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
- Positive aspects, concerns and consequences

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

- a story that began long time ago
- 10,000 B.C. neolitic revolution **cultivation and domestications**
- 8,000 B.C. **fermented bread** (ancient Egypt)
- 8,000 B.C. **cheese making** (the Middle East)
- 6,000 B.C. **wine production** (Egypt and the Middle East)
- 5,000 B.C. **brewing** (ancient Egypt)
- developed without any knowledge about existence of cells or enzymes



History of biotechnology

- 1665 Robert Hooke - term **the cell**
- 1675 Anton Van Leeuwenhoek - the father of **microbiology**
- 1839 Matthias Schleiden, Theodore Schwann and Rudolf Virchow - **the cell theory**

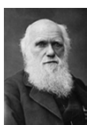


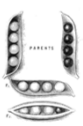




History of biotechnology

- 1822-95 Louis Pasteur – **germ theory, pasteurisation, vaccines**
- 1859 Charles Darwin - **evolutionary theory**
- 1866 Gregor Johann Mendel - **laws of inheritance**
- 1869 Johann Miescher - **discovery of DNA**
- 1900 rediscovery of Mendelism

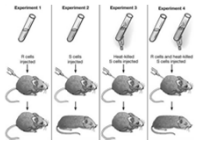




History of biotechnology

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



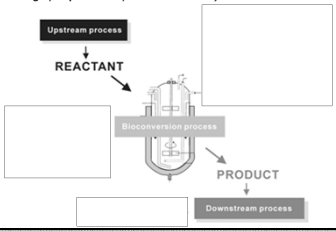



History of biotechnology

- 1944 Oswald Avery – **DNA the genetic carrier**
- 1952 Joshua Lederberg – **conjugation and plasmids** (Nobel Prize in 1958)
- 1953 James Watson nad Francis Crick, Maurice Wilkins and Rosalind Franklin – **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)



History of biotechnology

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  (interlink of chemical engineering, microbiology nad biochemistry)
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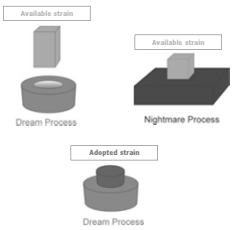
- development focused on **process technology**
  (bioreactor design, upstream, downstream)
- **biotransformation component**
  - natural strains - far from optimum
  - difficult to optimise
  - induced mutagenesis and selection (chemical mutagens, UV radiation)
  - limited by inherited properties of the strain

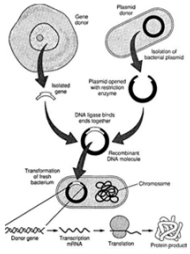


History of biotechnology

MOLECULAR BIOTECHNOLOGY REVOLUTION

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

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



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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History of biotechnology

MOLECULAR BIOTECHNOLOGY REVOLUTION

- 1973 Stanley Kohen and Herbert Boyer - development of **recombinant DNA technology**
- 1976 Herbert Boyer and Robert Swanson

Genentech
IN BUSINESS FOR LIFE

- 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

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)



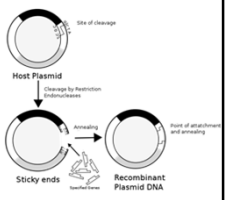
History of biotechnology

MOLECULAR BIOTECHNOLOGY REVOLUTION

- 1995 **first genome** sequenced (bacterium *Haemophilus influenzae*)
- 1996 complete **eukaryotic DNA sequence** THE ORGANISM?
- 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)

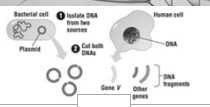

Molecular biotechnology

- **classical biotechnology** based on selective breeding
- **molecular** (modern) **biotechnology** („mol biotech")
  is revolutionary scientific discipline based on **gene manipulation**
- 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



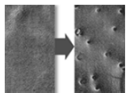
Basic concept of rDNA technology

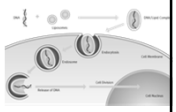
- **isolate** gene(s) of interest
- **modify** gene(s)
  - ✓ *protein engineering (**Lecture 3**)*
  - ✓ *metabolic engineering (**Lecture 4-5**)*
- **ligate** gene(s) into a vector
- **transform** host organism

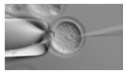


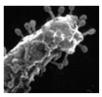
Techniques of DNA transfer

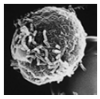
- **transformation** and **transfection**
- **direct methods**
  - **electroporation** (2.5 kV, 5 ms)
  - **chemical** transformation ($CaCl_2$)
  - **heat shock** (42°C)
  - **micro-injection**
  - **biolistic** delivery - „gene gun"
  - **liposomal** transfection
- **indirect methods**
  - **transduction**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

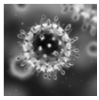












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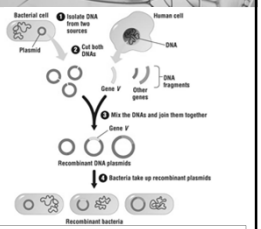
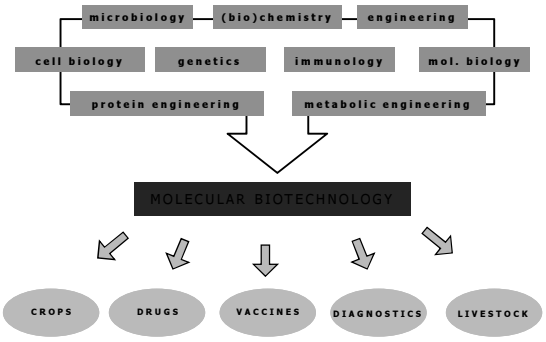
- **ligate** gene(s) into a vector
- **transform** host organism
- **select** transformed cells
- **culture** host organism
- **application** of gene product



Molecular biotechnology



Main fields of application

- **white** - industrial biotechnology (***Lecture 6***)
  - production of fine chemicals
  - production of proteins/enzymes

- **green** - agricultural biotechnology ***(Lecture 7)***
  - transgenic plants and animals
  - biofertilizers and biopesticides

- **red** - medical biotechnology ***(Lecture 8-10)***
  - developing new vaccines and drugs
  - tissue engineering and regenerative therapies
  - molecular diagnostics and pharmacogenomics
  - cell and gene therapy

- **grey** - environmental biotechnology ***(Lecture 11)***
  - 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 accurately **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