Applied Biotechnology Group - AUA

Developed in 2005 with the scope to:

Basic research in biotechnology

Use the results to promote applied research

Transform applied biotechnology into new products

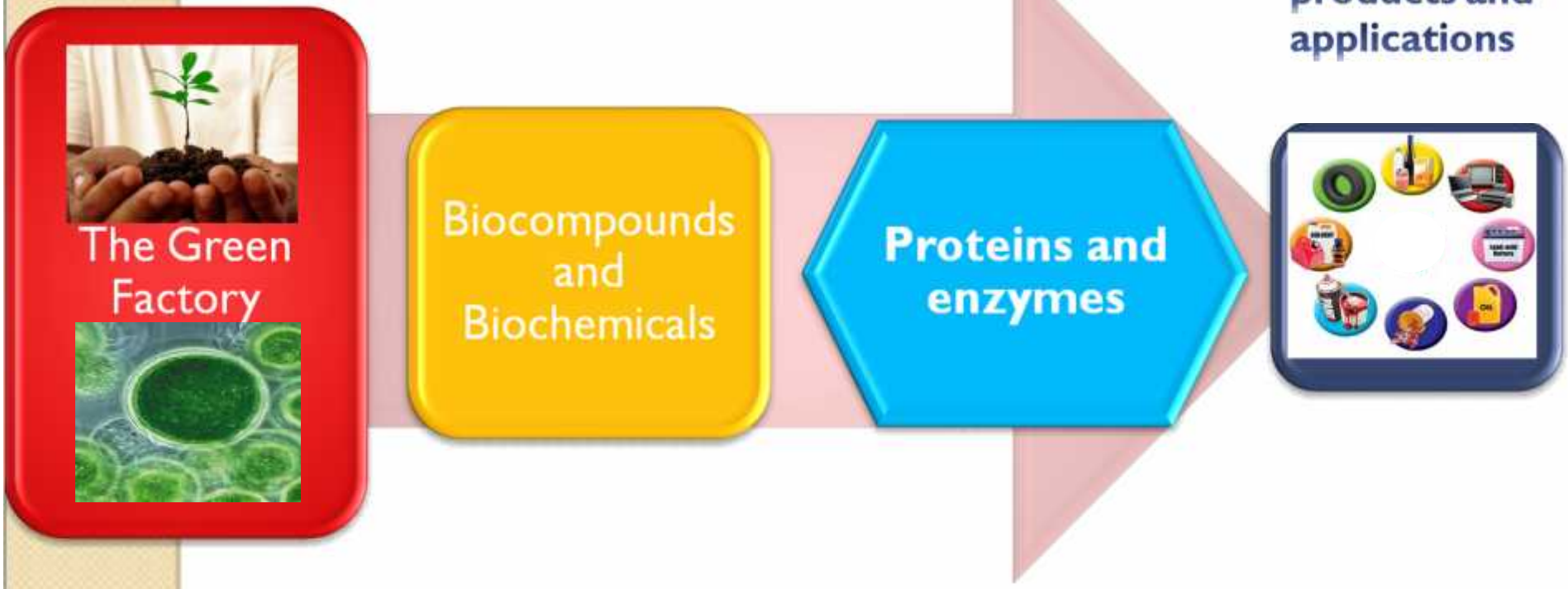
Agricultural University of Athens -AUA
Department of Agricultural Biotechnology

Applied Biotechnology Group

The group:

- Consists of about 15 scientists
  - Assoc. Prof. N. Lambrou
  - Assist. Prof. E. Flemetakis
  - Post-doc, postgraduate and undergraduate students
- The group have developed a broad network of national and international collaborations with both Academia and Industry
- Published more than 150 scientific publications and book chapters.

