LOSCHMIDT LABORATORIES

2. Introduction to Molecular Biotechnology

Outline

- Definition of biotechnology
- History of biotechnology
- Fundamentals of molecular biotechnology
- Basic concept of rDNA technology
- Methods of gene transfer
- Main fields of biotech applications
- Risks and positives

Definition of biotechnology

- **biotechnology** („biotech")
  bios – techne – logos
- **Kalr Ereky, 1917** – „biotechnology is a process by which raw materials could be biologically upgraded into socially useful products"
- „any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use"
  (*The United Nations Convention on Biological Diversity, 1992*)

History of biotechnology

- 1665 term **the cell**
- 1675 the father of **microbiology**
- 1839 - **the cell theory**





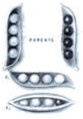


History of biotechnology

- 1822-95 **germ theory, vaccines**
- 1859 **evolutionary theory**
- 1866 **laws of inheritance**
- 1869 **discovery of DNA**
- 1900 rediscovery of Mendelism



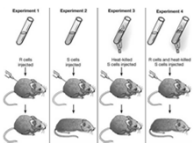





History of biotechnology

- 1902 **chromosome theory** of heredity
- 1910 **genes** are carried on chromosomes, basis of modern genetics (Nobel Prize in 1933)
- 1928 **bacterial transformation**



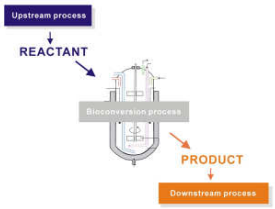



History of biotechnology

- 1944 **DNA the genetic carrier**
- 1952 – **conjugation and plasmids**
  (Nobel Prize in 1958)
- 1953 **structure of DNA**
  (Nobel Prize in 1962)
- 1967 Hargobind Khorana, Marshal Nirenberg, Robert Holley
  nucleotides carry the **genetic code**
  (Nobel Prize in 1968)

History of biotechnology

- 1970s biotechnology recognized as **scientific discipline**
  (interlink of chemical engineering, microbiology nad biochemistry)
- **traditional biotechnology** – based on fermentation
- development focused on **process technology**
  (bioreactor design, upstream, downstream)



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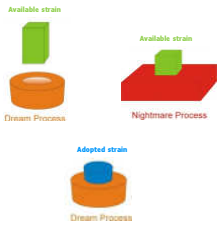
- **biotransformation component**
  - natural strains - far from optimum
  - difficult to optimise
  - induced mutagenesis and selection
    (chemical mutagens, UV radiation)
  - limited by inherited properties
    of the strain

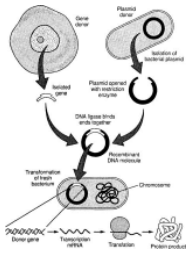


History of biotechnology

MOLECULAR BIOTECHNOLOGY REVOLUTION

- 1973 Stanley Cohen and Herbert Boyer - development of **recombinant DNA technology**

genetic engineering provided the means to create, rather than merely isolate, highly productive strains



History of biotechnology

MOLECULAR BIOTECHNOLOGY REVOLUTION

- 1973 Stanley Kohen and Herbert Boyer - development of **recombinant DNA technology**
- 1976 Herbert Boyer and Robert Swanson
- 1978 production of **human insulin** in *E. coli* by Genentec (recombinant "human" insulin approved by FDA 1982)
- 1981 production of recombinant **growth hormone**
- 1987 production of recombinant **tissue plasminogen activator** used to dissolve blood clots during myocardial infarction
- 1980-83 about 200 small biotechnological companies founded in US

Genentech
IN BUSINESS FOR LIFE

History of biotechnology

MOLECULAR BIOTECHNOLOGY REVOLUTION

- 1974 Rudolf Jaenisch - **first transgenic mammal** (a mouse)

animals and plants became targets to act as natural bioreactors

- 1982 first **recombinant animal vaccine** approved
- 1983 engineered Ti plasmid – **plant transformation**
- 1988 Kary Mullis - **PCR method** (Nobel Prize in 1993)
- 1994 first **genetically engineered food** aproved by FDA (tomato)

