Dissemination of antibiotic resistance genes from antibiotic producers to pathogens

It has been hypothesized that some antibiotic resistance genes (ARGs) found in pathogenic bacteria derive from antibiotic-producing actinobacteria. Here we provide bioinformatic and experimental evidence supporting this hypothesis. We identify genes in proteobacteria, including some pathogens, that appear to be closely related to actinobacterial ARGs known to confer resistance against clinically important antibiotics. Furthermore, we identify two potential examples of recent horizontal transfer of actinobacterial ARGs to proteobacterial pathogens. Based on this bioinformatic evidence, we propose and experimentally test a 'carry-back' mechanism for the transfer, involving conjugative transfer of a carrier sequence from proteobacteria to actinobacteria, recombination of the carrier sequence with the actinobacterial ARG, followed by natural transformation of proteobacteria with the carrier-sandwiched ARG. Our results support the existence of ancient and, possibly, recent transfers of ARGs from antibiotic-producing actinobacteria to proteobacteria, and provide evidence for a defined mechanism.
The aldehyde dehydrogenase, AldA, is essential for L-1,2-propanediol utilization in laboratory-evolved Escherichia coli

Most Escherichia coli strains are naturally unable to grow on 1,2-propanediol (PDO) as a sole carbon source. Recently, however, a K-12 descendent E. coli strain was evolved to grow on 1,2-PDO, and it was hypothesized that this evolved ability was dependent on the aldehyde dehydrogenase, AldA, which is highly conserved among members of the family Enterobacteriacea. To test this hypothesis, we first performed computational model simulation, which confirmed the essentiality of the aldA gene for 1,2-PDO utilization by the evolved PDO-degrading E. coli. Next, we deleted the aldA gene from the evolved strain, and this deletion was sufficient to abolish the evolved phenotype. On re-introducing the gene on a plasmid, the evolved phenotype was restored. These findings provide experimental evidence for the computationally predicted role of AldA in 1,2-PDO utilization, and represent a good example of E. coli robustness, demonstrated by the bacterial deployment of a generalist enzyme (here AldA) in multiple pathways to survive carbon starvation and to grow on a non-native substrate when no native carbon source is available.
Metabolic engineering with systems biology tools to optimize production of prokaryotic secondary metabolites

Metabolic engineering using systems biology tools is increasingly applied to overproduce secondary metabolites for their potential industrial production. In this Highlight, recent relevant metabolic engineering studies are analyzed with emphasis on host selection and engineering approaches for the optimal production of various prokaryotic secondary metabolites: native versus heterologous hosts (e.g., Escherichia coli) and rational versus random approaches. This comparative analysis is followed by discussions on systems biology tools deployed in optimizing the production of secondary metabolites. The potential contributions of additional systems biology tools are also discussed in the context of current challenges encountered during optimization of secondary metabolite production.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds, Korea Advanced Institute of Science & Technology
Authors: Kim, H. U. (Intern), Charusanti, P. (Intern), Lee, S. Y. (Intern), Weber, T. (Intern)
Number of pages: 9
Pages: 933-941
Publication date: 2016
Main Research Area: Technical/natural sciences

Publication information
Journal: Natural Product Reports
Volume: 33
ISSN (Print): 0265-0568
Ratings:
BFI (2018): BFI-level 2
BFI (2017): BFI-level 2
Web of Science (2017): Indexed Yes
BFI (2016): BFI-level 2
Scopus rating (2016): SJR 3.458 SNIP 3.136 CiteScore 9.33
Activities:
The next generation of ‘omics-based natural products discovery

CRISPR-Cas9 Based Engineering of Actinomycetal Genomes
Bacteria of the order Actinomycetales are one of the most important sources of pharmacologically active and industrially relevant secondary metabolites. Unfortunately, many of them are still recalcitrant to genetic manipulation, which is a bottleneck for systematic metabolic engineering. To facilitate the genetic manipulation of actinomycetes, we developed a highly efficient CRISPR-Cas9 system to delete gene(s) or gene cluster(s), implement precise gene replacements, and reversibly control gene expression in actinomycetes. We demonstrate our system by targeting two genes, actIORF1 (SCO5087) and actVB (SCO5092), from the actinorhodin biosynthetic gene cluster in Streptomyces coelicolor A3(2). Our CRISPR-Cas9 system successfully inactivated the targeted genes. When no templates for homology-directed repair (HDR) were present, the site-specific DNA double-strand breaks (DSBs) introduced by Cas9 were repaired through the error-prone nonhomologous end joining (NHEJ) pathway, resulting in a library of deletions with variable sizes around the targeted sequence. If templates for HDR were provided at the same time, precise deletions of the targeted gene were observed with near 100% frequency. Moreover, we developed a system to efficiently and reversibly control expression of
target genes, deemed CRISPRi, based on a catalytically dead variant of Cas9 (dCas9). The CRISPR-Cas9 based system described here comprises a powerful and broadly applicable set of tools to manipulate actinomycetal genomes.

