LOSCHMIDT LABORATORIES

Bi9540 Biotechnology and practical use of algae and fungi

Lecture 1 – Introduction to biotechnology

Course organization

- Lectures every week
- Materials in English, lectures in Czech

- Attendance is not obliogatory, yet highly recommended
- Activity during lessons (discussions etc.)

- Oral exam

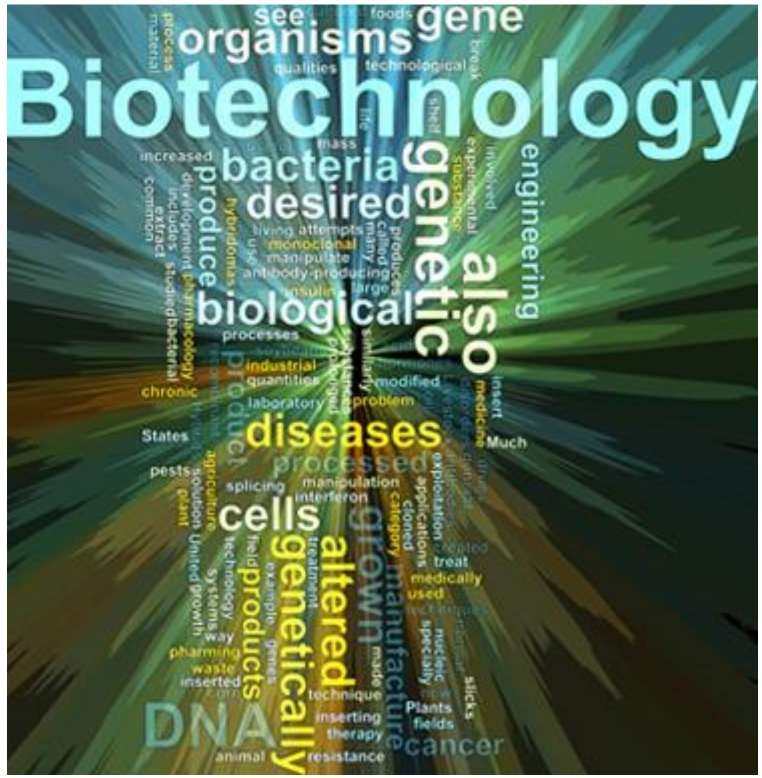
Lecturers

- Mgr. Petr Hrouda, PhD.
  - Dept. of Botany and Zoology, Fac. Sci. MU
  - Office A32/211

- Mgr. Lukáš Chrást
  - Loschmidt Laboratories, Dept. of Experimental Biology and RECETOX, Fac. Sci. MU
  - Office A13/311

Topics covered by Bi9540

- Introduction to biotechnology
- Physiology and cultivation of cyanobacteria, algae and fungi
- Toxic metabolites of fungi, poisonous toadstools and toxins of microscopic fungi, possibilities of risk elimination
- Cyanobacteria and algae as nutritional supplements, algae in traditional medicine
- Biofuel production using cyanobacteria and algae
- Gene and metabolic engineering of cyanobacteria and algae
- Yeasts and filamentous fungi in classic biotechnology
- Yeasts as expression system in molecular biotechnology
- Fungi in traditional medicine, antibiotics
- Exploitation of fungi for natural substances production, fungi as industrial chassis
- Fungi as biocontrol agents in agriculture, phytopathogenic fungi and their elimination
- Fungi as nutrition sources, cultivation of edible mushrooms


Biotechnology
organisms
gene
see
foods
qualities
technological
bacteria
desired
genetic
also
engineering
increased
mass
biological
produce
processes
industrial
quantities
laboratory
modified
problem
chronic
States
diseases
Much
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pests
splicing
manipulation
interferon
cells
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genetically
products
grown
manufacture
treat
medically
used
pharming
waste
inserted
technique
inserting
therapy
Plants
fields
DNA
animal
resistance
cancer

What is biotechnology?

- **Biotechnology** is the use of living systems and organisms to develop or make products, or "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use" (UN Convention on Biological Diversity, Art. 2)

- The term ‘biotechnology’ was coined by Karl Ereky in 1919

6 colours of biotechnology

- Red – medical
- White – industrial
- Green – agriculture
- Blue – marie/aquatic
- Black – bioterorism
- Grey – environmental

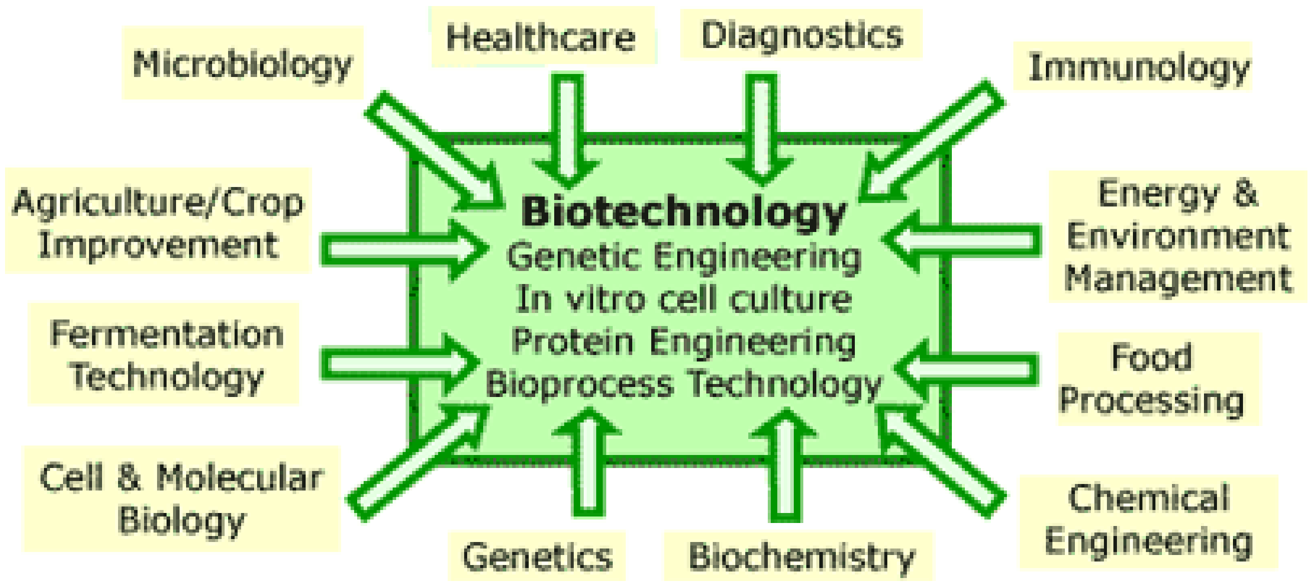
+ bioinformatics

Classical vs Modern

- Classical biotechnology
  - Traditional approach since archaic times
  - Usage of natural strains or domestication of wild species
  - Agriculture, beer, fermented products, cheese, etc.
- Modern (=molecular) biotechnology
  - Since late 70s and early 80s
  - Rapidly growing field, mostly important for medicine
  - Uses genetically modified organisms
  - Production of recombinant proteins, antibodies, molecular farming, bioremediation; GM crops and livestocks

