BUTE Faculty of Chemical Technology and Biotechnology
Department of Applied Biotechnology and Food Science

Biology, Biotechnology
2. Lecture

Áron Németh, PhD
Senior Lecturer
http://f-labor.mkt.bme.hu

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Chemical Engineering MSc

Content:

2.Lecture: Cell-biology

- Overview of microbiology, and of microbial physiology
- Type of industrial microbes,
- Main biochemical characteristics: aerobs and anaerobs,
- Basic microbial metabolism

Biochemistry for Chemical Engs.

Introduction

Biotechnology = applied (micro)biology

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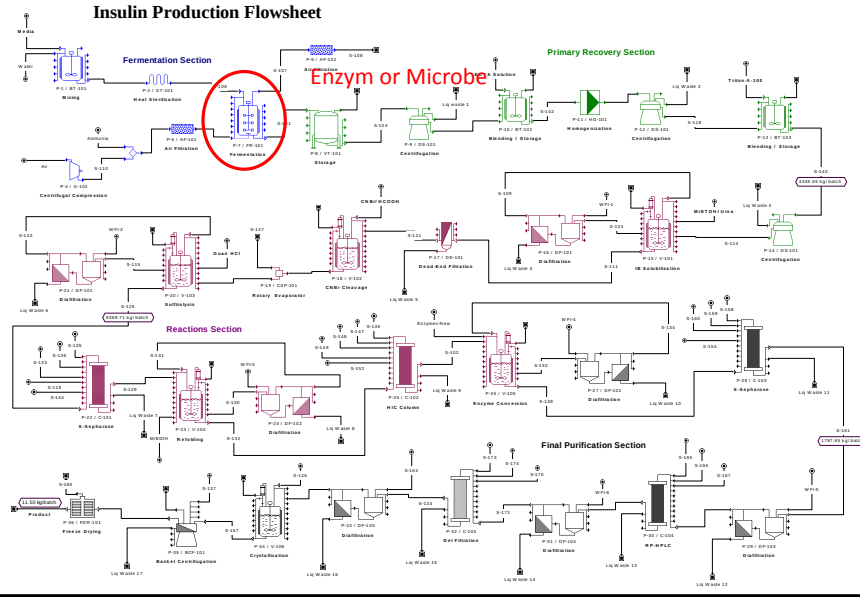
Biotechnology = applied (micro)biology



Introduction

Biotechnology = applied (micro)biology

Fermentation: the conversion of the given substrate by some living organism (or by their parts)

Living organism (or its part) = Microorganism or (its) enzym

Biotechnology is based on microorganism -> Microbiology

General Microbiology

Systematic (Detailed) Microbiology (Taxonomy)

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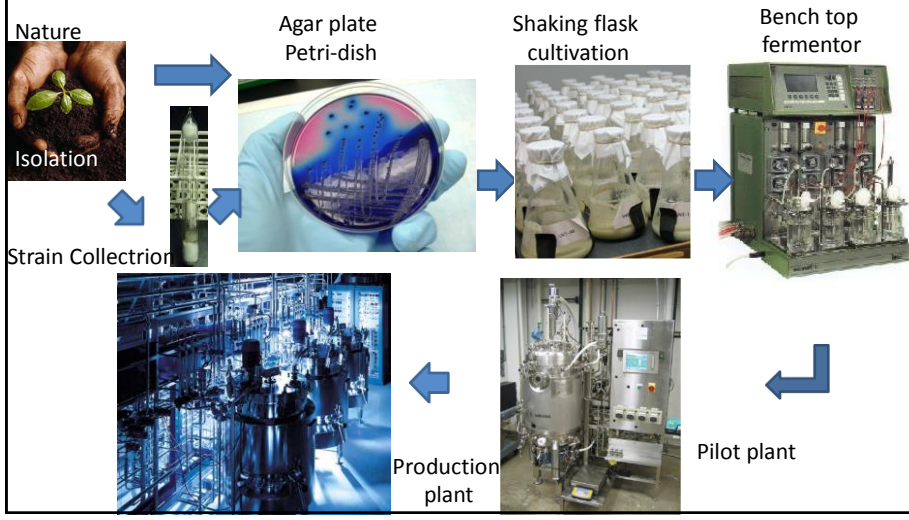
Microorganism : microscopic living organism, which is unvisible by eyes

Motto : „ It might look that I am doing nothing, but at a cellular level I am really quite busy."

