Yeasts in Biotechnology

Yeasts

- Yeasts have been used for fermentation of food and beverages since ancient time
- To date they are widely used for industrial production of chemicals, pharmaceutical, and proteins
- *Saccharomyces cerevisiae* is the most widely employed eukaryotic organism in biotechnologies
- Other yeasts in biotechnology: *Hansenuela polymorpha, Pichia pastoris, Kluyveromyces lactis, Yarrowia lipolytica = non-conventional yeasts*.
- These yeasts grow quickly and are adaptable to large-scale production.
- They do not produce endotoxin and perform partial glycosylation of proteins.

Food and Chemical Industry

- For decades, yests have been used for production of savoury flavours, enzymes, pigments, food acidulants.
- For novel applications appropriate strains have to be generated by genetic engineering, i.e. introducing of genes whose products are capable to synthesize the products and eventually secrete it in the medium

Metabolic engineering

- By introduction of appropriate gene, the yeast can consume starch, lactose, melibiose, xylose as a carbon source.
- This can be used for eliminating unwanted by-products in brewer’s, distiller’s and wine yeasts.
- Heterologous production of proteins

Production in Yests

- flavors, fragrances (e.g. limonene, nootkatone, menthol, camphor, cubebol), food colorants (carotenoids) or pharmaceuticals (e.g. taxol, bisabolol, lycopene, artemisinin).

- Production of variety of isoprenoids
- Production of hydrocortisone from ethanol

- Production of lipid compounds: sterols, biosynthesis of of different omega-3 and omega-6 unsaturated fatty acids

- K-kasein

Recombinant Biotherapeutics

- First product was human interferon – 1981
- Hepaptitis B surface antigen (HBAg) – the first genetically engineered vaccine –1982

To date HBAg is produced by different strategies in yests.

The greatest value from production of human insulin

The production covers about half of the insuline needed by the diabetics patients.

Production of pharmaceutics

- Production of human serum albumin (rHSA), which can serve as a carrier for synthetic heme protein, reversibly carrying oxygen.
- Albumin-anticancer drug conjugate, since there is preferential albumin uptake by tumor cells.
- Incorporation of drugs into albumin microspheres
- Galactosydated or mannosylated albumin targets conjugates to liver

Microsperes and nanoparticles can be derived from HSA for drug delivery

Environmeltal Technologies

- Using cell-surface display

System engineered to display enzymes, functional proteins, antibodies on the yeasts surface

Example: coupling of gene encoding the target protein with the secretion signal from C-terminal half of α-agglutinin with the glycosylphosphatidylinositol (GPI) anchor signal sequence (Ueda, tanaka 2002).

