LOSCHMIDT LABORATORIES

1. Introductory lecture

Organisation of the course

Outline

- Introduction of course
- Content of course and practical classes
- Lecturing and evaluation
- Recommended literature
- Biotechnology at MU
- **Excursion in Loschmidt Laboratories**

Introduction of the course

EXTENSIVE MULTIDISCIPLINARITY

PREREQUISITES:

- basic knowledge of microbiology, molecular biology, biochemistry, immunology and genetics

COURSE FOCUSE:

- the specific aspects of **modern biotechnology**
- examples of **up to date applications and discoveries** (industry, agriculture, pharmacy, biomedicine and environmental protection)
- the role of modern biotechnology in **sustainable living**

Sustainability

- **concept of sustainability**

with the aim to promote a necessary "… development that meets the needs of the present without compromising the ability of future generations to meet their own needs"

World Commission on Environment and Development, 1987

Sustainability

- **reduce waste** production and environmental impact
- reduce **consumption of resources** (e.g., materials, energy, air, water)
- increase the **recycling** and use of **renewable materials** (e.g., biomass)

Biotechnology

- **KEY TECHNOLOGY** of 21st century

ENVIRONMENTAL ASPECTS

- **natural** processes (bioprocesses)
- **sustainable** and resource efficient

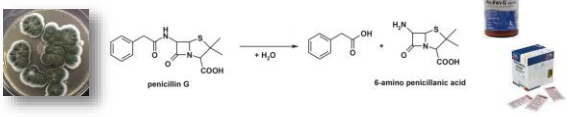
ECONOMICAL ASPECTS

- **1/3 of worldwide production** derived from bioprocesses in 2030
- biotechnology market 300 billion EUR*

Route to the Knowledge-Based Bioeconomy, 2007

Example of sustainable technology

- **hydrolysis of penicillin G**



CHEMICAL PROCESS (-40°C)		BIOCATALYSIS (+30°C)	
1000 t	penicillin G	1000 t	penicillin G
160 t	ammonia	45 t	ammonia
300 t	dimethylchlorosilane	10,000 m³	water
800 t	*N,N*-dimethylaniline	1 t	ENZYME
600 t	phosphopentachloride		(1 $/kg 6-APA)
4,200 m³	dichloromethane		
4,200 m³	*n*-butanol		

LECTURES

Organization info

Content of the course

2. Basics of Molecular Biotechnology

METHODOLOGICAL LECTURES

3. Methods of Gene Manipulations
4. Protein Engineering
5. Microfludics, Lab on a Chip

TECHNOLOGICAL LECTURES

6. Biofuels
7. Molecular Biotechnology in Industry
8. Environmental Molecular Biotechnology
9. Molecular Biotechnology in Agriculture
10. Molecular Biotechnology in Medicine I.
11. Molecular Biotechnology in Medicine II.

Lecturers

Doc. RNDr. Zbyněk Prokop, Ph.D. (UČO 23696)

- protein engineering, microfluidics
- biotechnological applications
- Loschmidt Laboratories, leader of research team
- co-founder of Enantis – 1st biotech spin-off at MU

Mgr. Sarka Bidmanova, Ph.D. (UČO 77580)

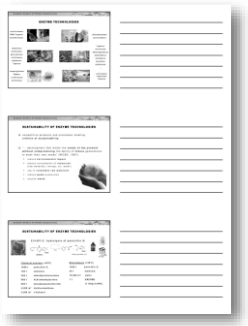
- bioanalytical devices for military and environment
- immobilization and characterization of enzymes
- Enantis and Loschmidt Laboratories, research specialist

Mgr. Táňa Koudeláková, Ph.D. (UČO 39790)

- molekulární biologie a proteinové inženýrství
- řízená evoluce, enzymové biotechnologie
- Loschmidt Laboratories, research specialist

Instructions

- bring printed copy of the slides as **handouts** for notes



Instructions

- bring printed copy of the slides as **handouts** for notes
- find all materials including printed version of the slides at **http://is.muni.cz/**
- **be on time**, come at least 5 min before lecture starts
- please,contact me if any problem with the lecture or material
- be **active** and participate in **discussions**

Lecturing system

- powerpoint slides as well as recommended literature in **English**
- lecturing, discussions and examination in **Czech**
- 2 hrs per week
- lecture part I. (45 min)
  BREAK (5 -10 min)
  lecture part II. (45 min)

