



## ENGINEERED STREPTOMYCES HOST FOR HETEROLOGOUS EXPRESSION OF BIOSYNTHETIC GENE CLUSTER FOR SECONDARY METABOLITES

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**Introduction.** The identification and detailed characterization of secondary metabolic gene clusters have proved to be an invaluable tool for elucidation of the biosynthetic mechanism of secondary metabolites. A critical requirement for these applications is the availability of the relevant biosynthetic gene clusters controlling the production of a secondary metabolite of interest as well as appropriate genetic systems for the *in vivo* manipulation of the corresponding genes in heterologous hosts. We constructed a model host for heterologous expression of biosynthetic gene clusters using engineered *Streptomyces avermitilis* (1). This pioneer heterologous expression system has been confirmed by the production of three exogenous biosynthetic gene clusters in heterologous genetically engineered hosts, large-deletion derivatives of *S. avermitilis*. We have shown the feasibility of using genetically engineered *S. avermitilis* as a heterologous host by the effective expression and production of 20 biosynthetic gene clusters for exogenous secondary metabolites.

**Methods.** The construction of the genomic library using integrating cosmid or BAC vector and *in vivo* cloning of the entire biosynthetic gene cluster by  $\lambda$ -RED or *recE/T* system are described in ref. 2. Conditions for vegetative culture and fermentation culture were described previously (1, 2).

**Results.** All the large-deletion derivatives (SUKA1 to 17) grew well on the sporulation medium and their morphological development on the medium was slightly faster than that of the wild-type strain. Large-deletion derivatives could grow on minimum medium without any supplements because the regions deleted from *S. avermitilis* chromosome included no essential genes. The growth rates of large-deletion derivatives until 24 hr incubation were similar and were faster than

the wild-type strain. Interestingly, the biomass of the wild-type strain increased until 24 hr but large-deletion derivatives continued to grow until 44 hr and their biomass contents were about 1.7-fold that of the wild-type strain. Thus, large-deletion derivatives had the ability to grow faster and increase their biomass. Among of twenty heterologous expressions of the entire biosynthetic gene clusters, production of bafilomycin B1, lactacystin, holomycin, pholipomycin and chloramphenicol was extremely efficient. The productivity of lactacystin, holomycin, pholipomycin and chloramphenicol in transformants of genetically engineered *S. avermitilis* derivatives was higher than that of original producer *Kitasatospora setae* KM-6054, *S. lactacystinaeus* OM-6159, *S. clavuligerus* ATCC 27065 and *S. venezuelae* ATCC 10712.

**Conclusions.** The feasibility of using large-deletion derivatives of *S. avermitilis* as a heterologous host has been shown by the effective expression of 18 intact biosynthetic gene clusters, two biosynthetic gene clusters controlled by an alternative promoter and three codon-optimized genes encoding plant terpenoid intermediates. Furthermore, because large-deletion derivatives of *S. avermitilis* no longer produced major endogenous metabolites, primary metabolism seemed to be efficiently exploited to generate precursors of exogenous biosynthetic gene clusters.

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### References.

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