Rhizopus oryzae glucoamylase can grow on starch

Baccillus sterarothermophilus α-amylase can grow on starch

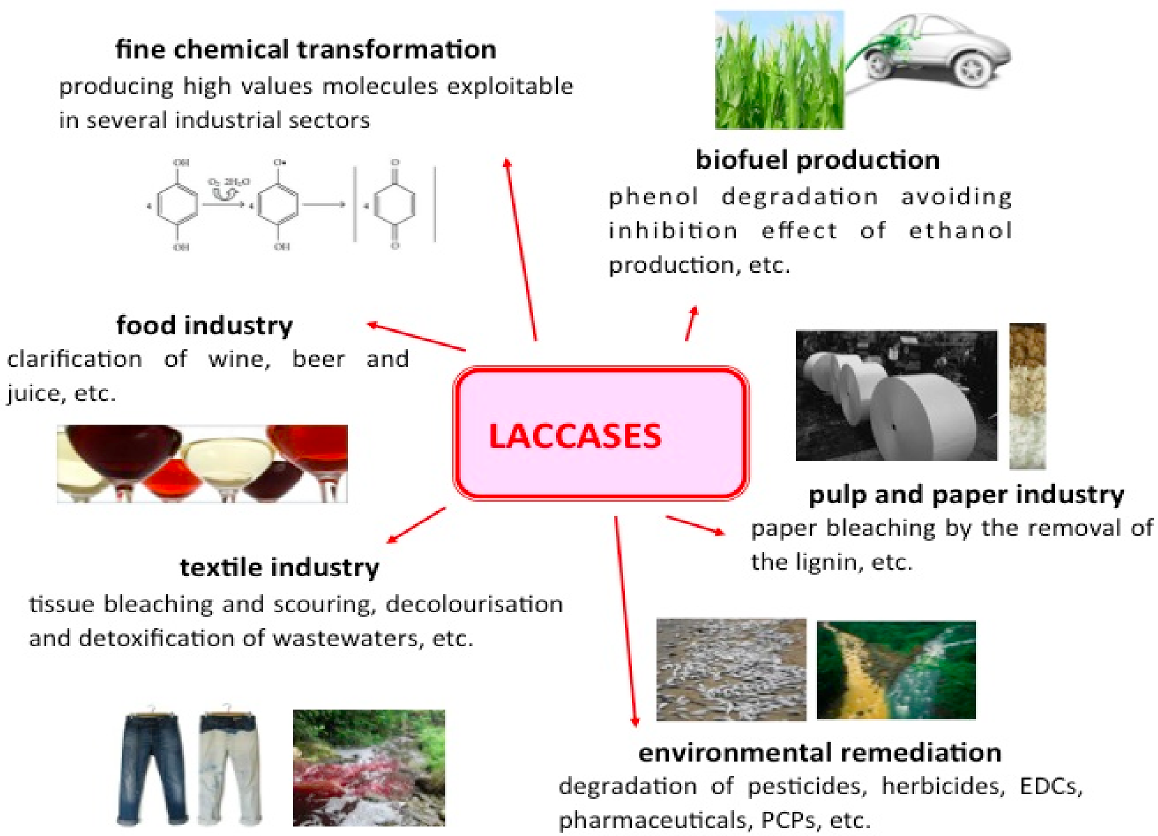
Aspergillus aceleatus β-amylase and CM cellulase cellulose as a carbon source

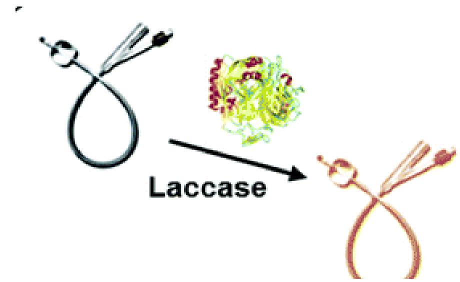
Rhizopus oryzae lipase (ROL)

coupled to α-agglutinin

Ueda, M., Tanaka, A. (**2000**) Cell-surface engineering of yeast: construction of arming yeast with biocatalyst. *J. Biosci. Bioeng.* **90**, 125-136.

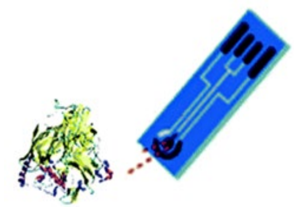
Production of oxidoreductases LACCASES in *Saccharomyces cerevisiae*





biomedical industry

tumor therapy



biosensors

for codeine, morphine or plant flavonoids

Klára Herkommerová

Laccases

Identified in:

- plants (formation of the cell wall; lignification)
- fungi (degradation of lignin in white-rot fungi)
- bacteria (UV-resistant spores in *Bacillus* sp.)
- insects (cuticule sclerotization)

Heterologous expression of three laccases in *Saccharomyces cerevisiae* and their potential in dye decolorization

- genes from different source of origin

- ***MtL*** from *Myceliophthora thermophilla*

  (the gene for MtL (mutated by directed evolution) was obtained from Bulter T. et al. (2003, Appl Env Microbiol 69(2):987)

