

Rational Engineering of Advanced *Clostridia* for Transformational Improvements in Fermentation

Project acronym: REACTIF

Project no: EIB.12.050

Name Dr. Preben Krabben

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Project partners

- *Green Biologics Ltd, United Kingdom*
- *University of Nottingham, United Kingdom*
- *University of Ulm, Germany*
- *Georg-August-University Göttingen, Germany*
- *Weyland, Norway*
- *Borregaard, Norway*

- *Total project budget: 2187000 Euros*

Introduction



- *Project objectives (problem to be solved)*
  - *Develop advanced clostridial production strains which can convert non-food cellulosic feedstocks to acetone and n-butanol.*
- *General project approach*
  - *Identification of genetic traits enriched in strains used commercially for butanol production over 40 years, but also new traits generated by adaptive laboratory evolution.*
  - *Transfer of genetic traits into production strains.*

Technical overview



- *Screening of cellulosic hydrolysate from Weyland and Borregaard.*
- *Evaluation of the performance of historical production strains on sucrose.*
- *Selection of mutants for faster utilization of xylose, co-utilization of xylose and glucose, and butanol tolerance.*
- *Sequencing of up to 28 different wild-type solvent producing clostridia strains and several evolved strains with new traits.*
- *Transcriptome analysis of solventogenesis in solventogenic clostridia and detection of possible anti-sense RNA regulation and small non-coding RNA.*

Technical overview



- *Development of a genetic modification protocol for a previously non-genetic tractable butanol production strains.*
- *Re-engineering of evolved traits into clean strain backgrounds.*

Summary



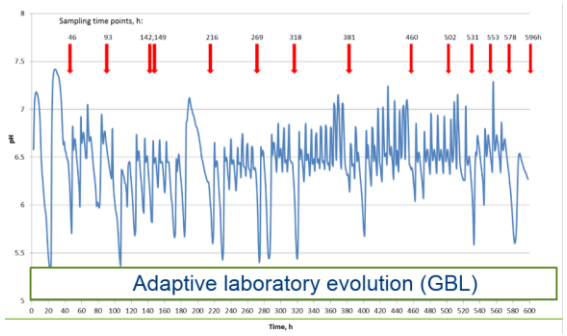
- *What was achieved*
  - *Traits identified which allows better conversion of sugars to butanol.*
  - *Adaptive laboratory evolution protocol, which only introduces few and beneficial mutations.*
  - *Better genetic understanding of Clostridial solvent producers.*
  - *Targeted genetic modification protocol developed for an industrial production strain, which was previously un-tractable.*
  - *Identification of a clostridial compatible hydrolysis process for lignocellulosic feedstocks.*

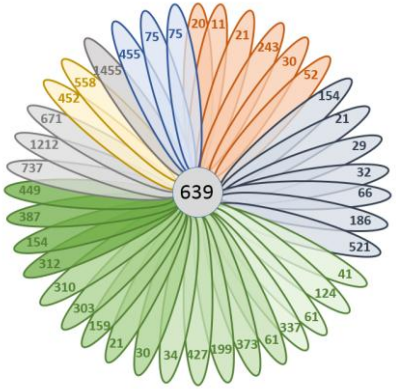
Summary



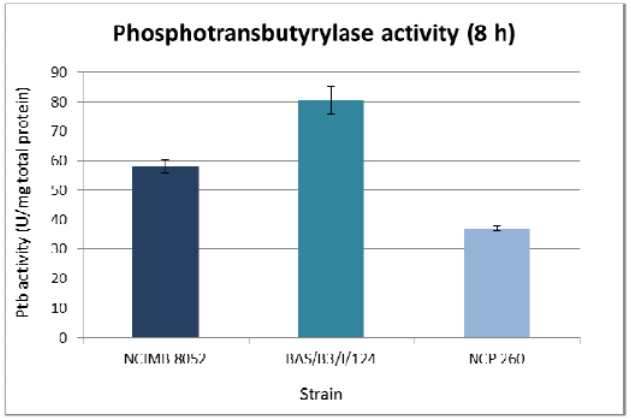
- *What are the plans for future (any follow-up projects?)*
  - *Start-up of commercial bio-butanol production*
  - *ButaNext: Next Generation Bio-butanol (Horizon 2020 LCE11)*
  - *W2Bu: Cost Competitive Conversion of Municipal Solid Waste to Advanced biofuels (ERA-Net + BESTF)*
  - *BIOFOREVER: BIO-based products from FORestry via Economically Viable European Routes (Horizon 2020)*

Project outcome





Genome comparison (G2L)



Enzyme activities (Ulm)

Clostridium transformation (CRG)

Project outcome

- *Implementation and exploitation of results*
  - *De-risking the cellulosic butanol production by initiating sugar to butanol at CMR.*

General Evaluation



- *The project has provided the means to work with world class research groups with the EU.*
- *So far 3 publications has been published, an additional publication accepted and one is in active preparation.*
- *Green Biologics is taken part in a University of Nottingham led Marie Sklodowska Curie Innovative Training Networks (ITN)*

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