



Metagenomics of soil microbial communities - challenges and perspectives

Małgorzata Łobocka



Department of Microbial Biology, Faculty of Agriculture and Biology
Warsaw University of Life Sciences
and

Department of Microbial Biochemistry, Institute of Biochemistry and Biophysics
of the Polish Academy of Sciences, Warsaw, POLAND

Institute of Biochemistry and Biophysics of the Polish Academy of Sciences

► Department of Bioinformatics

► Department of Biophysics

► Department of Genetics

► Department of Lipid Biochemistry

► Department of Microbial Biochemistry

► Department of Molecular Biology

► Department of Plant Biochemistry

► Department of Protein Biosynthesis

► Laboratory of Antimetabolites

► Laboratory of Biological NMR

► Laboratory of Microarrays

► Laboratory of DNA Sequencing and Oligonucleotide Synthesis

► Laboratory of Fungal Glycobiology

► Laboratory of Mass Spectrometry

► Laboratory of Mutagenesis and DNA Repair

► Laboratory of Plant Pathogenesis

► Laboratory of Systems Biology

► Laboratory of Molecular Biology (affiliated to the University of Gdańsk)

► Laboratory of Molecular Medicine (affiliated to the International Institute of Molecular and Cell Biology and the Nencki Institute of Experimental Biology)

► Department of Genetics (Warsaw University affiliated with IBB PAS)



<http://www.ibb.waw.pl/>

IBB PAS

IBB PAS – scientific reaserch

Current activities: 70 national grants, 22 targeted or structural grants, 6 international projects, 5 scientific networks, EU projects.

- ▶ Signal Transduction by Ubiquitination, a Matter of Location (MRTN-CT-2006-034555, “UbiRegulators”; 2006-2010)
- ▶ Evolution of the protein-interaction Networks: the SH3 network in Yeast (MRTN-CT-2006-036076, “PENELOPE”; 2007-2011)
- ▶ Preparation and identification of new HIV reverse transcriptase inhibitors targeted against HIV strains resistant to anti-HIV/AIDS drugs, LSHP-CT-2007-037760, “HIV ResInh”; 2007-2009)

Since 2004 - Center of Excellence
“Comparative genomics for health and environment”

Genomic projects at IBB PAS

- ▶ *Saccharomyces cerevisiae*
- ▶ *Paramecium tetraurelia*
- ▶ Potato
- ▶ Lactococcal, Staphylococcal and Enterobacterial phages and plasmids
- ▶ *Staphylococcus aureus* strains for phage propagation

Faculty of Agriculture and Biology, Warsaw University of Life Sciences



Fac. of Agriculture and Biology

Fac. of Veterinary Medicine

Fac. of Forestry

Fac. of Horticulture and Landscape Architecture

Fac. of Engineering and Environmental Sciences

Fac. of Wood Technology

Fac. of Animal Sciences

Fac. of Food Technology

Fac. of Human Nutrition and Consumer Sciences

Fac. of Production Engineering

Fac. of Agricultural Economics



Faculty of Agriculture and Biology

Faculty of Agriculture and Biology, Warsaw University of Life Sciences (SGGW)

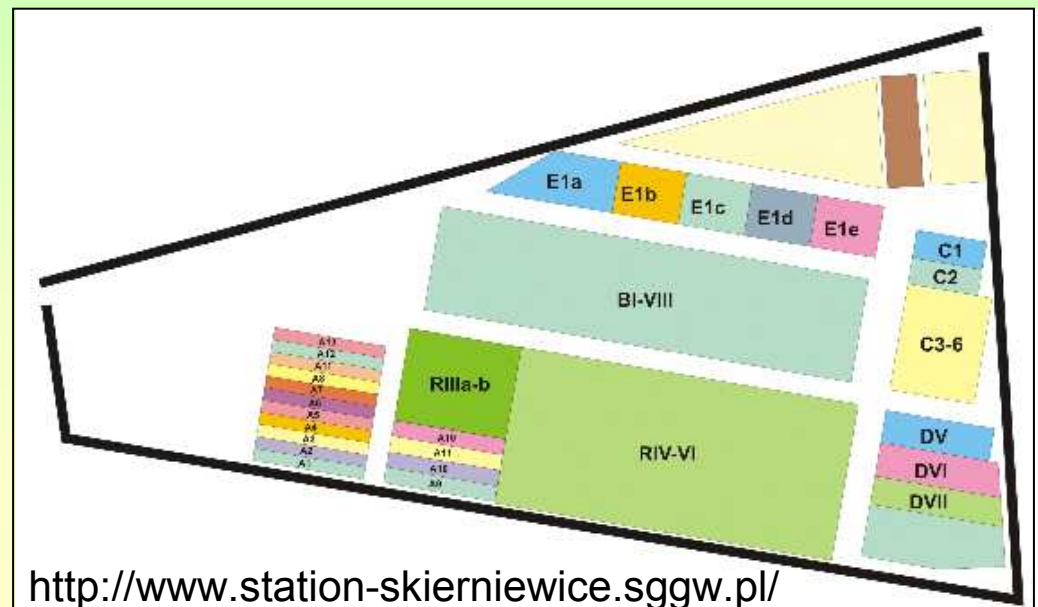
- ▶ Department of Agronomy
- ▶ Department of Biochemistry
- ▶ Department of Botany
- ▶ Department of Soil Environmental Sciences
- ▶ Department of Experimental Design and Bioinformatics
- ▶ Department of Microbial Biology
- ▶ Department of Plant Physiology
- ▶ **Experimental Station in Skierniewice**