Biotechnology

- Interdisciplinary approach is essential

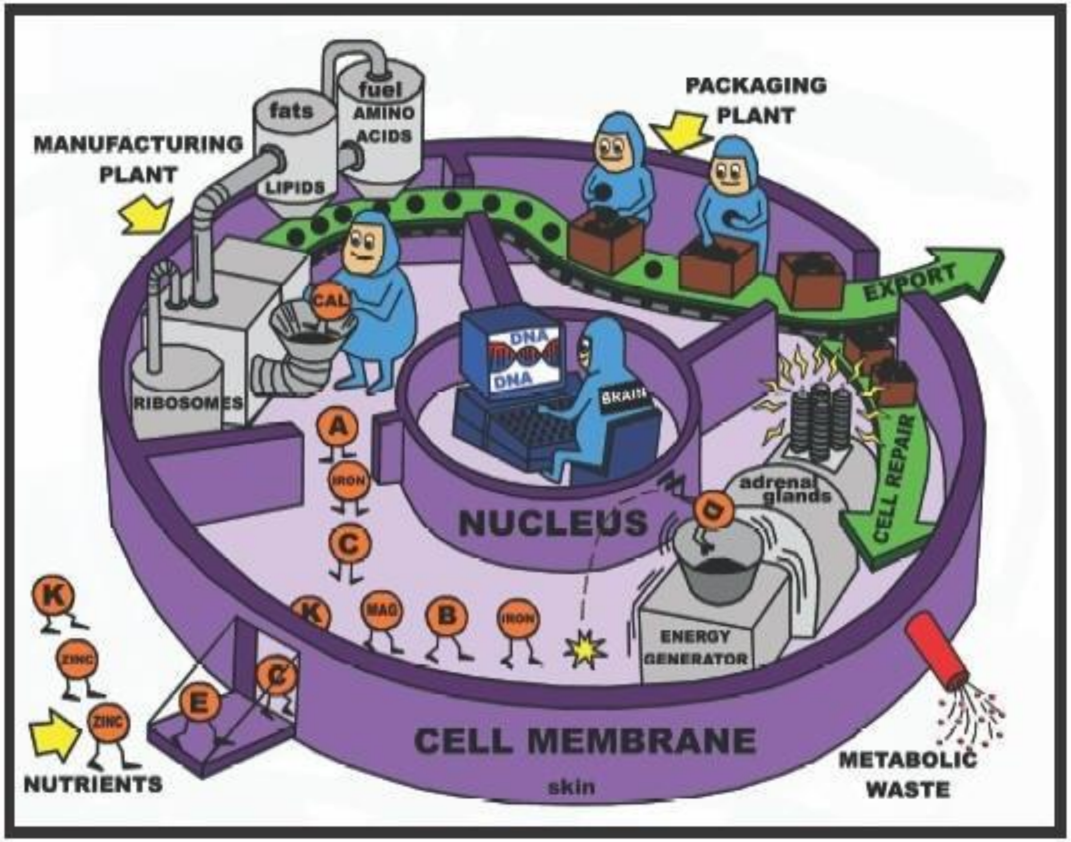


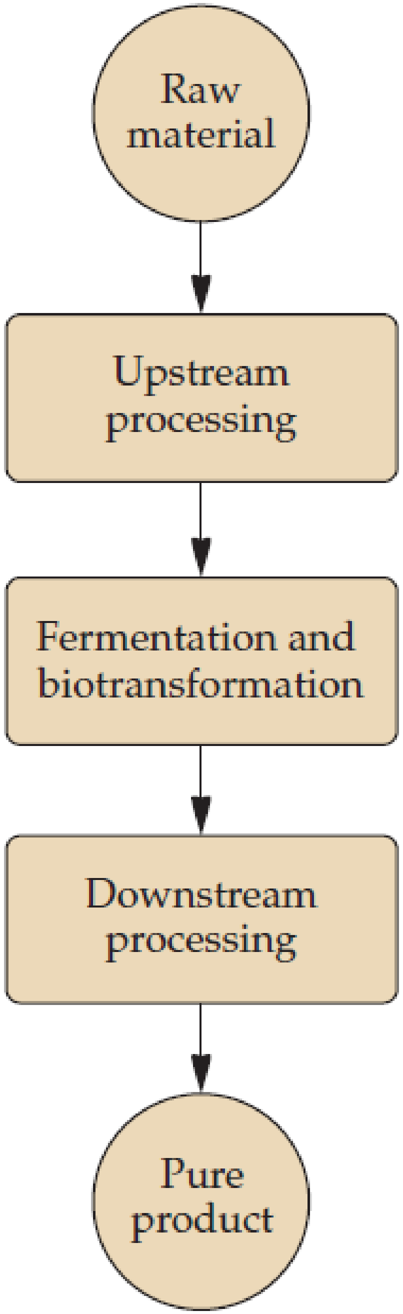
Levels of biotechnology

System	Examples	Utilisation
At the level of small molecules	Steroids, lipids, sugars vitamins, coenzymes	Drugs, packaging or encapsulating materials, nutrition, food, cleaning and detergency
Macromolecular level	Proteins, nucleic acids, polysaccharides	Catalysis, energy sources, copying and reproduction
Organelle level	Membranes, cell extracts, chloroplasts, mitochondria	Separation, energy transduction, *in vitro* biochemistry
Cells with walls removed	Protoplasts	Hybrid cells and cell fusion
Cells	All types of microbial, plant and animal cells	Biochemicals, genetic engineering, various purposes
Tissues	From plants and animals for use in medicine and surgery	Biochemicals of Biotechnology
Organs and organisms	Plants, fruitflies, nematodes, frogs, mice, rats and rabbits	Chemicals, biochemicals, toxins, immunochemicals, drug testing, transgenics

Cell to factory analogy

- Cells are pretty much working like a factory. Biotech makes an advantage of this fact