Comments:

1. There's no „stupid" microbes.
2. Microbes only want to live.
3. But we selfish serve and utilize them.
4. They give us in change profit and salary.
5. BUT: unvisible, thus often their accompany processes are also unvisible (for example: contamination)

Technology Development vs. Production – applied microbiology:



General Microbiology

1. Microbiology knowledge:

A. Structure of a microbial cell
B. Microscopic observations
C. Microecology

Detailed Microbiology

Taxonomic system:
-Phenotype based
-Genotype based

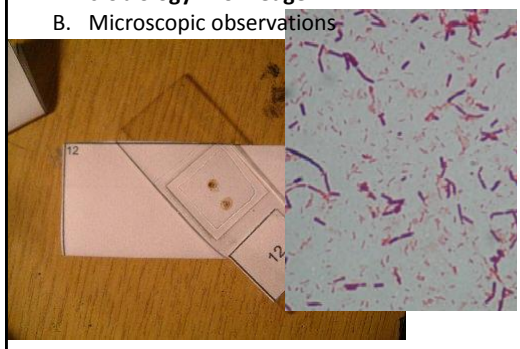
2. Microbiology operations:

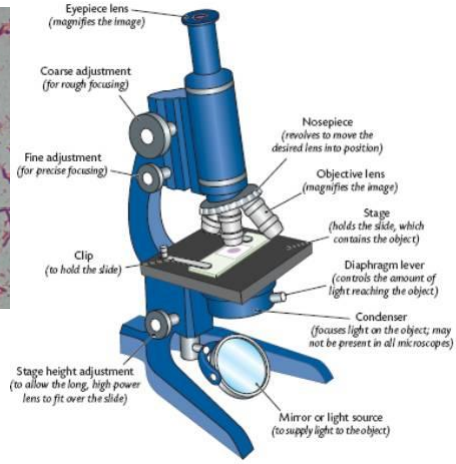
A. Media preaparation
B. Isolation
C. Screening
D. Identification
E. Preservation and maintenance
F. Subcultivation (transfering)
G. Strain imprvements

General Microbiology

1. Microbiology knowledge:

B. Microscopic observations





Bacteria: 1-2 µm 1000x

Yeasts: 10 µm 400x

Molds: 10 µm-1mm 100x

General Microbiology

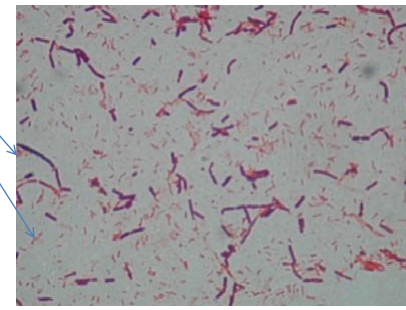
Microscope:

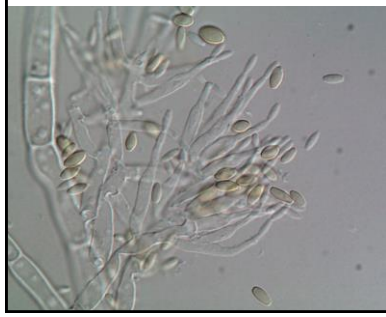
Bacteria: 1-2 µm 1000x

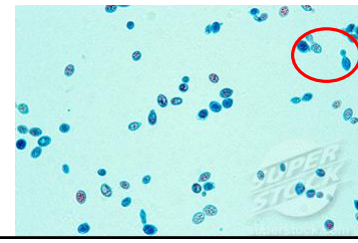
Yeasts: 10 µm 400x

Molds: 10 µm-1mm 100x

Gram+
Gram -







General Microbiology

Measurements of microbes:

1. OD-optical density (UV-Vis photometer, 600-660nm)
2. Turbidimetria (online)
3. Mikcroscope – cellcounting with Buerker chamber (10^6 pieces/ml)
4. Automatic Cellcounter (10^6 pieces/ml)
5. Cell Dry Weight (1-10 g/L)
6. Diluting-dispersing method (CFU/ml)

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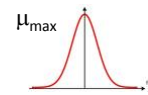
General Microbiology