Activities and Evaluation

- **reading** the original literature
  - review or book chapter for each lecture
  - „Lecture 02 (READING).pdf"
- **four progress written tests** during the lecturing period
  - at the beginning of lecture 4., 6., 8., and at the end of semester
  - each 10-12 questions from lectures and reading
  - questions a,b,c,d type, can be cumulative with multiple answers
  - duration 10 min
- **final written test** during examination period
  - 50 questions from entire course / 1 hour

Recommended literature

- M. Wink (Ed.) 2011: **An Introduction to Molecular Biotechnology**: Fundamentals, Methods and Applications, 2nd Edition, Willey-Blackwell
- B. R. Glick, J. J. Pasternak, C. L. Patten 2011: **Molecular Biotechnology: Principles and Applications of Recombinant DNA**, 4th Edition, ASM Press
- J. M. Walker, R. Rapley 2009: **Molecular biology and biotechnology**, 5th Edition, RSC Publishing
- A. Sonnino (Ed.) 2011: **Introduction to molecular biology and genetic engineering**, Food and Agriculture organization of the united Nations, Rome (pdf on IS materials)

PRACTICAL LESSONS

Organization info

Content of the course

1. **Design** of recombinant systems (LL, MU)
2. Preparation and testing of **microfluidic** chip (LL, MU)
3. **Fermentation** of recombinant microorganisms (LL, MU)
4. Preparation of enzymatic **biosensor** (Enantis)
5. **Biodegradation** of environmental pollutant by recombinant bacterium (LL, MU)
6. **Biocatalytic** preparation of pharmaceutical precursor (Enantis)
7. Preparation and transformation of **liposoms** (VRI)
8. Analysis of liposoms by DLS, TEM etc. (VRI)

Instructors

Loschmidt laboratories, MU
Táňa Koudeláková, Ph.D. (UČO 39790)
Lukáš Chrást, M.Sc. (UČO 269981)
Tomáš Buryška, M.Sc. (UČO 323660)

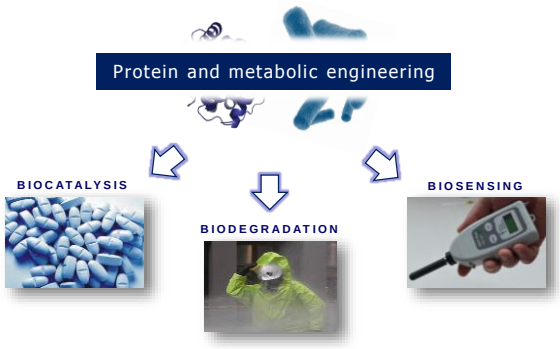
Enantis, Ltd.
Šárka Bidmanová, Ph.D.
Veronika Štěpánková, Ph.D.

Veterinary Research Institute
PharmDr. Josef Mašek, Ph.D.
Ing. Štěpán Koudelka, Ph.D.
MVDr. Pavel Kulich, Ph.D.
RNDr. Jana Plocková

Lecturing system

- **INTERACTIVE SYNOPSIS** available on IS
- 2 hrs per week (STARTS on Wednesday 12/10, A13 entrance 2.floor)
- **CAPACITY**: two groups of 10 students (10:00 – 12:00, 14:00 – 16:00)
- **LANGUAGE**: materials EN, spoken language CZ, protocols and essays either EN or CZ
- **ABSENCE (max. 1)**: official excuse in IS + substitute activity - written essay EN or CZ, two A4 pages, 1.5 spaced, TNR 12 (*template on IS*)
- **LECTURE ORGANISATION**
  - assignment (**HOMEWORK**)
  - theoretical introduction given by lecturer
  - experimental work in the laboratory
  - **protocol submitted in one week after each practical** (*template on IS*)

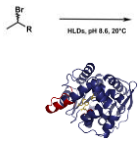
Loschmidt Laboratories, MU

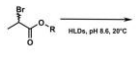


Koudelakova et al. 2012. Biotech. J.

Biocatalysis

Bradyrhisobium japonicum





Drugs

Feromones

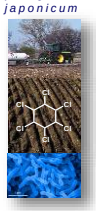
DSM BRIGHT SCIENCE. BRIGHTER LIVING.