A long-term fertilization experiment in Skierniewice, started at 1922, and conducted till today (the sixth oldest field experiment in the world)

Goals: estimation of influence of fertilization on yields and quality of crops; evaluation of nutrient losses from soil environment



Photo: owing to the kindness of prof. J. Chojnicki



<http://www.station-skierniewice.sggw.pl/>

Faculty of Agriculture and Biology – selected fields of research

- ▶ soil chemistry,
- ▶ soil biology, impact of climate change and antropogenic factors,
- ▶ gut microflora of farm animals,
- ▶ natural biocontrol factors in forestry and agriculture,
- ▶ plant endophytic microorganisms and their products,
- ▶ plant – pathogen interaction,
- ▶ plant physiology,
- ▶ modelling of protein structure

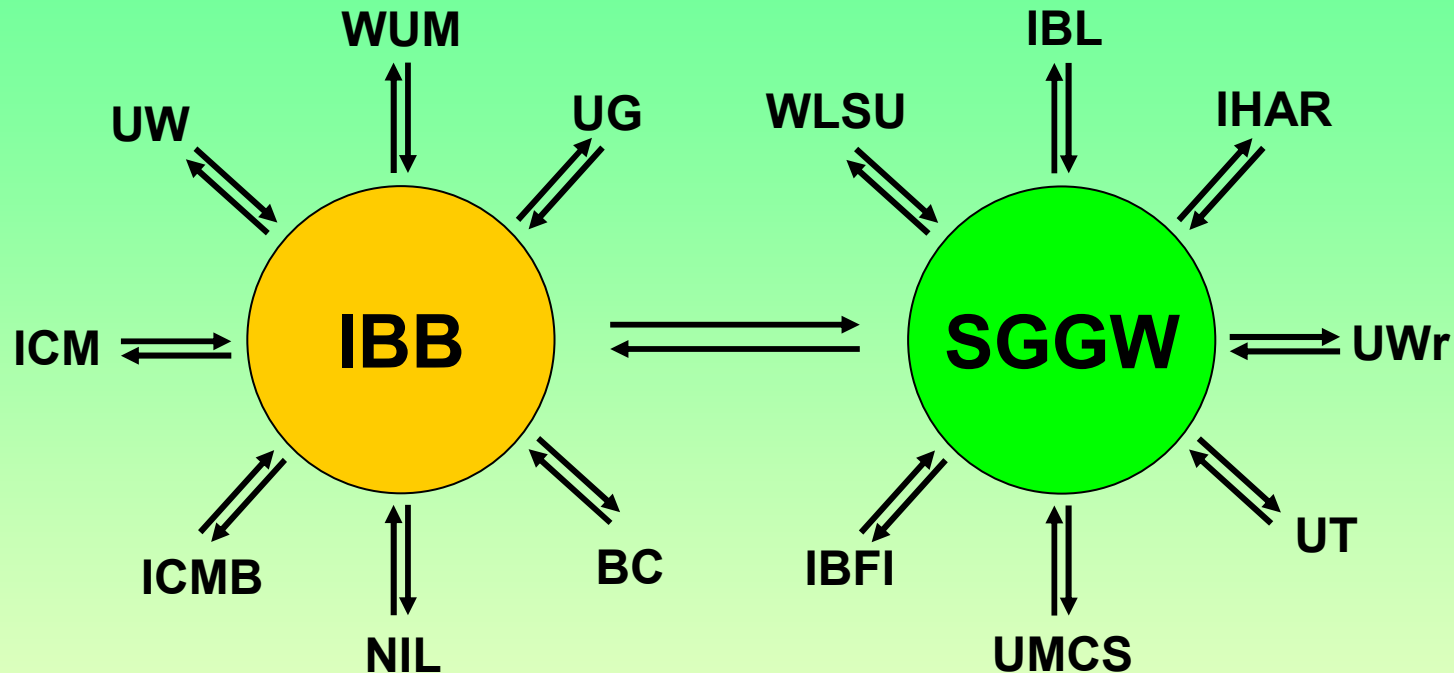
EU-funded projects at the Faculty of Agriculture and Biology

- ▶ Basis and development of molecular approaches to nematode resistance" (BIO4-CT96-0318, "ARENA").
- ▶ Production of plants resistant to pathogenic nematodes: infection limitation by nematode starvation" (QLK5-CT-1999-01501, "NONEMA").