1. Microbiology knowledge:

C. Mikroecology

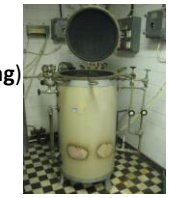


Livingplaces: air, water, soil
Life-kind: saprophyta, symbionic, commensalism, parasitism,
Life-conditions: temperature tolerancy (psychrophil, mesophilic, thermophylic)
pH tolerancy (acidophilic, neutrophilic, alkalophilic)
halophyilic (osmotolerancy)
Role of Microbes in biosphere: photosynthesis: fixing CO2, produce (bind) Energy
Degrading: C, N, P recycling

General Microbiology

2. Microbiology operations:

A. Prepearing media
Fluid media <-> Solid media (+agar, or gelrite)
In both: C-source: carbohydrates
N-Sources: proteins, aminoacids, oligopeptides, ammonium-salts
P-sources: phosphatides
+salts, vitamines, precursors etc.
Anaerobs: reducing components (DTT, NaSH)+O_2 indicator)
Bact.: organic N-source (protein), C-source also incl., sugar is not always neccessary
Yeasts+molds: N-source can be inorganic salt => much easier downstream, cheaper up and downstream
Media sterilization:
Physical methods (filtration, irradiation, **thermal handling**)
Chemical methods (decontaminating agents)
Biological methods (cellwall degrading enzymes)



Not all components is compatible, sometimes should them separate

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General Microbiology

2. Microbiology operations:

B. (Strain) Isolation
•Solid→ most frequent (soil), because largest diversity
-sample->onto petri dish
-sample->suspension in water->onto petri dish
-from surface of sample with steril cotton-wool
} Incubation
•Fluid →more dilute-> concentrate: preincubation or filtration+agar+incubation
•Air

Petris dishes with agar for isolation:
mixed culture in soil->selective preasure
additives to media for exm.: antibiotics->only funghi can grow
antifunghi compouds-> bact. grow,
little acidification: yeast

General Microbiology

2. Mcrobiology operations:

C. Screening

Two goals: 1) to find a producer strain among the isolates
2a) to find a better producer for an existing technology
2b) strain improvement (see later)

For these: examination of microbes (isolates) in large numbers
(100-1000 or even more) – lot of sample=>automatisation
BUT
Every case is unique (the key parameters, methode of detection etc.)->manual

Combination

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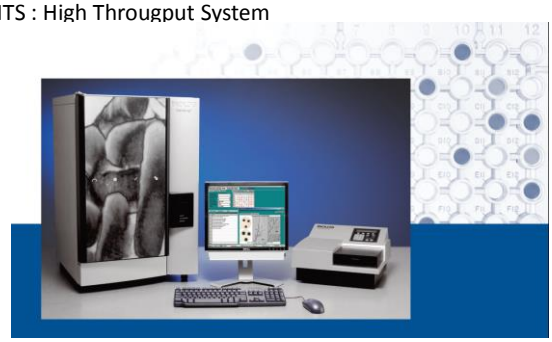
General Microbiology

2. Microbial operations:

C. Screening

-HTS : High Througput System



-manually: additions to agarplates: usually turbid/cleaned zone around the colony
For example.: CaCO3-> acid producers (cleaned zone)
szensitive microbe->antibiotic producer (cleaned z.)
oil emulsion+Ca->lipase producers (turbid zone)
protein aggregate->protease producers (cleaned z.)

General Microbiology

2. Microbiology operations:

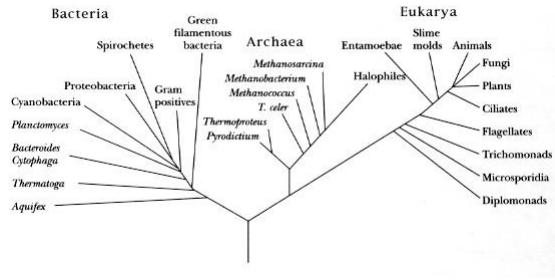
D. Identification

Goal: to identify, and rank into taxonomic system

-HTS system: automatised on both pheno/genotype

phenotype based: ~manual (see next)

genotype based: conserved geneom ->relationship (phylogenetic tree)



More detailed: in Detailed Microbiology

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General Microbiology

2. Mikrobiológiai műveletek:

D. Identification

-manual: on the basis of phenotype and biochemical charachteristaion, for example:

macroscopic: color, smell, shape of the colony

microscopic: cell shape, groupping of cells, motion-organs,

cellcorn, cellwall (Gram staining, other staining)

Biochemical tests: oxydase probe, aerob/anaerob dextróz consumption,

urease, hydrogensulphide etc

Genearl Microbiology

2. Microbiology opeartions:

E. Storage and maintenance

Maintenance of isolates:

-in active forms:

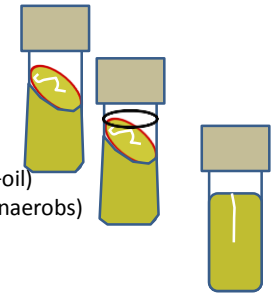
-freezdried in an ampul (lyophylised)

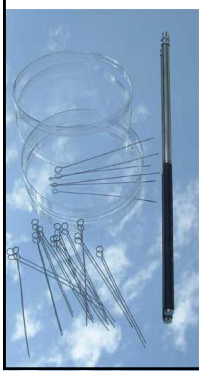
-slowed down in a fridge

-on agarslant in a testtube(+oil)

-punctured (sticked) agar (anaerobs)

-agar in petridish





-in inactive form: spores

General Microbiology

Microbiology operations:

F. Transfer- subcultivations

-daily usage-> maintenance in active form->but become aged->transfer to fresh
-starting an inoculum from maintenance culture (from solid to fluid)

Laminar box<> sterile box

Accessories:

-steril place
-steril loop
-bunsen flaming
-autoclave for sterilization
-sterile pipetta tip or glass-pipette
-sterile water (for suspension)

Sub-transfer: the new culture will be incubated, then stored in fridge. Next time wil be used.

General Microbiology

2. Microbiology operations:

G. Strain improvement

The charachterisation of a living organism is determined by genom =>
for improvement the genom should be changed=mutation

Phyisical mutagens:
-radiations (UV, gamma)
Chemical mutagens:
-DNA modifing compounds (carcinogen)

Dose:
- λ and intensity of radiation and time
- concentration+time

1. Mutatio->2.cultivations of mutants (isolation)->3.screening of mutants (which is better)->

4.Little better-> re-mutation

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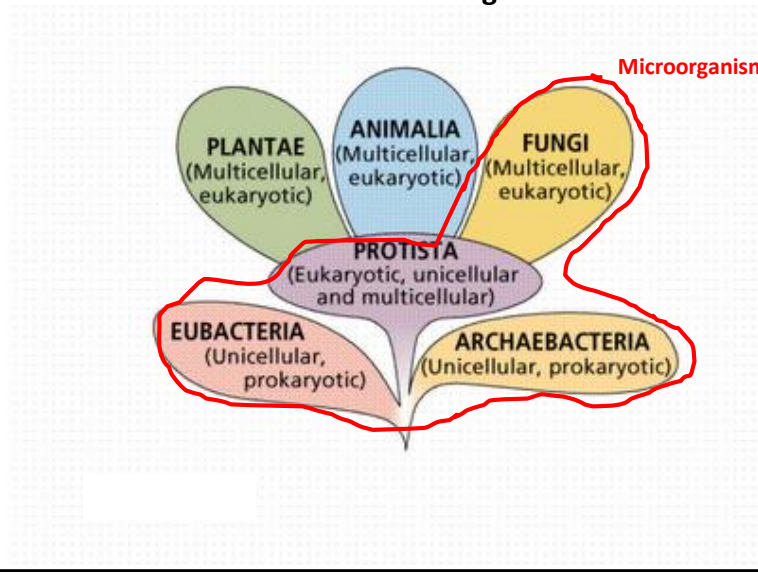
Részletes Mikrobiológia



