

Microbial evolution & classification

Definition of evolution

- **change in organism genotype and phenotype over time leading to improved or worsened fitness of evolved species**
- the latter species are not able to compete with the successful one and can become extinct because evolution **occured by natural selection**

Origin of life

- origin of life: some molecules in prebiotic period (CO_2 , NH_3 , CH_4 , N_2 , H_2O), in reducing atmosphere could react and some biochemical building blocks could be made: sugars, amino acids, purines and pyrimidines, nucleotides, thioesters, fatty acids and they can have polymerized to form polypeptides, polynucleotides...
- ??? Polymerization of the building blocks – anhydrous surfaces (clay, pyrite...) functioned as support for prebiotic polymerization

How organisms evolved?

- from common ancestor/unicellular procaryotic organism to complex multicellular organisms composed from eucaryotic cells
- the oldest known organism - around 3,6 billion years old stromatolitic microfossils – resemble simple rod-shaped phototrophic bacteria (Western Australia) (modern stromatolites – filamentous cyanobacteria)

Ancient and modern stromatolites



Some of the earliest fossils in the world come from around Australia (Pilbara Supergroup) and South Africa (Onverwacht and Nondweni groups) (1) and date to around 3.4 billion years old. These fossils consist of stromatolites, structures formed by bacteria interacting with sediment. This is at least what people assumed until some researchers suggested the structures were in fact of abiotic origin.

Why evolution so interests clinical microbiologist?

- Because concept of microbial species usually dramatically differ from concept of different, sexually reproducing higher organisms

Concept of microbial species

Microbial species is defined as a clonally related population which is descendant from an ancestor
or

Species of procaryotes – a collection of closely related (>97% 16S rRNA sequence homology) strains sufficiently different from all other strains to be recognized as a distinct unit