Development of high-added-value new products



Proteins and Enzymes

Applications

Food, energy industry

Food processing

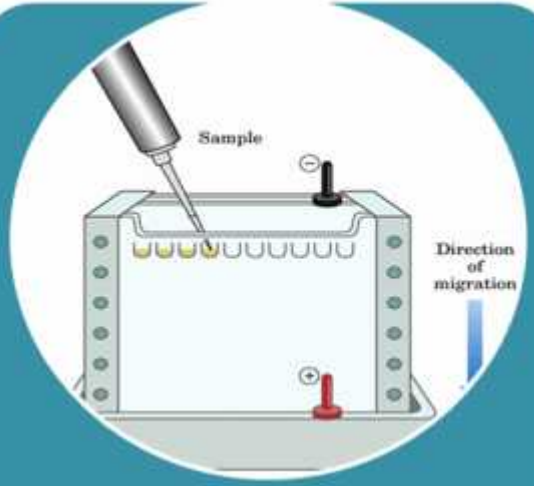
Biofuel production

Cosmetology, medicine

Anti-microbial agents

Detoxification

Anti-aging agents



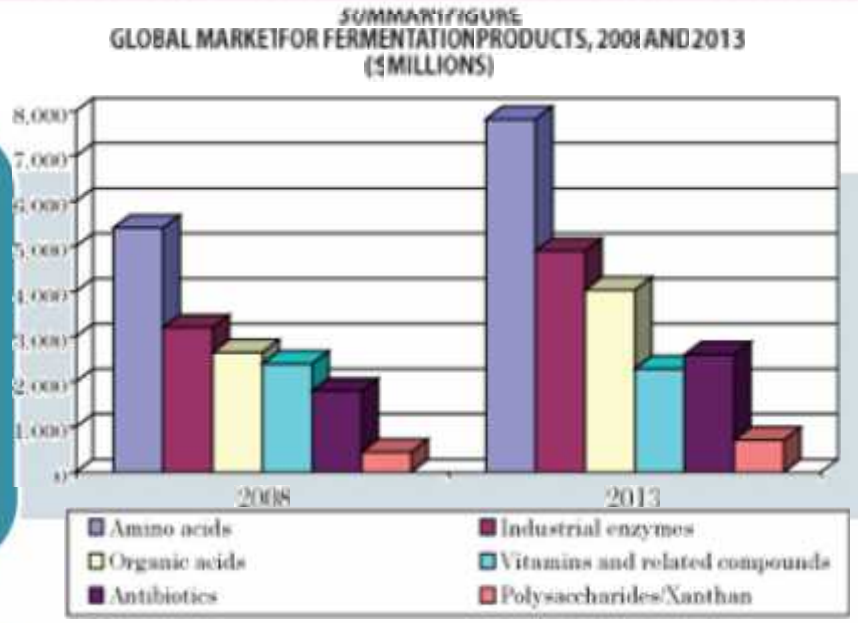
Analytical Biotechnology

Analytical enzymes

Molecular labels

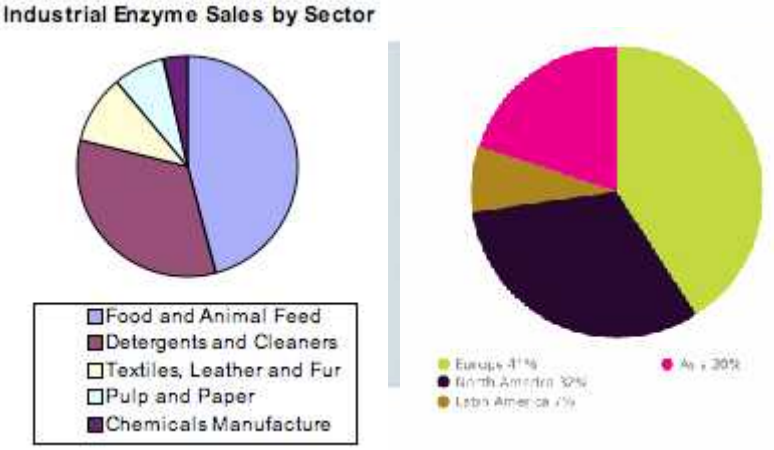
The global market for enzymes is expanding rapidly

The global enzyme market exceeds 10 billions USD 5.5% average annual increase

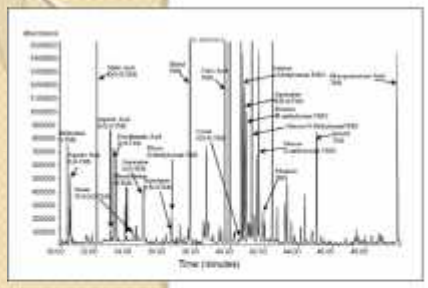


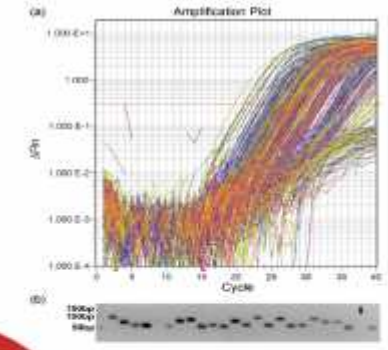
The principal market includes Food and animal Feed

