evropský sociální fond v ČR

EVROPSKÁ UNIE

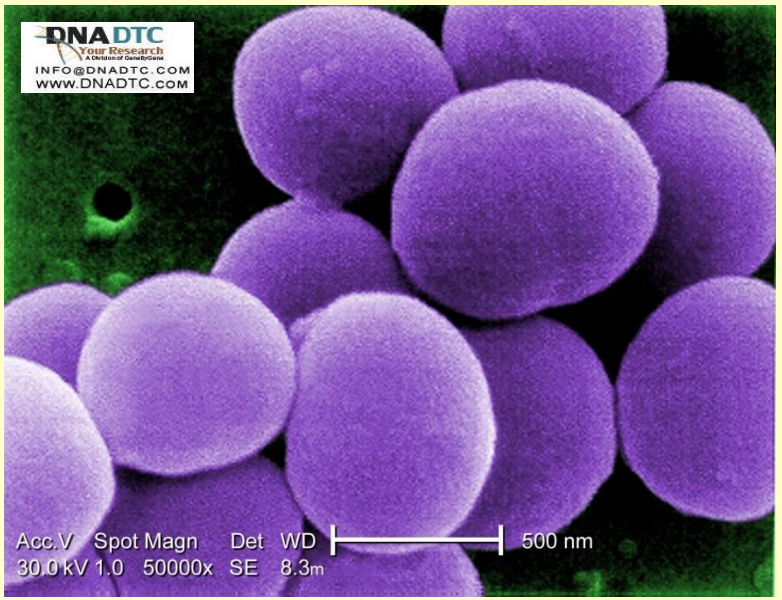
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY

OP Vzdělávání pro konkurenceschopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Konzervační genetika

MIKROORGANISMY



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Katedra buněčné biologie a genetiky

Univerzita Palackého, Olomouc

OPVK (CZ.1.07/2.2.00/28.0032)

Mikroorganismy ?

- Mikroorganismus (mikrob) je jednobuněčný, jen mikroskopicky pozorovatelný organismus. Často tvoří různé kolonie, shluky, případně i symbiotická společenstva s jinými organismy. Řadí se mezi ně převážně prokaryota (bakterie, archebakterie), ale patří k nim také např. plísně, kvasinky, některé řasy, prvoci.
- Viry

Mikroorganimy v průmyslu

- Výroba sýrů (*Lactobacillus, Streptococcus, Penicilium*)
- Pivo, víno, destiláty, chleba (*Saccharomyces*)
- Jogurt (*Lactobacillus*)
- Degradace biomasy (*Clostridium, Trichoderma*)
- Fermentované potraviny (*Pedicoccus, Monoscus, Neurospora, Penicilium*)
- Produkce aminokyselin, vitamínů, org. kyselin (*Bacillus, Aspergilus, Candida*)
- Produkce paliv – líh, bioplyn (*Clostridium, Saccharomyces*)
- Výroba biopolymerů
- Výroba enzymů (*Aspergilus, Trichoderma*)

Mikroorganismy v zemědělství

- Fixace dusíku (*Cyanobacteria, Rhizobium, Frankia*)
- Bakterie podporující růst rostlin (*Bacillus, Pseudomonas, Streptomyces* produkce fytohormonů, ACC deaminázy, rozpustnost fosforu)
- Biokontrola
  - *B. thuringiensis* kurstaki – larvy motýlů
  - *B. thuringiensis* israelensis – komáři, Eusimulium latipes (přenašeč Trypanozóm)
  - *B. thuringiensis* san diego – některé larvy brouků
  - *Microsporidium moscema* – sarančata
- Pesticidy (*Colletotrichum, Bacillus, Baculoviridae*)
- Fytoremediace