- ***TtL*** from *Trametes trogii*

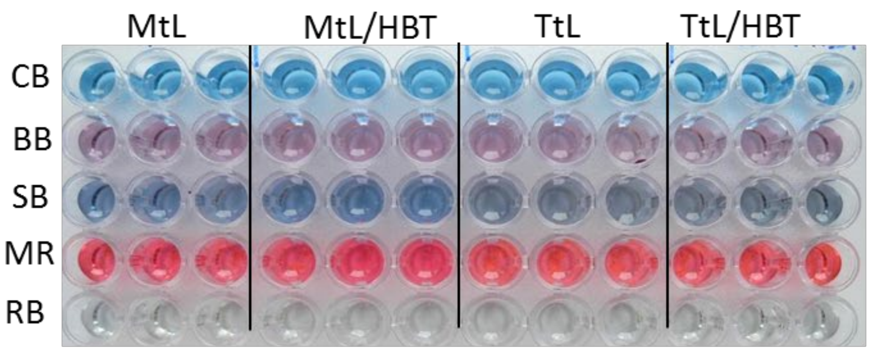
  (synthetic gene for TvL was ordered by Invitrogen, codons are optimized for *S. cerevisiae*)

- ***TvL*** from *Trametes versicolor*

  (synthetic gene for TtL was ordered by Invitrogen, codons are optimized for *S. cerevisiae*)

Decolorization by laccases (in cultivation medium)

- **dyes:** Coomassie Brilliant Blue (CB), Bromphenol Blue (BB), Saturn Blue (SB), Methyl Red (MR), Remazol Brilliant Blue (RB)



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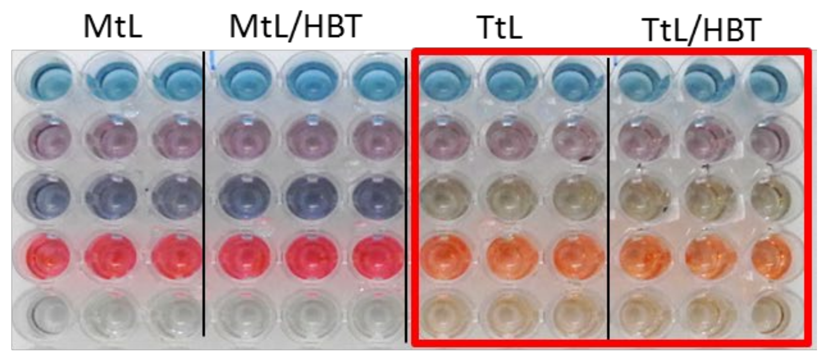
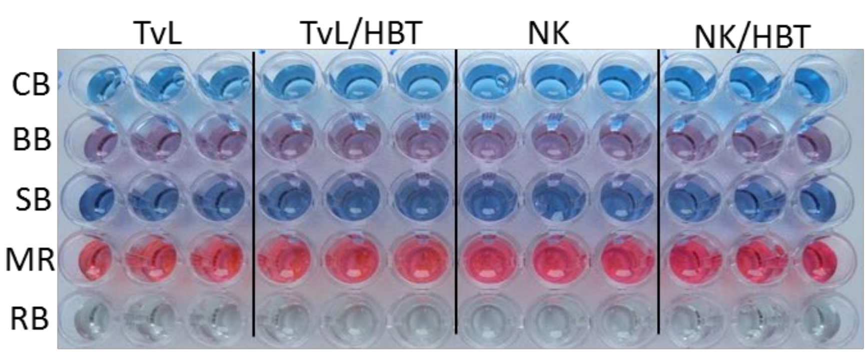