Europe dominates the market



Application of -omic technologies





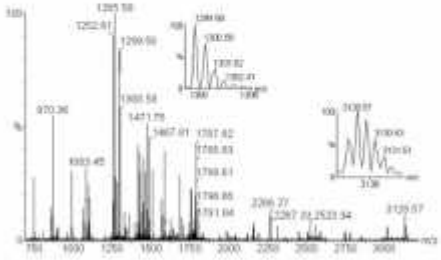
Metagenomics

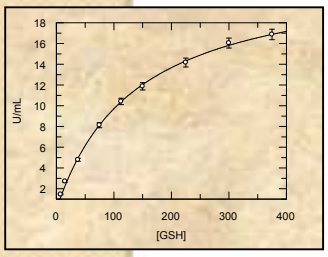
Transcriptomics

Proteomics

Catalomics

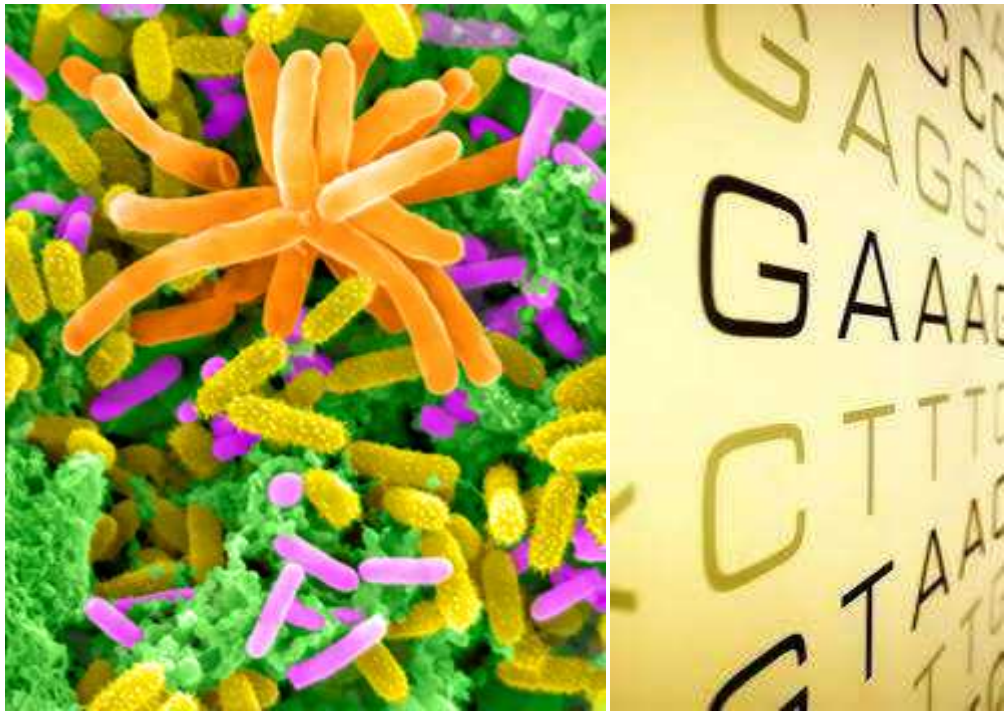
Metabolomics





Metagenomics

in environmental research

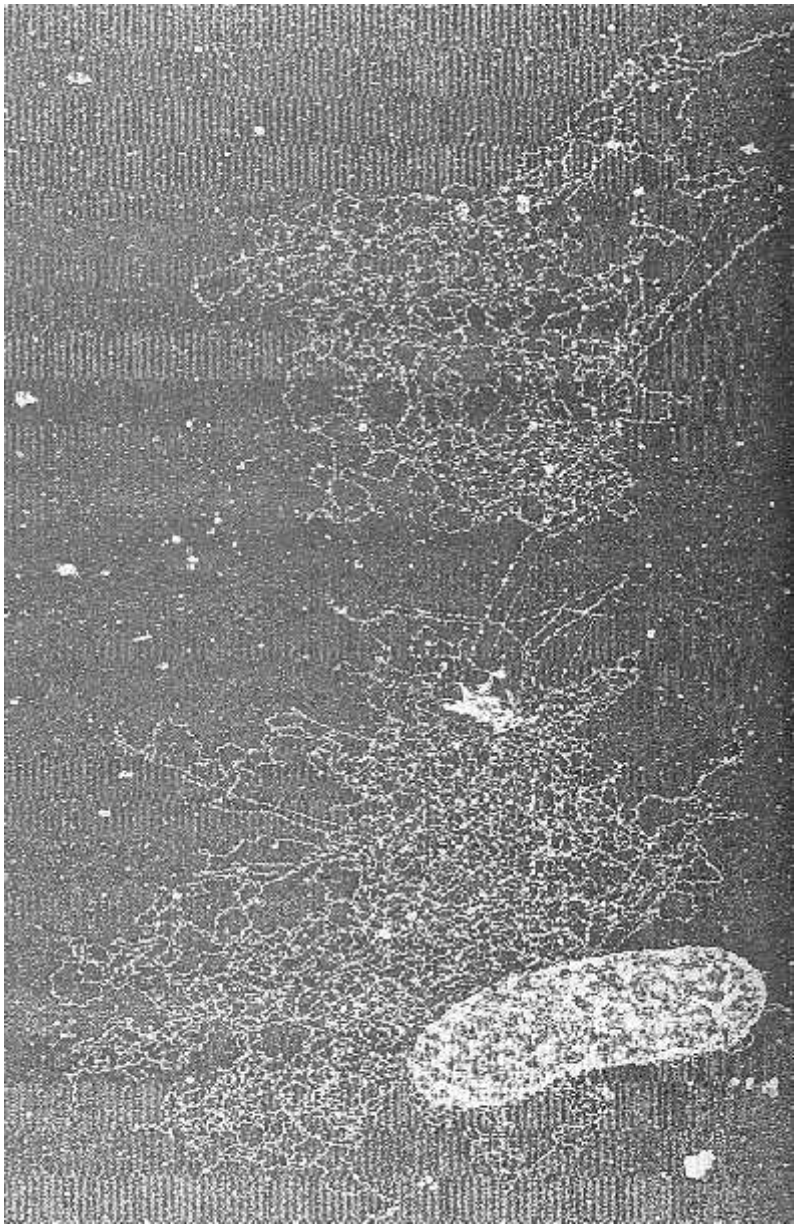


AQUAPHAGE 1rst Workshop

Heraklion 2011

What is Metagenomics?

Metagenomics is an emerging field in which the power of genomic analysis is applied to entire communities of microbes, bypassing the need to isolate and culture individual microbial species.



Metagenomics in action….

Extract all DNA from microbial community in sampled environment

DETERMINE WHAT THE GENES ARE

(Sequence-based metagenomics)

- Identify genes and metabolic pathways
- Compare to other communities
- and more…

DETERMINE WHAT THE GENES DO

(Function-based metagenomics)

- Screen to identify functions of interest, such as vitamin or antibiotic production
- Find the genes that code for functions of interest
- and more…

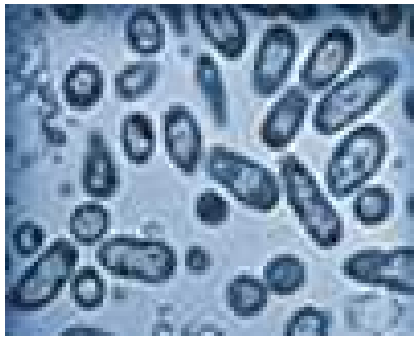
Why Metagenomics?

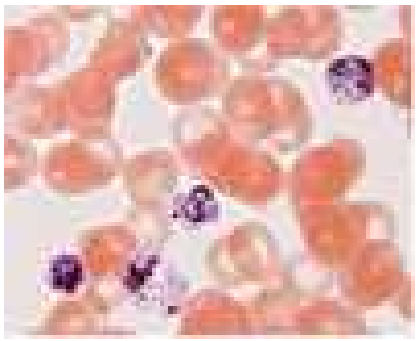
The tools of classical genomics and microbiology largely rely on isolating individual microorganisms.

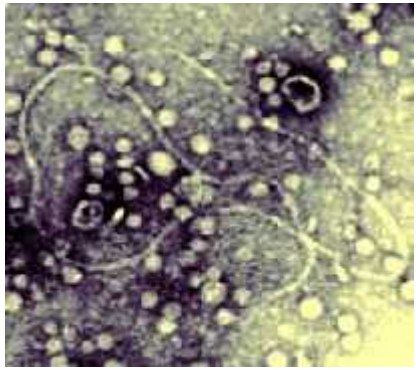
The vast majority of the microbial world has been inaccessible to science because merely less than 1% of the estimated millions of microorganisms can be cultured.