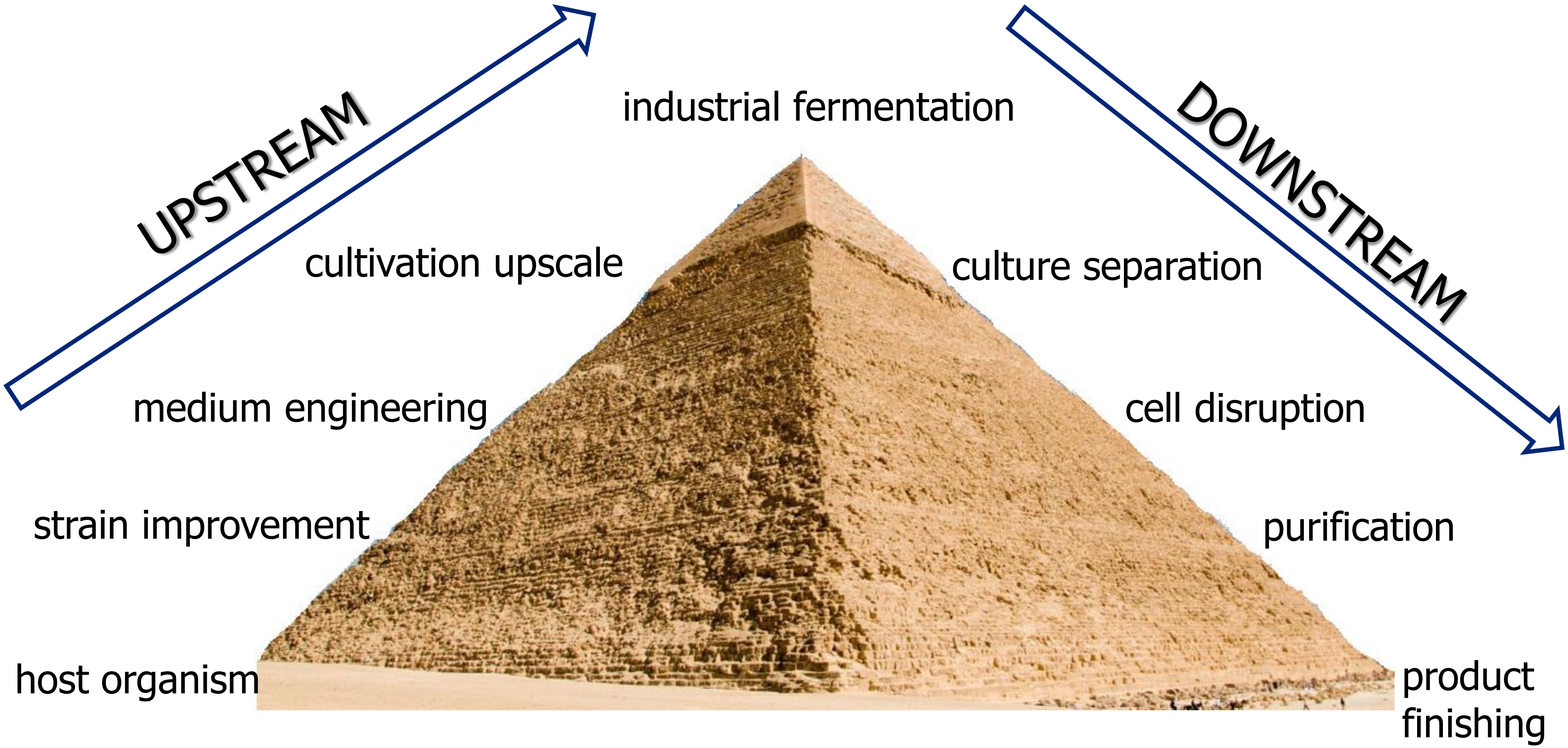




- Most important principle of all times
- Valuable products from raw material

- Always keep this in mind when designing a bioprocess, otherwise you lose money

Upstream And Downstream Processes

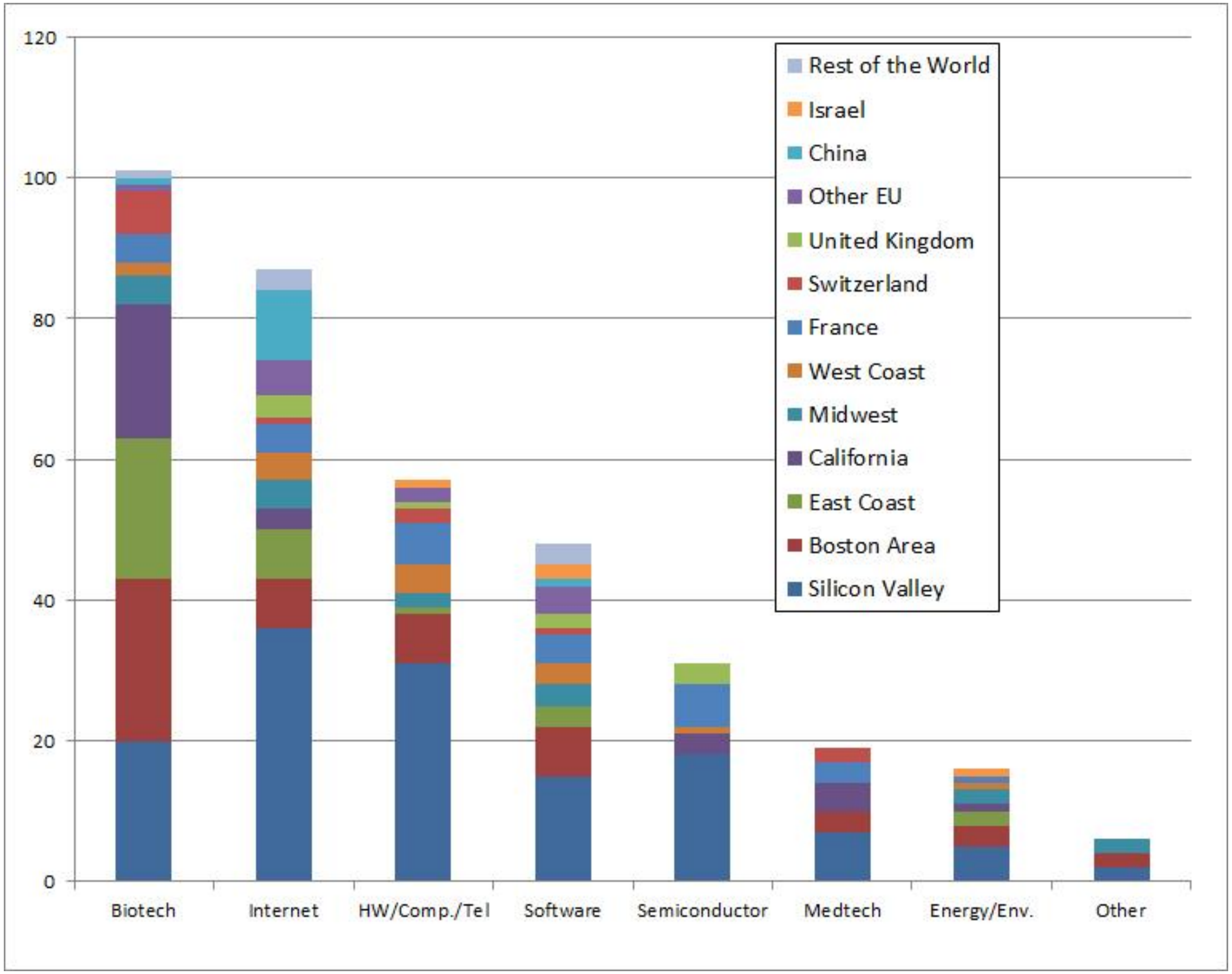


Biotechnology is growing

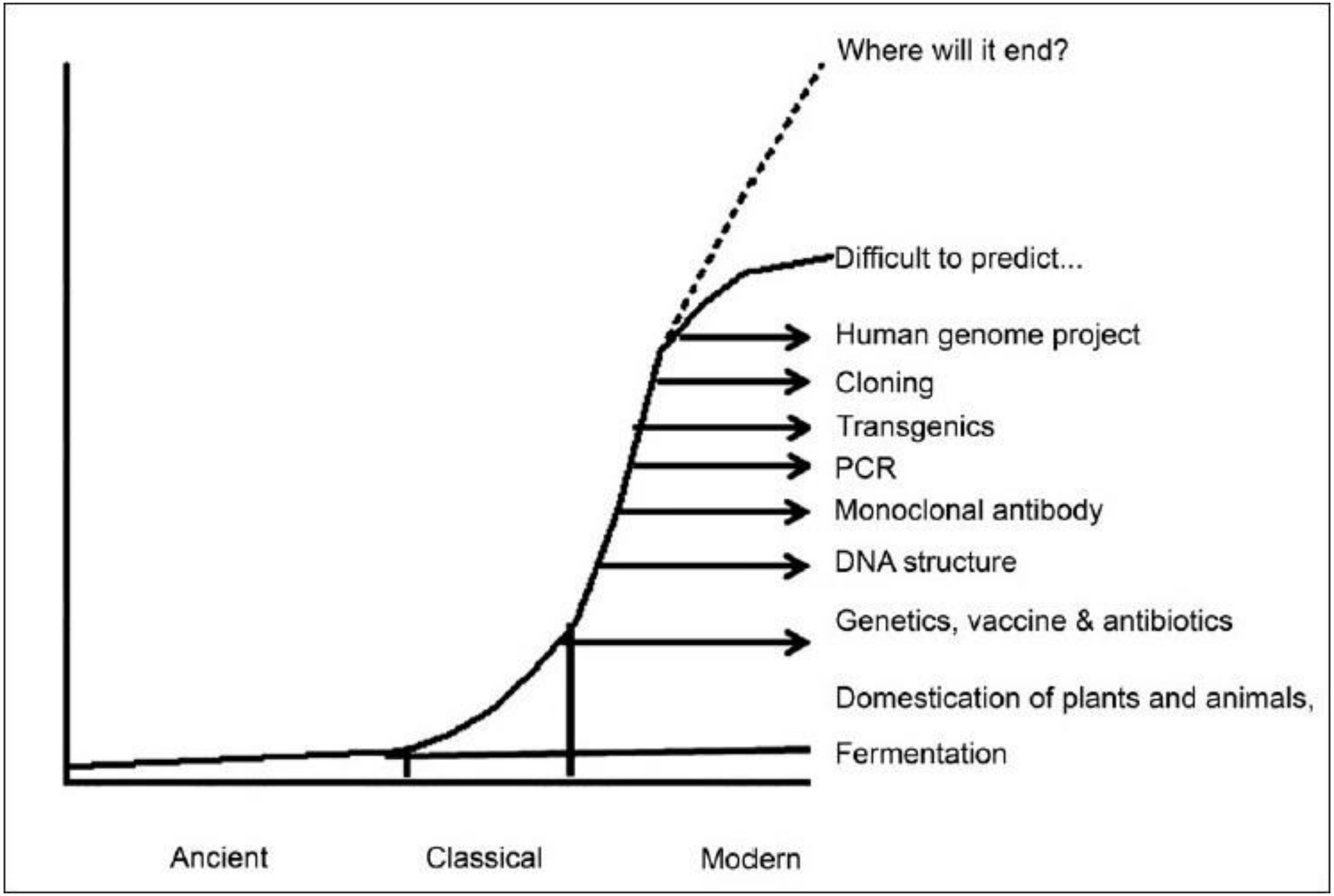
Field	Start-ups	Years to IPO	VC Amount ($M)	Series A ($M)	Sales ($M)	Income ($M)	Employees
Biotech	101	9	91	11.6	11	-16.5	71
Energy/Env.	16	7	208	4.9	50	-40.8	425
HW/Comp./Tel	57	8	99	6.9	87	-11.0	396
Internet	87	7	140	6.0	297	35.2	1213
Medtech	19	11	93	3.9	23	-12.6	184
Other	6	8	137	5.6	169	-15.0	391
Semiconductor	31	8	51	5.0	60	-5.6	404
Software	47	10	60	6.4	115	9.2	602
Overall	**364**	**8**	**103**	**7.6**	**114**	**-0.2**	**521**

Field	Founders' age	Founders %	Employee %	Investors %	IPO Shares
Biotech	45	7%	15%	56%	23%
Energy/Env.	38	8%	19%	56%	18%
HW/Comp./Tel	37	12%	27%	48%	15%
Internet	34	17%	22%	48%	15%
Medtech	41	8%	17%	54%	21%
Other	39	10%	21%	52%	20%
Semiconductor	37	13%	27%	43%	18%
Software	34	17%	27%	42%	17%
Overall	**38**	**12%**	**21%**	**50%**	**18%**

Biotechnology is growing



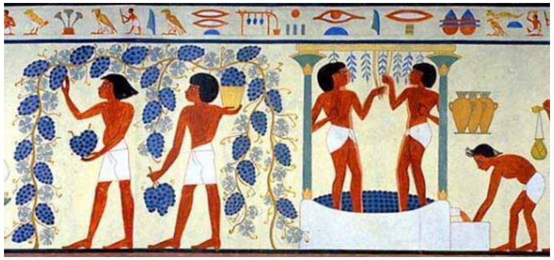
History of biotechnology



Ancient times (Biotechnology in BC)

- 10,000 BC – neolite – farming and domestication
- 8,000 BC – Egypt – fermented bread

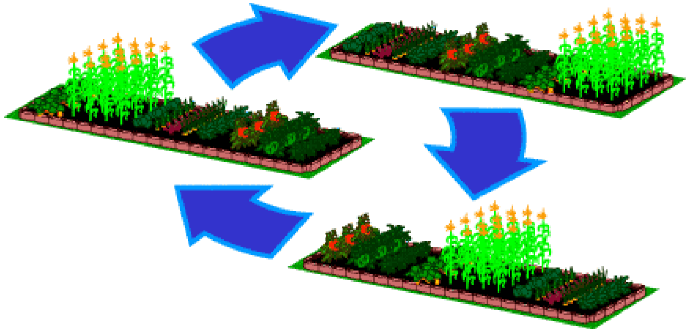
- 8,000 BC – Middle East – cheese production
- 6,000 BC – wine production



- 5,000 BC – brewing in Egypt
- 3,000 BC – all important crops of Old World domesticated
- 1,000 BC – all important crops of New World domesticated

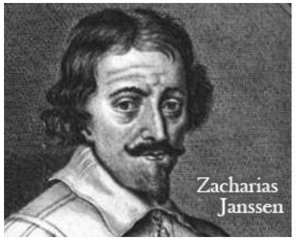
Ancient times (Biotechnology in BC)