Genomic projects

- ▶ *Pseudomonas syringae* bacteriophages (in collaboration with IBB PAS)

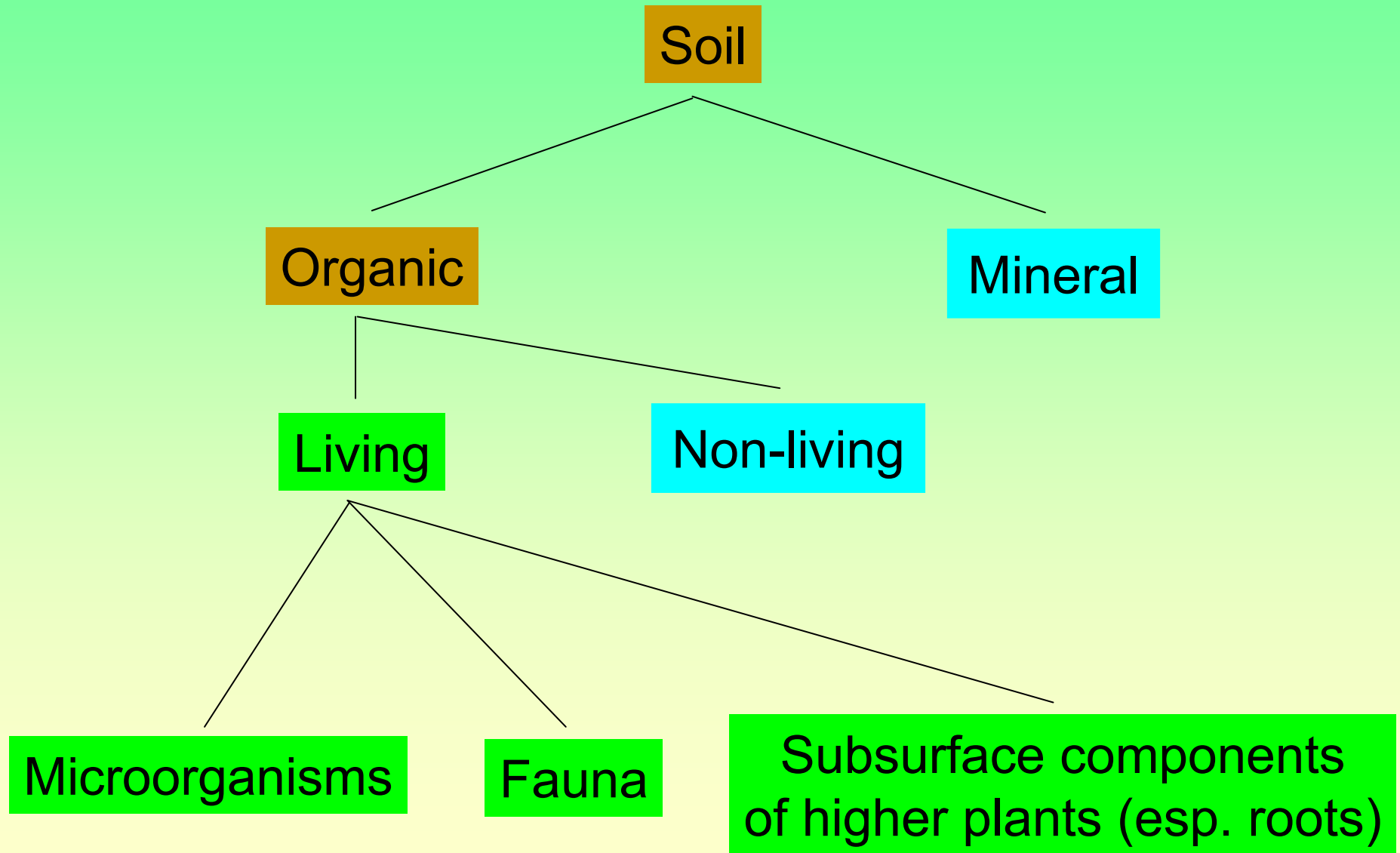
SGGW and IBB – networks of collaborations



How Polish, EU and world economy could benefit from the collaboration of these two institutions?