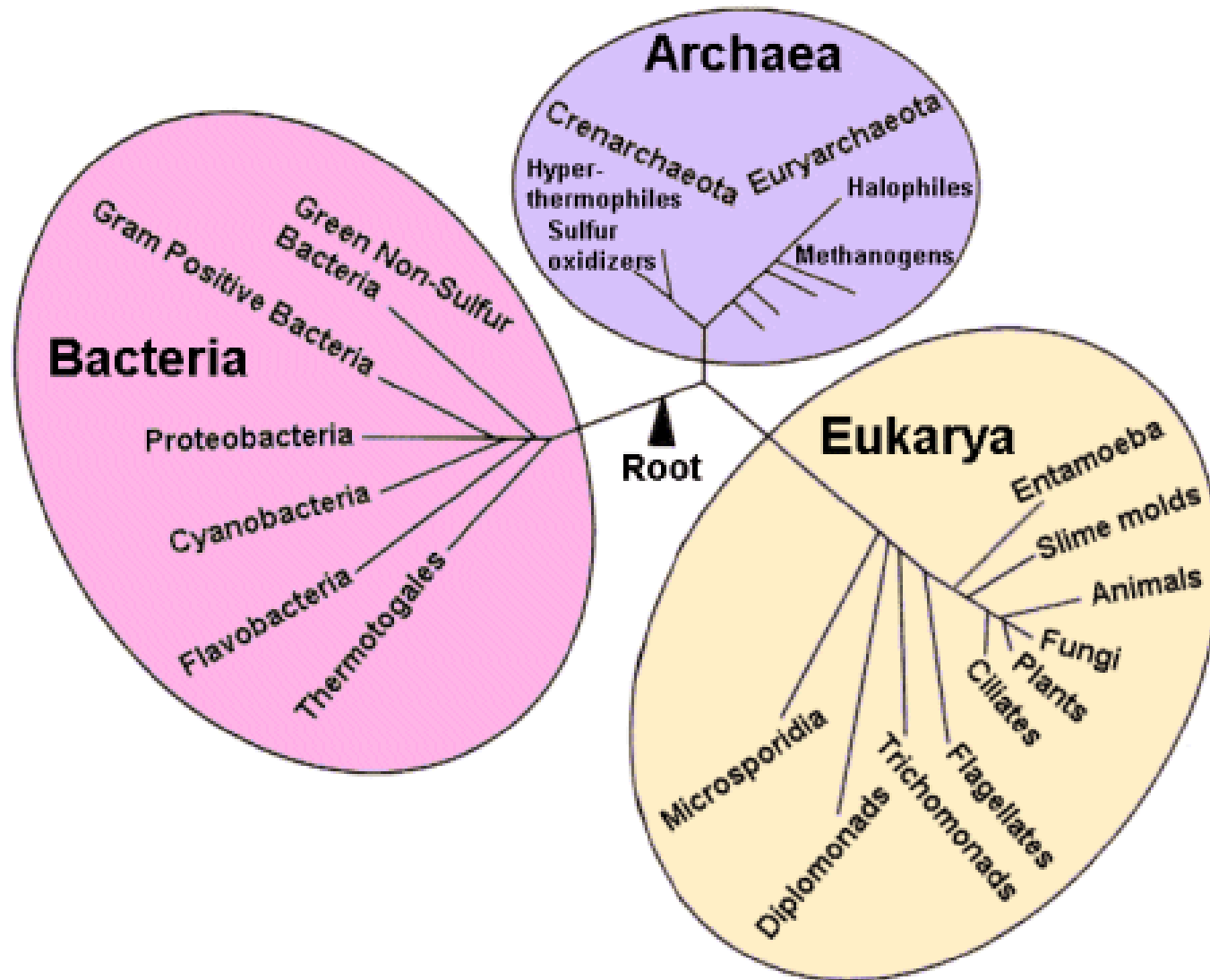
Consequences from the concept

- microorganisms multiply by binary fission –unicellular organism is divided into 2 by simple division
- even if **dothor cells should be identical with the mother cells** because of mutation and processes like DNA recombination gene transfer (conjugation, transduction, transformation) and gene rearrangement they **can differ** (usually silently)
- the DNA **differences could be transcribed to the following generations**
- **testing of species using testing their offspring is not possible so the classification differ**

Classification of microorganisms

- **main role of medical microbiology** founded upon the ability to **distinguish the microbes** and to **predict** their **pathogenicity** and **epidemiology** from *in vitro* characteristics
- most virulence factors are not usually tested in clinical laboratory
- classification use **taxons**/categories as e.g. family, genus, species...
- **Classification/Taxonomy is based on comparing taxons between each other**
- because of enormous number of newly described species situation in taxonomy start to be confusing

