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Reverse Engineering of Highly Efficient Multi-Purpose Heterologous Expression System through Comparative Functional Genomic Analysis of Pharmaceutical-Overproduction Actinomycete Strains

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Actinomycetes offer impressive arrays of sencondary metabolites; anticancer, antibiotics, immunosuppressant, anti cholesterol and so on (Fig. 1). The biosynthetic genes of secondary metabolites are typically clustered and thus readily amenable to genetic manipulation. Combinatorial biosynthesis involves the exploitation of nature's synthetic capabilities in a combinatorial mix-and-match fashion by interchanging natural product biosynthesis genes to create unnatural gene combination.

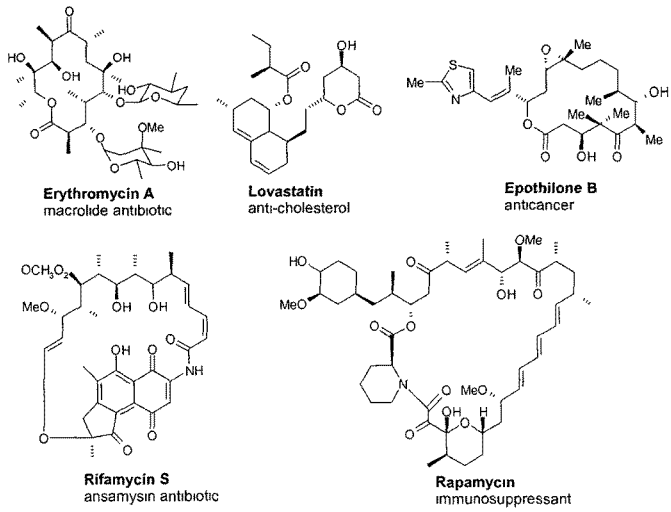

Figure 1. Examples of polyketide secondary metabolites Primary biological activity is indicated.

The reverse engineering of highly efficient multi-purpose heterologous expression system is to identify the genetic and the environmental factors of industrial high-producing actinomycete strain through comparative functional genomics analysis and its application for the reconstruction of heterologous actinomycete strain by combining the construction the heterologous expression system of gigantic biosynthetic gene clusters. The ultimate goal is to construct heterologous expression system with the high-efficiency and multipurpose towards the high-level production of valuable therapeutic drugs.

The present work is going to be developed the novel and universal ways for high-level production of useful natural products from the actinomycete strains in which genetic manipulation can be hardly performed. To achieve this aim, we are to construct heterologous expression system with the high-efficiency and multipurpose through the analysis based on the comparative functional genomics with the classically modified high-producing strain. The method of "Inverse metabolic engineering" strategy involving identification of a desired phenotype, determining the genetic or the particular environmental factors conferring that phenotype and endowing that phenotype on another strain or organism by directed genetic or environmental manipulation, addresses the drawbacks of classical approaches in strain improvements methods which are laborious and inaccessible for new strain engineering efforts. A serial study on the biosynthetic pathway, regulatory mechanism, protein expression pattern, precursor metabolism, and rate-determining step of secondary metabolite from a doxorubicin high producer *S. peucitius* will be applied to the inverse metabolic engineering and the expression system of gigantic biosynthetic gene clusters. *S. venezuelae* will be substituted for two universal heterologous expression hosts, *S. lividans* and *S. coelicolor*, to utilize its advantageous properties such as rapid growth and ease of genetic manipulation.

To enhance the productivity of new and existing therapeutic molecules from microorganism sources, the proposed work will involve a parallel application of functional genomics such as proteomics and metabolomics, and heterologous expression system of gigantic biosynthetic gene clusters. The developed technique will propose the generally applicable method for high production of bioactive molecules. The inverse metabolic engineering method is also applicable to eukaryotic system which has wide-spread implications for increasing the supply of valuable therapeutic agents. Through the study of industrially important strains other than the model strain *S. coelicolor*, such as *S. peucetius*, we should be able to generate knowledge on the mechanisms of the biosynthesis of commercially useful molecules and its regulatory mechanism.

References

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