**General information**

**State:** Published

**Organisations:** Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds, Chinese Academy of Sciences

**Authors:** Tong, Y. (Intern), Charusanti, P. (Intern), Zhang, L. (Ekstern), Weber, T. (Intern), Lee, S. Y. (Intern)

**Number of pages:** 10

**Pages:** 1020-1029

**Publication date:** 2015

**Main Research Area:** Technical/natural sciences

**Publication information**

**Journal:** ACS Synthetic Biology

**Volume:** 4

**Issue number:** 9

**ISSN (Print):** 2161-5063

**Ratings:**

Web of Science (2017): Indexed yes

Scopus rating (2016): CiteScore 4.7 SJR 2.736 SNIP 1.024

Web of Science (2016): Indexed yes

Scopus rating (2015): SJR 2.269 SNIP 1.049 CiteScore 4.41

Web of Science (2015): Indexed yes

Scopus rating (2014): SJR 3.783 SNIP 1.219 CiteScore 3.84

Web of Science (2014): Indexed yes

Scopus rating (2013): SJR 1.796 SNIP 0.859 CiteScore 3.42

ISI indexed (2013): ISI indexed yes

ISI indexed (2012): ISI indexed no

**Original language:** English

**CRISPR-Cas9, CRISPRi, DNA repair, Actinomycetes, Genome engineering**

**DOIs:**

10.1021/acssynbio.5b00038

**Relations**

**Activities:**

Tools for the genomics driven discovery and engineering of natural products

The next generation of ‘omics-based natural products discovery

In silico and CRISPR/Cas9-based tools for the metabolic engineering of actinomycetes

2nd European Conference on Natural Products

VAAM Workshop on the Biology of Bacteria Producing Natural Products

Source: PublicationPreSubmission

Source-ID: 110808989

Publication: Research - peer-review › Journal article – Annual report year: 2015

**Metabolic engineering of antibiotic factories: New tools for antibiotic production in actinomycetes**

Actinomycetes are excellent sources for novel bioactive compounds, which serve as potential drug candidates for antibiotics development. While industrial efforts to find and develop novel antimicrobials have been severely reduced during the past two decades, the increasing threat of multidrug-resistant pathogens and the development of new technologies to find and produce such compounds have again attracted interest in this field. Based on improvements in whole-genome sequencing, novel methods have been developed to identify the secondary metabolite biosynthetic gene clusters by genome mining, to clone them, and to express them in heterologous hosts in much higher throughput than before. These technologies now enable metabolic engineering approaches to optimize production yields and to directly manipulate the pathways to generate modified products.

**General information**

**State:** Published

**Organisations:** Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds

**Authors:** Weber, T. (Intern), Charusanti, P. (Intern), Musiol-Kroll, E. M. (Intern), Jiang, X. (Intern), Tong, Y. (Intern), Kim, H. U. (Intern), Lee, S. Y. (Intern)

**Number of pages:** 12

**Pages:** 15-26
Model-driven discovery of synergistic inhibitors against *E. coli* and *S. enterica* serovar Typhimurium targeting a novel synthetic lethal pair, *aldA* and *prpC*