Initiating and developing environmental studies with the use of modern “omics” technologies, especially in the field of forestry and agriculture

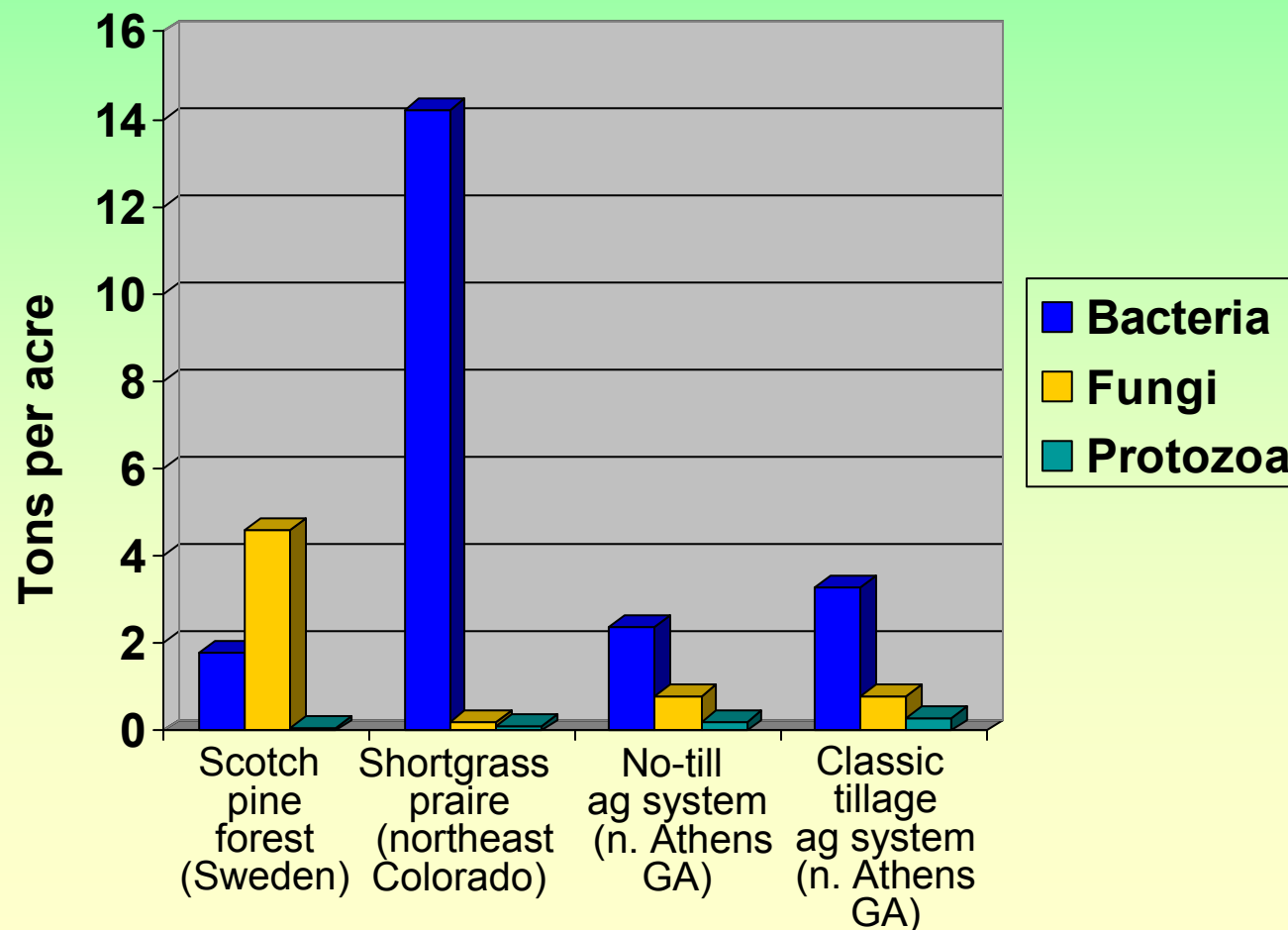
Soil as the biologically richest and most diversified environment



Differences in soil food webs of different ecosystems

Soil food web → the community of microorganisms living all or part of their lives in the soil.