Phylogenetic classification of living organisms



Phylogenetic bacterial classification

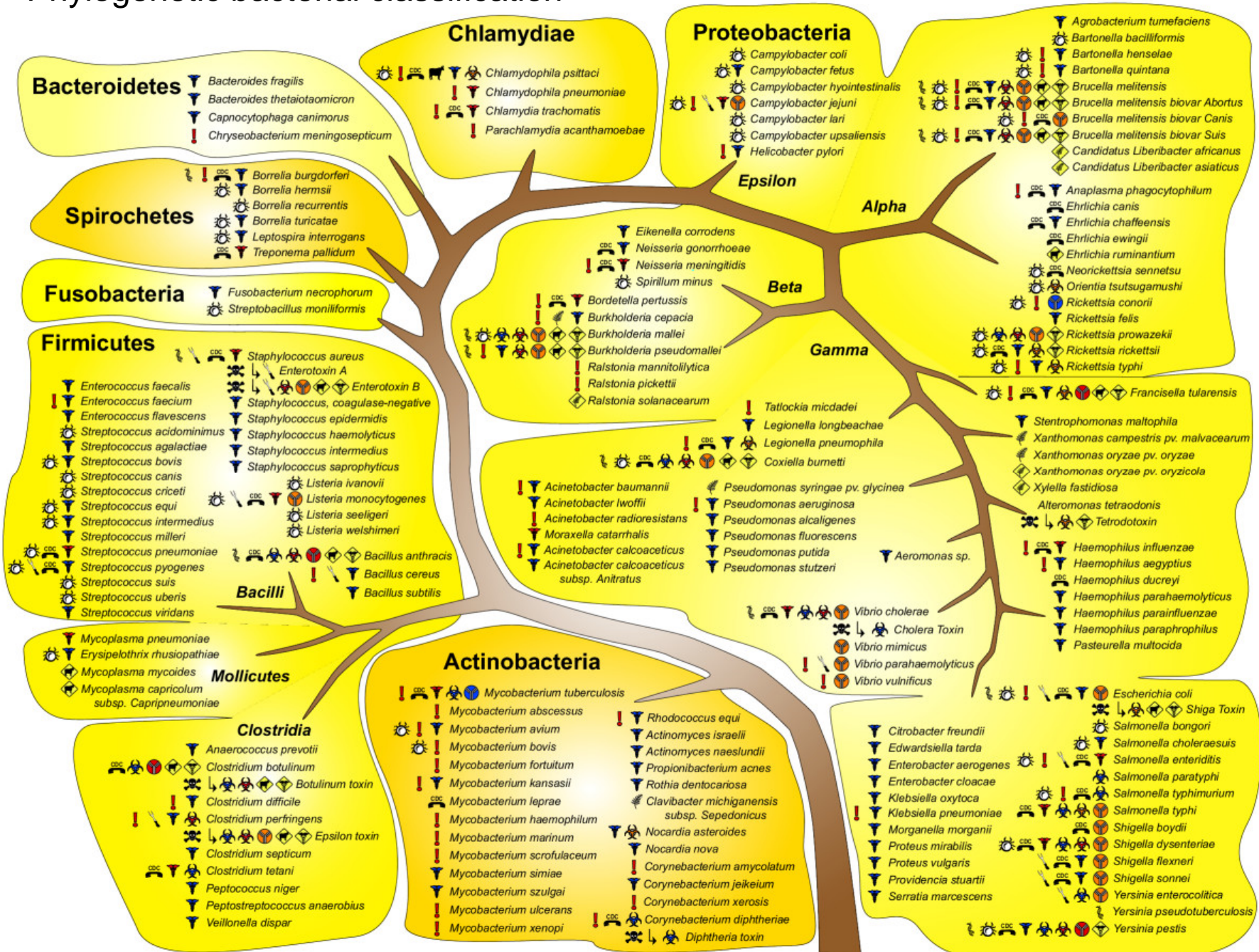
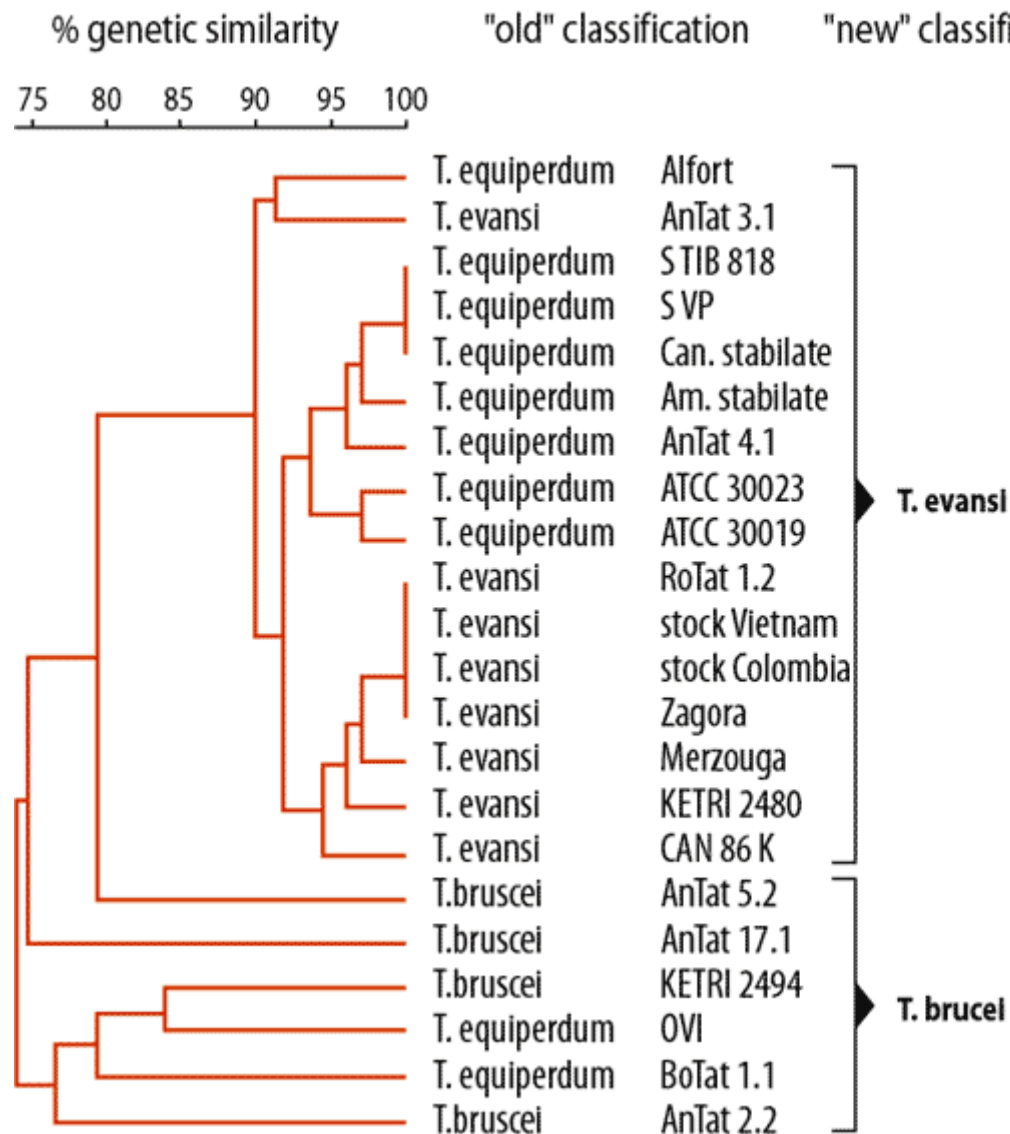