Mathematical models of biochemical networks form a cornerstone of bacterial systems biology. Inconsistencies between simulation output and experimental data point to gaps in knowledge about the fundamental biology of the organism. One such inconsistency centers on the gene *aldA* in *Escherichia coli*: it is essential in a computational model of *E. coli* metabolism, but experimentally it is not. Here, we reconcile this disparity by providing evidence that *aldA* and *prpC* form a synthetic lethal pair, as the double knockout could only be created through complementation with a plasmid-borne copy of *aldA*. Moreover, virtual and biological screening against the two proteins led to a set of compounds that inhibited the growth of *E. coli* and *Salmonella enterica* serovar Typhimurium synergistically at 100-200 μM individual concentrations. These results highlight the power of metabolic models to drive basic biological discovery and their potential use to discover new combination antibiotics.
Antibiotic development, Bacterial metabolism, Drug discovery, Metabolic reconstruction, Model-based drug target discovery, Pathway gap filling, Synthetic lethality, Systems biology

Electronic versions:
Model_driven_discovery_of.pdf
DOIs:
10.3389/fmicb.2015.00958

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Source: FindIt
Source-ID: 2281847996
Publication: Research - peer-review › Journal article – Annual report year: 2015

Systems biology-guided identification of synthetic lethal gene pairs and its potential use to discover antibiotic combinations
Mathematical models of metabolism from bacterial systems biology have proven their utility across multiple fields, for example metabolic engineering, growth phenotype simulation, and biological discovery. The usefulness of the models stems from their ability to compute a link between genotype and phenotype, but their ability to accurately simulate gene-gene interactions has not been investigated extensively. Here we assess how accurately a metabolic model for Escherichia coli computes one particular type of gene-gene interaction, synthetic lethality, and find that the accuracy rate is between 25% and 43%. The most common failure modes were incorrect computation of single gene essentiality and biological information that was missing from the model. Moreover, we performed virtual and biological screening against several synthetic lethal pairs to explore whether two-compound formulations could be found that inhibit the growth of Gram-negative bacteria. One set of molecules was identified that, depending on the concentrations, inhibits E. coli and S. enterica serovar Typhimurium in an additive or antagonistic manner. These findings pinpoint specific ways in which to improve the predictive ability of metabolic models, and highlight one potential application of systems biology to drug discovery and translational medicine.

General information
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds, Cairo University, University of California, Albany Molecular Research Inc., Albany Molecular Research Singapore Research Centre, Pte. Ltd.
Number of pages: 12
Publication date: 2015
Main Research Area: Technical/natural sciences

Publication information
Journal: Scientific Reports
Volume: 5
Article number: 16025
ISSN (Print): 2045-2322
Ratings:
BFI (2018): BFI-level 1
BFI (2017): BFI-level 1
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 4.63 SJR 1.625 SNIP 1.401
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 2.057 SNIP 1.684 CiteScore 5.3
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.103 SNIP 1.544 CiteScore 4.75
Web of Science (2014): Indexed yes
Streptomyces species continue to attract attention as a source of novel medicinal compounds. Despite a long history of studies on these microorganisms, they still have many biochemical mysteries to be elucidated. Investigations of novel secondary metabolites and their biosynthetic gene clusters have been more systematized with high-throughput techniques through inspections of correlations among components of the primary and secondary metabolisms at the genome scale. Moreover, up-to-date information on the genome of Streptomyces species with emphasis on their secondary metabolism has been collected in the form of databases and knowledgebases, providing predictive information and enabling one to explore experimentally unrecognized biological spaces of secondary metabolism. Herein, we review recent trends in the systems biology and biotechnology of Streptomyces species.

**General information**
State: Published
Organisations: Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds, Network Reconstruction in Silico Biology, Korea Advanced Institute of Science & Technology
Authors: Hwang, K. (Ekstern), Kim, H. U. (Intern), Charusanti, P. (Intern), Palsson, B. (Intern), Yup Lee, S. (Intern)
Pages: 255-268
Publication date: 2014
Main Research Area: Technical/natural sciences

**Publication information**
Journal: Biotechnology Advances
Volume: 32
Issue number: 2
ISSN (Print): 0734-9750
Ratings:
BFI (2018): BFI-level 2
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 11.05 SJR 2.681 SNIP 3.146
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 2.919 SNIP 3.432 CiteScore 10.56
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 2.922 SNIP 3.757 CiteScore 10.24
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 2.936 SNIP 4.028 CiteScore 10.71
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
Genome-scale metabolic reconstructions of multiple Escherichia coli strains highlight strain-specific adaptations to nutritional environments.