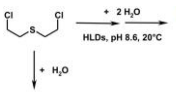
ZENTIVA SPOLEČNOST SKUPINY SANOFI

Prokop, Z., et al. 2004. WO 2006079295 *Prokop, Z., et al. 2010. Angew. Chem. 49: 6111–6115*

Biodegradation

Sphingobium japonicum





+ 2 H_2O

HLDs, pH 8.6, 20°C

+ H_2O

toxic

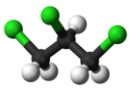
Pacific Northwest NATIONAL LABORATORY

Bundeswehr

Foster-Miller

Prokop, Z., et al. 2005. WO 2006128390 *Prokop, Z., et al. 2006. Biotech. J. 1: 1370-1380*

Biodegradation



- **1,2,3-trichloropropane (TCP)**
  - chemical building block, fumigant, solvent
  - persistent water and soil contaminant
  - toxic, carcinogenic

Biodegradation

Rhodococcus

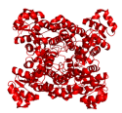
Agrobacterium

rDNA

Escherichia

$CO_2 + H_2O$

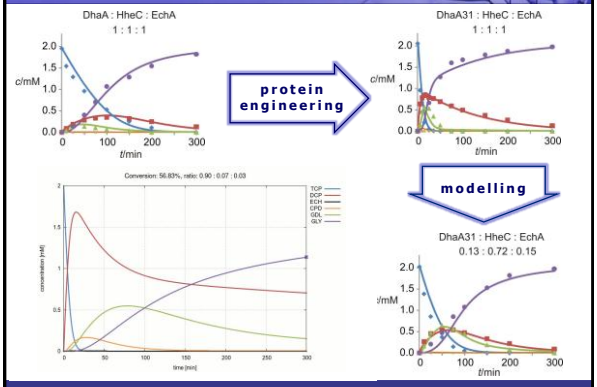
haloalkane dehalogenase



haloalcohol dehalogenase

epoxide hydrolase

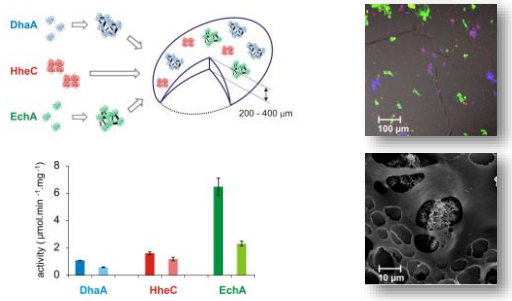
Biodegradation



Pavlova, et al. 2009. Nature Chem. Biol. 5: 727-733 Dvorak, et al. 2014. ChemBioChem. 15: 1891-1895

Immobilization of pathway

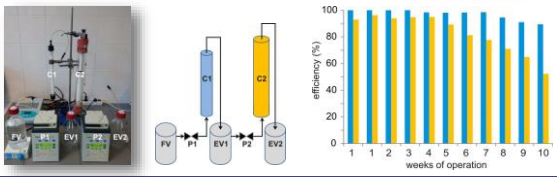
- **LentiKats®** - polyvinil alcohol particles
- **CLEA®** - cross-linked enzyme agregates



Dvorak, et al. 2014. ACS Environ. Sci. Tech. DOI 10.1021/es500396r

Immobilization of pathway

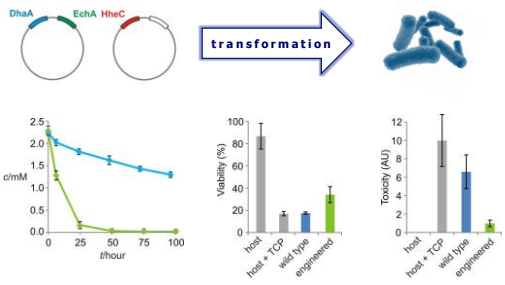
- **packed bed reactor**
  - column 1 (50 ml, DhaA)
  - column 2 (100 ml, HheC + EchA)
- **2.5 months** at 22°C
- **97% efficiency** for TCP
- **9.7 g** of TCP degraded in total



Dvorak, et al. 2014. ACS Environ. Sci. Tech. DOI 10.1021/es500396r

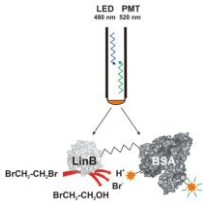
Biodegradation

- **optimized synthetic pathway** assembled in *E. coli*
  - pairs of duet vectors (pETDuet, pCDF, pACYC)