Diagram of classified microorganisms

- **similarity of each species (its characters) to each other** could be explained by its position in multi-dimensional space
- because of **visual and graphical limitations how to describe multi-dimensional space** (e.g. 100 dimensional) some mathematical methods using measuring similarity between the species and some methods for creating **clusters** of related strains are used



Homology tree based on RAPD results, analysed by UPGMA cluster analysis. The *T. evansi* and eight out of ten *T. equiperdum* are grouped in one cluster with 90-100% similarity. A second more heterogenous group (74-83% similarity) harbours all *Trypanosoma brucei* and two *T. equiperdum*. This result gives rise to a new hypothetical classification in which there are only two species (*T. evansi*, *T. brucei*) instead of three (*T. evansi*, *T. brucei*, *T. equiperdum*).

Which classification in clinical microbiology is preferred

- **phylogenetic** classification – depend on evolutionary relatedness (today based on analyzing „molecular clock“ molecules)
- **phenetic** classification – depend on relatedness of genotypic (similarity of DNA composition and relatedness) and phenotypic (morphology – colonies, cells, enzymes production characters)
- border between both could not be sharp
- **preferred is phenetic classification**

Nomenclature of microorganisms

- **each species has its own name created corresponding with binominal nomenclature** (Swedish botanist and physician, Carl Linnaeus, 1707-1778)
- rule:
 1. name – genus (e.g. Staphylococcus)
 2. name – species (aureus)

Staphylococcus aureus – written in italic !

