Enzyme Engineering: a key technology for innovation in bio-economy

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The bio-based economy

Increasing demand for products made from renewable raw materials by biotechnological production processes

Technology Push
Industrial biotechnology

Economic Value
resources / cost

Market Pull
sustainability
Enzymes have found their way into processes and products in several application areas:

**Enzyme uses in consumer-near applications**

- **Bioprocessed chemicals**
  - Renewable chemicals / biopolymers
  - Food & Feed Additives
  - Small molecule APIs
  - Other fine & specialty chemicals

- **Industrial enzymes**
  - Food enzymes
  - Feed enzymes
  - Enzymes for cleaning and washing
  - Other industrial applications
Increasing value by enzyme engineering

- Adaptation of catalysts to **cost-efficient reaction profiles**
- Directed evolution of **de novo enzyme activities** for addressing cheap and sustainable raw materials or non-natural molecules
- Developing new products by **artificial enzymes** with previously non-existing catalytic properties, replacing costly product alternatives

**c-LEcta** as enzyme specialist contributes to **innovative, efficient** and **market-oriented opportunities** of its large industry partners through a broad technology platform and expertise in commercial, IP and regulatory aspects at all project stages.
Company overview

c-LExcta is a technology company in the field of **White / Industrial Biotechnology** located in Leipzig, Germany.

We are specialized on the development and implementation of biotechnological solutions for industrial applications based on customized **Enzymes** and microbial **Strains**.

Processes for the production of **bioprocesed chemicals** and **industrial enzymes** developed by c-LExcta are implemented at industry partners and licensees.

The company’s proprietary technologies and products are protected by currently about **40 patents and patent applications**.

Under an ISO9001-certified quality management system c-LExcta runs a **pilot scale Enzyme production and scale-up facility** and cooperates with contract manufacturers for industrial scale enzyme production.
c-LExcta is a spin-off from University of Leipzig and currently employs **50 highly qualified employees**.

We recently moved into a new, state-of-the-art research and production facilities with 2,300 m² (BioCube) in order to set the basis for a further dynamic company growth.

c-LExcta was founded about 8 years ago and could establish itself as a technology partner for the industry in the market place. We successfully cooperate with a number of well known companies like **BASF, DSM, Uhde ThyssenKrupp** or **Evonik**.
Technology platform

Innovation and economic value of a process are achieved on every technological level.
Microbial Libraries
Genomic DNA of approx. 80-100 pooled strains
Recombinant expression libraries (overall > 55 Mio. clones)
High-Throughput Cluster-Screening
→ Highly efficient identification of new enzymes in activity-based screens

Genomic Libraries
Genomic DNA of individual high potential strains

Microbial Libraries
Genomic DNA of approx. 80-100 pooled strains

Metagenomic Libraries
Pooled genomic DNA of environmental probes

>7,000 strains from various habitats (prequalified actinomycetes, bacteria from tree canopy, marine bacteria)

Biodiversity – Source for new enzymes
Biodiversity – Source for new enzymes

Microbial Libraries

Genomic Libraries
Genomic DNA of individual high potential strains

Metagenomic Libraries

Identity to database enzymes

Cluster Screening

Cluster Screening

c-LEcta’s biodiversity collection provides a reliable source for new and protectable enzyme entities
Engineering - Optimized enzymes for non-natural processes

**Gene Library**

Enzyme variant with improved properties

**Cluster-Screening***

**Gene**

**c-LEcta-Mutagenesis***

mutagenesis

**Gene Library**

Enzyme Library

**Activity-based Screening**

according industry process requirements in *E. coli, Bacillus, Pichia* hosts

**Mutagenesis**

combining randomized, focussed, homology-based and recombinatorial approaches

* patented / patent pending
Overview c-LEcta enzyme engineering

Enzyme classes
- Alcohol dehydrogenases
- Aldolases
- Transaminases
- Nitrilase
- Lipases
- Dismutases
- Phosphorylases
- ...

Application areas
- Food & Feed Enzymes and Additives
- Small molecule APIs
- fine & specialty chemicals
- ...

Optimization targets
- Substrate scope
- Stereo-selectivity
- Regio-selectivity
- Temperature and pH activity/stability
- Solvent stability
- Process stability
- Productivity
- High substrate concentration performance
- Expression yield
- ...

Improved processes are routinely developed in many areas through efficient enzyme engineering by c-LEcta
Examples

- De novo enzyme design
- Improved Fructose-6-phosphate aldolase
- Nitrilase for API synthesis
Enzyme engineering: *de novo* enzyme design

Developing an ammonia-lyase for β-amino acid synthesis

**Target application**
- Providing building blocks for bioactive compounds and fine chemicals

**Structure-based approach with randomization at 4 sites**
- Cluster Screening (10,000-50,000 variants per plate)
- HPLC analysis of product formation with crotonic acid as model substrate

>3 mutations necessary for initial activity

Identification of active variant possible only by efficient screening in a combinatorial library

**After screening of 300,000 clones:**
- Hit cluster C6
- Other cluster
Enzyme engineering: Aldolase

Improved Fructose-6-phosphate Aldolase (FSA) for fagomine synthesis

\[
\begin{align*}
\text{R} & + \text{HO-CH-OH} & \rightarrow & \text{R} \text{-OH} \\
\text{Dihydroxyacetone (DHA)} & \rightarrow & \text{FSA-Variant} & \rightarrow & \text{PA4-23-P2-A2 OD5}
\end{align*}
\]

Optimization demands
- Increased activity on non-natural aldehyde substrate
- Process performance (substrate inhibition, process stability)

Structure-based approach with directed library design at 7 sites
- Cluster Screening (~65,000 variants)
- HPLC screening for product formation at high substrate concentrations

Simultaneous multi-parameter optimization in one screening
Enzyme Engineering: Nitrilase

Engineering of a nitrilase for the synthesis of an API intermediate

Optimization demands:
Activity → activity screen
process stability → thermostability screen
high substrate concentration → process condition screen

Increase of activity

Increase of stability

higher process productivity

Process with optimized enzyme is currently in scale-up for +100 ton synthesis
Summary

c-LEcta’s biodiversity collection provides a broad access to novel enzyme candidates and activities, allowing setup of a strong IP position.

Our enzyme optimization expertise allows quick and process-oriented development of enzyme candidates towards cost-efficient industry processes.

c-LEcta is open for collaborations with industry partners in guiding biotechnological innovation to the market.
Thank you for your attention!

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