TECHNISCHE UNIVERSITÄT DRESDEN

DRESDEN concept

Chair of Bioprocess Engineering

Engineering of DHB resistance

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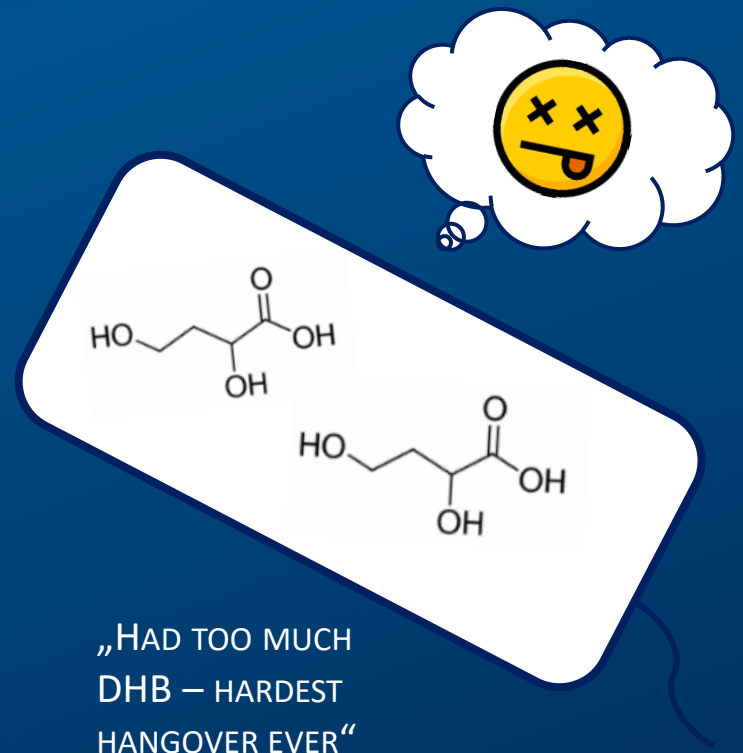


Context and motivation

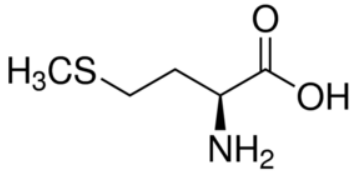
Sustainable production of amino acids

Most amino acids are currently synthesized by fermentation processes

— Exception: (L)-methionine

— Important supplement for animal nutrition

— Production volume reached 1 million tons in 2014



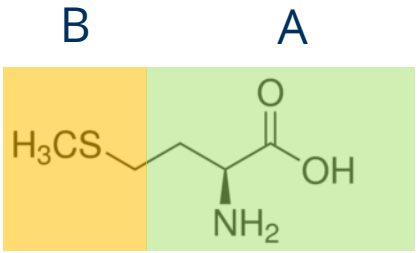
Methionine production is dominated by petroleum based synthesis

