Engineering of actinomycetes using CRISPR/Cas9 technologies

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Actinomycetes historically have been – and still are – among the most important producers of Natural Products with anti-microbial activities and therefore are an excellent source for the discovery of novel bioactive molecules that could serve as leads for novel drugs to treat infectious and other diseases.
While this group of organisms was studied for many decades, genome analyses indicate that the genetic potential of these bacteria to synthesize secondary metabolites is far beyond the number of molecules observed in traditional chemical screenings. While advances in genome sequencing and genome mining software like antiSMASH (http://antismash.secondarymetabolites.org) [1-3] help scientists in identifying promising biosynthetic pathways, it still remains challenging to genetically engineer streptomycetes and apply systems metabolic engineering approaches to induce expression of these gene clusters or optimize the production in these strains [4].

Here, we present a CRISPR/Cas9-based toolkit for streptomycetes and other actinomycetes that allows (i) the generation of a library of mixed-size deletion mutants around a defined target site, (ii) the highly efficient generation of mutants in specific targeted genes, and (iii) the repression of transcription using a "dead-Cas9" variant of the CRISPR system [5].
In addition, we present CRISPy-web (http://crispy.secondarymetabolites.org), a publicly accessible web-service to design single guide-RNAs (sgRNAs) for a wide variety of CRISPR applications [6]. In contrast to most other guide-RNA design tools, CRISPy-web is not limited to pre-selected model organisms but can be used to identify suitable Cas9 target regions in any user-submitted microbial genome.

Keywords: *Streptomyces*, actinomycetes, CRISPR, metabolic engineering, sgRNA, bioinformatics

References:

[1] *Weber T, Blin K, Duddela S, Krug D, Kim HU, Bruccoleri R, Lee SY, Fischbach MA, Müller R, Wohlleben W, Breitling R, Takano E, Medema MH*. antiSMASH 3.0-a comprehensive resource for the genome mining of biosynthetic gene clusters. Nucleic Acids Res 2015; 43: W237-W243
[2] *Blin K, Medema MH, Kazempour D, Fischbach M, Breitling R, Takano E, Weber T.* antiSMASH 2.0 – a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res 2013; 41: W204-W212
[3] *Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R*. antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res 2011; 39: W339-W346
[4] *Weber T, Charusanti P, Musiol-Kroll EM, Jiang X, Tong Y, Kim HU, Lee SY*. Metabolic engineering of antibiotic factories: new tools for antibiotic production in actinomycetes. Trends Biotechnol 2015; 33: 15-26

[5] *Tong Y, Charusanti P, Zhang L, Weber T, Lee SY*. CRISPR-Cas9 based engineering of actinomycetal genomes. ACS Synth Biol 2015; 4: 1020-1029
[6] *Blin K, Pedersen LE, Weber T, Lee SY*. CRISPy-web: An online resource to design sgRNAs for CRISPR applications. Synth Syst Biotechnol 2016, doi: 10.1016/j.synbio.2016.01.003

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