

# Development of hosts and production process for precision fermentation with emerging safety aspects

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## VTT – *beyond the obvious*

VTT is a visionary research, development and innovation partner for companies and society and one of the leading research organisations in Europe.

Our role is to promote the utilisation and commercialisation of research and technology in business and society.

**Through science and technology, we turn global challenges into sustainable solutions.**

**261 M€**

turnover and other  
operating income

**2,213**

employees

**43%**

of the net turnover  
from abroad

**32%**

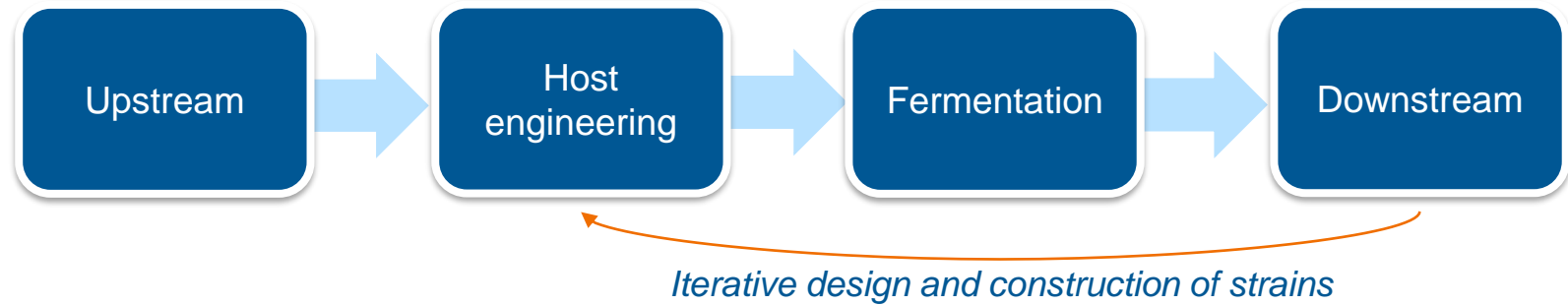
a doctorate or a  
licentiate's degree

Establishment year

**1942**

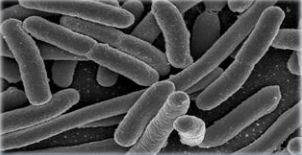

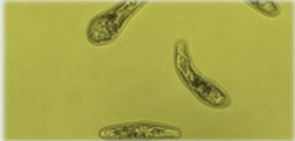
Steered by Ministry  
of Economic Affairs  
and Employment

# Precision fermentation process development



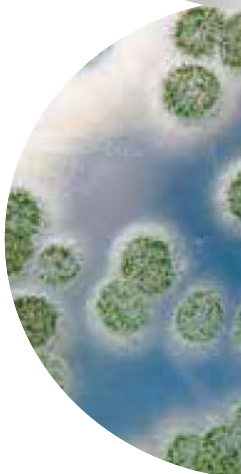
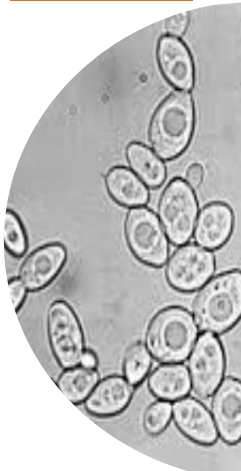
- Target molecule, protein lipid, oligosaccharide
- Host strain, best suitable for target molecule
- Expression of target molecule
- Modification of metabolic pathways
- Modifications to enhance of target molecule yield
- Submerged vs. solid state, reactor type
- Selection of feedstock and media components
- Process optimization to improve yields
- Separation
- Purification
- Concentration
- Quality analysis
- Product formulation

# Advantages and disadvantages of different production hosts

Host	Pros	Cons
Bacteria 	<ul style="list-style-type: none"> <li>• Simplest genome</li> <li>• Good molecular tools, plasmids</li> <li>• Fast growth</li> <li>• Simple media components</li> <li>• Scalable</li> </ul>	<ul style="list-style-type: none"> <li>• Mutation rate</li> <li>• General acceptance</li> <li>• Lack of post-transcriptional modifications (proteins)</li> <li>• Down stream processing</li> </ul>
Yeast and filamentous fungi 	<ul style="list-style-type: none"> <li>• High product yields</li> <li>• Robustness</li> <li>• Scalable, several commercial scale food grade systems operating</li> <li>• Eukaryotic</li> <li>• Secretion capacity of proteins</li> </ul>	<ul style="list-style-type: none"> <li>• Strain construction laborious</li> <li>• Cost of media components</li> <li>• Possible fungal pathogenicity</li> </ul>
Microalgae 	<ul style="list-style-type: none"> <li>• Robustness</li> <li>• Produce many unique molecules</li> <li>• Heterotrophic growth</li> </ul>	<ul style="list-style-type: none"> <li>• Poorly characterized organisms</li> <li>• Fewer molecular tools</li> <li>• cell wall structure may hinder product recovery</li> </ul>

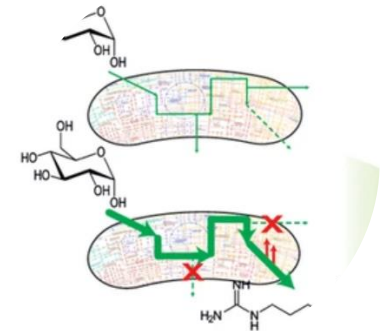
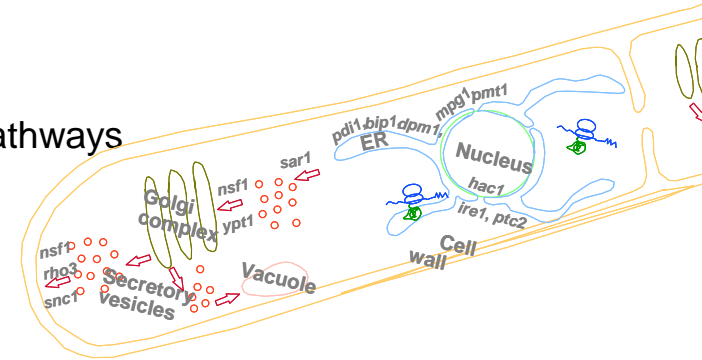
# Selection of production host