7 days



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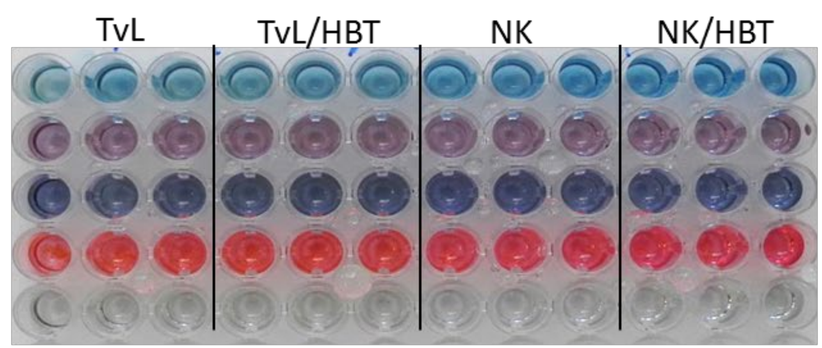
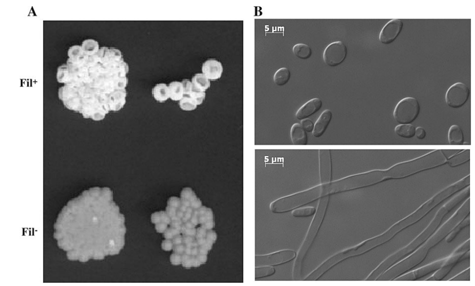


7 days

Yarrowia lipolytica



- *Y. lipolytica* is a good host for the industrial bioproduction of chemicals, fuels, foods, and pharmaceuticals.

