Engineering *Corynebacterium glutamicum* with a comprehensive cosmid library and phage-based vectors

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Meet *Corynebacterium glutamicum*

an **actinobacterium**, like ***Streptomyces* spp.**,

useful for the **food & feed** industries...

Discovery of *Corynebacterium glutamicum.* This organism was firstly isolated from a soil sample from a Tokyo Zoo (Kinoshita *et al.,* 1957). Ironically, the industrial production of L-lysine by *C. glutamicum* provides the essential amino acid to feed poultry and pigs. Painting: "Girl feeding pigs" by Richard Westall (1800).

Molecular tools for *C. glutamicum.* One of the first genetic manipulations of *C. glutamicum* was transformation with a modified plasmid (Santamaría *et al.,* 1984). The dawn of genetic engineering in *C. glutamicum* revisits the primitive yet essential first steps in agriculture for Humanity. Photography: tools from the Bronze Age.

but also as **heterologous host,**

thanks to its **genetic ameaneability**

and the availability of **molecular tools**

e.g. plasmids and programmable endonucleases.

Since the current technologies of

gene editing and gene delivery in *C. glutamicum*

often require

Lasting selection, e.g. expression plasmids

Long preparations periods, e.g. designing repair templates

Laborious mutant screening, e.g. after Cas9 gene editing

We developed

A NOVEL PLATFORM FOR GENE EDITING AND PHAGE-BASED INTEGRATIVE SYSTEMS

for *Corynebacterium glutamicum*

Featuring dedicated molecular tools

Cosmid library of *C. glutamicum*, representing >94% of the chromosome

Markerless **gene knock-out,** using cosmids modified with excisable markers

Presenting a new microbial factory

***Corynebacterium glutamicum* BCA strain**

with a reduced metabolic footprint

with integration sites for plasmids based in the actinophages φBT1 and φC31

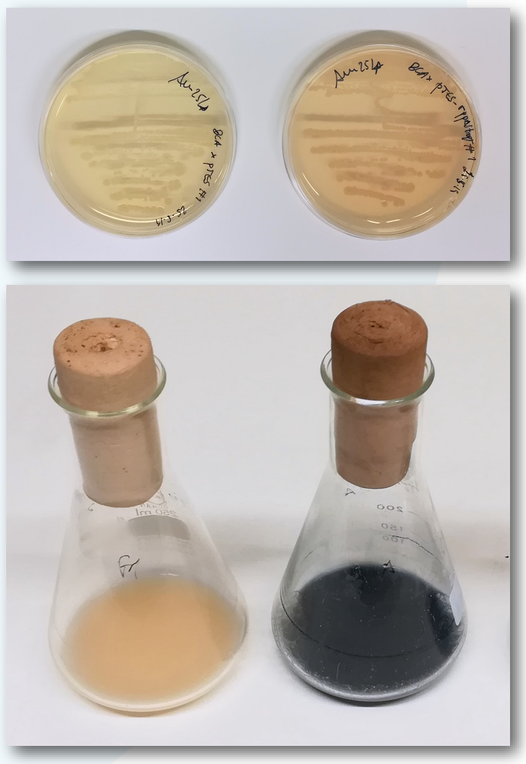
Showcasing the production of specialized metabolites

40 mg L^{-1} polyketide flaviolin, in 72 hours

sustained by a synthetic *rppA* gene, delivered via φC31-based system

7.3 g L^{-1} non-ribosomal peptide indigoidine, in 72 hours

sustained by a modified *bpsA* gene, delivered via φBT1-based system



Heterologous production of specialized metabolites in *C. glutamicum* BCA strain. Strains producing the red pigment flaviolin (top right) and blue pigment indigoidine (bottom right) compare to the control strains (top and bottom left). CC-BY Filipe Marques

Keywords

Actinobacteria

Genome editing

Natural products

Heterologous expression

Summary

There is a need for efficient methods of genetic manipulation of actinobacteria.

We developed gene editing and delivery systems to engineer the industrial host *Corynebacterium glutamicum*.

As a result, we were able to implement the heterologous production of two classes of specialized metabolites.

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