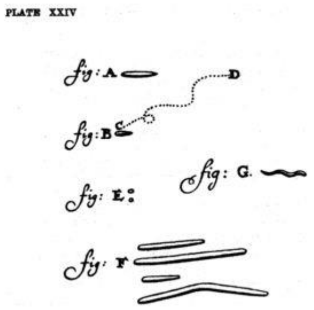
- 700 BC – Assyria – artificial pollination of date palm
- 500 BC – The Chinese use moldy curds as antibiotic treatment
- 250 BC – Greece – crop rotation
- 100 BC – China – powdered chrysanthemum as insecticide



Pre-20th century biotechnology

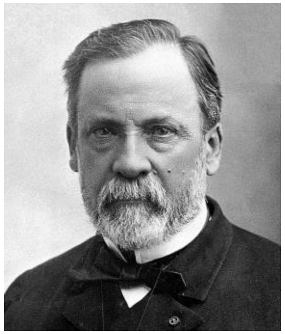
- 1590 Janssen invents the microscope
- 1663 Hooke discovers cells
- 1675 A. van Leeuwenhoek – father of microbiology – discovers bacteria and protozoa
- 1797 Jenner – smallpox vaccine

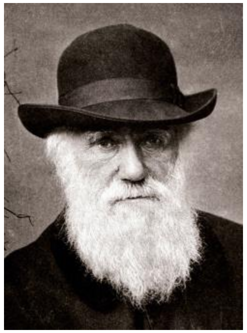




Pre-20th century biotechnology

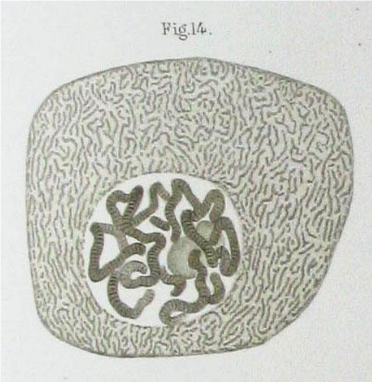
- 1833 The cell nucleus is discovered
- 1839 Schleiden, Schwann, Virchow – cell theory
- 1855 *Escherichia coli* is discovered
- 1855 Pasteur proves yeast are living organisms
- 1859 Darwin proposes theory of evolution
- 1865 Mendel publishes laws of inheritance

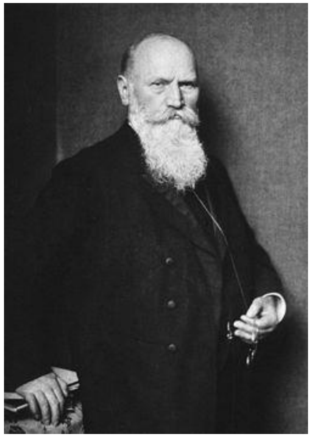


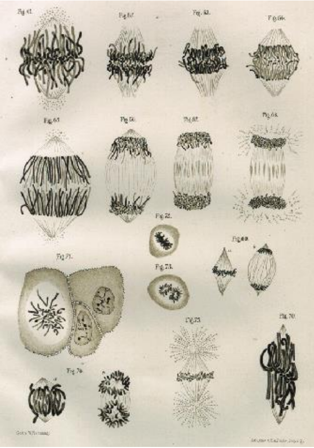


Pre-20th century biotechnology

- 1869 Miescher discovers DNA
- 1879 Flemming discovers chromatin
- 1883 Rabies vaccine is developed
- 1888 Waldeyer discovers the chromosome
- 1900 Mendelism is rediscovered



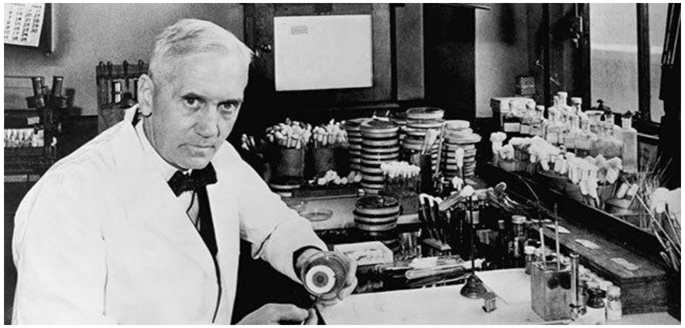


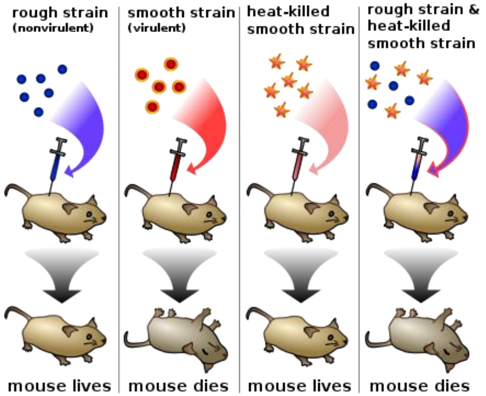


Early 20th century

- 1910 Morgan – genes are carried on chromosomes
- 1915 Twort discovers bacteriophages
- 1919 The term ‘biotechnology’ is coined
- 1928 Fleming discovers penicillin
- 1928 Griffith – bacterial transformation

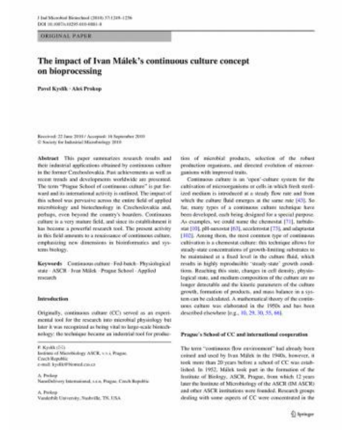




20th century biotechnology

- 1944 Avery – DNA is building blocks of genes
- 1953 Watson, Crick, Wilkins, Franklin – structure of DNA
- 1954 Development of cell culturing techniques
- 1956 Fermentation process is perfected
- 1961 Nucleotides carry the genetic code



J Ind Microbiol Biotechnol (2010) 37:1249–1256

ORIGINAL PAPER

The impact of Ivan Málek's continuous culture concept on bioprocessing

Pavel Kyslík · Aleš Prokop

Received: 22 June 2010 / Accepted: 16 September 2010
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Abstract This paper summarizes research results and their industrial applications obtained by continuous culture in the former Czechoslovakia. Past achievements as well as recent trends and developments worldwide are presented. The term "Prague School of continuous culture" is put forward and its international activity is outlined. The impact of this school was pervasive across the entire field of applied microbiology and biotechnology in Czechoslovakia and, perhaps, even beyond the country's borders. Continuous culture is a very mature field, and since its establishment it has become a powerful research tool. The present activity in this field amounts to a renaissance of continuous culture, emphasizing new dimensions in bioinformatics and systems biology.

Keywords Continuous culture · Fed-batch · Physiological state · ASCR · Ivan Málek · Prague School · Applied research

Introduction

Originally, continuous culture (CC) served as an experimental tool for the research into microbial physiology but later it was recognized as being vital to large-scale biotechnology: the technique became an industrial tool for production of microbial products, selection of the robust production organisms, and directed evolution of microorganisms with improved traits.

Continuous culture is an 'open'-culture system for the cultivation of microorganisms or cells in which fresh sterilized medium is introduced at a steady flow rate and from which the culture fluid emerges at the same rate [43]. So far, many types of a continuous culture technique have been developed, each being designed for a special purpose. As examples, we could name the chemostat [71], turbidostat [10], pH-auxostat [63], accelerostat [73], and adaptastat [102]. Among them, the most common type of continuous cultivation is a chemostat culture: this technique allows for steady-state concentrations of growth-limiting substrates to be maintained at a fixed level in the culture fluid, which results in highly reproducible 'steady-state' growth conditions. Reaching this state, changes in cell density, physiological state, and medium composition of the culture are no longer detectable and the kinetic parameters of the culture growth, formation of products, and mass balance in a system can be calculated. A mathematical theory of the continuous culture was elaborated in the 1950s and has been described elsewhere [e.g., 10, 29, 30, 55, 66].