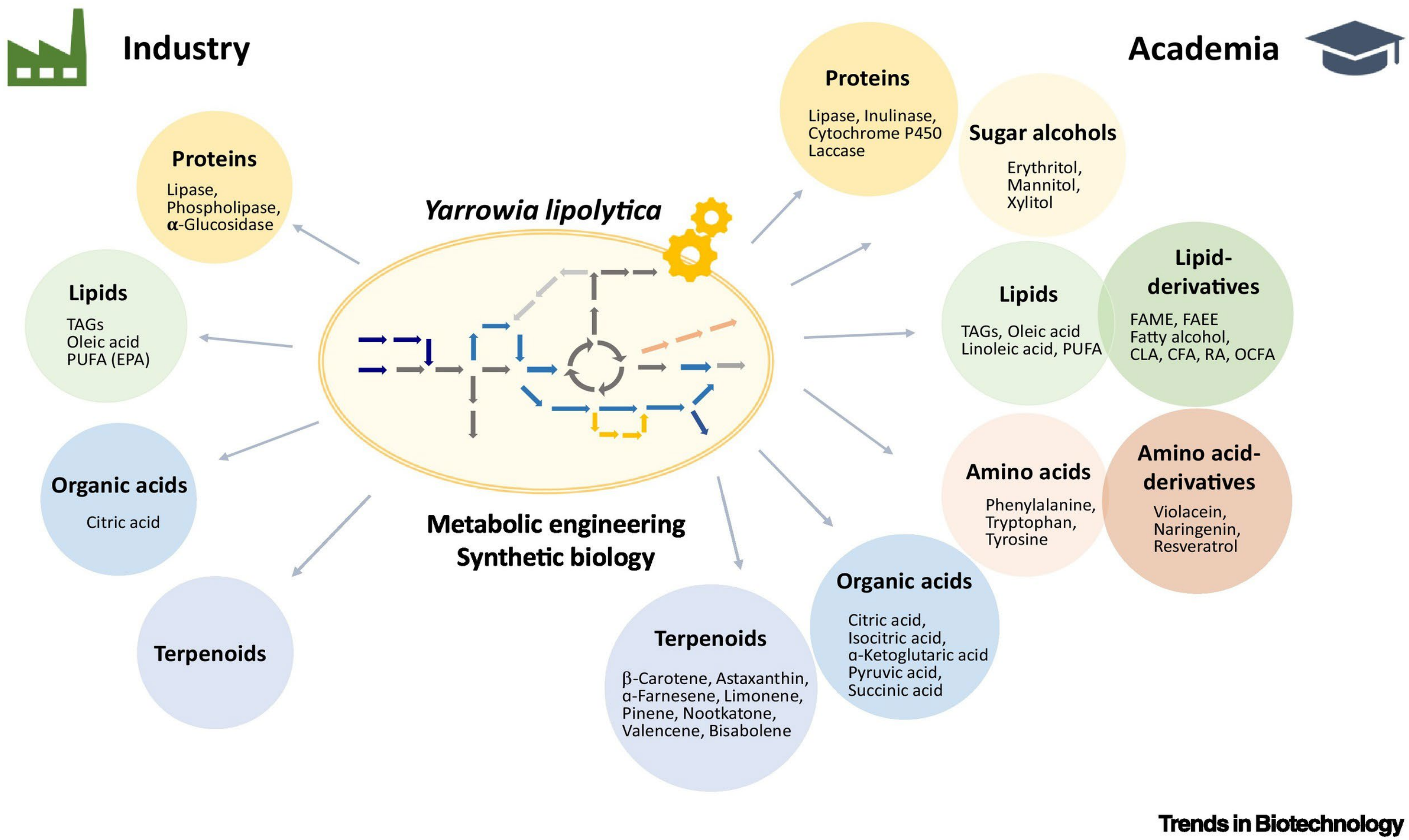
- *Y. lipolytica* has many features that are desired at an industrial scale, such as safety, robustness, efficient and stable genetic modifications, capacity to use a variety of substrates, and ability to grow at very high cell density.