By allowing access to a community's genome without relying on isolation and culture, metagenomics transcends the limitations of classical genomics and microbiology.

Metagenomics gives scientists access a vast array of microorganisms that have not previously been studied







Applications…

- Earth Science and Global Cha
- Human Health

- Agriculture

- Environmental Remediation
- Energy

- *and more...*

Future challenges

Development of improved tools and methods:

- Scientists must determine how to best sample an environment, how many times samples should be taken, and whether a sample is representative of the environment.
- Improved DNA extraction techniques could help ensure that the DNA extracted from a sample adequately represents the entire community's genome and has little or no contamination.
- Studying the proteins and metabolites (the products of cellular processes) generated by a microbial community will help describe how the community operates and interacts with its habitat.

Future challenges

Data management and bioinformatics needs:

- Metagenomics data should be stored in databases that use common standards and are freely accessible to all.
- Databases should include specialized tools that enable different scientists to analyze the data in different ways.

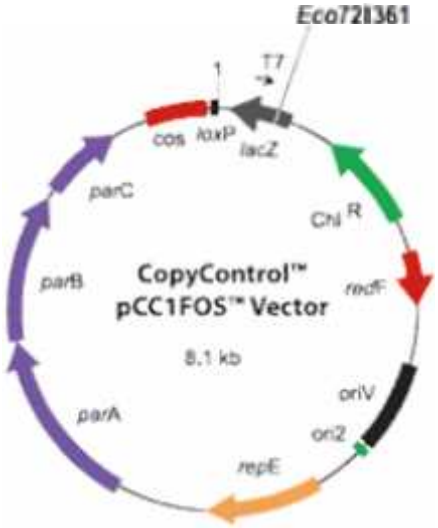
Future challenges

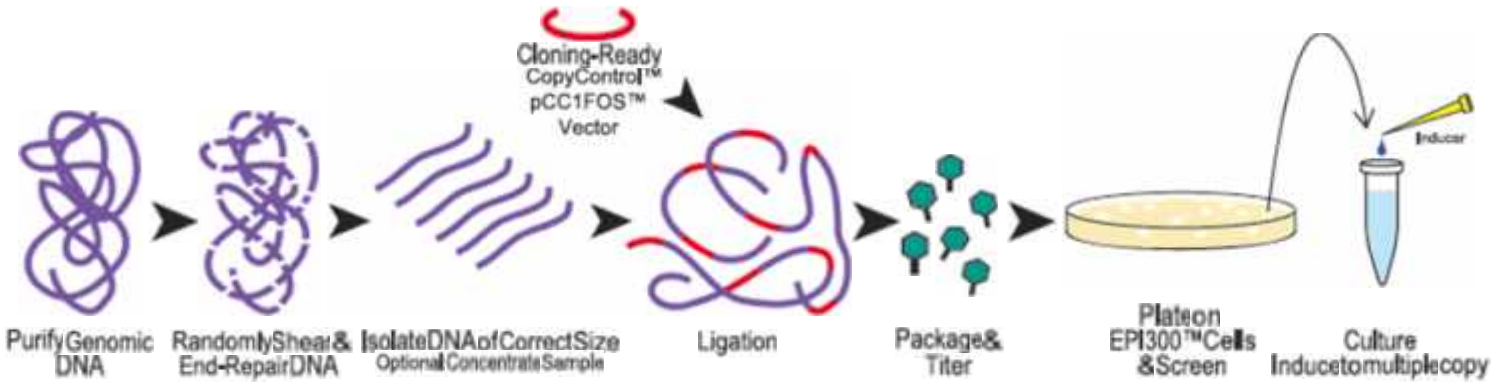
Institutional frameworks:

- A broad range of fields; including chemistry, genetics, microbiology, biochemistry, pathology, ecology, evolution, soil and atmospheric sciences, geology, oceanography, statistics, computer sciences, database development, mathematics, engineering, and others have applicability to metagenomics.
- Research organizations and Universities will need to rapidly evolve their educational, administrative, and mentoring structures to facilitate interdisciplinary collaboration to help metagenomics reach its full potential.

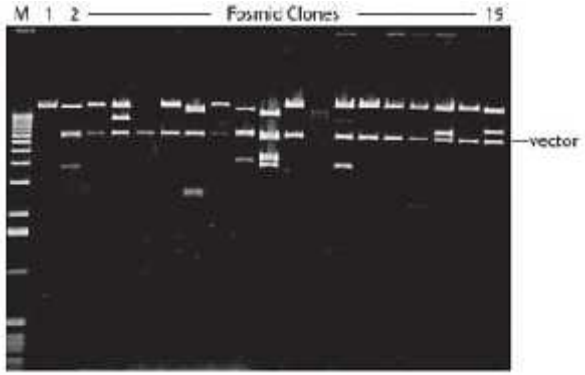
Construction of metagenomic libraries

- Isolation of high-molecular-weight genomic DNA from environmental samples
- Mechanical random shearing for DNA molecules



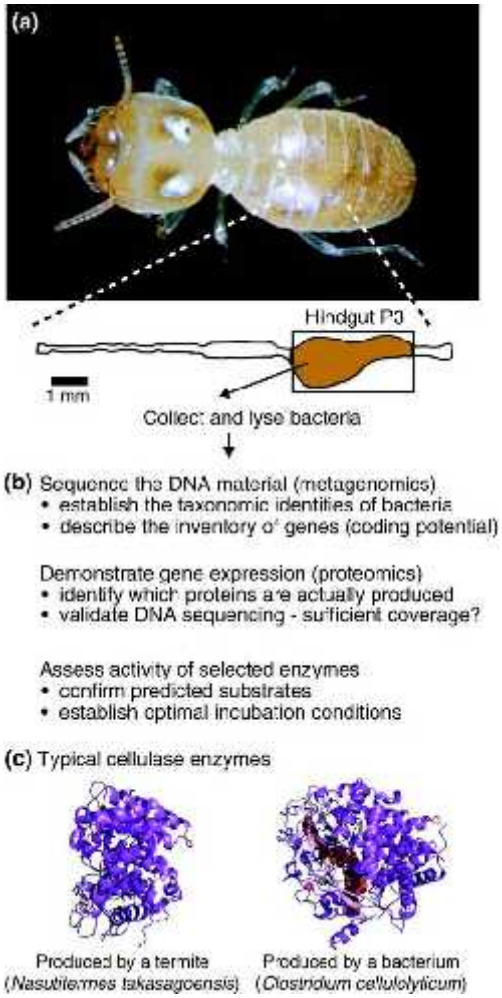


- End-repair and size-select DNA fragments (40kb)
- Ligation into the vector
- In-vitro packaging into λ particles
- Plating the original library into a suitable *E. coli* host.