Biomass of different microorganisms in soils of four exemplary ecosystems

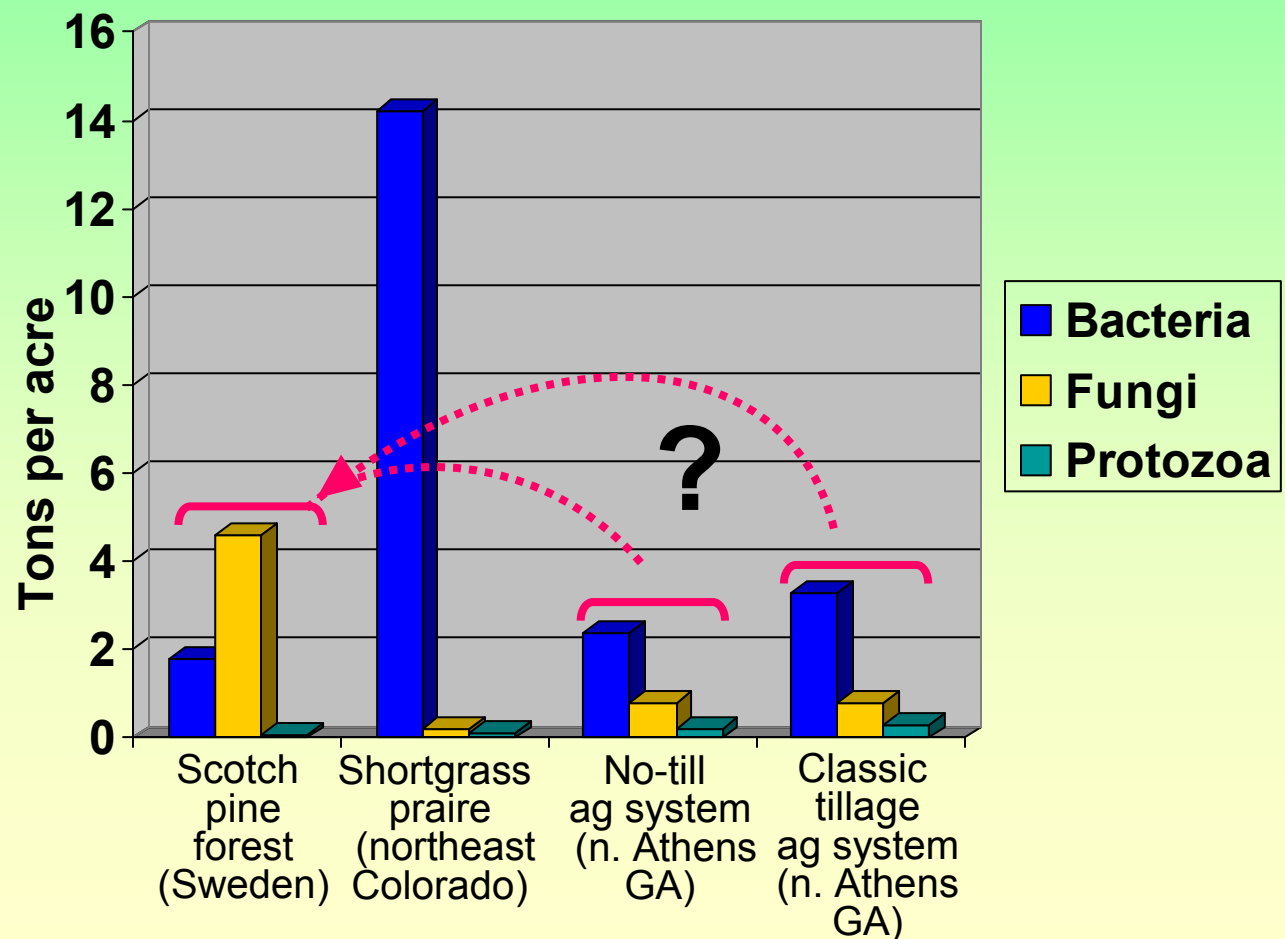


Differences in soil food webs of different ecosystems

Soil food web → the community of microorganisms living all or part of their lives in the soil.

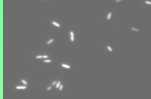
Biomass of different microorganisms in exemplary soils of different ecosystems

How to introduce forests in post-agricultural regions?



Who is there?

Traditional way of soil food web assays:

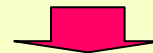
- ▶ Counting (direct and plate counts) 
- ▶ Measurements of activity levels (respiration, nitrification rates, decomposition rates)
- ▶ Measurements of cellular constituents (biomass, enzymes, phospholipids and other lipids, DNA and RNA)

