A Perspective for Assembling SALP (Streptomyces Artificial Linear Plasmid): A Potential New Genetic Tool of Gene Manipulation System for Producing Bioactive Secondary Metabolites in Streptomyces

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Streptomyces genus is a very important industrial bacterial and a major natural source (about 2/3) of bio-active secondary metabolites including antibiotics used today [1].

Genetic systems for combinatorial biosynthesis of bioactive products including antibiotic have been developed in Streptomyces genus. Several shuttle vectors, including circular plasmid, cosmid, BAC (Bacterial Artificial Chromosome)/PAC (P1-derived Artificial Chromosome) between E. coli-Streptomyces have been used for manipulation of Streptomyces gene cluster for producing bioactive chemicals. Usually normal circular plasmids can carry only 1–20 kb DNA size. Cosmids are able to carry 37 to 52 kb of DNA, it was reported that cosmids have been successfully used for construction of Streptomyces genome libraries to clone biosynthetic gene clusters [2-7]. Nonetheless, most biosynthetic gene clusters for natural products are larger than the average capacity of common cosmids vectors, thus, vectors with large capacity such as BAC or PAC have been used to construct E. coli-Streptomyces artificial chromosomes for heterologous production of secondary metabolites [8-14]. The DNA fragments insertion loading capacity for BAC/PAC is from 100 kb up to 100-300 kb in E. coli cell, the large capacity of BAC/ PAC allows the gene manipulation of complete biosynthesis gene cluster of secondary metabolites in bacteria E. coli cell. However, E. coli is not an ideal host for heterologous gene overexpression and production of Streptomyces biosynthesis gene cluster due to its lower genomic GC content compare to Streptomyces's. In addition, BAC capacity can be up to 100 kb and more, but the copy number usually is low. Therefore, a shutter capability for the vector to introduce BAC back to Streptomyces host is required for efficient production. Intergeneric conjugation is a major approach for manipulating DNA shuttered between Streptomyces and E. coli [11].

Different from most other eu-bacteria, the plasmids and chromosomes in Streptomyces species are usually linear [15,16], some Streptomyces species harbor multiple linear plasmids up to three [17]. The sizes of linear plasmids range are from 12 kb [18] to 1800 kb [6]. More and more linear plasmids in Streptomyces have been identified. Some of natural endogenous giant linear plasmids have been discovered carrying gene clusters for the production of secondary metabolites including antibiotics biosynthesis clusters [19-21].

In this editorial, the possibility for developing SALP: Streptomyces Artificial Linear Plasmid, a new genetic tool for gene manipulation system based on the backbone elements of linear plasmid in Streptomyces is discussed.

Streptomyces species harbor linear plasmids varied in number and size. Linear plasmids can interact with chromosome, genetic exchanges between chromosome and linear plasmids including some giant linear plasmids occur via recombination, transposition etc. [16,21]. Linear plasmids share some common functional mechanism with chromosome and are able to recruit chromosome encoded protein to perform functions such as replication as linear mode in Streptomyces [22]. The capacity of linear plasmid could be very big, up to 1.8 M, those endogenous linear plasmids are stable expressed In vivo, and a majority of them replicate as high copy number. A certain number of linear plasmids are capable of self-conjugative transfer. The mechanism of linear plasmid conjugation is different from that of circular plasmid, it was reported that in addition to classic tra gene which was identified as essential transfer gene for Streptomyces circular plasmid, more gene loci and/or elements are required for linear plasmid efficient conjugation transfer [9,11,23-26]. Genes involved in efficient transfer usually co-transcribe in an operon cluster, a six co-transcribed genes including a tra gene encoding Tra-like DNA translocase has been identified for SL2 which is a 50 kb endogenous linear plasmid in extensively-studied model strain: Streptomyces lividans [24,25].

To construct an ideal shuttle SALP vector, several essential factors should be taken into account: capacity for carrying large size DNA; replication in Streptomyces with high efficiency; efficient intergenetic conjugation transfer. Due to its natural characters, endogenous linear plasmid in Streptomyces is capable of serving as a potential source for constructing new genetic tools to manipulating bioactive secondary metabolites gene cluster. First of all, Streptomyces linear plasmids derived from Streptomyces and are able to replicate and express stably In vivo. Secondly, the capacity is big: endogenous giant linear plasmids whose size varied from hundreds to thousands kb have been isolated and characterized, some of them carry natural antibiotics synthesis gene cluster. Thirdly, some linear plasmids carry conjugation transfer operon that allows them efficiently transfer between different host species.

In summary, more and more linear plasmids in Streptomyces have been identified and characterized. Accumulated fundamental studies on linear plasmid reveal their functions and molecular mechanism. Those findings provide insights into molecular basis of essential elements required for genetic manipulation system.

Acknowledgments

I would like to thank Prof. Zhongjun Qin and Prof. Guoping Zhao who are experts in this field, for their support and suggestions.

References


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Received December 15, 2012; Accepted December 17, 2012; Published December 19, 2012


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Biochem Anal Biochem ISSN:2161-1009 Biochem, an open access journal Volume 2 • Issue 1 • 1000e132