Prague's School of CC and international cooperation

The term "continuous flow environment" had already been coined and used by Ivan Málek in the 1940s, however, it took more than 20 years before a school of CC was established. In 1952, Málek took part in the formation of the Institute of Biology, ASCR, Prague, from which 12 years later the Institute of Microbiology of the ASCR (IM ASCR) and other ASCR institutions were founded. Research groups dealing with some aspects of CC were concentrated in the

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Springer

Biotechnology in the 1970s

- Further development of process technology
- Biotechnology recognized as independent discipline
- 1973 Cohen et al. – recombinant DNA technology

Proc. Nat. Acad. Sci. USA
Vol. 70, No. 11, pp. 3240–3244, November 1973

Construction of Biologically Functional Bacterial Plasmids *In Vitro*

(R factor/restriction enzyme/transformation/endonuclease/antibiotic resistance)

STANLEY N. COHEN*, ANNIE C. Y. CHANG*, HERBERT W. BOYER†, AND ROBERT B. HELLING†

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Biotechnology in the 1980s

- 1981 First GM plant reported
- 1981 First successfully cloned mice
- 1982 Humulin approved by FDA
- 1983 First artificial chromosome
- 1983 Engineered Ti plasmid
- 1985 Production of HBV vaccine
- 1988 Mullis – PCR method
- 1988 US Congress funds the Human Genome Project
- 1989 Microorganisms used to clean up oil spill

Biotechnology since 1990s

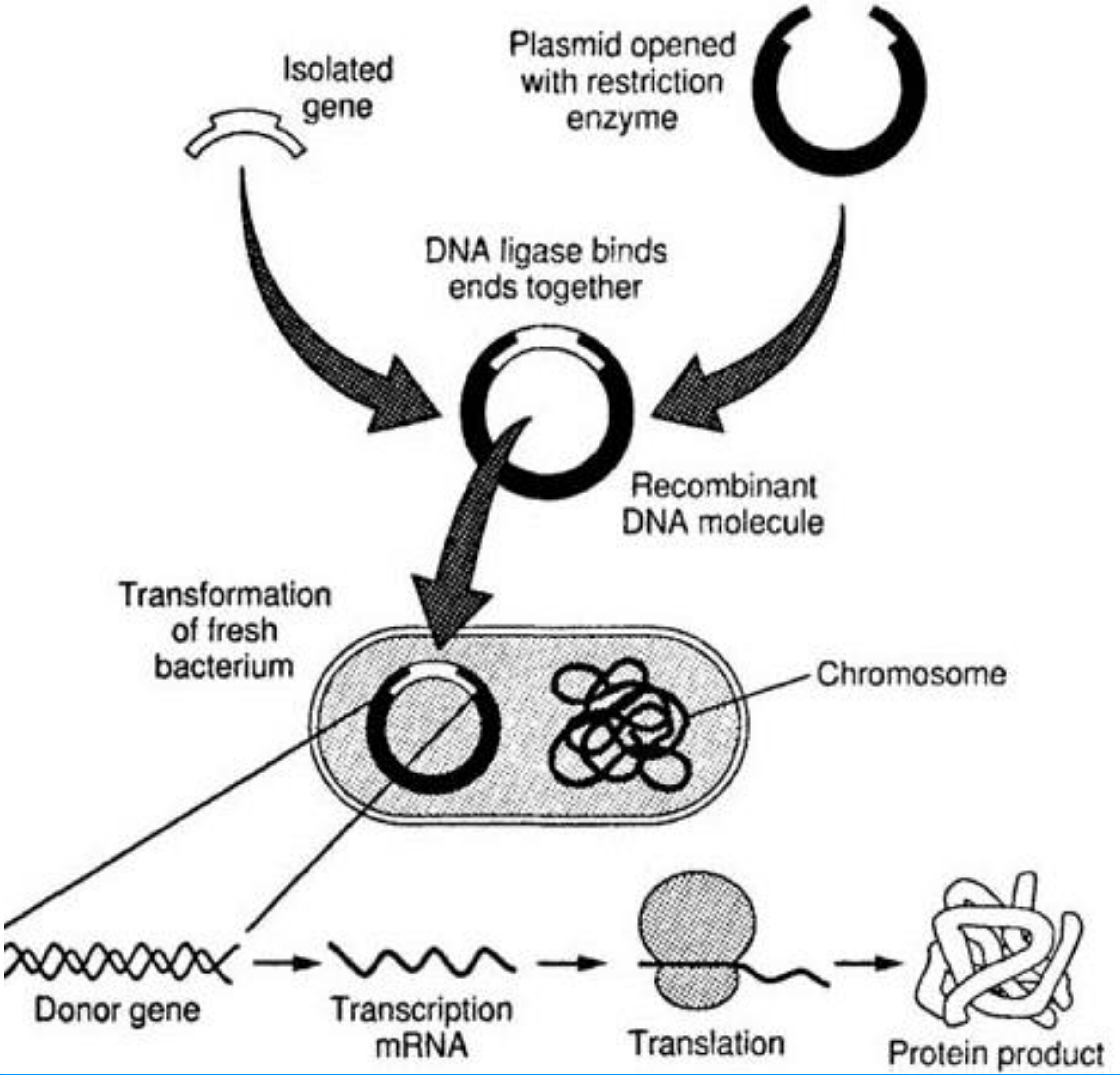
- 1994 First GM food approved by FDA
- 1997 Dolly is born
- 2000 Development of Golden rice
- 2001 Human genetic code published
- Drugs produced in GM animals
- Molecular farming

GMO

- Genetically modified organisms
- Deliberate artificial changes in genome (insertions, deletions of genes) achieved by recombinant DNA technology
- Breeding, crossing, mutagenesis etc. products are not GMO
- Transgenic organism

- Essential for preserving the mankind

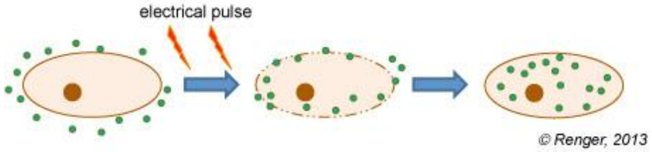
Concept of recombinant dna technology

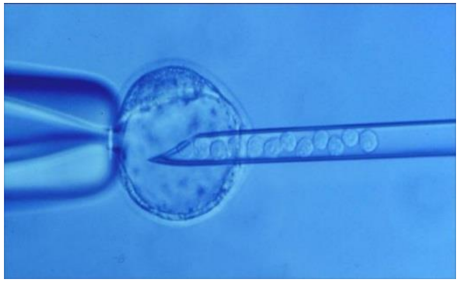


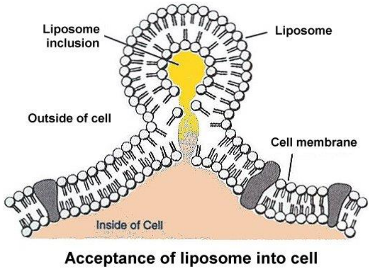
http://fhs-bio-wiki.pbworks.com/f/1265935367/8363.nfg021.jpg

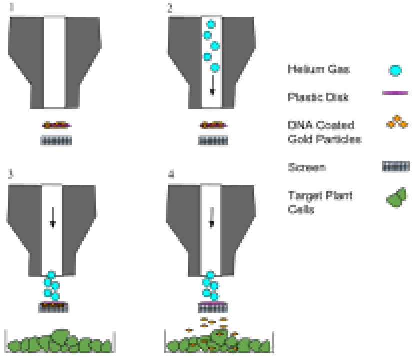
Techniques of DNA transfer

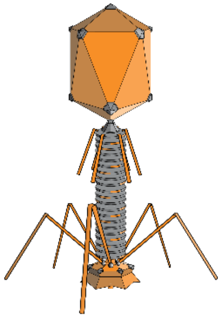
- Transformation/transfection
  - Chemical transformation
  - Electroporation (1.6-2.5 kV, 5 ms)
  - Micro-injection
  - Biolistic delivery
  - Liposomal transfection
  - T-DNA transfer
  - Transduction
  - Viral infection











