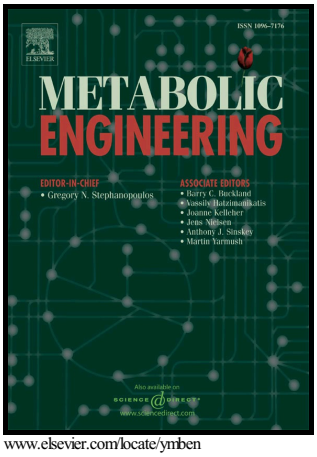

www.elsevier.com/locate/ymben

Secondary metabolites overproduction through transcriptional gene cluster refactoring

Liliya Horbal, Filipe Marques, Suvd Nadmid, Marta V. Mendes, Andriy Luzhetskyy

PII: S1096-7176(18)30281-7
DOI: https://doi.org/10.1016/j.ymben.2018.09.010
Reference: YMBEN1471

To appear in: *Metabolic Engineering*

Received date: 13 July 2018
Revised date: 17 September 2018
Accepted date: 17 September 2018

Cite this article as: Liliya Horbal, Filipe Marques, Suvd Nadmid, Marta V. Mendes and Andriy Luzhetskyy, Secondary metabolites overproduction through transcriptional gene cluster refactoring, *Metabolic Engineering,* https://doi.org/10.1016/j.ymben.2018.09.010

Secondary metabolites overproduction through transcriptional gene cluster refactoring

Liliya Horbal[1], Filipe Marques[1,3,4], Suvd Nadmid[1], Marta V. Mendes[3,4], Andriy Luzhetskyy[1,2]*

[1]*Department Microbial Natural Products, Actinobacteria Metabolic Engineering Group, Saarland University, Campus C2.3, 66123, Saarbrücken, Germany*

[2]*Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS) Helmholtz Center for Infectious Research (HZI)*

[3] *i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal*

[4] *IBMC - Instituto de Biologia Molecular e Celular, Universidade do Porto, Porto, Portugal*

*E-mail: a.luzhetskyy@mx.uni-saarland.de

ABSTRACT

We present a random rational approach enabling the construction of overproducing strains in two steps. The approach first involves creating a library of clusters of interest, in which native promoters are substituted with randomly generated constitutive synthetic promoters, and then expressing this library in an appropriate host strain. This strategy is fast, easy to use, accounts for the architecture of a cluster and completely decouples the expression of a gene cluster from complex native regulatory networks. The strategy was applied to improve the production of a macrocyclic peptide, bottromycin, which possesses antibacterial activity against multidrug-resistant bacteria and is a blueprint for a new class of antibacterials. We successfully optimized the expression of genes in operons and created several variants of the bottromycin gene cluster that provide 5-50 fold higher titres of bottromycin than the natural one, thus resulting in the identification of several new bottromycin derivatives not previously described. Moreover, due to the higher bottromycin yield, bottromycin derivatization was performed via the biosynthetic engineering of the gene cluster. The abovementioned features make this generic