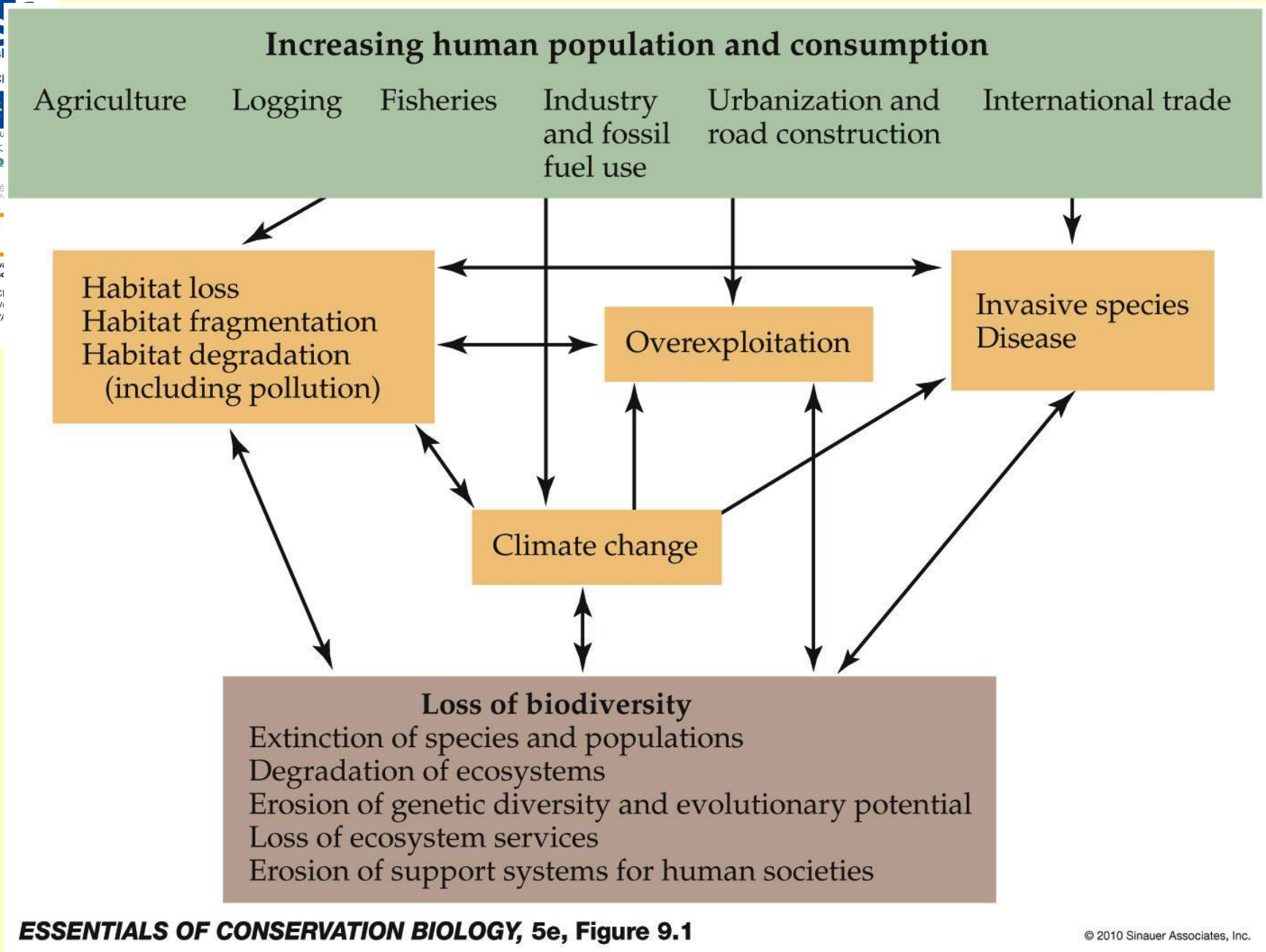
Mikroorganismy v medicíně

- Antibiotika (*Streptomyces, Penicillium*)
- Protinádorové látky
- Imunomodulátory

Mikroorganismy jako patogeny

- *Streptococcus pyogenes*
- *Neisseria meningitidis*
- *Mycobacterium tuberculosis*
- *Escherichia coli*
- *Yersinia pastis*

- *Plum pox virus*



***ESSENTIALS OF CONSERVATION BIOLOGY*, 5e, Figure 9.1**

Počet druhů

Table 4. Known and estimated total number of species on Earth (WCMC, 1992).

Groups	Known number of species	Estimated total number of species	% known species
Insects	950,000	8,000,000	11.9
Fungi	70,000	1,000,000	7.0
Arachnids	75,000	750,000	10.0
Nematodes	15,000	500,000	3.0
Viruses	5,000	500,000	1.0
Bacteria	4,000	400,000	1.0
Plants	250,000	300,000	83.3
Protozoans	40,000	200,000	10.0
Algae	40,000	200,000	10.0
Molluscs	70,000	200,000	35.0
Crustaceans	40,000	150,000	26.7
Vertebrates	45,000	50,000	90.0
World Total	**1,700,000**	**12,500,000**	**13.6**

Počet eukaryotních druhů

- When applied to all five known eukaryote* kingdoms of life on Earth, the approach predicted:
- 1) ~7.77 million species of animals (of which 953,434 have been described and cataloged)
- 2) ~298,000 species of plants (of which 215,644 have been described and cataloged)
- 3) ~611,000 species of fungi (moulds, mushrooms) (of which 43,271 have been described and
- cataloged)
- 4) ~36,400 species of protozoa (single-cell organisms with animal-like behavior, eg. movement,
- of which 8,118 have been described and cataloged)
- 5) ~27,500 species of chromists (including, eg. brown algae, diatoms, water moulds, of which 13,033 have been described and cataloged)
- Total: 8.74 million eukaryote species on Earth.

Proč konzervovat mikroorganismy?

- Konzervace mikroorganismů jako biologických zdrojů má nesmírný význam pro život lidí a jejich aktivity. Zahrnuje globální snahy o identifikaci, konzervaci a generování dat o jejich ekosystémech, společenstvech a populacích.

Proč konzervovat mikroorganismy?

- Většina biomasy a diverzity na Zemi je tvořena mikroorganismy
- Mikroorganismy hrají klíčovou úlohu v biogeochemických cyklech
- Poskytují nové metabolity pro průmyslové využití

- Proto je nutné zachovat mikrobiální ekosystémy, společenství a druhy mimořádného významu, jejich přirozené prostředí a gene pool (genofond)

Cíl konzervace mikroorganismů

Konzervovat mikroorganismy v životaschopném stavu, bez morfologických, fyziologických a genetických změn s možností využití v budoucnosti (důležité kmeny pro výzkum a průmysl).