- The production host is selected based on the ability to produce good quality target molecule in highest possible level in a cost effective process
- Several well known microbial hosts producing compounds for food applicaitons exist
- Bacteria
  - *E. coli*
- Yeast and filamentous fungi
  - *Aspergillus sp, Trichoderma reesei*
  - *Yarrowia lipolytica, Cryptococcus curvatus*
- The increase in the number of precision fermentation target molecules has increased the demand for new production hosts
  - whole genome sequencing and phenotypic
    - Check for lack of genes/gene clusters encoding for known toxins, virulence related genes
    - antibiotic resistances, antimycotic resistance
    - Genetic stability
    - Pathogenicity



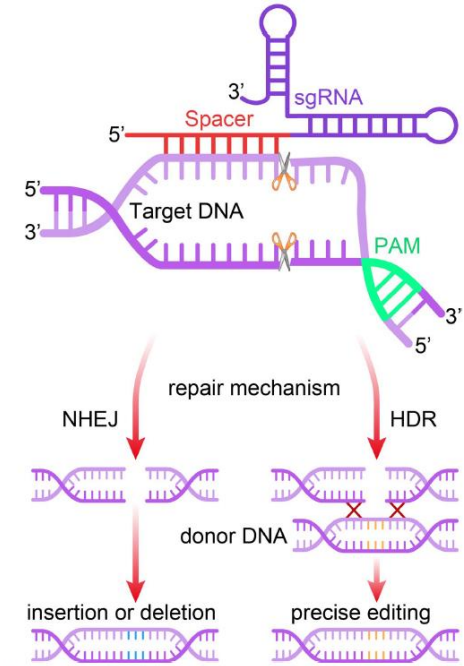
# Modification of hosts

- Improvement of target yield
  - expression cassette design and copy number
  - modification of e.g. chaperone expression
  - Secretion carrier molecules
  - Modification of metabolic pathways, construction of novel pathways
  - Modifications to improve carbon flux
  - Improvement of host tolerance against target molecule
- Reduction of background
  - simplified down stream processing
- Modifications that affect quality of target molecule
  - Engineering e.g. the glycosylation pathway
  - Host proteases
  - Host lipases, esterases, deletion of enzymes oxidating fatty acids
- Modifications related to bioprocess optimization
  - Reduction of foaming
  - Engineering for improved yield, heat, C-source utilization



# Genetic modification tools for host development

- Transformation
  - Selection marker
  - Vector backbone
  - Promoters, terminators, other regulatory elements
    - Endogenous or exogenous
- Random- or targeted integration to genome
  - Homologous recombination
  - Cas9/CRISPR
    - Stable or transient expression of Cas9 or use of Cas9 protein
- Regulatory RNAs
  - e.g. lncRNA for down regulation of gene expression
- Mutagenesis



Zhang et al. Mol Cancer (2021) 20:126

# Bioprocess design

- High target molecule yield and quality, low level background
- Food grade bioprocess facility and equipment, use of correct material in all parts of equipment
- Culture media components food grade
  - Glucose most common carbon source
  - Nitrogen source inorganic or organic
  - Water
  - Vitamins, minerals, trace elements
  - Antifoam
- In the future we possibly need to use cheaper raw materials as media components to provide energy and carbon source for the micro-organism
  - Chemical purity
    - Food industry side streams
    - Agricultural side streams
      - Cellulosic side streams, fruit- or grain peels etc.
    - Other

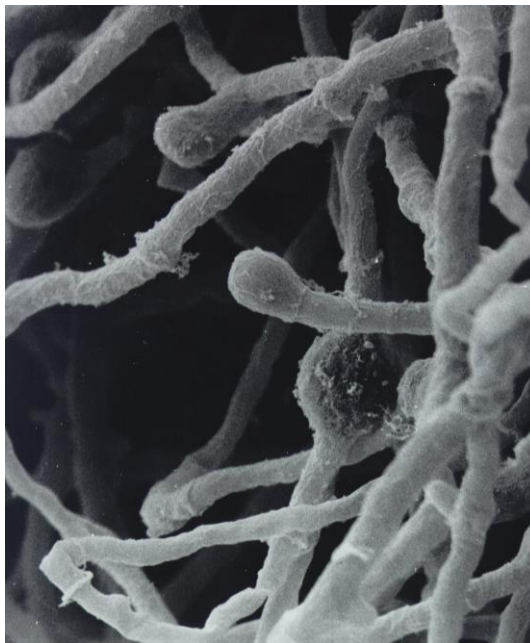




# Down stream processing and purification

- Cost effective in large scale
- Minimal loss of target molecule
- Quality of target molecule not changed
- Purity of target protein, lipid or carbohydrate
  - Toxins
  - DNA/RNA
  - Impurities from isolation/purification process e.g resin or solvents
  - Antifoam residues
- Final composition of end product known
- Allergenicity, chemical purity, digestibility, microbiological- and toxicity analyses
  - Main protein and impurities
- Similarity to natural product?
  - Amino acid sequence, glycosylation, phosphorylation, N-terminus, mass
    - Variation in the natural product
  - Mixtures of carbohydrates, lipids





# bey<sup>0</sup>nd

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