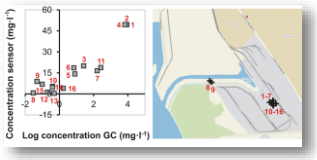


Kurumbang, et al. 2014. ACS Synth. Biol. 3: 172−181

Biosensing

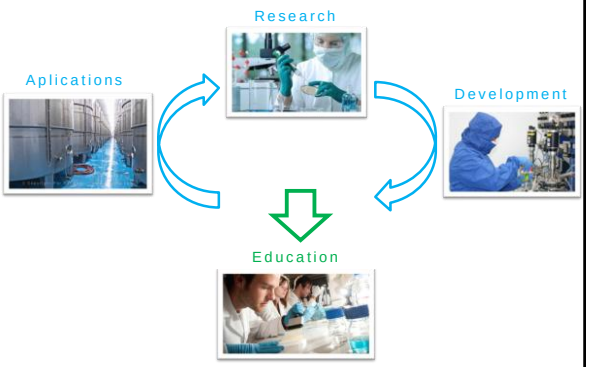


Sampled site	Concentration GC (mg·l⁻¹)	Response sensor (mV·s⁻¹)
1	1.68	2003.8
2	1.60	956.3
3	1.46	670.2
4	1.13	529.9
5	0.96	752.4
6	1.08	901.6



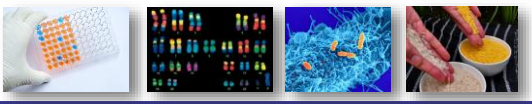
Bidmanova, et al. 2010. Anal. Bioanal. Chem. 398:1891–1898

Modern Biotechnology at MU



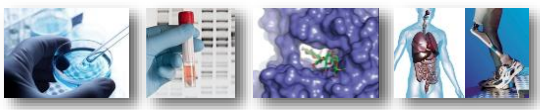
Molekulární biotechnologie Bi7430

- Období: podzim (každoročně)
- Rozsah: přednáška 2 hodiny/týden, cvičení 2 hodiny/týden
- Přednášky: Doc. Prokop, Dr. Koudeláková, Dr. Bidmanová
- Cvičení: Dr. Bidmanová, Dr. Koudeláková, Dr. Štěpánková, Mgr. Buryška, Mgr. Chrást
- Osnova:
  - proteinové a metabolické inženýrství
  - genetické inženýrství rostlin a živočichů
  - molekulární diagnostika a moderní vakcíny
  - buněčná a genová terapie a regenerativní medicína
  - molekulární biotechnologie v průmyslu a zemědělství



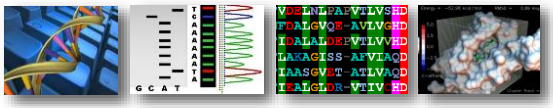
Proteinové inženýrství Bi7410

- Období: jaro
- Rozsah: přednáška 1 hodina/týden
- Vyučující: Mgr. Radka Chaloupková, Ph.D.
- Osnova:
  - strukturně-funkční vztahy proteinů
  - metody exprese a purifikace rekombinantních proteinů
  - metody strukturní a funkční analýzy proteinů
  - racionální design, semi-racionální design a řízená evoluce
  - příklady využití proteinového inženýrství



Bioinformatika Bi5000+Bi9060+Bi9061

- Období: podzim
- Rozsah: přednáška 2 hodiny/týden, cvičení 2 hodiny/týden
- Vyučující: prof. Mgr. Jiří Damborský, Dr., doc. RNDr. Roman Pantůček, Ph.D.,
- Osnova:
  - bioinformatické databáze a jejich prohledávání
  - analýza nukleotidových a proteinových sekvencí
  - hledání a identifikace genů
  - analýza a předpověď struktury proteinů



Strukturní biologie Bi9410

- Období: podzim
- Rozsah: přednáška 2 hodiny/týden, cvičení 2 hodiny/týden
- Vyučující: Mgr. David Bednář
- Osnova:
  - struktura, stabilita a dynamika biologických makromolekul
  - makromolekulární interakce a komplexy
  - stanovení a předpověď struktury, identifikace důležitých oblastí
  - stanovení vlivu mutace na strukturu a funkci proteinu
  - aplikace v biologickém výzkumu, návrhu léčiv a biokatalyzátorů