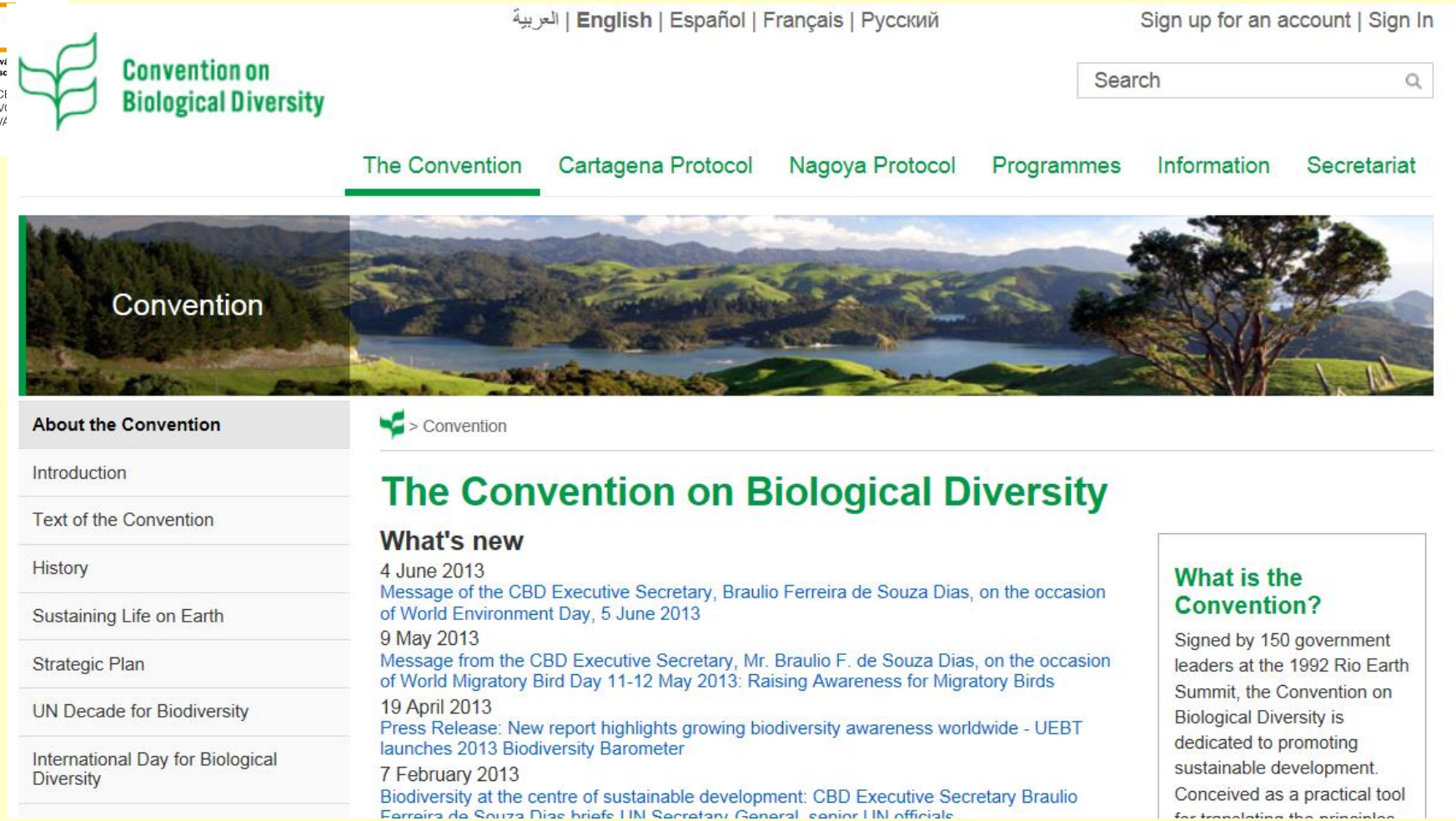
Úloha konzervační genetiky

- Minimalizovat inbreeding a **ztrátu genetické diversity**
- **Vybrat a charakterizovat zájmové populace**
- Objasnit strukturu populace
- **Objasnit taxonomické nejasnosti**
- Definovat management pro daný druh
- Způsob rozmnožování (genetic pollution)
- **Detekovat a definovat invasivní druhy**
- Definovat místa a genotypy vhodné pro reintrodukci
- Využití ve forenzní konzervační genetice
- Odhadnutí velikost populací a poměru pohlaví
- Odhadnutí příbuzenských vztahů; analýza rodokmenů
- **Poznání mezipopulačních vztahů**
- **Využití v managementu populací v** zajetí nebo **kultuře**
- **Poznání genových center druhů** a vztahů v nich
- Zahrnutí genotoxických studií
- Zvýšit reprodukční kapacitu

Mikroorganismy a konzervační biologie

- Infekční choroby
  - hrozba pro druh
  - životaschopnost populace
  - ovlivňuje chovy, reintrodukci
  - vyžaduje ozdravné programy
- *In-situ* konzervace mikroorganismů
- *Ex-situ* konzervace mikroorganismů

Úmluva o biologické rozmanitosti



http://www.cbd.int/

Úmluva o biologické rozmanitosti

Article 9. Ex-situ Conservation

Each Contracting Party shall, as far as possible and as appropriate, and predominantly for the purpose of complementing in-situ measures:

(a) Adopt measures for the ex-situ conservation of components of biological diversity, preferably in the country of origin of such components;

(b) Establish and maintain facilities for ex-situ conservation of and research on plants, animals and micro- organisms, preferably in the country of origin of genetic resources;

(c) Adopt measures for the recovery and rehabilitation of threatened species and for their reintroduction into their natural habitats under appropriate conditions;

(d) Regulate and manage collection of biological resources from natural habitats for ex-situ conservation purposes so as not to threaten ecosystems and in-situ populations of species, except where special temporary ex-situ measures are required under subparagraph (c) above; and

(e) Cooperate in providing financial and other support for ex-situ conservation outlined in subparagraphs (a) to (d) above and in the establishment and maintenance of ex- situ conservation facilities in developing countries.

Sbírky mikroorganismů

Professor Frantisek Kral (1846–1911) of Prague was the first to realize the importance of Culture Collections: he collected cultures which he made available for a fee to other workers (*Figures 1a,b*). This Collection was later transferred to the University of Vienna in 1915 by Professor Ernst Pribram. The next oldest Collection, Centraalbureau voor Schimmelcultures (CBS), was founded in 1906 and still exists at Baarn, The Netherlands.

Typy genových zdroj

Table 8. Types of genetic resources collection

Plant genetic resource collections	Animal genetic resource collections	Microbial genetic resource collections
- Seed samples - Spore samples - Pollen samples - Field gene banks - Embryos - Tissues - Cell suspensions etc.	- Embryos and semen under cryogenic storage - Groups of living animals kept under supervision in an exclusive area - Networks of breeding groups	- Cultures of algae, bacteria, fungi - Protozoa and viruses as well as materials derived from them in the form of cDNA, plasmids, vectors, cell lines etc.

Sbírky mikrooganismů

- Typové kmeny – referenční kmeny
- Modelové organismy
- Mikroorganismy v zemědělství, průmyslu , medicíně

World Federation for Culture Collections

WDCM WORLD DATA CENTRE FOR MICROORGANISMS

Culture Collections Information Worldwide

Home Browse Search Statistics

A B C D E F G H I J K L M N O P Q R S T U V W X Y Z

Canada Chile China Colombia Cuba Czech

There are 13 collections in Czech

Acronym	WDCM Number	Collection	Region
CAPM	WDCM 181	Collection of Animal Pathogenic Microorganisms	Europe
CAUP	WDCM 486	Culture Collection of Algae of Charles University in Prague	Europe
CCALA	WDCM 905	Culture Collection of Autotrophic Organisms	Europe
CCBAS	WDCM 558	Culture Collection of Basidiomycetes	Europe
CCC	WDCM 959	Culture Collection of Clavicipitaceae	Europe
CCDM	WDCM 878	Culture Collection of Dairy Microorganisms Laktoflora	Europe
CCF	WDCM 182	Culture Collection of Fungi	Europe
CCM	WDCM 65	Czech Collection of Microorganisms	Europe
CCMAC	WDCM 645	Culture Collection of Macromycetes (Basidiomycotina and Ascomycotina)	Europe
CNCTC	WDCM 130	Czech National Collection of Type Cultures	Europe
DBM	WDCM 654	Department of Biochemistry and Microbiology	Europe
DMUP	WDCM 658	Microbiology and Biophysics Charles University	Europe
RIBM	WDCM 655	Research Institute for Brewing and Malting	Europe

http://www.wfcc.info/ccinfo/collection/col_by_country/c/420/

MICRENS

MIRCEN (Microbial Resources Centres) program of UNESCO is a network of existing academic and/or research institutes in developed and developing countries.

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http://www.wdcm.org/

http://gcm.wfcc.info/

Nomenclatura x barcoding

- International Committee on Taxonomy of Viruse (ICTV)
- International Committee on Systematics of Prokaryotes (ICSP)

International Committee on Systematics of Prokaryotes (ICSP)

The Role of the ICSP, by H. G. Trüper and B. J. Tindall.

