Technical Implementation

Evolva has multiple technological capabilities, all associated with getting yeast to make valuable products. Some of the key ones are:

Combinatorial Genetics. *Evolva can create billions of different yeast cells expressing multiple new gene combinations.*

We have an array of technologies that allow us to rapidly insert and express tens to hundreds of genes in billions of individual yeast cells in a highly combinatorial fashion. This allows us to find those combinations that are necessary to make (biosynthesise) a given ingredient in the most efficient way. The same approach can create novel pathways that generate diverse small molecules for drug discovery. The genes that we use are either sourced from various species (in compliance with the CBD) or constructed de novo based on online databases or other sequence data.

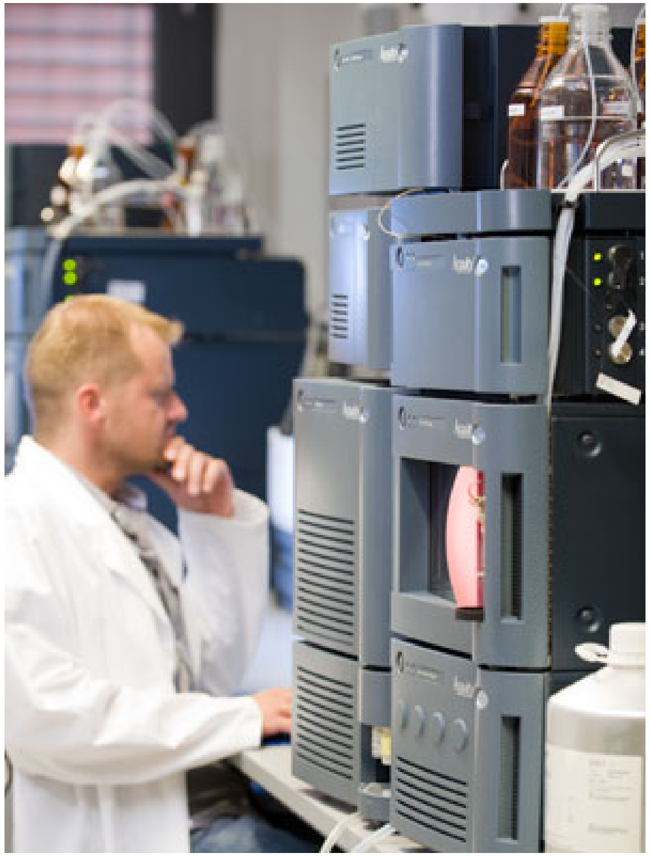
Screening and Analytical Technologies. *Evolva has an array of advanced screening tools that can select those yeast cells that produce desirable ingredients from a background of large number of cells.*

We have both function-led and structure-led screening tools that allow us to rapidly identify which yeasts are making desired ingredients and/or which have acquired desired functions (as a result of making certain ingredients).

- Function-led screens are very high throughput and we have used such screens to discover novel molecules with potential utility against cancer and infectious diseases. The approach can also be used to find new functionalities for food ingredients.
- Structure-led screens use state-of-the-art capabilities that combine ultra-high performance liquid chromatography, time-of-flight mass spectroscopy, NMR and informatics. They are primarily used to elaborate production pathways for known ingredients.

Pathway Optimisation Technologies. *Evolva has a number of tools that can improve the efficiency with which yeast produces the desired product, which results in a lower cost as well as other benefits.*

Once a biosynthetic route has been established, you need to improve it with respect to purity of product, efficiency of feedstock conversion, speed of production and final titer. The more these elements are optimised, the lower the cost of production of the ingredient. Evolva has the ability to rapidly assemble multi-step biosynthetic pathways in the yeast genome, allowing us to both create the production pathways in the fastest manner and optimise gene combinations in one-step processes. We also optimise pathways using a combination of enzyme co-factor balancing, metabolic engineering and pathway flux analysis.



Decoration Technologies. *Evolva has proprietary technologies that allow it to enhance the properties of ingredients, as well as their economics.*

We have multiple collections of enzymes that allow us to "decorate" ingredients and hence enhance their properties.