Current molecular biotechnology

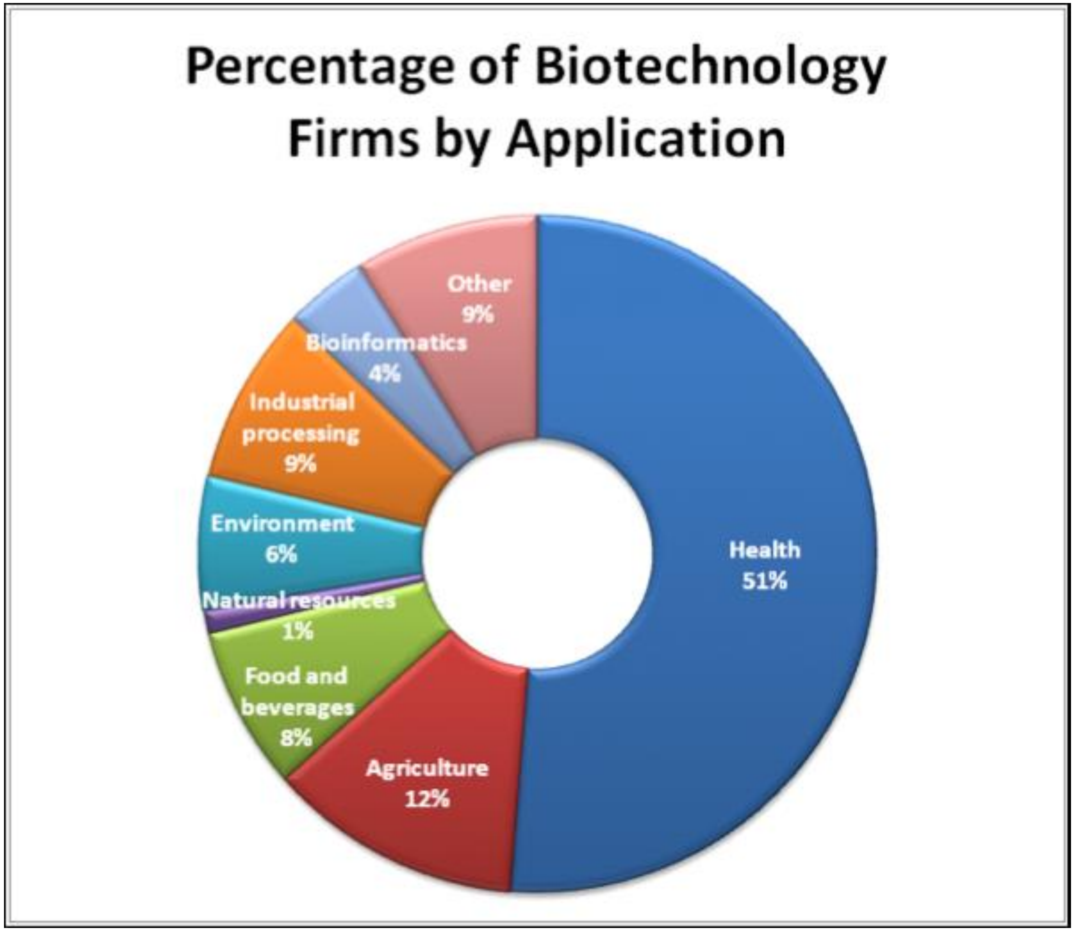


Table 3 The 20 top-selling biopharmaceutical products in 2013

Ranking	Product	Sales ($ billions)[a]	Year first approved	Company	Patent expiry (EU)	Patent expiry (US)
1	Humira (adalimumab; anti-TNF)	11.00	2002	AbbVie & Eisai	2018	2016
2	Enbrel (etanercept; anti-TNF)	8.76	1998	Amgen, Pfizer, Takeda Pharmaceuticals	2015	2028
3	Remicade (infliximab; anti-TNF)	8.37	1998	J&J, Merck & Mitsubishi Tanabe Pharma	2015	2018
4	Lantus (insulin glargine)	7.95	2000	Sanofi	2014	2014
5	Rituxan/MabThera (rituximab; anti CD20)	7.91	1997	Biogen-IDEC, Roche	2013	2016
6	Avastin (bevacizumab; anti-VEGF)	6.97	2004	Roche/Genentech	2019	2017
7	Herceptin (anti-HER2)	6.91	1998	Roche/Genentech	2014	2019
8	Neulasta (pegfilgrastim)	4.39	2002	Amgen	2015	2014
9	Lucentis (ranibizumab; anti-VEGF)	4.27	2006	Roche/Genentech, Novartis	2016	2016
10	Epogen/Procrit/Eprex/ESPO (epoetin alfa)	3.35	1989	Amgen, J&J, KHK	Expired	2013
11	Novolog/Novorapid (insulin aspart)	3.13	1999	Novo	2015	2015
12	Avonex (IFN-β-1a)	3.00	1996	Biogen Idec	2015	2015
13	Humalog mix 50:50 (insulin lispro)	2.61	1996	Lilly	2015	2014
14	Rebif (IFN-β-1a)	2.59	1998	Merck Serono	2015	2013
15	Aranesp/Nesp (darbepoetin α)	2.42	2001	Amgen, KHK	2016	2024
16	Advate/Recombinate (Octocog α)	2.37	1992	Baxter		
17	Levemir (insulin detemir)	2.15	2004	Novo	[Levemir]	2014
18	Actrapid/Novolin (insulin)	2.02	1991	Novo	2017	
19	Erbitux (cetuximab; anti-EGF)	1.92	2004	Bristol-Myers Squibb, Merck Serono	2014	2016
20	Eylea (aflibercept; anti-VEGF)	1.88	2011	Regeneron, Bayer	2020	2021

[a]Financial data from LaMerie Business Intelligence. J&J, Johnson & Johnson

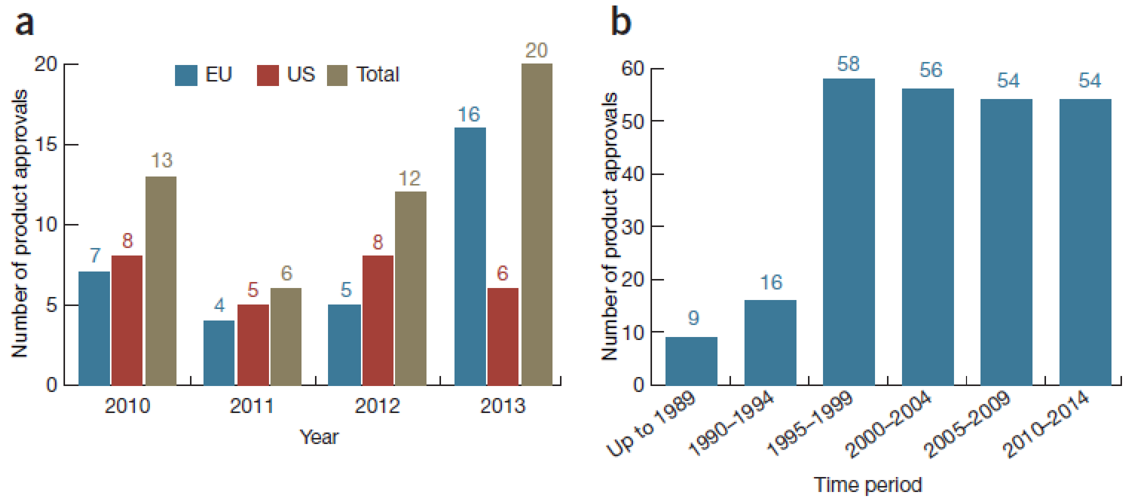


Figure 1 Approvals by region and by date. (**a**) Approvals in US and EU for each of four years in this study period. (**b**) Approval numbers over the indicated periods. Note that both regions experienced a lull in approvals, but in different years. In several instances, the same product has been registered in both regions, but in different years (**Table 1**), hence the yearly totals appear greater than the cumulative number of individual products approved from 2010-2013.

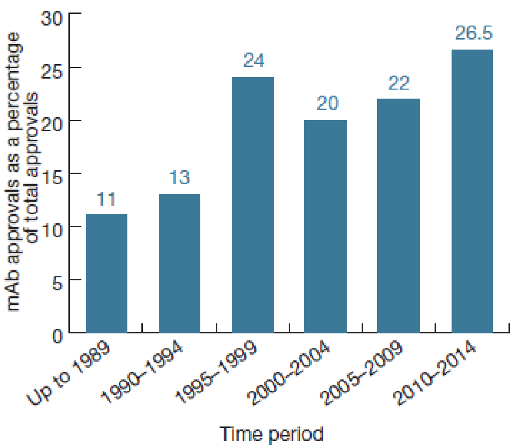


Figure 2 mAbs approved within the indicated periods, expressed as a percentage of total biopharmaceutical product approvals within the same period. Fc-based fusion products are not categorized as mAbs in these data.