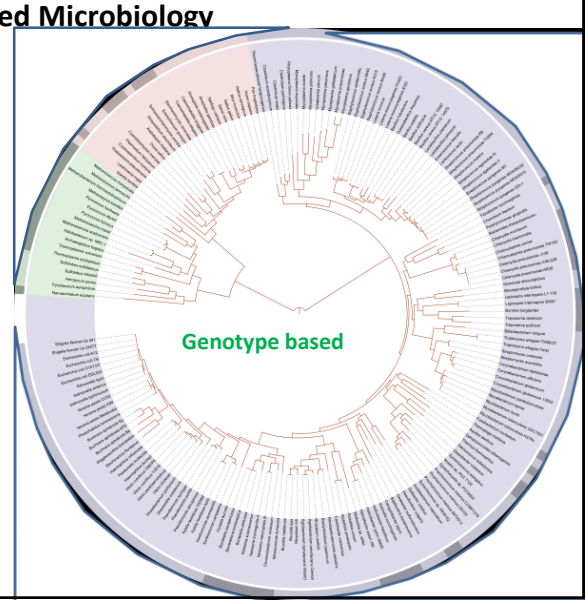
Detailed Microbiology

A) Procaryotes

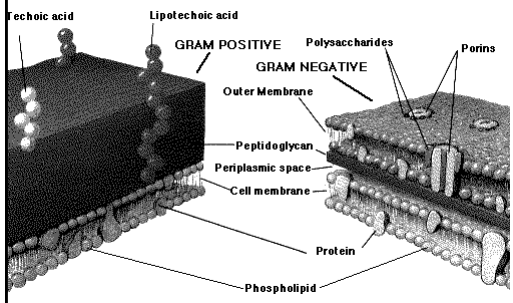
Bergey I. edition (years of 1980-90
4 division
I. Gram (-) bacteria
II. Gram (+) bacteria
III. Bacteria without hard cellwall
IV. Archeabacteria

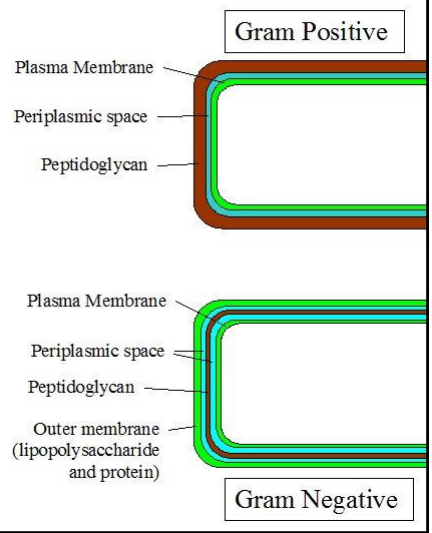
Phenotype+Biochemistry based

Bergey II. edition (from 2001)
2 domains 25 phylum
I. Archea
1. A1 phylum
2. A2 phylum
II. Eubacteria
1. B1 phylum
…
23.B23 phylum



Detailed Microbiology





On the basis of cellwall:
1. Archea: no mureinlayer
or pseudomurein
2. Gram (-) bacteria
3. Gram (+) bacteria

On the basis of cellshape
1. Sphere(Coccus)
2. Rod shaped (bacillus)
3. Twisted (spirohaeta)

Detailed Microbiology

A) Procaryotes

Archea

Gram (-)
- Coccus
- Rod shaped:
  - -aerob — Pseudomonas, Brucella, Legionella
  - -facultative anaerobs — Enterok :Escherichia, Klebsiella, Salmonella
  - -anaerob

Gram (+)
- Coccus — Staphylococcus, Streptococcus
- Rod shaped:
  - -endospore forming — Bacillus (aerob), Clostridium (anaerob)
  - -without spores — Lactobacillus
  - - unshaped without spores — Corynebacterium, Streptomyces, Actinomyces

B) Parabiotes (viruses, prions)

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Detailed Microbiology

C) Eucaryotes

Protists
- Amoebas,
- Monera

Funghi
- Bazidomycetes
- Ascomycetes
- Zygomycetes

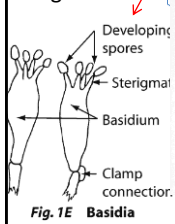
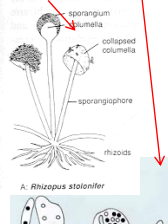
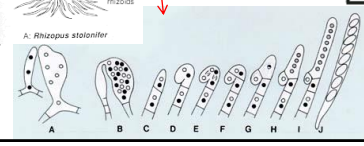
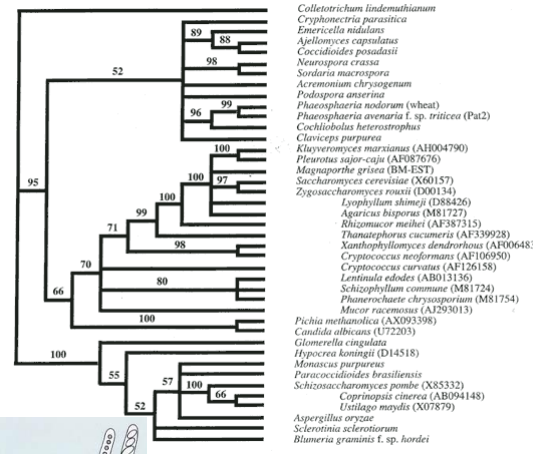


Fig. 1E Basidia







Perfect and Imperfect funghi =
Yeast and filamentous form

Potential of Biotechnology

What can be produced by microbes?