Genome-scale models (GEMs) of metabolism were constructed for 55 fully sequenced Escherichia coli and Shigella strains. The GEMs enable a systems approach to characterizing the pan and core metabolic capabilities of the E. coli species. The majority of pan metabolic content was found to consist of alternate catabolic pathways for unique nutrient sources. The GEMs were then used to systematically analyze growth capabilities in more than 650 different growth-supporting environments. The results show that unique strain-specific metabolic capabilities correspond to pathotypes and environmental niches. Twelve of the GEMs were used to predict growth on six differentiating nutrients, and the predictions were found to agree with 80% of experimental outcomes. Additionally, GEMs were used to predict strain-specific auxotrophies. Twelve of the strains modeled were predicted to be auxotrophic for vitamins niacin (vitamin B3), thiamin (vitamin B1), or folate (vitamin B9). Six of the strains modeled have lost biosynthetic pathways for essential amino acids methionine, tryptophan, or leucine. Genome-scale analysis of multiple strains of a species can thus be used to define the metabolic essence of a microbial species and delineate growth differences that shed light on the adaptation process to a particular microenvironment.
Issue number: 50
ISSN (Print): 0027-8424
Ratings:
BFI (2018): BFI-level 2
BFI (2017): BFI-level 2
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 8.56 SJR 6.321 SNIP 2.629
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 6.767 SNIP 2.682 CiteScore 8.84
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 6.853 SNIP 2.725 CiteScore 8.86
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 6.989 SNIP 2.73 CiteScore 9.5
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 6.792 SNIP 2.682 CiteScore 9.49
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 6.771 SNIP 2.636 CiteScore 9.31
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 6.769 SNIP 2.529
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 6.913 SNIP 2.544
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 6.899 SNIP 2.445
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 6.766 SNIP 2.441
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 6.734 SNIP 2.434
Web of Science (2006): Indexed yes
Scopus rating (2005): SJR 6.784 SNIP 2.551
Web of Science (2005): Indexed yes
Scopus rating (2004): SJR 7.026 SNIP 2.622
Web of Science (2004): Indexed yes
Scopus rating (2003): SJR 7.018 SNIP 2.501
Web of Science (2003): Indexed yes
Scopus rating (2002): SJR 7.183 SNIP 2.471
Web of Science (2002): Indexed yes
Scopus rating (2001): SJR 7.192 SNIP 2.463
Web of Science (2001): Indexed yes
Scopus rating (2000): SJR 7.731 SNIP 2.475
Web of Science (2000): Indexed yes
Scopus rating (1999): SJR 8.271 SNIP 2.446
Projects:

**Integration of Informatics and Metabolic Engineering for the discovery of Novel Antibiotics**

Novo Nordisk Foundation Center for Biosustainability

New Bioactive Compounds

Network Reconstruction in Silico Biology

Research Groups

Bacterial Cell Factory Optimization

Fundación MEDINA

Korea Advanced Institute of Science and Technology (KAIST)

Period: 01/03/2017 → 31/03/2023

Number of participants: 12

Acronym: iimena

Project participant:

Weber, Tilmann (Intern)

Palsson, Bernhard (Intern)

Charusanti, Pep (Intern)

Jiang, Xinglin (Intern)

Damborg, Mie (Intern)

Durczak, Oliwia (Intern)

Kontou, Eftychia Eva (Intern)

Lizak, Dawid Mariusz (Intern)

Beck, Charlotte (Intern)

Kjiproski, Darko (Intern)

Rasmussen, Birte Kastrup (Intern)

Project Manager, organisational:

Lohmann, Ricarda (Intern)

Financing sources

Source: Forsk. Private danske - Fonde

Name of research programme: Novo Nordisk Foundation Challenge Program

Web address: http://www.novonordiskfonden.dk

Amount: 58,832,942.00 Danish Kroner

Year of approval: 2017

Relations

Activities:

Lectures on antibiotics biosynthesis: polyketides, aminoglycosides, RiPPs and others

Generation of click-able kirromycin derivatives by exploiting the substrate promiscuity of the discrete acyl transferase KirCII

In silico and experimental approaches to understand and engineer the biosynthesis of antibiotics

In silico and experimental approaches to understand and engineer the biosynthesis of antibiotics

Publications:

Dissemination of antibiotic resistance genes from antibiotic producers to pathogens

Press / Media items:

Video and Blog-post / interview at sciencenews.dk on iimena project (NNF Challenge Grant)