The ICSP, formerly the International Committee on Systematic Bacteriology (ICSB), is the body that oversees the nomenclature of prokaryotes, determines the rules by which prokaryotes are named and whose Judicial Commission issues Opinions concerning taxonomic matters, revisions to the Bacteriological Code, etc.

Because of the increasing problem of unsolicited email, the ICSP web pages have been redesigned to try to prevent spammers from obtaining email addresses from the site. Click on a name for contact details, including email addresses. A full list of contact details for ICSP officers and members of those ICSP subcommittees for which information is available can be found on the Contacts page. To obtain an email address from this page, simply highlight the two parts of the address (together with the @ sign) and copy and paste the text into your email program. Then, remove any spaces from around the @ sign.

Last modified 16 January 2013

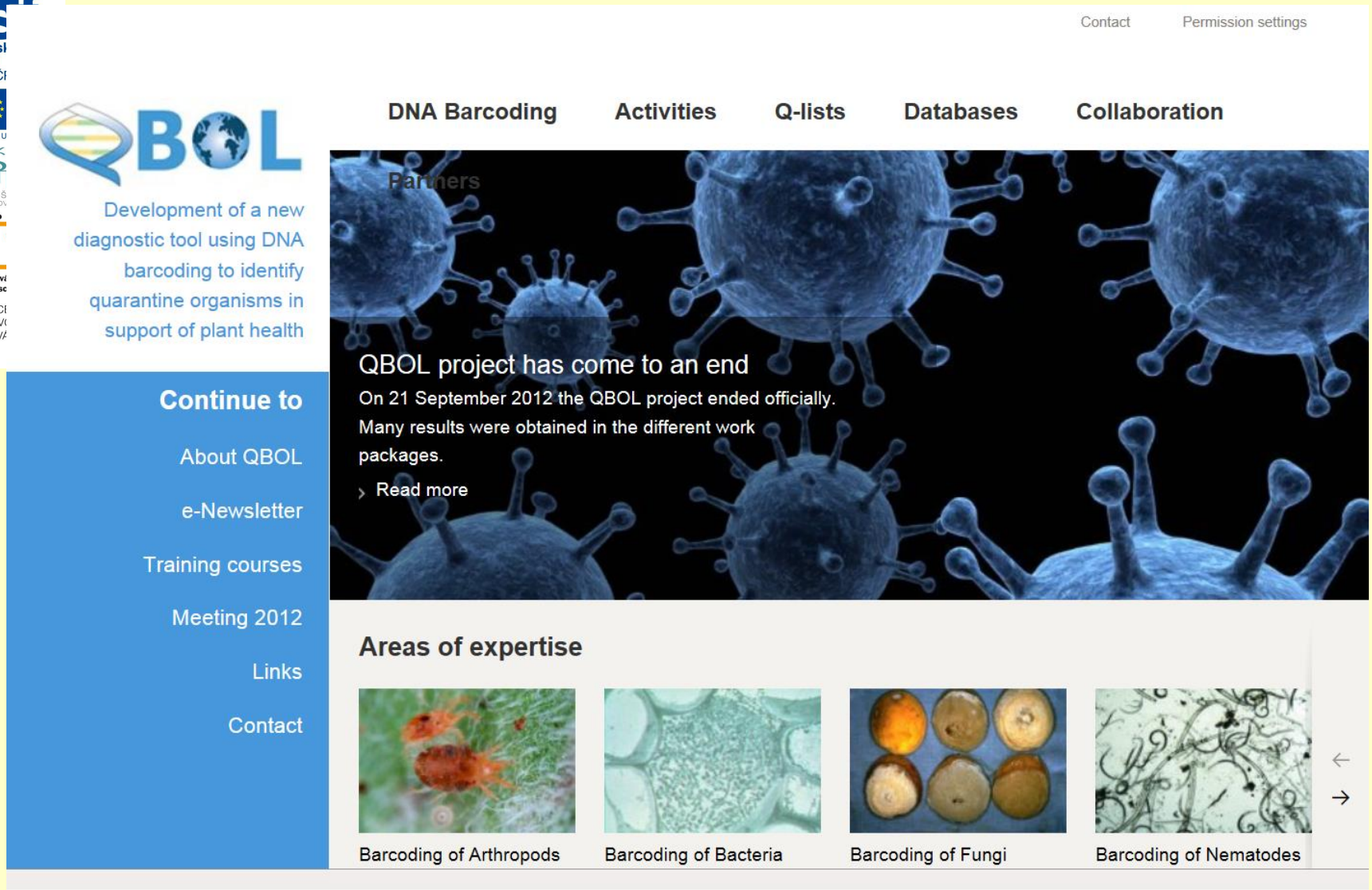
Taxonomic subcommittees

Some subcommittees have home pages listing their current membership (together with information on meetings, minutes, minimal standards papers and other subcommittee activities) and lists of taxa covered.

***Aeromonadaceae*, *Vibrionaceae* and related organisms**	Subcommittee home page	Taxa covered
Genera *Agrobacterium* and *Rhizobium*	Independent web page	
***Bacillus* and related organisms**	Subcommittee home page	Taxa covered
Bifidobacterium, *Lactobacillus* and related organisms	Subcommittee home page	Taxa covered
Genus *Brucella*	Subcommittee home page	Taxa covered
Burkholderia, *Ralstonia* and related organisms	Subcommittee home page	Taxa covered
***Campylobacter* and related bacteria**	Subcommittee home page	Taxa covered
Clostridia and *Clostridium*-like organisms	Subcommittee home page	Taxa covered
Comamonadaceae and related organisms	Subcommittee home page	Taxa covered
Family *Enterobacteriaceae*	Subcommittee home page	Taxa covered

http://www.the-icsp.org/

http://ictvonline.org/virusTaxonomy.asp?bhcp=1



http://www.qbol.org/en/qbol.htm

http://www.barcodeoflife.org/

Metody konzervace bakterií

In-vivo, často vysoké nároky na podmínky kultivace

- Vysušení (silikagel, $CaCl_2$, lyofilizace)
- Cryopreservation -70 °C, **tekutý dusík -196 °C**

Fenotypová chrakterizace

Phenotypic Characterization

When bacterial strains are initially received by ATCC, they are analyzed for characteristic colony and bacterial morphology; this is observed via colonial growth and Gram staining, respectively. Additionally, samples are tested for culture purity on blood agar plates. The presence of more than one colony type is often, but not always, indicative of a mixed culture.