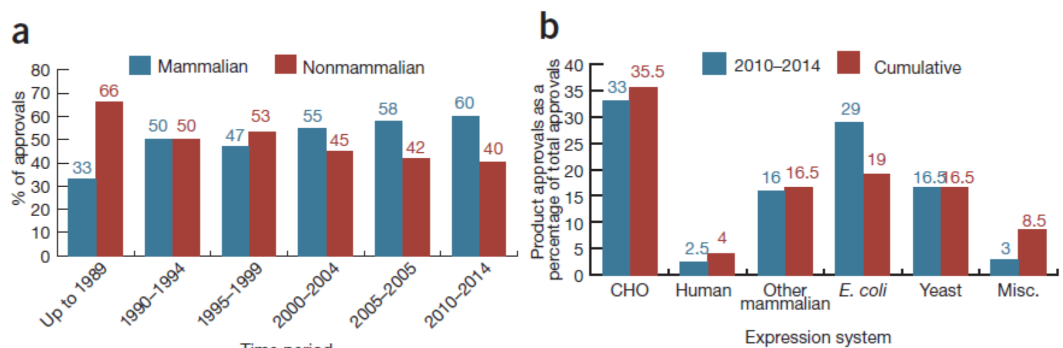
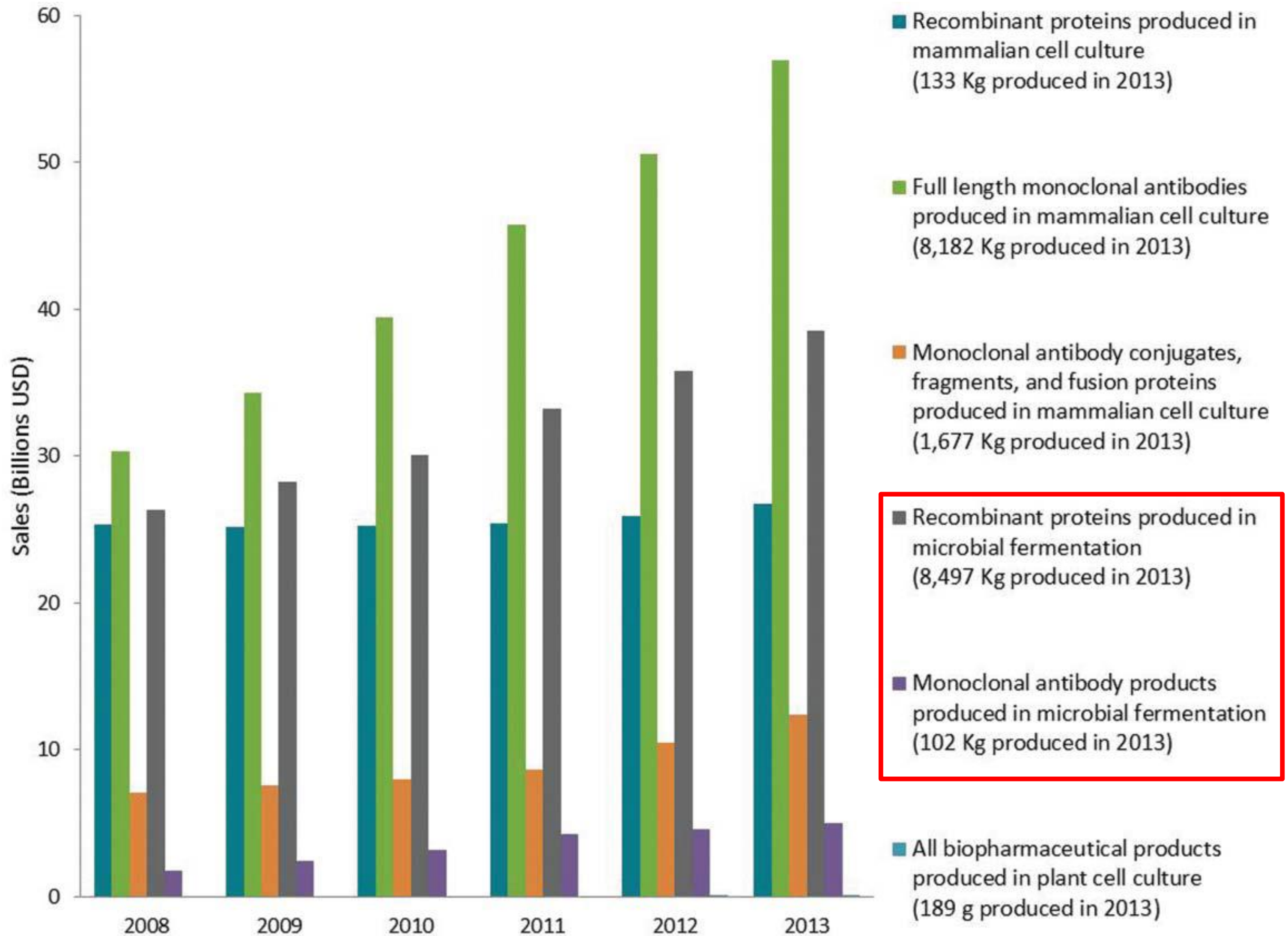
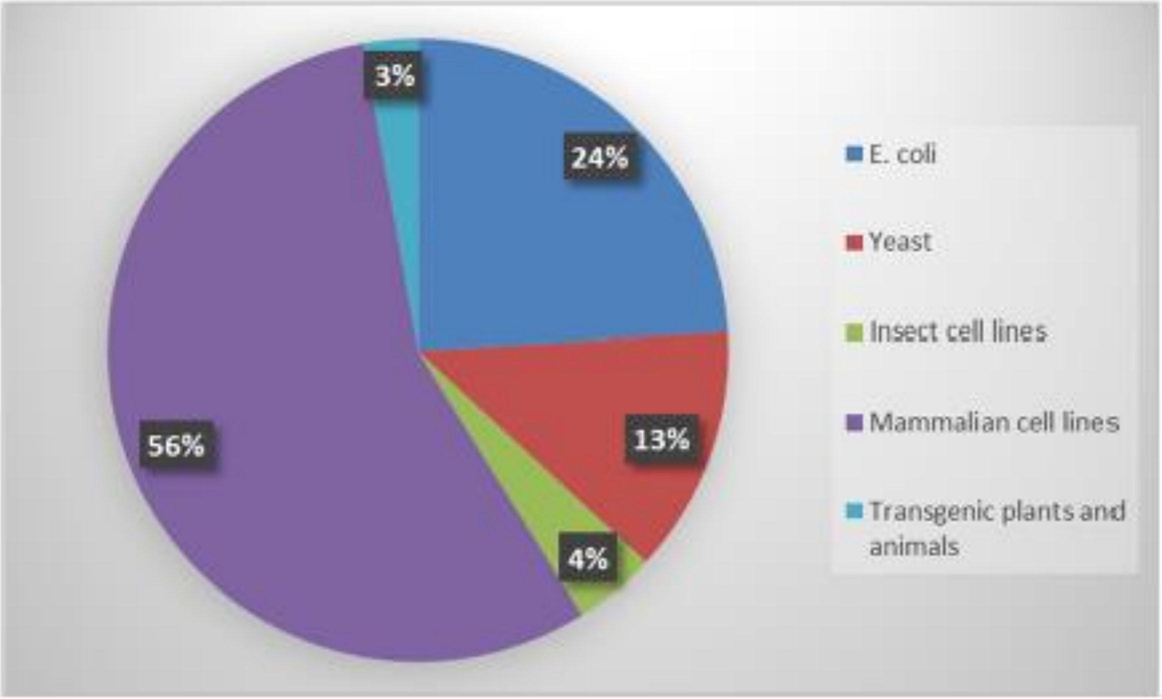


Figure 5 Expression systems used to manufacture biopharmaceutical products. (**a**) Relative application of mammalian versus nonmammalian-based expression systems in the production of biopharmaceuticals approved over the indicated periods. Each data set is expressed as a percentage of total biopharmaceutical product approvals for the period in question. (**b**) Product approvals, cumulative (1982–2014) and for period of this study (2010–July 2014) in the context of expression systems employed. Each data set is expressed as a percentage of total biopharmaceutical product approvals for the period in question.

Monoclonal antibodies



Ecker et al. (2015) mAbs 7: 9-14.



Percentage of biopharmaceuticals produced in different expression systems [5-13].

Why fungi and algae??

- Eukaryotic orgamisms
  - Post-translational modifications
- Easier for cultivation than mammalial cells or tissue cultures
- Several genomes sequenced
- Most important for classical biotechnology
- Components of traditional medicine
- Natural sources of useful compounds and enzymes
- Sustainability

Edible mushrooms

Table 1. China's production of edible mushrooms.

Year	Production		Values
	Production in China (1000 tons)	Share of World products (%)	(in billion Yuan)
1978	60	6	
1986	586	27	
1994	2641	54	
2000	6636	64	23
2001	7818	66	32
2002	8764	71	41
2003	10,387	73	48
2004	11,600	68	46
2005	13,340	70	48
2006	14,000	70	64
2007	16,820	75	80
2008	17,300	80	82
2009	20,203	80	110
2010	22,012	80	141
2011	25,717	80	149

Sources: The edible Fungi association of China [18] and Wu *et al.* [19].

- Production of edible mushrooms in Taiwan 2009/10

Common name	Scientific name production (MT)	Estimated (US$/kg)	Unit price
Golden mushroom	*Flammulina velutipes*	24,000	0.8-1.3
Shiitake	*Lentinula edodes*	28,000	3.1-3.5
King oyster mushroom	*Pleurotus eryngii*	12,000	2.6-3.2
Bunashimeji	*Hypsizigus marmoreus*	2,000	Bottle:4.8-9.7
			Bag:2.3-3.2
Phoenix tail mushroom	*Pleurotus sajor-caju*	10,000	1.8-2.7
Jew's ear	*Auricularia spp.*	3,000	1.3-1.9
Common white mushroom	*Agaricus bisporus*	2,000	3.2-4.5
Straw mushroom	*Volvariella volvacea*	1,000	1.3-1.7
Summer oyster mushroom	*Pleurotus cystidiosus*	600	0.4-0.6
Yuhuanmo	*Pleurotus citriopileatus*	300	0.6-1.1
Brown swordbelt	*Agrocybe spp.*	300	Bottle:3.2-4.8
			Bag:2.9-3.9
Sunny mushroom	*Agaricus braziliensis*	350	7.1-9.7
Oyster mushroom	*Pleurotus ostreatus*	100	2.6-3.2
Shaggy cap	*Coprinus comatus*	50	4.0-5.2
Lingzi	*Ganoderma spp.*	15(dry)	15.4-21.5

Edible mushrooms

- Wholesale prices of mushrooms (EUR/kg) in 2008

	Agaricus	*Pleurotus*
China	0,50	0,34
USA	1,00	1,77
Canada	1,43	-
Netherlands	1,50	3,00
Poland	1,00 - 1,40	2,10
France	0,65 - 1,60	2,50
Spain	1,20	2,00 - 3,50
Italy	1,20 - 1,50	0,30 - 3,00
CZE	1,80 - 1,90	2,20 - 3,00

Luxury food

- Truffles

- Black truffle – 2,500 USD/kg
- White truffles (*T. magnatum*) is the world's priciest food costing 6,000 – 10,000 USD/kg.