- *Y. lipolytica has* capacity to accumulate lipids, it can yeast and pseudo-hyphae forms, it can degrade hydrophobic carbon sources (fatty acids, triglycerides, alkanes, alkenes, etc.

- Promissing products in Y. lipolytica: lipids, terpenoids, amino acids, proteins, and sugar alcohols

- In the past few years, metabolic engineering has expanded the range of applications of this yeast to the manufacture of more than 100 products.

Products from *Y. lipolytica*



Trends in Biotechnology DOI: (10.1016/j.tibtech.2022.07.006)
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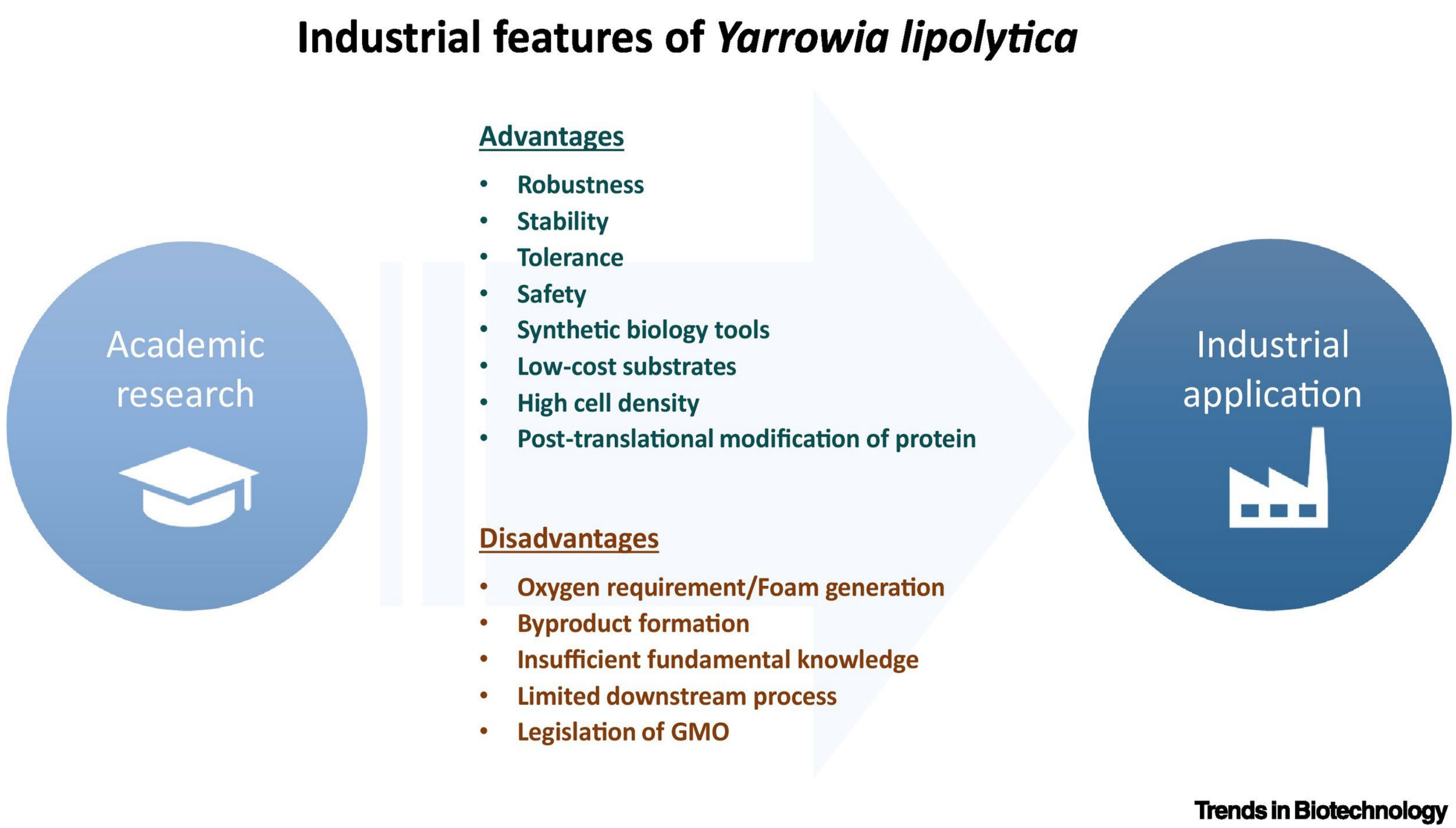
Y. lipolytica



Trends in Biotechnology DOI: (10.1016/j.tibtech.2022.07.006)
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Y. lipolytica commercial products in industry

- **Polyunsaturated fatty acids (PUFAs):**
- eicosapentaenoic acid (EPA, 20:5, n-3) and docosahexaenoic acid (DHA, 22:6, n-3), also known as long-chain omega-3 fatty acids, have a global market valued at US$2.49 billion in 2019. *EPA* is a US FDA-approved prescription drug for reducing triglyceride levels. As a supplement, people most commonly *use EPA* for heart disease.
- DuPont de Nemours and Company developed a metabolic engineering strategy in *Y. lipolytica*, which expressed heterologous Δ6 desaturase, C18/20 elongase, Δ5 desaturase, and Δ17 desaturase, enabled production of up to 40% EPA.

Y. lipolytica commercial products in industry

- **Commercial recombinant enzymes from *Y. lipolytica*:**
- phospholipase enzyme Lipomod 833L2 by Biocatalysts Ltd., the lipase obtained by *LIP2* gene overexpression by Mayoly, or the human acid α-glucosidase OXY2810 Oxyrane (Skotan SA).