Millions for research into antibiotic resistance and better drugs
Scientists solve 30-year old mystery on how resistance genes spread
Research program on new antibiotics receives 58 M DKK

Press clippings:

**Millions for research into antibiotic resistance and better drugs**
Tilmann Weber, Pep Charusanti, Sang Yup Lee & Bernhard Palsson
26/01/2017 → 26/12/2017

**Description**
DTU Press release on NNF Challenge grants, including IIMENA
Network Reconstruction in Silico Biology, Novo Nordisk Foundation Center for Biosustainability, Big Data 2 Knowledge, New Bioactive Compounds

**Media coverage (2)**

**Novo-millioner skal gøre lægemidler bedre**
26/12/2017
medwatch.dk (National), Denmark, Web
LONNI PARK LYNGE
http://medwatch.dk/secure/Medicinal___Biotek/article9319828.ece
Tilmann Weber, Pep Charusanti, Sang Yup Lee & Bernhard Palsson
Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds, Big Data 2 Knowledge, Network Reconstruction in Silico Biology

**GODT NYT I KAMPEN MOD ANTIBIOTIKARESISTENS**
30/01/2017
Dansk Kemi (National), Denmark, Web
http://www.kemifokus.dk/godt-nyt-i-kampen-mod-antibiotikaresistens/
Tilmann Weber, Pep Charusanti, Sang Yup Lee & Bernhard Palsson
Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds, Big Data 2 Knowledge, Network Reconstruction in Silico Biology

**Media contributions (2)**

**Millions for research into antibiotic resistance and better drugs**
26/01/2017
DTU Homepage (International), Denmark, Web
Viebeke Hempler
http://www.dtu.dk/english/news/nyhed?id=39201475-593e-41c9-b63e-08c790731768
Tilmann Weber, Pep Charusanti, Sang Yup Lee & Bernhard Palsson
Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds, Big Data 2 Knowledge, Network Reconstruction in Silico Biology

**SPIILDEVAND FRA 100 LANDE SKAL BIDRAGE TIL BEGRÆNSE ANTIBIOTIKA-RESISTENS**
26/01/2017
NNF Homepage (International), Denmark, Web
http://novonordiskfonden.dk/da/content/spildevand-fra-100-lande-skal-bidrage-til-begraense-antibiotika-resistens
Press release on 2017 NNF Challenge Grants by Novo Nordisk Foundation
Tilmann Weber, Sang Yup Lee, Pep Charusanti & Bernhard Palsson
Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds, Big Data 2 Knowledge, Network Reconstruction in Silico Biology

**Relations**
Projects:
Integration of Informatics and Metabolic Engineering for the discovery of Novel Antibiotics
Press / Media

**Researchers hunt for tomorrow's antimicrobial agents in the Christiania topsoil**
Pep Charusanti
13/11/2017

**Description**
Description of research activities carried out by the New Bioactive Compounds (NBC) section. Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds

Media contribution (1)

Researchers hunt for tomorrow's antimicrobial agents in the Christiania topsoil
13/11/2017
DTU (International), Denmark, Web
Anne Lykke
Description of research done by the NBC group. Written for a general, non-scientific audience.
Pep Charusanti
Press / Media

Scientists solve 30-year old mystery on how resistance genes spread
15/06/2017
Description
Press release on our Nature Communication paper on the dissemination of antibiotic resistance genes; Press release / article covered by multiple news outlets, blogs and individual tweeters
(https://www.nature.com/articles/ncomms15784/metrics )
Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds, Research Groups, Bacterial Synthetic Biology, Department of Biotechnology and Biomedicine

Media contribution (1)

Scientists solve 30-year old mystery on how resistance genes spread
15/06/2017
DTU Biosustain Homepage (International), Denmark, Web
Anne Wärme Lykke
http://www.biosustain.dtu.dk/english/nyhedsbase/2017/06/antibiotic-genes?id=9b924680-693f-47e2-8d7f-03c6cecfe473
Novo Nordisk Foundation Center for Biosustainability, New Bioactive Compounds, Department of Biotechnology and Biomedicine, Bacterial Synthetic Biology, Research Groups

Relations
Research outputs:
Dissemination of antibiotic resistance genes from antibiotic producers to pathogens
Projects:
Integration of Informatics and Metabolic Engineering for the discovery of Novel Antibiotics
Press / Media