Functional metagenomics

- Screen microbial communities for genes of interest:
  - Glycosyl-hydrolases (cellulases)
  - Proteases
  - Detoxifying enzymes
    - Aromatic compounds
    - Pesticides etc.
  - Lytic enzymes
    - Gram^{+} bacteria
    - Gram^{-} bacteria

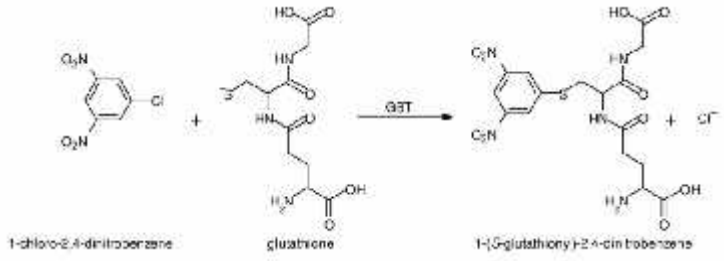


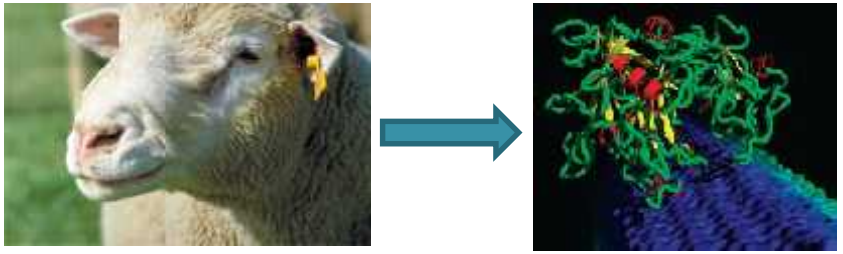
(Chaffron and von Mering, 2007)

(**For a review**: Brady et al. *Nat. Prod. Rep. 2009 26:1488-1503*)

Key-aspects for functional metagenomics screens

- Start the screen at the right place:
  - Screen for enzymes involved in xenobiotic degradation in enviromental samples containing the target substrate.
  - Screen for hydrolytic activities in rumen microbiata

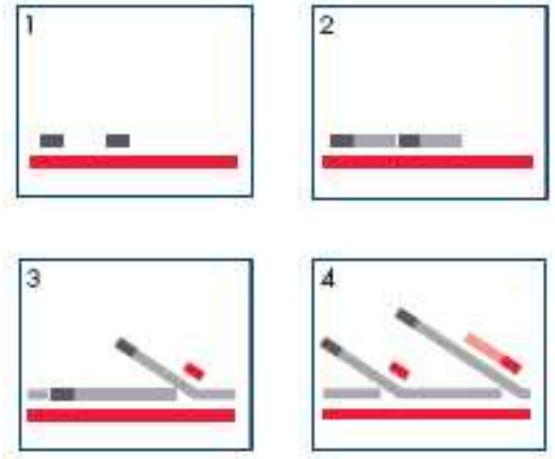




- Careful selection of the environment:
  - Increases screening efficiency
  - Reveals novel biocatalysts with unique structural or kinetic properties
    - cold- or heat- adapted enzymes

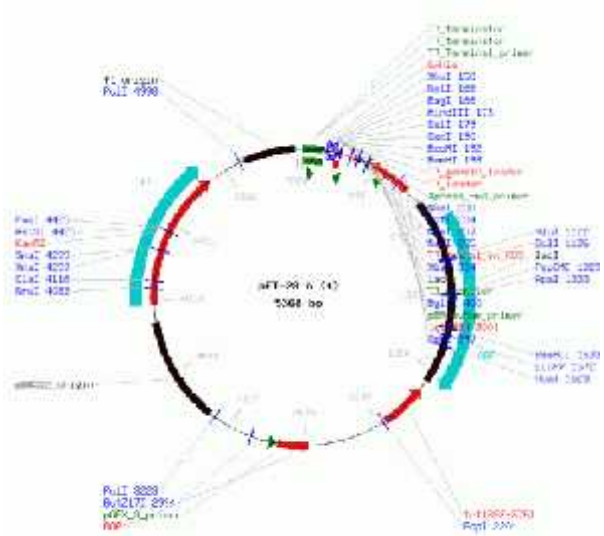
Key-aspects for functional metagenomics screens

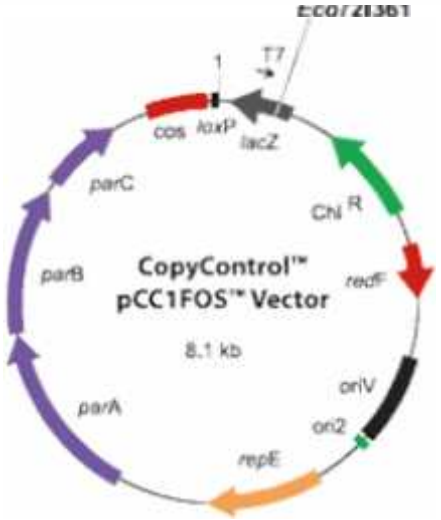
- Maximizing genomic representation:
  - Enrichment cultures
  - Selective substrate utilization using Stable Isotope Probing (**SIP**) (^{13}C, ^{15}N, ^{18}O)
    - Genomic DNA is fractionated by density centrifugation before library construction.
  - Whole genome amplification:
    - Single-cell library construction
    - Multiple Displacement Amplification (**MDA**)
    - Linker-Amplified Shotgun Library (**LASL**)
    - Expressed-Linker-Amplified Shotgun Libraries (**E-LASLs**)