- **Citric acid** is widely used by the food industry as additives, preservatives, anticoagulants, antimicrobial agents, fine chemicals. The global market volume of citric acid is greater than 2 million tons, and its value is estimated to reach US$6.28 billion by 2030. Due to easy cultivation, high conversion rate, and tolerance to high product concentrations, *Y. lipolytica* has been proposed as an alternative citric acid producer to *Aspergillus niger.* DSM, Akad Wissenschaften DDR, and OrganoBalance GmbH, own approximately 40 patents on citric acid production by *Y. lipolytica*

Y. lipolytica commercial products in industry

- Carotenoids have a number of applications in the food processing, animal feed, pharmaceutical, and cosmetics industries. There is already an enormous global market, which is estimated to be US$1.57 billion in 2022 and reach a valuation of US$2.09 billion by 2027
- Microbia (now part of DSM) brought several carotenoid production strains to pilot scales, and a GRAS self-affirmation has been demonstrated for the β-carotene produced with *Y. lipolytica*

Production of pheromones in *Y. lipolytica*

- Pheromones are mostly produced by chemical synthesis, which is a complicated and costly process and production is uneconomical for use in large-scale agriculture.
- Use of cell factories, i.e. enzymes involved in pheromone biosynthesis (fatty acid desaturases and fatty acid reductases) introduced in engineered yeasts *S. saccharomyces* and *Y. lipolytica*
- *Y. lipolytica is suitable host for pheromone precursors*
- BioPhero company, spin-out from the Technical University of Denmark
- **Cotton Bollworm (***Helicoverpa armigera, černopáska bavlníková);* **Diamondback Moth (***Plutella xylostella,* ***zápředníček polní,*** cabbage, broccoli, and cauliflower*);* **Rice Stem Borers (***Chilo suppressalis, travařík* and *Scirpophaga Incertulas);* **Fall Armyworm (***Spodoptera frugiperda,* blýskavka kukuřičná*)*

GMO problem

- Almost all products from *Y. lipolytica* are the consequence of genetic and metabolic engineering and are therefore made by genetically modified organisms (GMOs).
- Legislation around the use of GMOs for various applications will therefore be relevant, and additional studies supporting the long-term safety will be beneficial.

Bioethanol

- The mostly used biofuel worldwide

- The global production near 56 billion liters per year
- USA and Brazil are currently the world ’ s largest producers of ethanol,
- Bioethanol is used directly pure or blended with gasoline to produce „gasohol“.

- Different generation of biothanol:
- First generation – produced from sucrose (sugar cane, suger beet, fruits)
- Second generation: produced from lignocellulose biomass
- Third generation: derived from algae biomass

Production of bioethanol in yeasts

Some Yeast spp. have ability to catabolize six-carbon molecules into two carbon molecules (glucose to ethanol) without a final oxidation into CO_2

Important role of alcohol dehydrogenase (Adh1):

catalysis of acetaldehyde reduction to ethanol during glucose fermentation

Searching and engineering of Adh1 to improve its properties

Yeast producers:
Saccharomyces cerevisiae, Pichia stipitis, Kluyveromyces fagilis

Limits: growth inhibition by EtOH ≥ 20%, higher temperature, ability to ferment pentose sugars

Lignocellulose and Bioethanol

- Lignocellulosic materials contain two types of polysaccharides, cellulose and hemicellulose, bound together by a third component lignin.
- It is necessary to perform pre-treatment – mechanical, chemical, biological: ozonolysis, acid or alkaline hydrolysis, wet oxidation .
- Next step: hydrolysis with cellulases and hemicellulases

- Fermentation
- Ethanol separation by distillation or by sorbent and stripping by a dry gaz

Fermentation

- *Saccharomyces cerevisiae* has several disadvantages: limited substrate utilization range, specially its inability to ferment xylose.
- *Saccharomyces* and *Pichia* species have been genetically engineered to ferment glucose, xylose and arabinose sugars

- Some identified xylose fermenting microorganisms are *Pachysolen tannophilus, Pichia stipitis, Candida shehatae* yeast strains and the most widely applied recombinant bacterial strains are *E. coli, Klebsiella oxytocoa* and *Zymomonas mobilis.*

- Genetically engineered microorganism that can convert pentoses as well as hexose sugars simultaneously to ethanol can greatly improve ethanol production efficiency and reduce the production cost.