— High metabolic production costs in wild type *E. coli*

Establishment of a two step process for microbial production

1) Production of a **functional carbohydrate** in *E. coli* **(A)**

2) Chemical incorporation of sulphur to form (L)-methionine **(B)**



Context and motivation

Sustainable production of amino acids

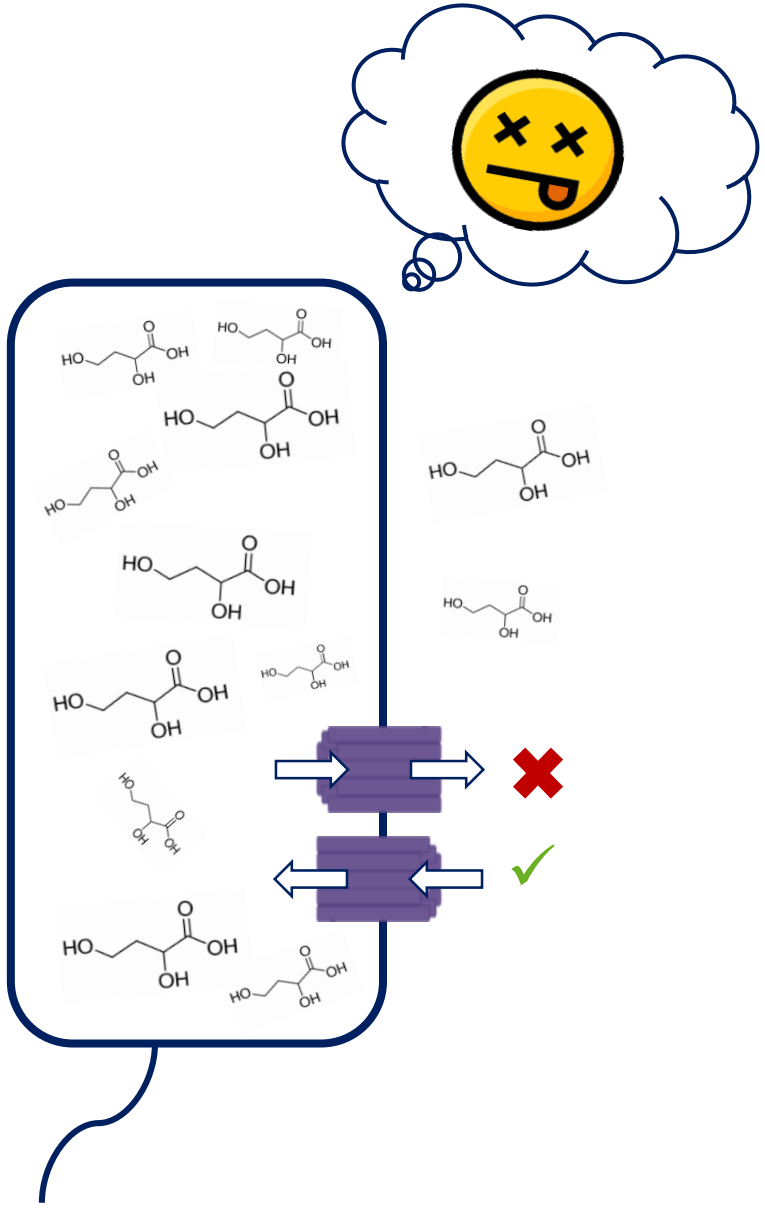
Functional carbohydrate is 2,4-Dihydroxybutyrate (DHB)

— Naturally not produced in *E. coli*

— Produced via a synthetic pathway

— Accumulation of DHB is toxic

— No transporter proteins known to export DHB



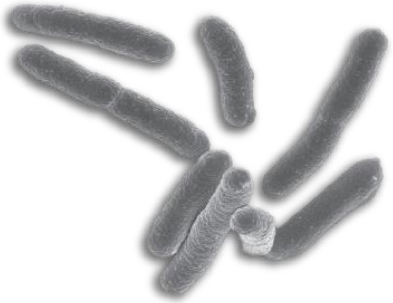
Central idea of the project

— Identification of a DHB exporting permease

— Strain engineering to reduce the toxic effect of DHB

Applied techniques

…mainly molecular biological methods



Strain engineering via homologous recombination

— Deletion of genes in the *E. coli* genome

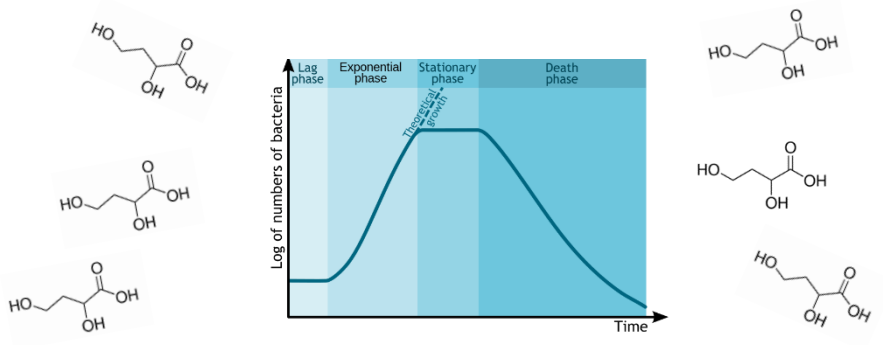
— Overexpression of genes via promoter exchange

Molecular biology

— Plasmid construction via PCR / Gibson assembly

— Creation of genomic libraries of various species

— DeepSequecncing analysis



Growth experiments

— Determination of specific growth rates

State of the project
Engineering DHB resistance

✓ **Successful construction and application of genomic libraries**

— *E. coli* strains harboring genomic library plasmids were able to grow at much higher DHB concentrations

✓ **Identification of genes which contribute to DHB resistance**

— Introduction of additional gene copies resulted in a significant increase in DHB tolerance in *E. coli*

— Partial elucidation of the toxic effect of DHB

— **More clones need to be screened and new libraries have to be tested!**

Want to join…?

Here’s what you could do

Learn the basic stuff - construction of genomic libraries

— DNA of various bacteria will be used used to screen a lot of genes which are not native to *E. coli*

Functionalization of libraries of distantly related bacteria of *E. coli*.

— … cause there actually is some kind of language barrier

Making the screening procedure big via DeepSequencing approach

— Allows to screen thousands and millions of clones