One particular focus is *glycosylation* (the process of attaching glucose or other sugars to molecules). Glycosylation allows us to:

- Make ingredients (such as stevia and saffron) whose natural properties depend on their glycosylation patterns.
- Improve the bioavailability of certain molecules, hence improving their effectiveness in nutritional or pharmaceutical use (or allowing a reduced amount of the relevant molecule to have the same effect as the larger amount).
- Improve manufacturing efficiencies by orders of magnitude, resulting in reductions in the relevant ingredient's manufacturing cost.

Cytochrome P450 enzymes transfer molecular oxygen to -CH, -NH or -SH bonds. Reactions can be very diverse, e.g. hydroxylations, epoxidations, N-, O- and S-dealkylations, deaminations, desulphurations and so on. We in particular use them to:

- ensure our ingredient production pathways are as effective as possible (P450 enzymes are part of most plant metabolite pathways).
- "activate" molecules containing only carbon and hydrogen, in order to diversify basic scaffolds for functionality testing.

Other decoration technologies allow us to functionalise molecules for further chemical derivatisation, alter lipophilicity or stability, etc.

Explain more about the benefits (www.evolva.com/technology/benefits)

SCIENTIFIC PAPERS

ANGEWANDTE CHEMIE (HTTP://ONLINELIBRARY.WILEY.COM/DOI/10.1002/ANIE.201410002/ABSTRACT)

This paper describes the “Discovery and Reconstitution of the Cycloclavine Biosynthetic Pathway” and demonstrates the potential for producing complex fungal alkaloids by fermentation in yeast.

MICROBIAL CELL FACTORIES (HTTP://WWW.MICROBIALCELLFACTORIES.COM/CONTENT/13/1/95)

The important ergot alkaloid intermediate chanoclavine-I produced in the yeast Saccharomyces cerevisiae by the combined action of EasC and EasE from Aspergillus japonicus

ACS SYNTHETIC BIOLOGY (HTTP://WWW.EVOLVA.COM/SITES/DEFAULT/FILES/ATTACHMENTS/ACS-SYNBIO-FEB2014.PDF)

Yeast Synthetic Biology Platform Generates Novel Chemical Structures as Scaffolds for Drug Discovery. This documents the “chemical space” that is created by Evolva’s platform - diverse low molecular weight compounds with a high degree of novelty compared to both synthetic and natural product scaffolds.

MICROBIAL CELL FACTORIES 2013 (HTTP://WWW.MICROBIALCELLFACTORIES.COM/CONTENT/PDF/1475-2859-12-31.PDF)

Reconstruction in yeast of a fungal polyketide pathway, for production of the natural pigment rubrofusarin. Provides a rare example of successful expression in yeast of a large iterative non-reducing polyketide synthase.

MICROBIAL CELL FACTORIES 2009 (HTTP://WWW.MICROBIALCELLFACTORIES.COM/CONTENT/PDF/1475-2859-8-45.PDF)

Article about Evolva’s Technology. Yeast artificial chromosomes employed for random assembly of biosynthetic pathways and production of diverse compounds in Saccharomyces cerevisiae

APPLIED & ENVIRONMENTAL MICROBIOLOGY (HTTP://AEM.ASM.ORG/CGI/CONTENT/FULL/75/9/2765?VIEW=LONG&PMID=19286778)

Heterologous pathways for biosynthesis of vanillin from glucose established in fission yeast and baker's yeast. The Article for free download (http://aem.asm.org/cgi/content/full/75/9/2765?view=long&pmid=19286778) .

PHYTOCHEMISTRY (HTTP://WWW.NCBI.NLM.NIH.GOV/PUBMED/19261311)

Altered substrate specificities of family 1 UDP-glycosyltransferases gained by swapping of enzyme domains. The abstract of the Article (http://www.ncbi.nlm.nih.gov/pubmed/19261311) .

LABORWELTEN (WWW.EVOLVA.COM/SITES/DEFAULT/FILES/ATTACHMENTS/LW6_2011_EVOLVA.PDF)

Introductory overview article on Evolva technology in German language. Article can be downloaded here (www.evolva.com/sites/default/files/attachments/lw6_2011_evolva.pdf) .

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