In addition to purity and morphology, strains are also examined for consistency in sugar metabolism, antibiotic susceptibility, and/or broad-spectrum biochemical reactivity. To confirm the bacterial species identity based on these associated phenotypic traits, ATCC commonly uses appropriate API® strip tests and Vitek® cards. Both aforementioned techniques employ over 20 different biochemical tests in reaction cells to assess the growth and viability of the bacterial strain[30]. The API® strip test characterizes bacterial identity through a manual, micro-method that generates a seven-digit code based upon the reaction of the individual biochemical assays. This generated code is the identifier number linked to a specific bacterial species. In contrast, the Vitek® system provides an automated, computer-based method of species identification via the measurement of light attenuation associated with each biochemical reaction[30, 31]. For this latter system, ATCC requires a ≥ 90% probability of organism identification.

Genotypová charakterizace

Genotypic Characterization

ATCC genotypically confirms bacterial species through both ribotyping and 16S ribosomal RNA (rRNA) sequencing. Ribotyping is a method of bacterial identification that is based upon differences in genomic material. This method is possible through the use of the RiboPrinter® system, which analyzes discrete-sized genome fragments generated by restriction digestion of rRNA operons[32, 33]. To confirm the identity of the bacterial strain, the respective riboprint is compared against a database of known organisms. ATCC requires a ribotype match of ≥ 0.85 for species confirmation.

In addition to ribotyping, ATCC also examines bacterial identity through 16S rRNA gene sequencing. These sequences are commonly used to analyze bacterial phylogeny and taxonomy as they are highly conserved and universal among prokaryotic species. In general, the 16S rRNA gene sequence is composed of both variable and conserved regions, the comparison of these sequences can allow for differentiation at the genus level[34, 35].

Ribotyping. A method of identification involving the fingerprinting of genomic DNA restriction fragments.

Informace o mikroorganismech

- Mikrofotografie
- Fotografie příznaků choroby
- HPLC
- MALDI TOF
- Metabolické profily
- Informace o genomu, informace o sekvencích

Genetická charakterizace

- Genetická diversita
  - Genetická variabilita mezi jedinci v populaci
  - Genetická variabilita v rámci druhu
  - Genetická variabilita mezi druhy
- Zdrojem genetických dat jsou vzorky biologických materiálů
- Genetická data jsou shromažďovány s cílem zachovat genetickou diverzitu pro budoucnost

Kontrola kvality

Metody biochemie, bakteriologie, mykologie, ekologie a molekulární genetiky

Získání anatomicko-morfologických, fyziologických, biochemických a genetických dat

Molekulárně-genetické metody

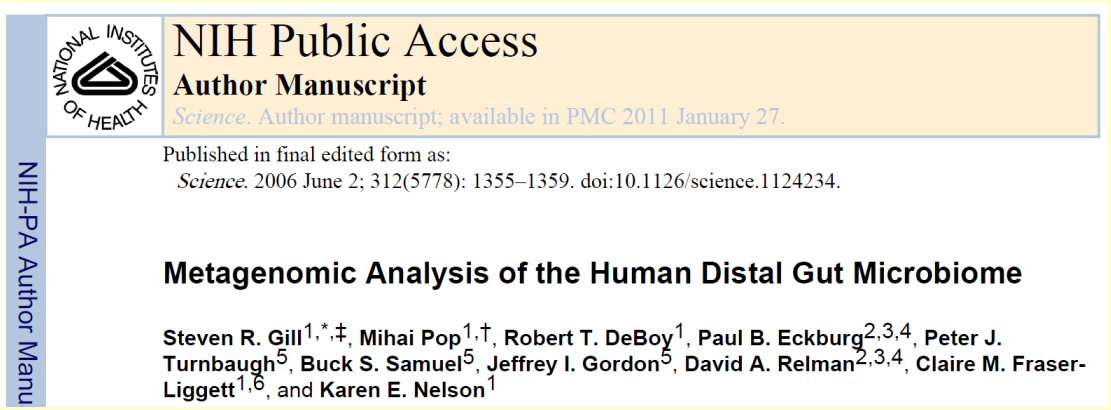
- PCR (Polymerase Chain Reaction)
- RAPD (Random Amplified Polymorphic DNA)
- AFLP (Arbitrary Fragment Length Polymorphism)
- ARDRA (Amplified Ribosomal DNA Restriction Analysis)
- SSCP (Single Strand Conformation Polymorphism)
- Analýza mikrosatelitů
- Sekvencování

- RFLPs (Restriction Fragment Length Polymorphism)
- Karyotypizace velikost a počet chromozómů

Kvalita izolované DNA!?

Metagenomika

- Struktura mikrobiálních společenství
  - druhové složení
  - reakce na stres
- Nové poznatky o procesech v ekosystémech
  - fyziologický stav
  - tok energií
  - tok látek



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Metagenomic Analysis of the Human Distal Gut Microbiome

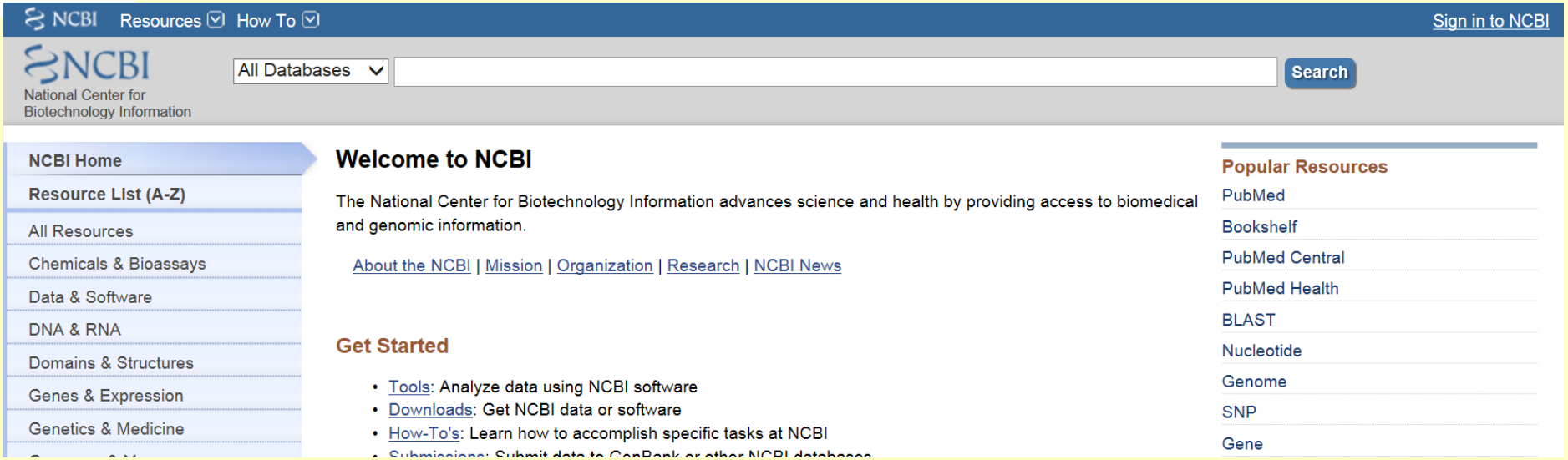
Steven R. Gill[1,*,‡], **Mihai Pop**[1,†], **Robert T. DeBoy**[1], **Paul B. Eckburg**[2,3,4], **Peter J. Turnbaugh**[5], **Buck S. Samuel**[5], **Jeffrey I. Gordon**[5], **David A. Relman**[2,3,4], **Claire M. Fraser-Liggett**[1,6], and **Karen E. Nelson**[1]