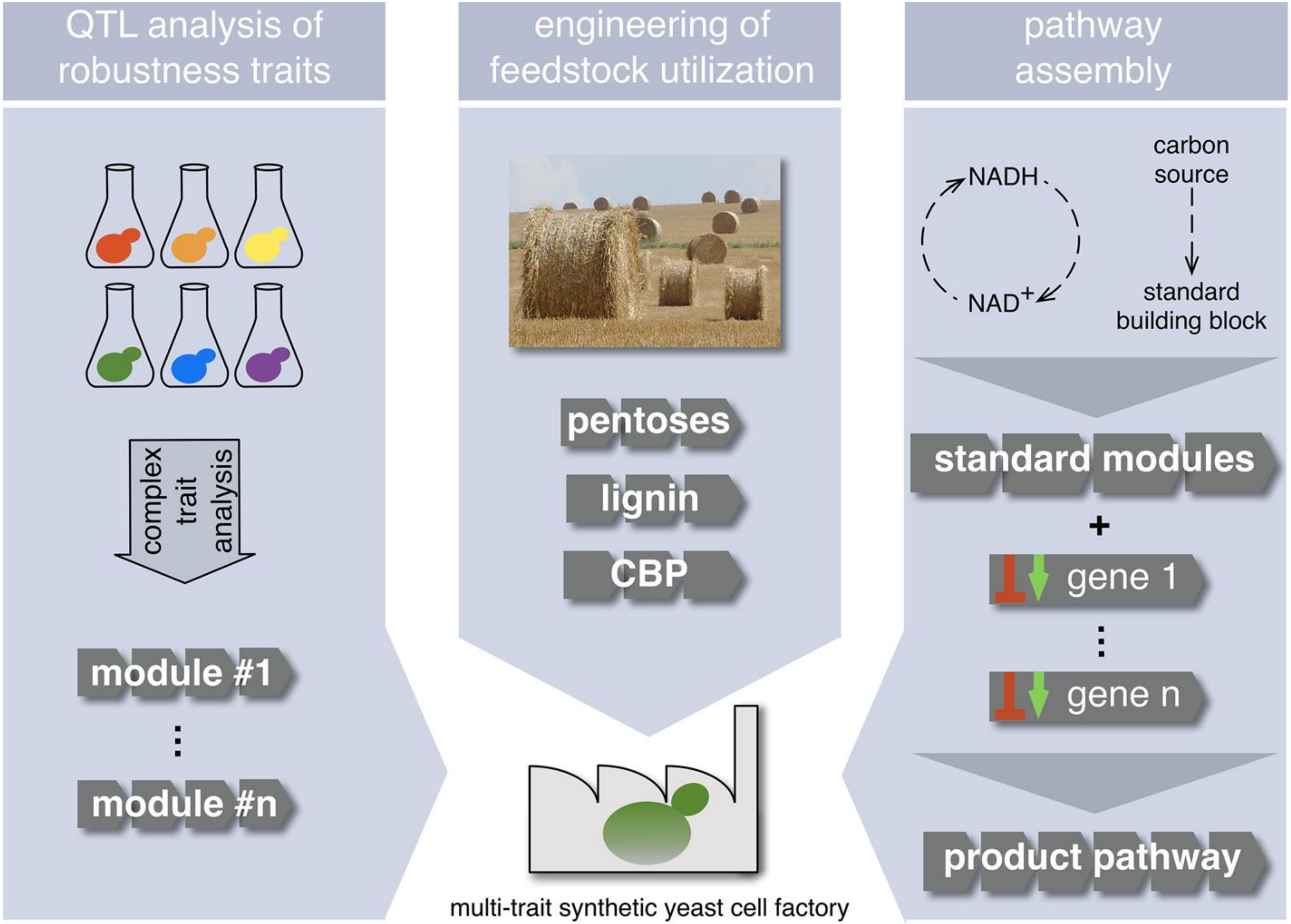
Pharmaceuticals in yeast

Table 1 Comparison of human insulin production systems [70]

Source	*E. coli*	*E. coli*	*S. cerevisiae*	*P. pastoris*
Destination of product	Cytoplasm	Secreted	Secreted	Secreted
Biomass cell dry weight (g/l)	80, in bioreactor with fed-batch culture	1.2, in shake flask with batch culture	5, in shake flask with batch culture	59, in bioreactor with fed-batch culture
Typical spec. growth rate (1/h)	0.08– 0.12	not specified	< 0.33	<0.03
Typical spec. production rate (mg/gh)	14.2	3.4	0.21	0.375
Product concentration (g/L)	4.34	0.009	0.075	3.075
Productivity (mg/l h)	1,085	4.01	1.04	17
Reference	[71]	[72]	[19]	[73]

Table 2 Some of the biopharmaceuticals produced by *S. cerevisiae* [2]

Type	Protein	Therapeutic application	Leader sequence	Titer
Hormones	Insulin Precursor	Diabetes	Synthetic	80 mg/L
	Glucagon	Diabetes	α-Factor	17.5 mg/L



QTL analysis of robustness traits
complex trait analysis
module #1
⋮
module #n
engineering of feedstock utilization
pentoses
lignin
CBP
multi-trait synthetic yeast cell factory
pathway assembly
NADH
NAD+
carbon source
standard building block
standard modules
+
gene 1
⋮
gene n
product pathway

Yeast in biotechnology

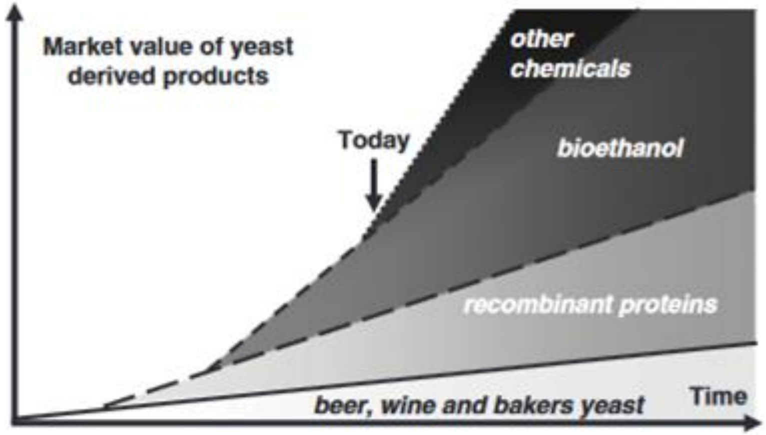
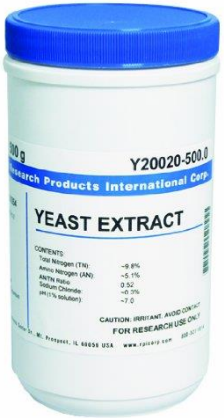
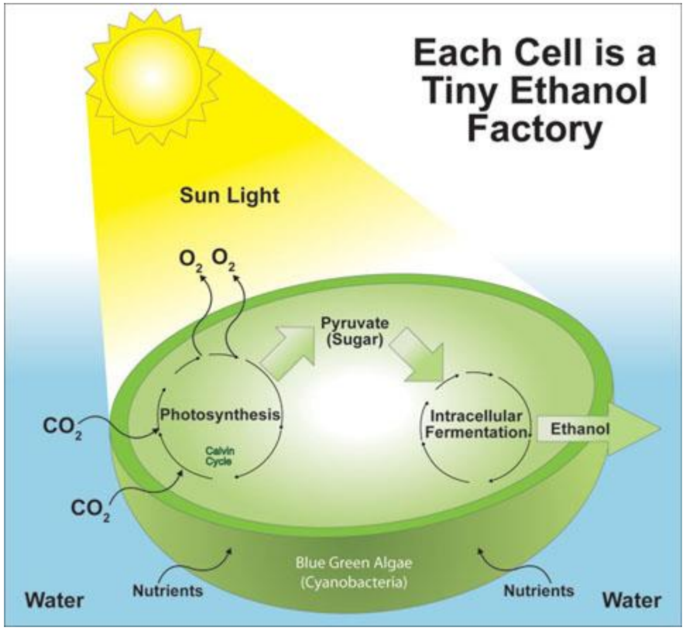


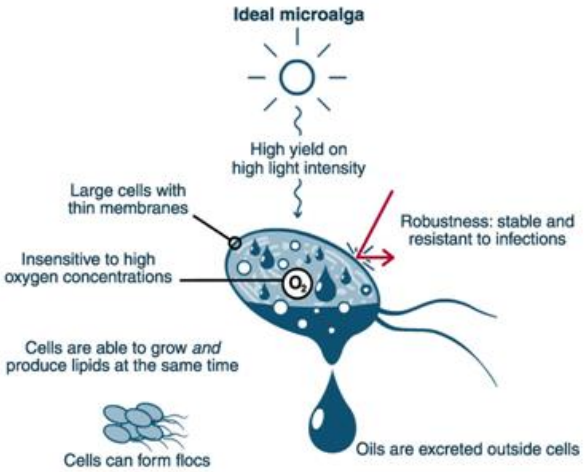
Fig. 1. Illustration of the growing market of yeast biotechnology. The use of *Saccharomyces cerevisiae* for the production of recombinant proteins is expected to grow substantially as more and more products can be produced using yeast as an expression system. The bioethanol market is expected to increase much beyond the current level. Yeast is also expected to be exploited for the production of a wide range of other chemicals in the future.



Algae and cyanobacteria in biotechnology

- Food and dietary supplements, livestock feed
- Biofuels
- Bioplastics
- Bioremediation





Advantages of algal biotechnology

- Cost-effective, eco-friendly process
- Photosynthetic organisms, nonpathogenic
- High nutritional value
- Algae are able to fix CO_2 -> wastewater treatment