1) Primery metabolites:
   produced under normal living conditions of microbes

2) Secondary metabiolites:
   metabolites of microbes not coupled to life (antibiotics)

3) Bioconversions product

4) (recombinant) proteins, enzymes

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Potential of Biotechnology

What can be produced by microbes?

1) Primary metabolites:

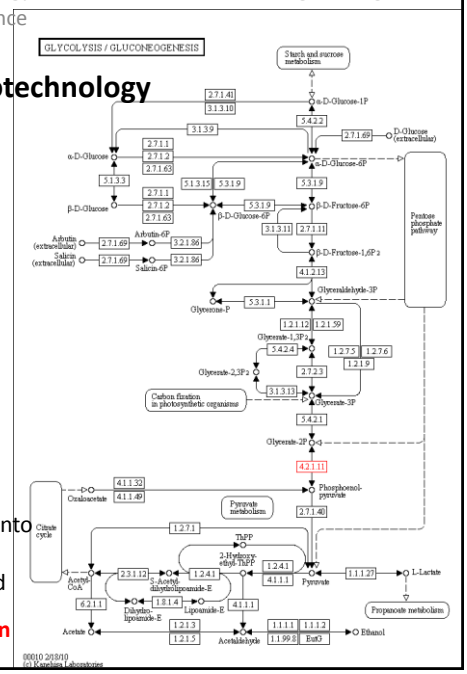
Energy production: **glycolisis+TCA**
glucose/fructose/other sugars

Chemical energy: ATP v. $NADH_2$

Direct Energy utilization

Converted into ATP and regenerated

Terminal oxydation



Potential of Biotechnology

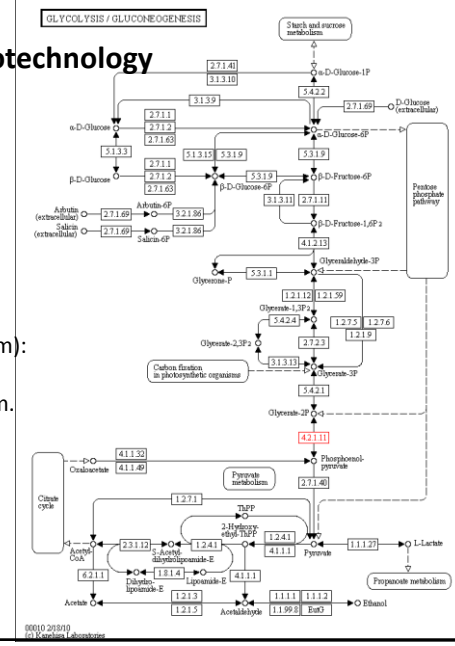
What can be produced by microbes?

1) Primary metabolites:

A) Glycolisis+Terminal oxydation

It needs O_2 – aerob metabolism

Without oxigyen(anaerobic metabolism):
Alternative NADH regeneration,
Like: pyruvate->LA reduction NAD form.
or Ac-CoA->AcOH

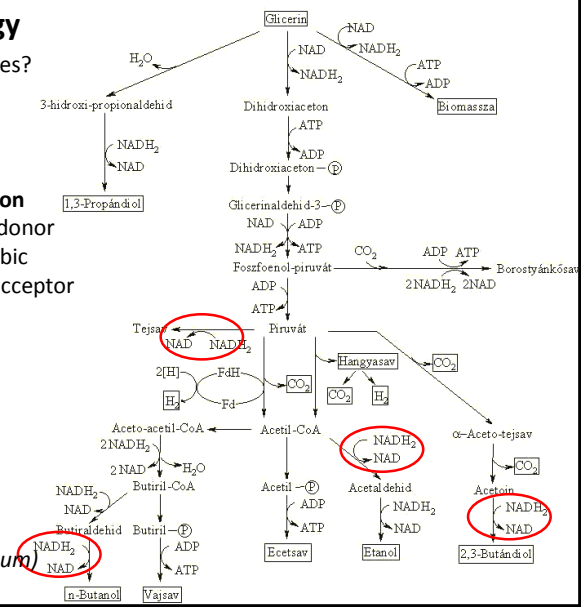


Potential of Biotechnology

What can be produced by microbes?