Databáze sekvencí

- NCBI, EMBL
- BLAST

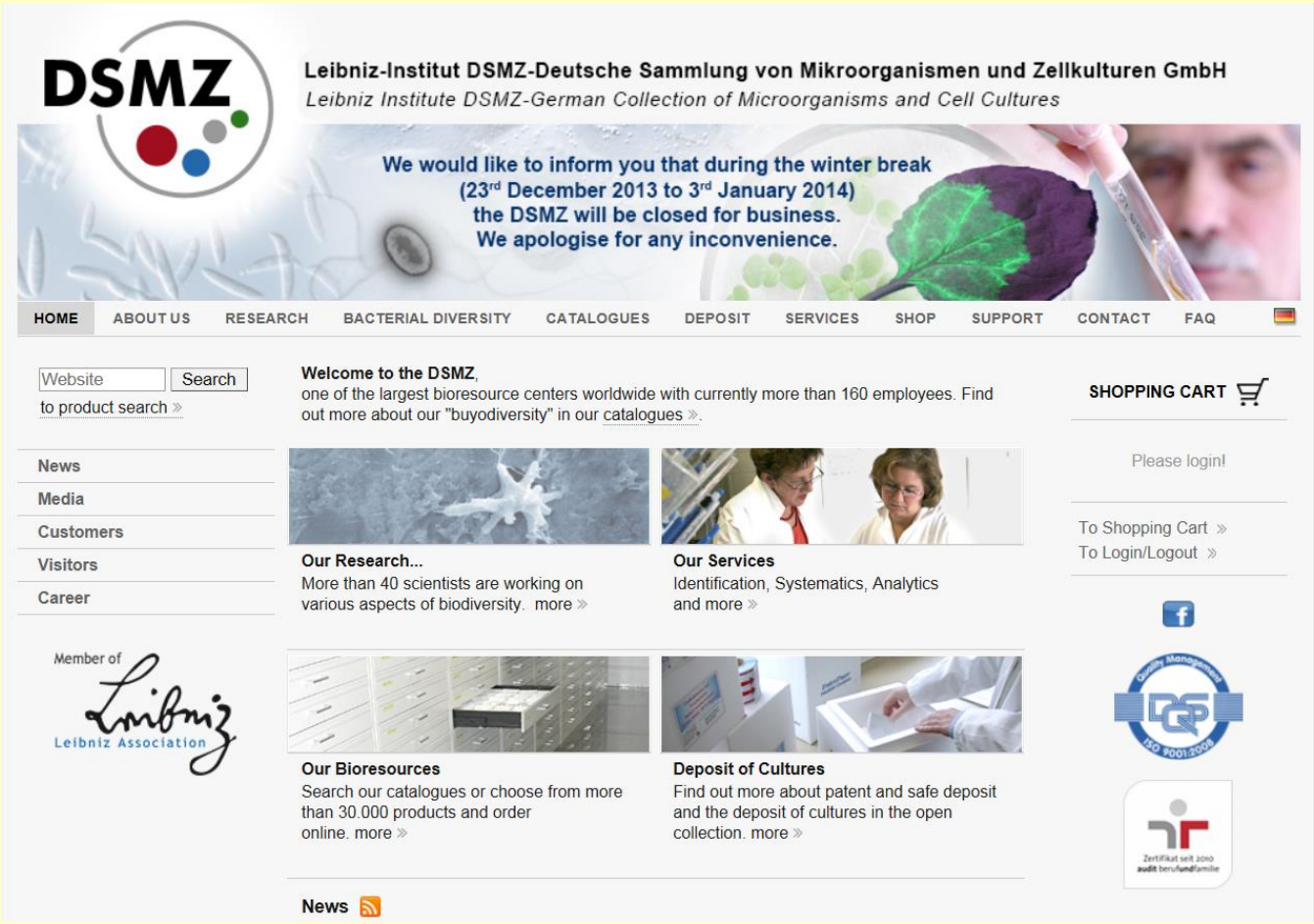
- **16S** a 18S rRNA

National Center for Biotechnology Information



http://www.ncbi.nlm.nih.gov/

DSMZ



http://www.dsmz.de

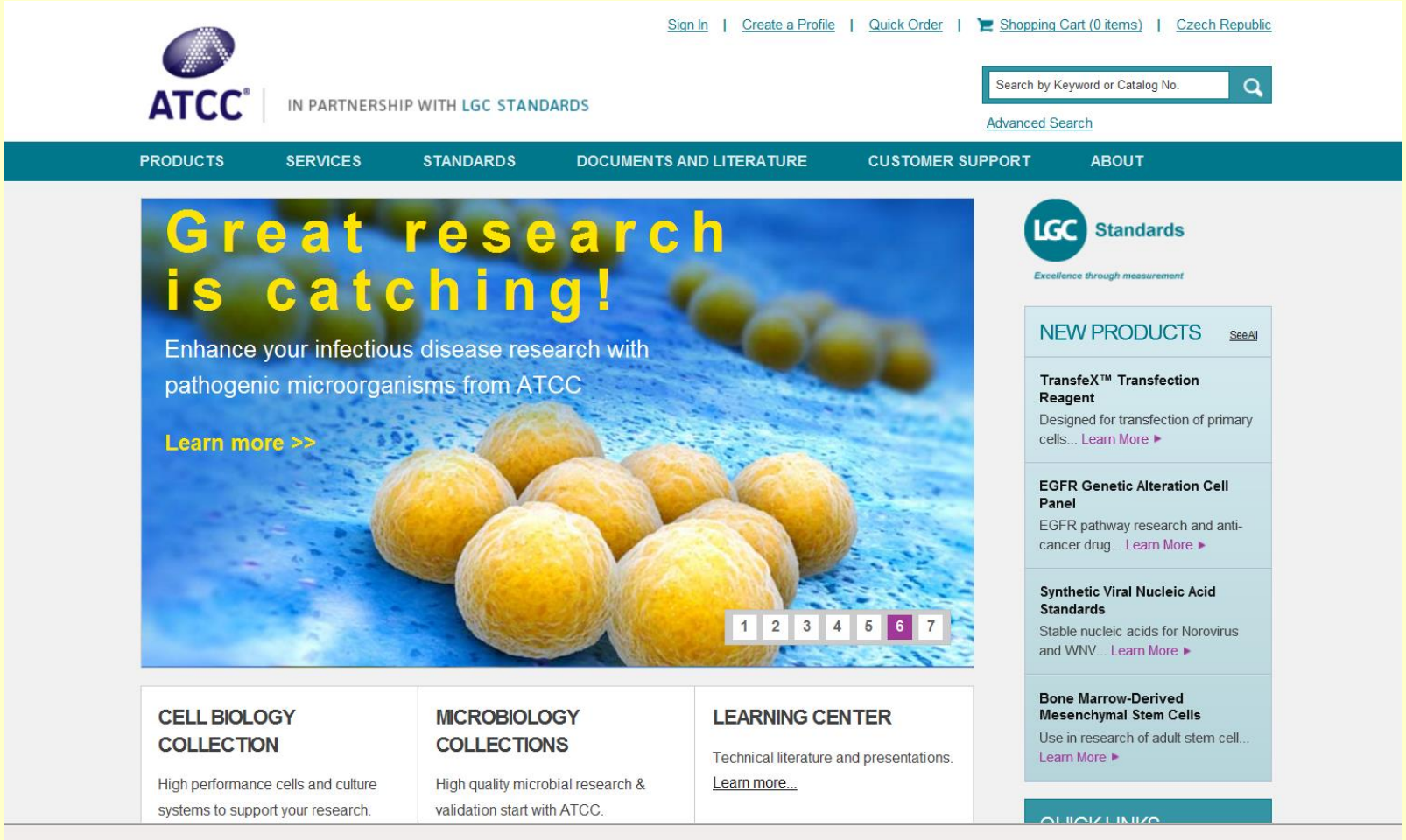
DSMZ – kontrola kvality

- Příslušnost k druhu – podobnost NT sekvence 16/18S rRNA je mezi 99,8 a 100%
- Pokud je podobnost sekvencí nižší (99%) porovnává se struktura RNA
- Pokud je podobnost pod 99 %, je opakováno sekvencování, případně požadováno zaslání nového kmene
- Specifické genomické a metabolické vlastnosti musí být identické