Key-aspects for functional metagenomics screens

- Selecting the expression system
  - Functional metagenomic expression systems involve:
    - Vectors (host range, insert size, copy number and transformation efficiency)
    - Host organisms that express the encoded enzyme activity
- Small-insert libraries (<15 kb inserts)
  - Vectors: high copy number plasmids
    - High transformation efficiency
    - Restricted to single locus activity screens
- Large-insert libraries
  - Vectors: low copy number Cosmids, Fosmids, Bacterial Artificial Chromosomes (BACS)
    - Fosmids libraries: between 32-45 kb inserts
    - Low transformation efficiency: use of phage transduction systems
    - Allows multilocus activity screens





Key-aspects for functional metagenomics screens

- Selecting the screening host
  - *E. coli* is the dominant screening host
    - Stable replication of a vast array of low/high copy number vectors
    - High transformation and infection efficiencies
    - Minimal expression of toxic proteins

Major limitation:

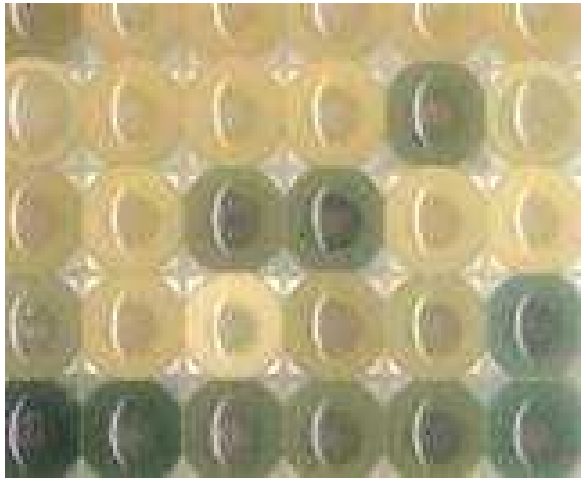
E. coli can support expression of only 40% of the genes of interest

  - *Host should be selected on the basics of:*
    - phenotypic trait of interest
    - taxonomic composition of the metagenome

Host	DNA delivery	Screen phenotypic trait
Agrobacterium tumefaciens	transformation	Pigmentation, morphology
Caulobacter vibrioides	transformation	Enzyme assays
Rhizobium leguminosarum	conjugation	Enzyme assays
Escherichia coli	transformation	Pigmentation, morphology, antibiotics enzyme assays
Pseudomonas putida	conjugation	Pigmentation, enzyme assays
Xanthomonas camp	conjugation	Pigmentation, enzyme assays
Pseudomonas fluorescens	conjugation	Pigmentation, enzyme assays

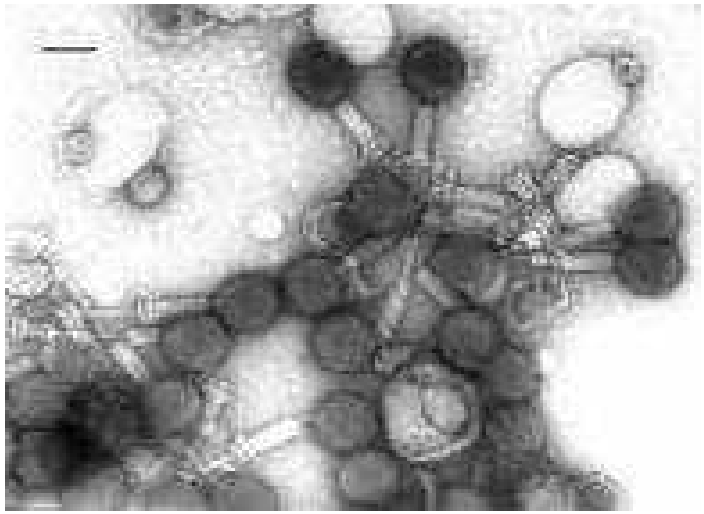
Key-aspects for functional metagenomics screens

- Availability and development of effective screening procedures
  - *Genetic selection and survival*
    - Cell lysis
    - Antibiotic resistance
    - Heavy metal/pollutants resistance
    - Complementation of genes mutated or missing in the host
  - *Changes in colony color, morphology or clearing*
  - *Direct assays of enzyme activities*
    - Amenable to high-throughput automation on 96-well or 384-well plates

