Engineering of genetic tools to optimize heterologous gene expression in *Arthrospira platensis* (Spirulina).

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Matrix Genetics: High value products team

Cyanobacteria

Recombinant DNA

Arthrospira platensis (Spirulina)

Spirulina extract

Natural color

Biopharmaceutical (Vaccine)
Three strains have been transformed at Matrix Genetics, indicating our approach to genetically modify Spirulina is versatile.

Arthospira sp.  
Arthospira platensis  
Arthospira platensis

Generally recognized as safe (GRAS)
Matrix technology introduced stable genetic modification.

Arthrospira chromosome

\[ \text{cpcB} \quad \text{cpcA} \]

Genotyping

Extracts from strains cultured for > 6 months

\[ +\text{cpcB} \quad \text{cpcA} \]

10-L photobioreactor

WT

Transformed

C-phycoecyanin

Allophycocyanin

\[ \text{WT} \quad \text{Transformed} \]

\% (w/w)
Promoter engineering to optimize heterologous gene expression

Transcriptome analysis by RNA-seq revealed transcriptional levels and transcription start sites of the individual Spirulina genes.

Heterologous gene expression

- Non-toxic, and well tolerated
  - Strong promoter
- Toxic, and/or reduced growth rate
  - Medium/weak promoter

Transcriptome by RNA-seq
Activities of promoter sequences taken from the *Arthrospira* chromosome

RNA-seq data helps to utilize native promoters for heterologous gene expression.
Spirulina as an oral vaccine platform

▼ Capable of accumulating antigen at a high level (>> 0.1 % of dry biomass), because a content of total protein is 60 % of dry biomass.

▼ Safe to be orally administered: no purification is required.

▼ Costs << 1 cent/dose.

▼ Might enhance the immunogenicity of recombinant vaccines, because the whole biomass contains bacterial DNA, RNA and glycolipid (which all can stimulate the innate immunity of host cells).
Spirulina as an oral vaccine platform

Disease-causing viruses/bacteria

Viral surface protein

Protein domain

Epitope

Immunogenic vaccine adjuvant/carrier expressed in Spirulina

Live stock
Three types of vaccine adjuvants/carriers can stimulate the immune responses.

- Viral-like particle
- Bacterial flagellin
- Non-toxic subunit of enterotoxin
Vaccine adjuvants/carriers

All the three types of vaccine carriers have been successfully expressed under a constitutive promoter in Spirulina.

- Bacterial flagellin
- Non-toxic subunit of enterotoxin
- Viral-like particle

Engineered variants
Antigen-displaying adjuvant expressed in Spirulina

The viral-like particles well tolerated insertion of antigenic sequences in Spirulina cells.

Western blotting
Matrix technology facilitates stable genetic modification of Spirulina.

Promoters derived from the Spirulina chromosome (identified through transcriptome analysis) are capable of driving heterologous gene expression.

Three types of vaccine adjuvant/carrier proteins can be expressed constitutively in Spirulina.
Thank you!