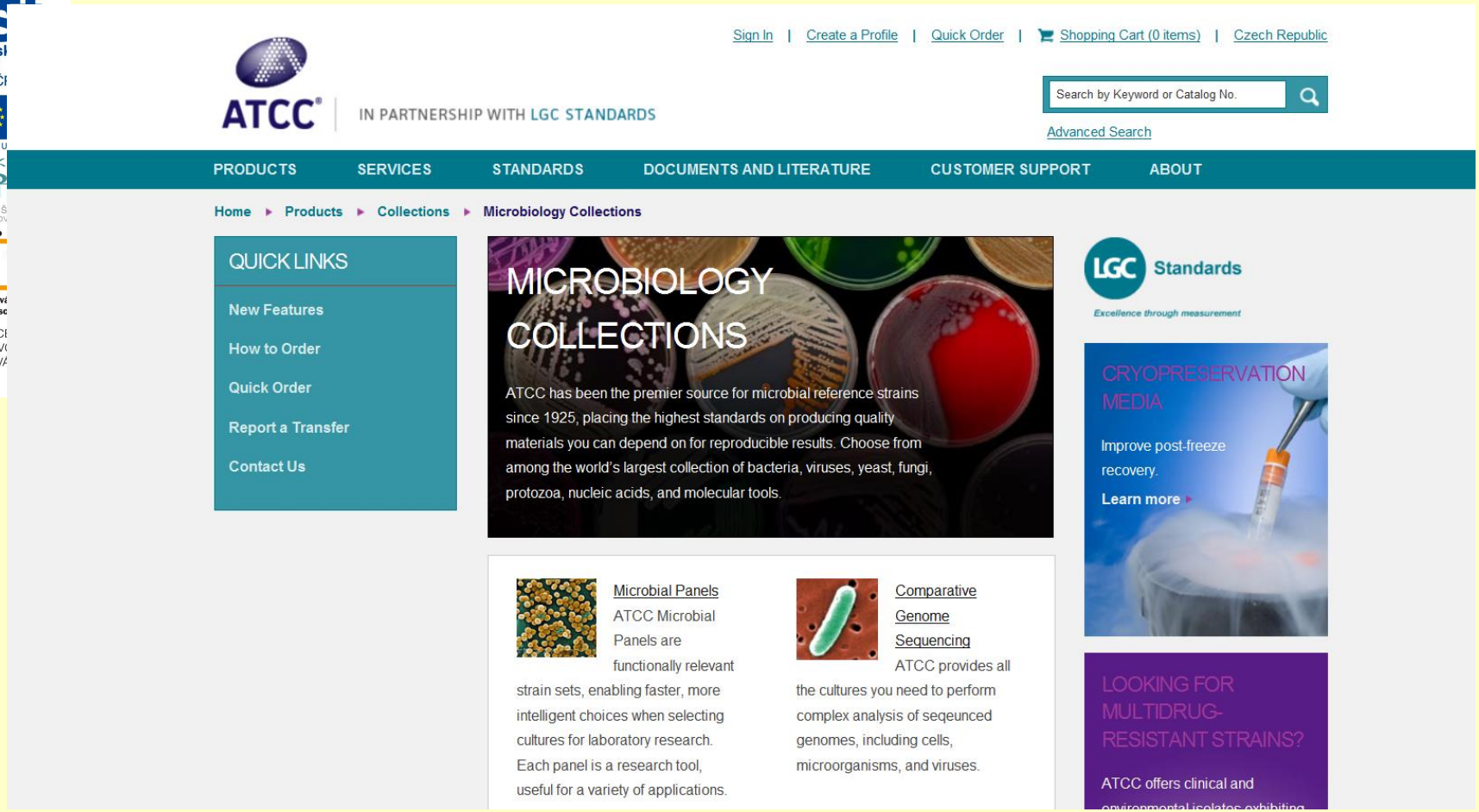
DSMZ – kontrola

- RiboPrint technology (Qualicon, Dupont, Wilmington)

ATCC
American Type Culture Collection



http://www.lgcstandards-atcc.org



http://www.lgcstandards-atcc.org/Products/Collections/Microbiology_Collections.aspx

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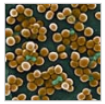
ATCC provides fully characterized strains necessary for the microbiological analysis of food products to identify and prevent the spread of foodborne disease.