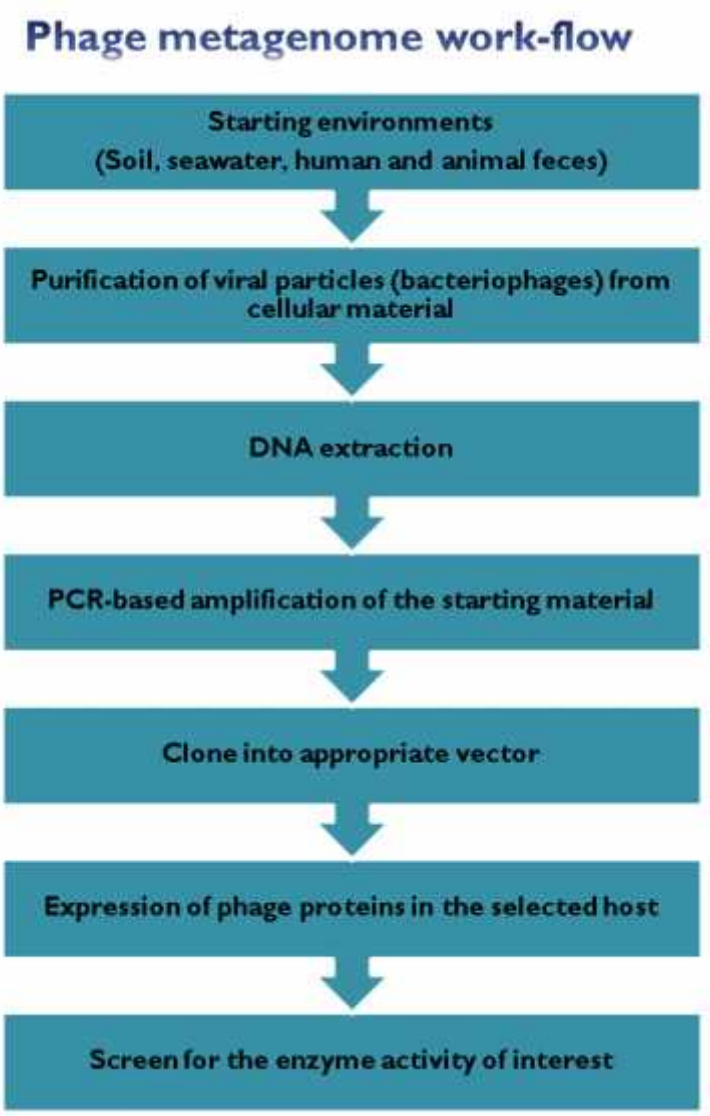


Functional metagenomics as a new tool for Blue-Biotechnology

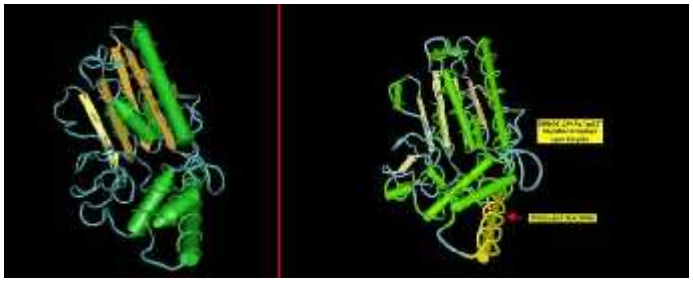
- Viral metagenomes have been indentified as a very promising source of recombinant proteins with high biotechnological value
- Phages represents the largest untapped genetic resource



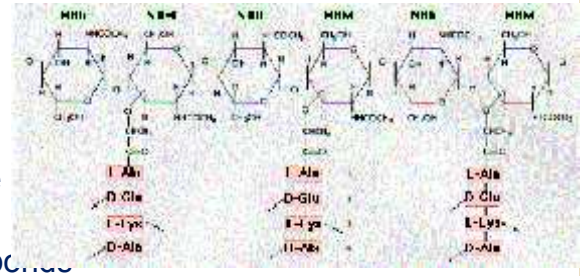
Paul et al.
College of Marine Science
University of South Florida



Phage lytic enzymes (endolysins, lysins)



- **Peptidoglycan hydrolases**
- Expressed late in the infective cycle of ds-DNA phages
- Responsible for the disruption of the bacterial cell envelope and the release of the progeny phage particles
- Phage lysins (especially Gram-positive) represent a very diverge group of proteins
  - **Glycosyl-transferases** → Target the polysaccharide backbone
  - **Alanine amidases** → Target the initial L-alanine of the peptapeptide
  - **Endopeptidases** → Target the subsequent peptide bonds



- Gram-negative lysins
  - Consist of a single enzymatic domain
- Gram-positive lysins
  - Multidomain enzymes: N-terminal lytic domain - C-terminal binding domain

Phage lysins represent fascinating targets for functional metagenomics

- Challenges towards the identification of new phage lysins through functional metagenomics
- General problems
  - Protein expression and solubility
- Specific problems
  - ***Clonal toxicity***
    - *E. coli* is generally tolerant to cytoplasmic lysins
    - Toxic effects due to the presence of Holins (phage membrane-permeabilizing proteins, often encoded by genes adjusted to lysins)
    - Significant loss of positive lysins
  - ***Target bacterial species***
    - Lysin-encoding clones are selected by their ability to kill the phage host bacterium
    - In metagenomic screens the large number of host bacteria make the choice of the screening host difficult.

Identification of phage lysins using functional metagenomics

Screening for active lysins using a two-step process.

Schmitz et al. 2010. Applied and Environmental Microbiology 76:7181-7187

Collection of fecal specimens from various domestic and wild animals

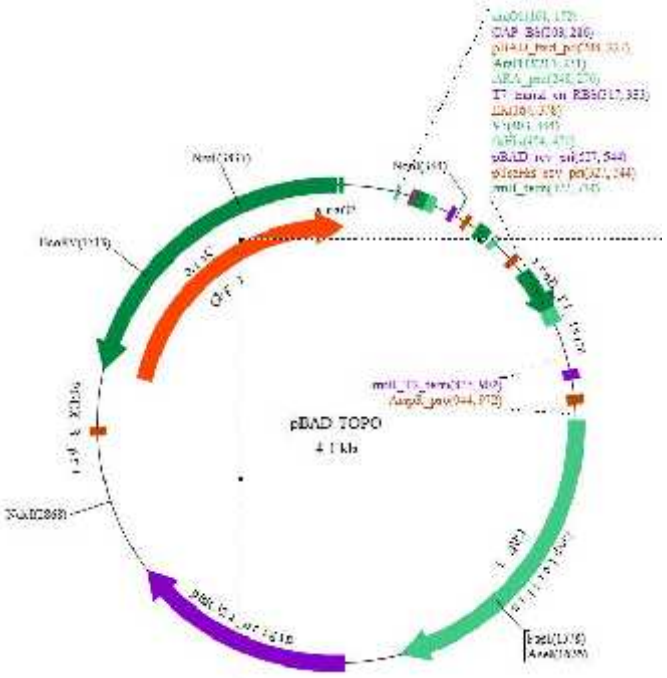
Isolation of a collective phage population