Problem



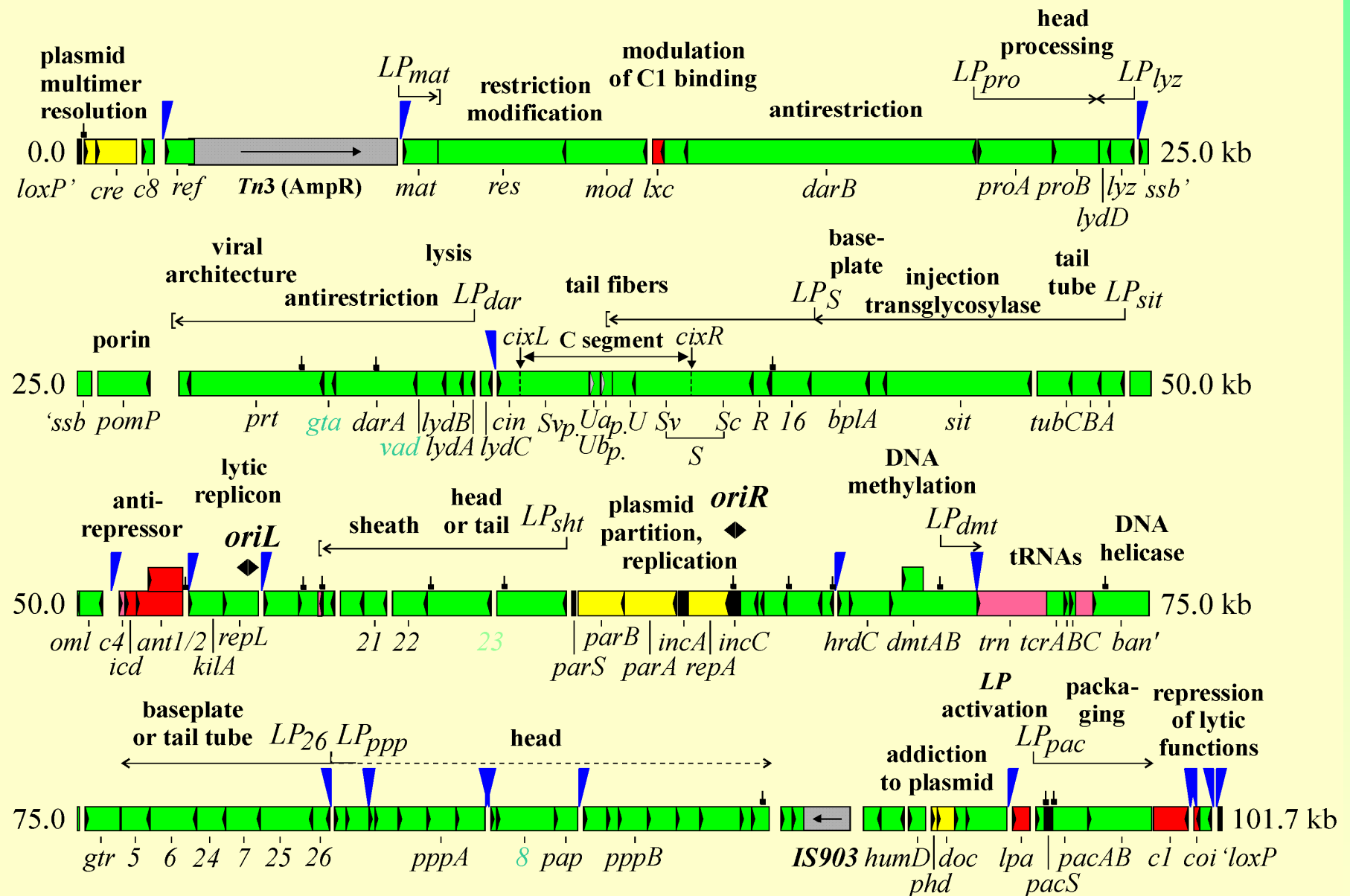
Over 99% soil microorganisms are unculturable!!!

Solution



Metagenomic approach to study soil microbial communities

Genomic versus metagenomic approach – a P7 phage example

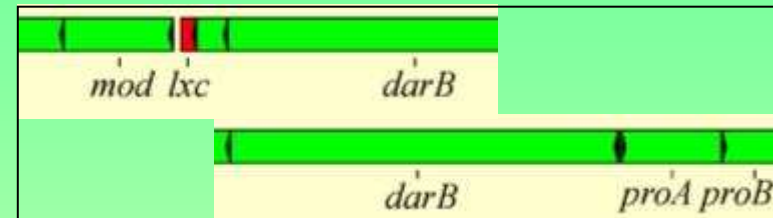


Łobocka et al. 2006. GenBank acc. No. AF503408

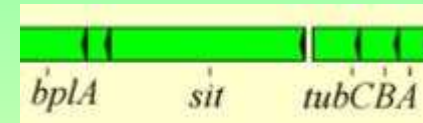
Genomic versus metagenomic approach – a P7 phage example

Metagenomic data that can be compared to a model sequence

Single reads that form contig:

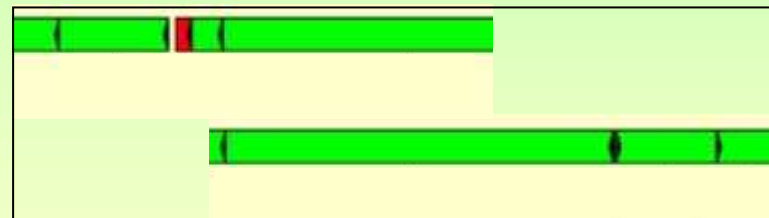


Non-overlapping reads:



Metagenomic data that can not be compared to a model sequence

Single reads that form contig:



