

THE FULL NUCLEOTIDE SEQUENCE OF BAFILOMYCIN SYNTHETIC GENE CLUSTER FROM *KITASATOSPORA CHEERISANENSIS* KCTC2395

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Introduction. Bafilomycin is a plecomacrolide antibiotic which shows a growth inhibitory activity on fungal plant pathogens produced from *Kitasatospora cheerisanensis* KCTC2395. The genome sequence including the sequence of bafilomycin synthesis gene cluster from *K. cheerisanensis* KCTC2395 was secured by three different types of next-generation sequencing method and gap in the complete sequence of the bafilomycin biosynthesis gene cluster was filled up by PCR amplification compare to *Kitasatospora seate*. Among the five PKS genes, the dehydratase domain in Bfms5 and amide synthetase gene were disrupted to confirm that the gene cluster involved in the biosynthesis of bafilomycin.

From this study, the full genome sequence of *K. cheerisanensis* KCTC2395 and the gene cluster for biosynthesis of bafilomycin from this strain are reported.

Methods. To secure the full nucleotide sequence of *K. cheerisanensis* KCTC2395, three different types of next-generation sequencing method containing Roche 454 pyrosequencing, Illumina GAII-X sequencing, PacBio RS sequencing platform were employed. For filling up the gap of bafilomycin gene cluster, 46 sets of PCR primers were designed compared to consensus sequence of other bafilomycin producers. The sequence was analyzed by NCBI-BLAST search. The gene disruption experiment was performed following the protocol of John Innes Centre [4], and for conjugal transfer was according to the Choi et al. [3]. Bafilomycin in culture broth of wild type and mutants were assayed for antifungal activity on *Rhizoctonia solani*.

Results. The obtained genome sequence was composed of 363 contigs with total length of 7.5 Mbp, and shows 73.15 % of (G+C) content. Among the genes, 116 genes include polyketide synthase(PKS), non-ribosomal peptide synthetase, and cytochrome P450 were related to the production of secondary metabolites. Based on the genome sequence, around 46.8 kb sequence of the gap in the complete sequence of the bafilomycin biosynthesis gene

cluster was filled up by PCR amplification. The total length of bafilomycin gene cluster is around 90 kb contains PKS, acyl-CoA ligase, amide synthase, 5-aminolevulinate synthase, methoxy malonate biosynthesis protein genes and etc. for biosynthesis of bafilomycin. The putative gene cluster was highly homologous with the bafilomycin biosynthetic gene cluster from *K. setae*. The dehydratase domain of module12 of PKS gene and amide synthetase gene were disrupted using apramycin cassette and antimicrobial activity on *R. solani* was examined.

Conclusions. Bafilomycin biosynthesis gene cluster of *K. cheerisanensis* is composed to five modular PKS genes from BfmS1 to BfmS5 that include 12 modules. Also other functional genes that acyl-CoA ligase, amide synthase, 5-aminolevulinate synthase, methoxymalonate biosynthesis protein, and etc. are presumed to be help for post-PKS tailoring were identified by sequence analysis. According to the sequence analysis of this gene cluster, the bafilomycin biosynthetic gene cluster is much similar to the gene cluster of bafilomycin produced by *K. setae*. From the antimicrobial activity assay of wild type and mutants, the 90kb gene cluster is involved in the biosynthesis of bafilomycin in *K. cheerisanensis*.

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