Extraction of phage DNA

Construction of a expressible-linker amplified shotgun library (E-LASL)

Ligation of amplified inserts into a arabinose inducible pBAD plasmid

Transformation of E. coli cells



Identification of phage lysins using functional metagenomics

Step 1: identification of active holins in a metagemonic library

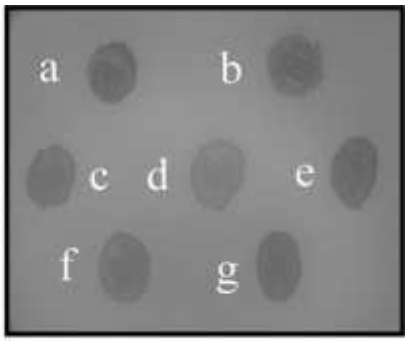
Plated *E. coli* trasformants were induced by arabinose

Hemolytic zones appear on around colonies expressing holins

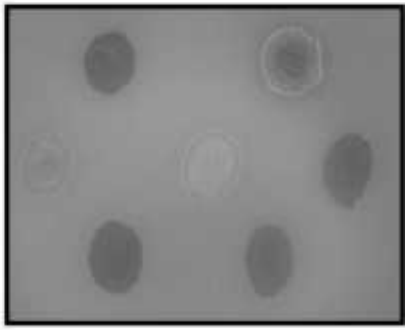
No cell lysis was observed in colonies expresing only lysins without the adjucent holins

Initial screen: 200.000 colonies
502 preliminary hits
52 clones sequenced
17 clones encoded complete holin/lysin pairs
18 clones with no homology to holins and lysins

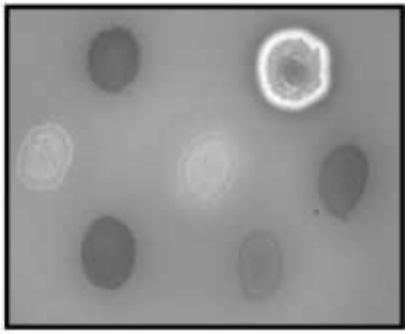
Pre-Induction



6 hrs.
Post-Induction



24 hrs.
Post-Induction



Schmitz et al. 2010.

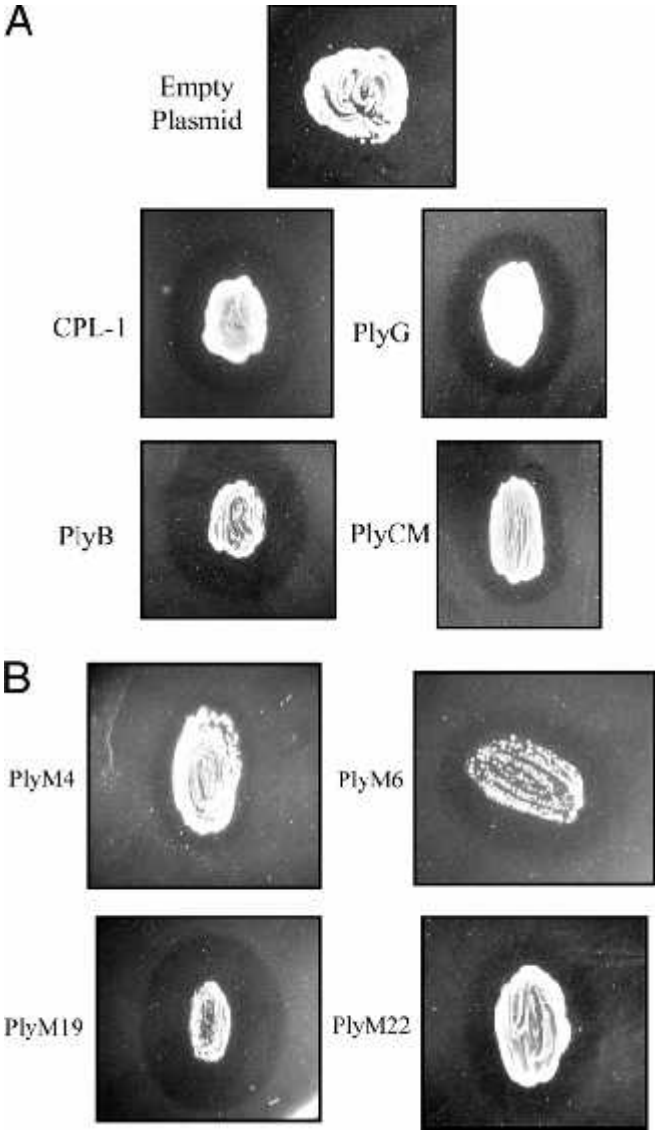
Identification of phage lysins using functional metagenomics

Step 2: identification of active lysins

The 502 initial hits where plated onto arabinose containing agar and tested for their ability to lyse a soft-agar overlay of autoclaved *P. aeruginosa*

Colonies containing active lysins could form distinct overlying clearing zones.

26 active lysins could be identified



Schmitz et al. 2010.

The screen could identify

26 active lysins containing various catalytic and binding domains

- 20 typical Gram-positive lysins
  - N-terminal catalytic domain
    - 8 type-2 amidases
    - 12 type-3 amidases
  - C-terminal binding domain
- 3 typical Gram-negative lysins
  - Muramidase catalytic domain
- 3 atypical lysins architecture

Schmitz et al. 2010.

Thus functional metagenomics lysins screens could

- Help with the identification of novel lysins with antibiotic activity
- Identify enzymes optimized for diverge biochemical conditions (temperature, pH, ionic strength etc)
- Complement the identification of by sequence-based metagenomics

Thank you!!!

Dr Emmanouil Flemetakis

E-mail: mflem@aua.gr