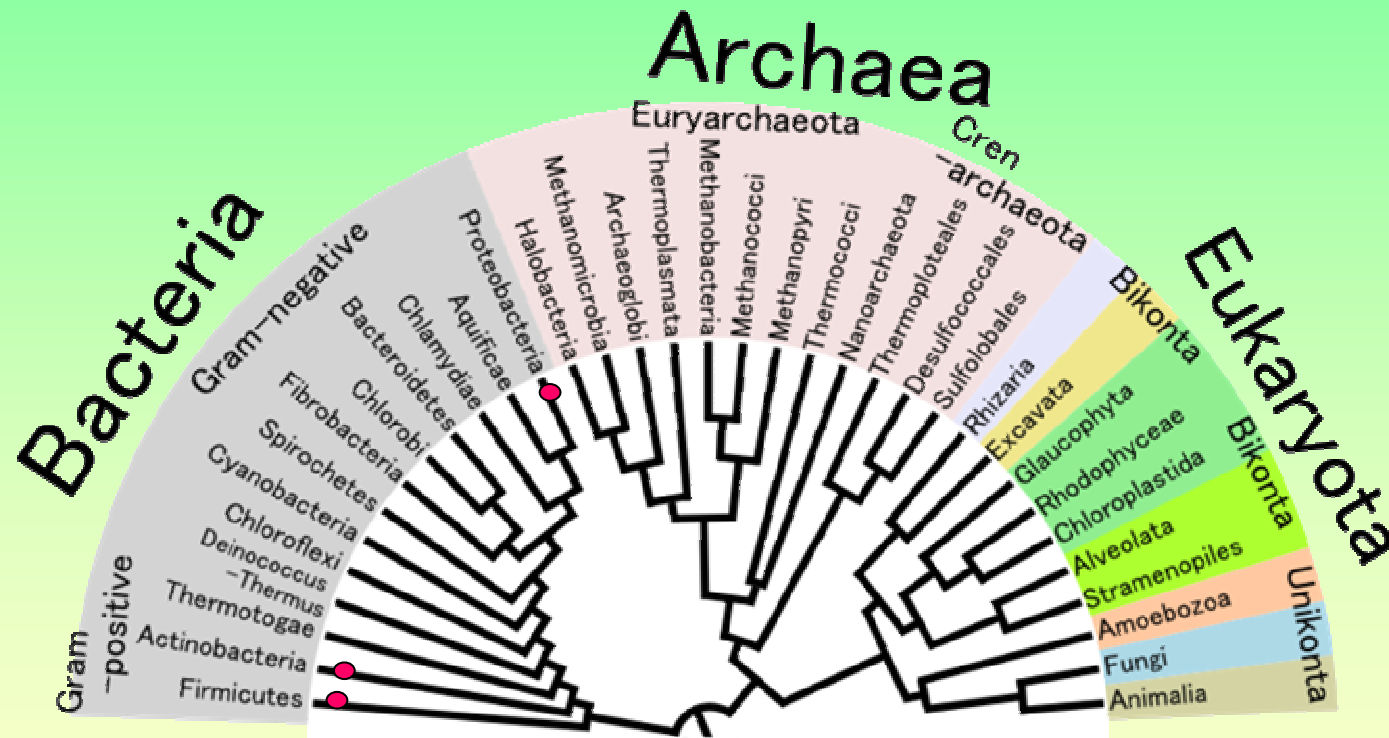
Non-overlapping reads:



Sources of information: homologies, relative locations

Problems with a representative coverage of existing prokaryotic genome sequences

- ▶ 75% of nearly 1000 bacterial and archeal strains of sequenced genomes belong to just three bacterial phyla (according to NCBI, August 2009)



http://en.wikipedia.org/wiki/File:Phylogenetic_Tree_of_Life.png (modified)

- ▶ GEBA (Genomic Encyclopedia of Bacteria and Archea) -
Joint Genome Institute + Deutsche Sammlung von Mikroorganismen und Zellkulturen
Geal – to sequence genome of at least one culturable Prokaryot of each type

Strategies of soil metagenome studies

Initial common steps:

- ▶ DNA isolation and amplification

Further alternative or additive steps:

- ▶ Global sequencing (shot-gun metagenomics)
- ▶ Amplification and sequencing of 16S rDNA i 18S rDNA
- ▶ Amplification and sequencing of selected genes that encode desired functions (use of bioinformatic analysis and degenerated primers)

The same batch of DNA can be used for many purposes by different laboratories, and stored frozen for much further use.

Metadata analysis

Challenges

- ▶ Difficulties in DNA isolation
- ▶ Complexity (experience from OMT and HMB project)
- ▶ Requirement of world-wide collaborative efforts
- ▶ Dependence on powerful international computer centers
- ▶ Costs – a need to develop cheap sequencing technologies

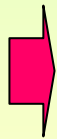
Expected benefits

- ▶ Understanding of ecosystem functions
- ▶ Basics for establishing or restoration of sustainable environments
- ▶ Development of successful methods for biocontrol of pathogens
- ▶ Access to the enormously large and diversified gene pool
- ▶ Preservation of DNA from “disappearing” local environments