1) Primary metabolites:

Respiration <> Fermentation
organic e⁻donor organic e⁻donor
Aerob/anaerobic Anaerobic
INorganice⁻acceptor organic e⁻acceptor



Several products,
Different fermentaion:
-lactic (*Lactobacillusok*)
-acetic (*Acetobacterek*)
-alcoholic (Yeasts: *S. cerevisiae)*
-butyric (*Clostridium butyricum)*
-aceton-butanol ferm. (*C. acetobutylicum)*

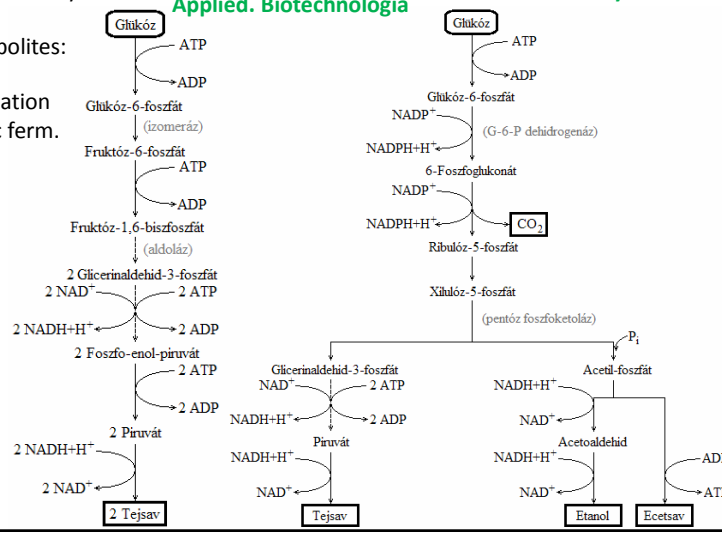
Potential of Biotechnology

What can be produced by microbes?

1) Primary metabolites:

Like: Lactic fermentation
Homo/hetero lactic ferm.

Applied. Biotechnológia **Food Industry**



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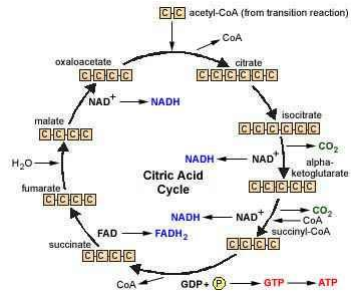
Potential of Biotechnology

What can be produced by microbes?

1) Primary metabolites:

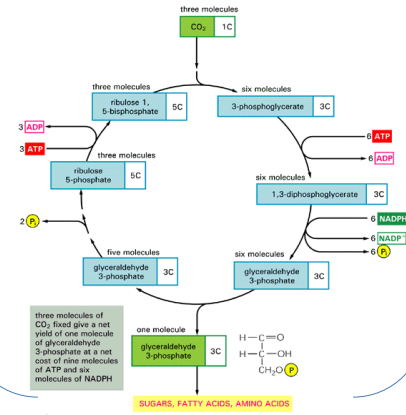
B) TCA-cycle (mitocondria)



MIKROBES-degradation

Breaking the cycle, its compounds can be produced if the strain have anaplerotic pathways

Calvin ciklus - kloroplaszt



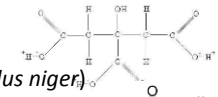
PLANTS-synthesis

Potential of Biotechnology

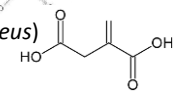
What can be produced by microbes?

1) Primary metabolites:

B) TCA-cycle (mitocondria)

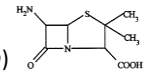


1. Citric acid production with filamentous funghi (*Aspergillus niger*)
2. Itaconic acid production with filamentous funghi (*Aspergillus terreus*)
3. Amino acids production: Lys, Glu (*Corynebacterium*)



2) Secondary metabolites:

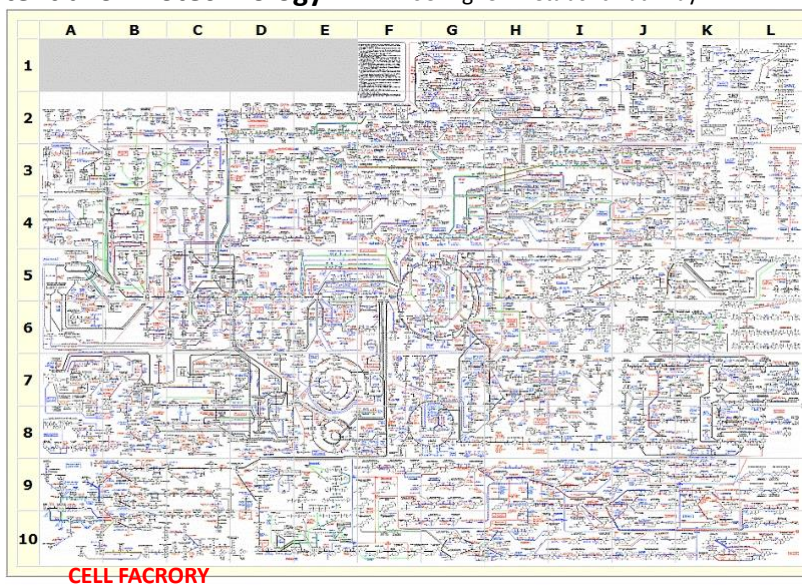
-penicilins (Penicillin G, 6APA, *Penicillium crysogenum*)
-cephalosporins (Cephalosporin C, *Cephalosporium acremonium*)
- Fumagilins (*Aspergillus fumigatus*)



The goal with Sec. Met. Prod of microbes:
to surpress under limiting conditions the competitors (=bacteria)

Potential of Biotechnology

Boeringher Metabolic Pathway



CELL FACRORY

Potential of Biotechnology

What can be produced by microbes?

3) Bioconversion products

Previous: *de novo* fermentations = synthesis (mostly natural reactions)

This:one point reaction in chemical synthesis routes:

-because of good yield

-stereospecific

-more simple to change a spec. functional group then totalsynthesis

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Article

Characterization of Hydrocortisone Biometabolites and 18S rRNA Gene in *Chlamydomonas reinhardtii* Cultures

Younes Ghasemi [1,*], Sara Rasoul-Amini [2], Mohammad Hossein Morowvat [1], Mohammad Javad Raee [1], Mohammad Bagher Ghoshoon [1], Fatemeh Nouri [1], Narges Negintaji [1], Rezvan Parvizi [1] and Seyed Bagher Mosavi-Azam [1]

[1] Department of Pharmacognosy and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

[2] Department of Medicinal Chemistry, and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran

* Author to whom correspondence should be addressed; E-mail: ghasemiy@sums.ac.ir; Tel.: +98-711-2425305; Fax: +98-711-2426070

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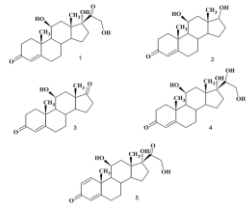
Abstract: A unicellular microalga, *Chlamydomonas reinhardtii*, was isolated from rice paddy-field soil and water samples and used in the biotransformation of hydrocortisone (**1**). This strain has not been previously tested for steroid bioconversion. Fermentation was carried out in BG-11 medium supplemented with 0.05% substrate at 25°C for 14 days of incubation. The products obtained were chromatographically purified and characterized using spectroscopic methods. 11β,17β-Dihydroxyandrost-4-en-3-one (**2**), 11β-hydroxyandrost-4-en-3,17-dione (**3**), 11β,17α,20β,21-tetrahydroxypregn-4-en-3-one (**4**) and prednisolone (**5**) were the main products of the bioconversion. The observed bioreaction features were the side chain degradation of the substrate to give compounds **2** and **3** and the 20-ketone reduction and 1,2-dehydrogenation affording compounds **4** and **5**, respectively. A time course study showed the accumulation of product **2** from the second day of the fermentation and of compounds **3**, **4** and **5** from the third day. All the metabolites reached their maximum concentration in seven days. Microalgal 18S rRNA gene was also amplified by PCR. PCR products were sequenced to confirm their authenticity as 18S rRNA gene of microalgae. The result of PCR blasted with other sequenced microalgae in NCBI showed 100% homology to the 18S small subunit rRNA of

Figure 1. Chemical structure of substrate hydrocortisone (**1**) and the biotransformed products 11β,17β-dihydroxyandrost-4-en-3-one (**2**), 11β-hydroxyandrost-4-en-3,17-dione (**3**), 11β,17α,20β,21-tetrahydroxypregn-4-en-3-one (**4**) and prednisolone (**5**).



Potential of Biotechnology

What can be produced by microbes?

4) Proteins – enzyms (recombinant)

-for bioconversion

-for detergents in washing

-for food industry

-for therapies

-for organic catalysis (in hetero phase system)

-for agriculture: endotoxin of *B. thueringhinesis*

Extra<>Intracellulare