Quality Control and Media Testing
ATCC provides various microbiological strains for use in routine media sterility testing and growth promotion



Health and Medical Quality Control
ATCC has several quality control strains for use by pharmaceutical corporations or in personal care for strain identification

http://www.lgcstandards-atcc.org/Products/Cells_and_Microorganisms/Bacteria/Quality_Control_Strains.aspx

Zdroje varibility a proměnlivosti virů

- Mutace
- Rekombinace
- Reasortment

Hostitelská adaptace virů

Interakce virus – hostitel je klíčový mechanismu udržující genetickou diverzitu patogena i hostitele

Multihost Experimental Evolution of a Plant RNA Virus Reveals Local Adaptation and Host-Specific Mutations

Stéphanie Bedhomme,*[1] Guillaume Lafforgue,[1] and Santiago F. Elena[1,2]

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[2]The Santa Fe Institute, Santa Fe, New Mexico

***Corresponding author:** E-mail: stebed@upvnet.upv.es.

Associate editor: Oliver Pybus

Abstract

For multihost pathogens, adaptation to multiple hosts has important implications for both applied and b
the applied level, it is one of the main factors determining the probability and the severity of emerging d
At the basic level, it is thought to be a key mechanism for the maintenance of genetic diversity both in hc
species. Using *Tobacco etch potyvirus* (TEV) and four natural hosts, we have designed an evolution ex
strength and novelty are the use of complex multicellular host organism as hosts and a high level of replic

Journal of General Virology (2007), 88, 2839–2845 DOI 10.1099/vir.0.82814-0

Adaptation of plum pox virus to a herbaceous host (*Pisum sativum*) following serial passages

Christopher M. Wallis,[1] Andrew L. Stone,[2] Diana J. Sherman,[2] Vernon D. Damsteegt,[2] Fred E. Gildow[3] and William L. Schneider[2]

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Plum pox virus (PPV) populations from peaches are able to adapt consistently to herbaceous hosts, characterized by a reduction in time to symptom development, increases in inoculation efficiency and increased titres. PPV adaptation was studied by using pea (*Pisum sativum*) as an alternative host. Two isolates of PPV from peaches were inoculated and passaged in peas ten

Hostitelská adaptace virů

Virology 374 (2008) 256–260

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Rapid Communication

Genetic diversity and purifying selection in West Nile virus populations are maintained during host switching

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ABSTRACT

To investigate differential evolutionary rates and selective forces of WNV in hosts and vectors, we measured the genetic diversity that arose during alternating passage in mosquitoes and birds. Within-host genetic diversity was monitored in each of three experimentally passed replicates, and the complete genome sequence of each WNV strain was determined after passage. The intrahost genetic diversity that arose during alternating passage was significantly greater than the diversity generated during chicken-only passage and

Zdroje genetické variability bakterií

- Mutace
- Rekombinace
- HGT (Horizontal Gene Transfer)
  - Transformace
  - Transdukce
  - Konjugace

Recipient obvykle špatně adoptuje nové geny

Adaptace bakterií

- Transformace DNA
- Patogeneze
- Produkce toxinů
- Rezistence
- Virulence
- Ztráta pohyblivosti
- Clustered regularly interspaced short repeats system (CRISPR)
- Komenzální kolonizační faktor (ccf)

Genetická stabilita bakterií

Při uchovávání lyofilizované kultury baktérií v 5 °C po dobu jednoho roku klesla jejich životnost. Produkce bacteriocinu nebyla ovlivněna.

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Exploration and conservation of bacterial genetic resources as bacteriocin producing inhibitory microorganisms to pathogen bacteria in livestock

Chotiah S

Abstract

Exploration and conservation of microorganisms producing bacteriocin was done as the primary study towards the collection of potential bacteria and its application in improving livestock health condition and inhibit food borne pathogens. Diferent kinds of samples such as beef cattle rectal swab, rumen fluids, cow's milk, chicken gut content, goat's milk were collected at Bogor cattle slaughter houses, poultry slaughter houses, dairy cattle and goat farms. A total of 452 bacterial isolates consisted of 73 Gram negative bacteria and 379 Gram positive bacteria were isolated from samples collected and screened for bacteriocin activity. Determination of bacteriocin activity with bioassay using

Komenzální kolonizační faktor (ccf)

Ztráta ccf *Bacteroides fragilis* vede k narušení kolonizace střeva a omezuje se HGT.

LETTER

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Bacterial colonization factors control specificity and stability of the gut microbiota

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Mammals harbour a complex gut microbiome, comprising bacteria that confer immunological, metabolic and neurological benefits[1]. Despite advances in sequence-based microbial profiling and myriad studies defining microbiome composition during health and disease, little is known about the molecular processes used by symbiotic bacteria to stably colonize the gastrointestinal tract. We sought to define how mammals assemble and maintain the *Bacteroides*, one of the

to the same species (marked by an antibiotic resistance gene) are resistant to super-colonization and clear the challenging strain (Fig. 1c). This novel observation of 'colonization resistance' by the same species is conserved in three other *Bacteroides* (Supplementary Fig. 1b–d), regardless of the antibiotic resistance markers used (Supplementary Fig. 1e), but not in *Escherichia coli* (Supplementary Fig. 1f). As conventional mice typically harbour 10^{11}–10^{12} c.f.u. per gram of caecal content[14]

Stabilita bakteriálního genomu

- Stabilita znaků nesených plazmidy je mnohem nižší
  - omezení replikace plazmidů
  - nerovnoměrná distribuce plazmidů
  - variabilita roste v neselekčním prostředí
- Opakované subkultivace mohou vést ke kontaminaci, ztrátě životaschopnosti, genotypovým a fenotypovým změnám
- ATCC nedoporučuje více než 5 pasáží dodaných originálních kultur
- Lyofilizace a kryogenní konzervace jsou preferovány

Stabilita bakteriálního genomu

- Mutace DNA mohou být vyvolány poškození cytoplazmatické membrány při zamrazování a rozmrazování
- Poškození cytoplazmatické membrány při zamrazování a rozmrazování má vliv pouze na životnost