Current large metagenomic projects

- ▶ Rhizosphere
- ▶ Great prairies
- ▶ Permafrost
- ▶ European sites
 - Rothamsted Park Grass Experiment (Penny Hirsch; Tim Vogel)
 - Metagenomics of disease suppressing soils (METACONTROL; J. D. Van Elsas, Wageningen, Holandia)
- ▶ Puerto Rico soils (Terry Hazen)

Park Grass,
Rothamsted



<http://www2.cnrs.fr/en/1600.htm>

Terragenome as the European metagenomic initiative

Consortium of scientists from 23 countries which are interested in soil metagenomics

Timothy Vogel – Environmental Microbial
Genomics Group,
Microsystems and Microbiology
Laboratoire Ampère, UMR CNRS 5005
Ecole Centrale de Lyon

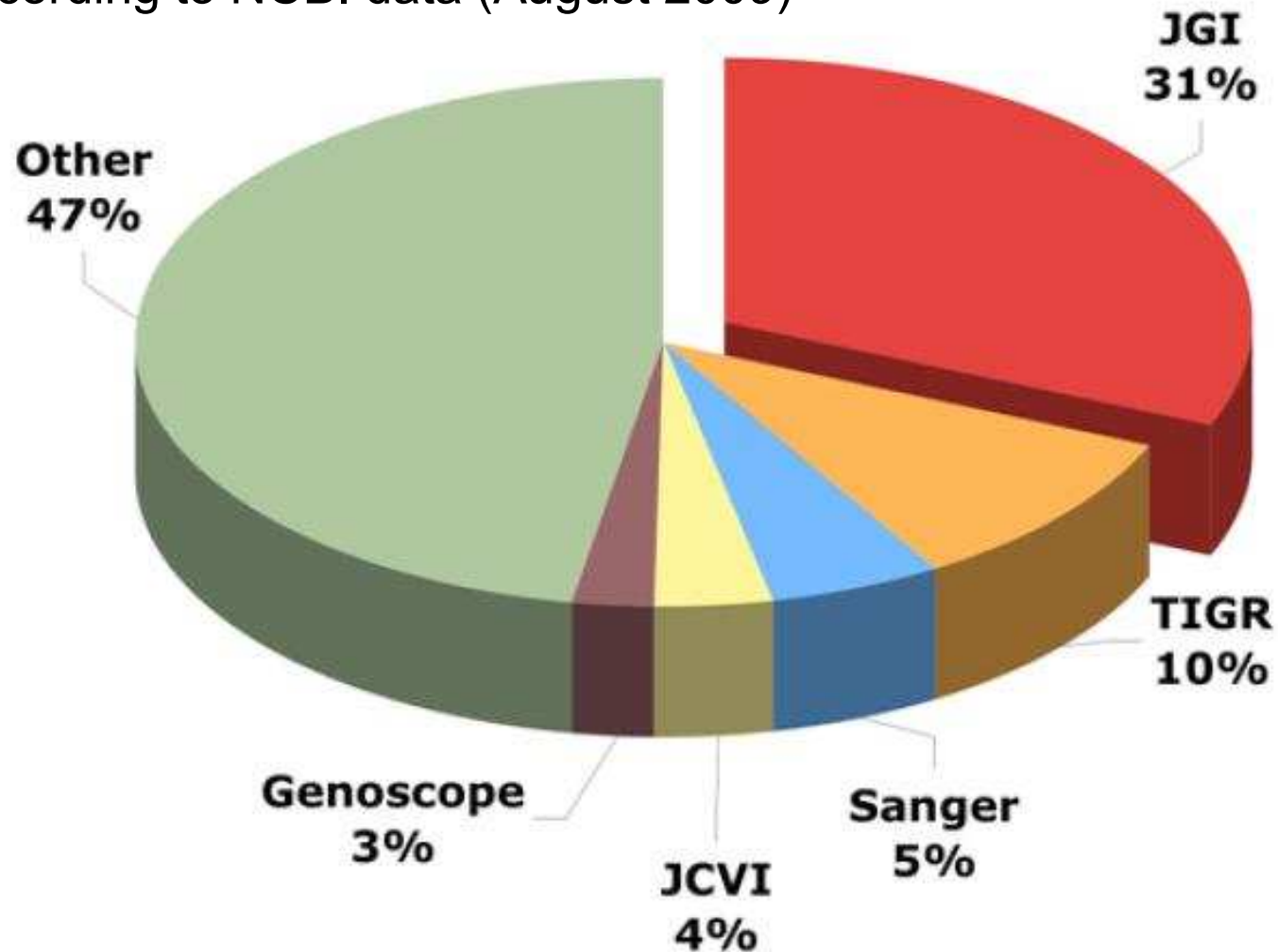


<http://www.terragenome.org/about/>

Thank you for your attention

Contribution of different institutions in the sequencing of 939 accessible Prokaryotic genomes

According to NCBI data (August 2009)



Sequencing technologies and the accessible sequence data

According to DOE report