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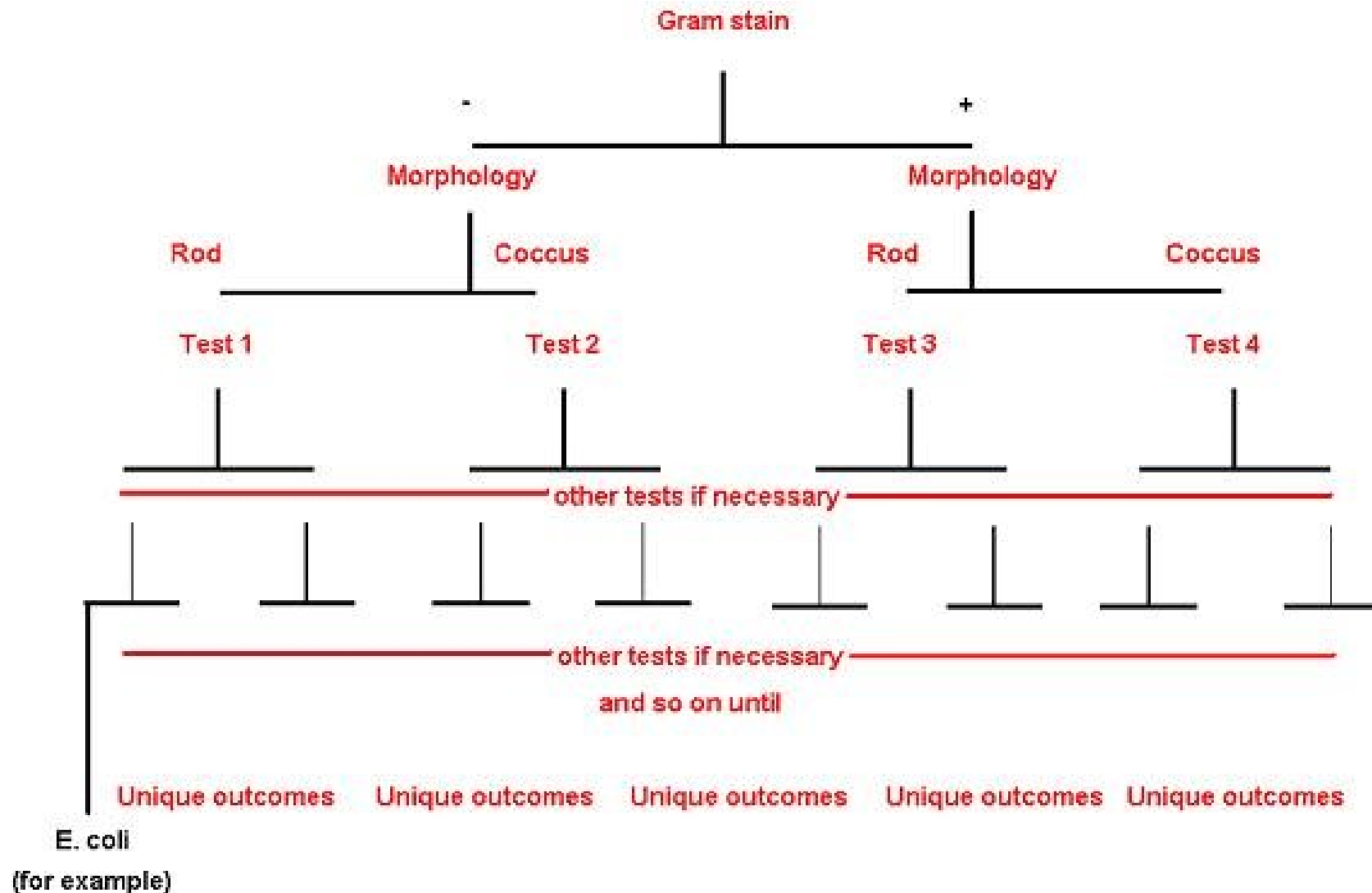
Microbial identification

- only if an **organism** is already **classified** it **could be identified** or we must know „which species we wish to prove“
- **so we have to know**
 - its name
 - its characteristics (morphological and physiological)
 - its position between closely related species

Identification – practical approach

1. INTUITIVE - colony morphology and few additional tests by experienced microbiologist
2. „KEY“ IDENTIFICATION – using dichotomous key to identify on species level
3. NUMERICAL IDENTIFICATION – based on defining tables of expected frequency of positivity in a series of tests for each species
 - reaction pattern of an analysed isolate is compared to this table
 - this comparison could be intuitive or calculated using computer programs

Dichotomous key/algorithm for bacterial identification



Identification – practical steps

1. **Microscopic morphology**
2. **Colonial morphology**
3. **Biochemical tests** (fermentation & oxidation sacharides & metabolism of organic acids, proteins, aminoacids lipids, tolerance to pH, temperature – **nutritional and physiological interactions of the organisms with its environment**)
4. **Toxic/virulence factor characterization** (enterotoxins, hemolysins...)
5. **Chemotaxonomy** (cell & cell wall structure...)
6. **Characterization of DNA** (fingerprinting, sequencing, hybridization)

phenotype

genotype

Identification – practical steps

1. Viruses

- using electron microscopy
- preferred identification using characterization of DNA or RNA using amplification methods (mostly based on PCR format)
- serological characterization/identification viral species

2. Fungi

- colony morphology
- DNA methods

3. Parasites

- unicellular – using microscope and using DNA methods
- multicellulars – mostly using phenotypical characters

References

- **Murray et al. Medical Microbiology, 2007**
- **Jawetz, Melnick and Adelbergs Medical Microbiology, 2007**
- **web references**