1922) produces the bafilomycin, an antifungal plecomacrolide antibiotic. We cloned and sequenced an 84-kb region, including polyketide synthase (PKS) region, methoxymalonate genes, flavensomycinate genes, and other putative regulatory genes. In the middle part of sequence, five different PKS was included. In downstream of PKS region, the genes for methoxymalonate biosynthesis were located, among which a gene for FkbH-like protein was assumed to play an important role in the production of methoxymalonyl-CoA from glyceryl-CoA. Further the genes encoding flavensomycinyl-ACP biosynthesis for the post-PKS tailoring were also found in the upstream of PKS region.
Here we report the full sequence of bafilomycin biosynthesis gene from S. griseus DSM 2608 and the involvement of this gene cluster in bafilomycin was confirmed by gene disruption experiments.

Methods. The cosmid library was constructed in SuperCos1 and screening of bafilomycin synthesis gene was performed with three 32P-labelled ketosynthase(KS), aminolevulinate synthase(ALS) and FkbH probes following the protocols. [1] DNA sequencing was done by Genotech Co., INC and analyzed by using FramePlot version2.3.2 [5] and NCBI-BLAST program. The gene disruption experiment was performed following the protocol of John Innes Centre. [2],[4]

Results. The total length of 87.3kb of complete gene cluster was analyzed. From the sequence analysis, 58.5kb of five genes for BafSI to BafSV were confirmed to be type I PKS gene responsible for the biosynthesis of main macrolacton backbone of bafilomycin. And two genes for transcription regulators, SARP, and LuxR family were also found. To confirrmed whether the gene cluster is involved in the biosynthesis of bafilomycin, the module 12 of PKS and FkbH like protein gene in S. griseus DSM 2608 was disrupted. After cultivation of wild type and two disruptants, antimicrobial activity assay on *Rizhoctonia solani* was examined. And it showed that the gene cluster is bafilomycin biosynthetic gene cluster in *S. griseus*.

Conclusion. The five different PKS genes, was assumed to be involved in the biosynthesis of plecomacrolide backbone including 16-membered macrocyclic lactone based on its chemical structure and modular organization. All the modules showed high similarity with typical type I PKS genes. However, the starting module of PKS gene was confirmed to be specific for isobutyrate by sequence comparison of acyltransferase domain. In downstream of PKS region, the genes for methoxymalonate biosynthesis were located, among which a gene for FkbH-like protein was assumed to play an important role in the production of methoxymalonyl-CoA from glyceryl-CoA. Further the genes encoding flavensomycinyl-ACP biosynthesis for the post-PKS tailoring were also found in the upstream of PKS region.

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