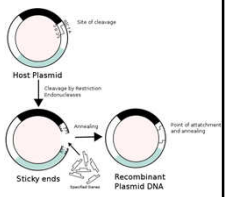
History of biotechnology

MOLECULAR BIOTECHNOLOGY REVOLUTION

- 1995 **first genome** sequenced (bacterium *Haemophilus influenzae*)
- 1996 complete **eukaryotic DNA sequence**
- 1996 commercial planting of **GMO crops** begins
- 1997 Ian Wilmut – **nuclear cloning** of a mammal
- 1998 first **antisense drug** approved by FDA
- 1999 *Drosophilia* genome sequenced
- 2000 *Arabidopsis* genome sequenced
- 2000 development of „**golden rice**"
- 2001 **human genome sequenced**
- 2009 first drug produced in genetically engineered animal (a goat)

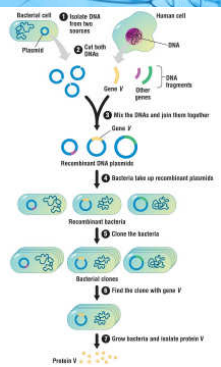
Molecular biotechnology

- **classical biotechnology** based on selective breeding
- **molecular** (modern) **biotechnology** („mol biotech")
  is revolutionary scientific discipline based on
  gene manipulation ***(Lecture 3)***
- the ability to transfer specific units of genetic information from one organism to another
- **recombinant DNA (rDNA) technology**
- **modern genetic engineering**
  enable create rather then isolate
  highly productive organisms



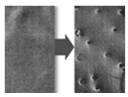
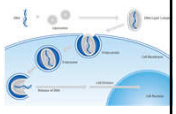
Basic concept of rDNA technology

- **isolate** gene(s) of interest
- **modify** gene(s)
  - ✓ *protein engineering* (***Lecture 4***)
  - ✓ *metabolic engineering* (***Lecture 5,6***)
- **ligate** gene(s) into a vector
- **transform** host organism
- **select** transformed cells
- **culture** host organism
- **application** of gene product

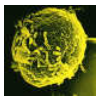


Techniques of DNA transfer

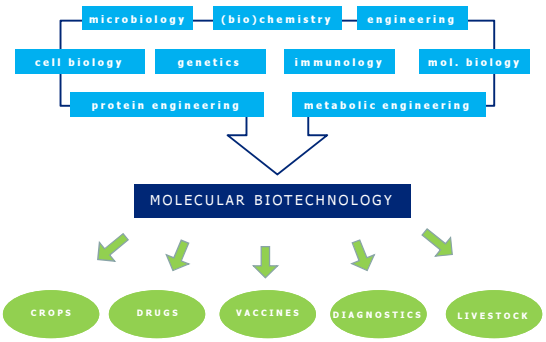
- **transformation** and **transfection**
- **direct methods**
  - **electroporation** (2.5 kV, 5 ms)
  - **chemical** transformation ($CaCl_2$)
  - **heat shock** (42°C)
  - **micro-injection**
  - **biolistic** delivery - „gene gun"
  - **liposomal** transfection
- **indirect methods**
  - **transduction** (bacteriophage)
  - viral and bacterial vectors






Molecular biotechnology



Main fields of application

- **white** - industrial biotechnology ***(Lecture 8)***
  - production of fine chemicals
  - production of proteins/enzymes
- **green** - agricultural biotechnology ***(Lecture 9)***
  - transgenic plants and animals
  - biofertilizers and biopesticides
- **red** - medical biotechnology ***(Lecture 10-11)***
  - developing new vaccines and drugs
  - tissue engineering and regenerative therapies
  - molecular diagnostics and pharmacogenomics
  - cell and gene therapy
- **grey** - environmental biotechnology ***(Lecture 12)***
  - biosensing and bioremediation
- **blue** - marine and aquatic




Pros and cons

- **safety and ethical concerns** of molecular biotechnology
  - do we have a right to move genes, creating new life forms ... „playing God"?
  - will transgenic organisms be harmfull to other organism or environment?
  - should humans be genetically engineered?

- **positive aspects** of molecular biotechnology
  - opportunities to accuratelly **diagnose, prevent and cure** a wide range of infectious and genetic **diseases**
  - **increase crop yield and resistence** to insects and diseases, environmental stress (e.g., drought, heat, cold)
  - develop microorganisms that **produce chemicals in sustainable manner**
  - facilitate **removal of pollutants and waste** materials from environment