Propagation of bacteria in vitro

The growth and reproduction of bacteria is a series of biochemical and physical processes.

The **growth cycle** consists of the following parts:

- cell growth - coordinated formation of macromolecule and cellular components;
- septum formation;
- cell division.

Generation time is the time between two divisions.

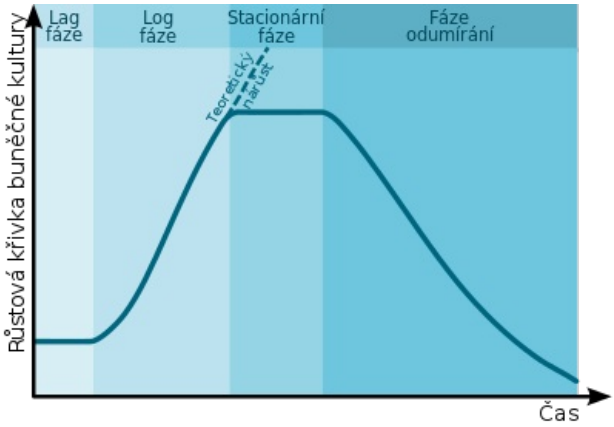
Doubling time is the time it takes for the number of bacteria to double.

Under ideal conditions, the division of bacteria follows a **geometric series**, but in reality this is not the case for the following reasons:

- nutrient depletion (or nutrients not reaching everyone);
- inhibition by the products of its metabolism.

Growth curve

The growth curve shows the dependence of the number of living cells (on a logarithmic scale) on the age of the culture. We describe the following parts of the growth curve:



Growth curve of bacterial culture

- **lag-phase** - delay in division at the beginning, enzyme synthesis, volume increase, length of the phase depends on the state of the inoculum (short if cells from the growth phase are used), the beginning of division has an unstable rate;
- **logarithmic (exponential) phase** - constant rate of division, depends on microbe species, temperature, environmental composition
  - specific growth rate is the growth rate per unit biomass;
- **stationary phase'** - cells hardly divide anymore, the amount of waste products increases, cells neither increase nor decrease, the length of the phase depends on the type of bacteria and the nature of the environment
  - **x = Y × s**
  - **x** is the concentration of biomass produced, **s** is the concentration of nutrient consumed, **Y** is the growth yield (informs about the physiology of the growing bacterium);
- **death phase (autolysis)** (neisserie or pneumococcus), induction of enzymes destroying the cell wall, disruption of the balance between lytic action and wall synthesis;
  - sometimes the velocity is constant (the only adverse factor), but usually several factors are at work, so the kinetics vary;
  - The specific death rate is the rate of cell death per cell.

In **continuous cultivation** there is a maintenance in logarithmic phase, nutrient supply and waste removal are needed. Continuous cultivation is carried out in apparatus called **fermenters'**, it is used, for example, to produce ATB, and occurs naturally in the GIT. Under natural conditions, bacteria usually multiply more slowly due to limited supply, suboptimal temperature and the action of other microbes. In practice, bacterial growth is monitored by *measuring* the increase in cell mass in the following ways:

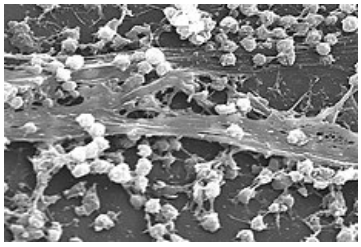
- spectrophotometrically;
- cell counting (microscopically in a counting chamber);
- by culture on a constricted culture medium in a petri dish.

Biofilm

Biofilm is a structure formed by bacteria which serves for their **adherence, communication and protection**. It can also be a factor in pathogenicity and virulence. It adheres to inert or inorganic surfaces (wet surfaces in nature, implants, catheters, cannulas) and living (epithelial cells). It is a **complex structure** with channels (water brings nutrients and removes waste). Reminiscent of tissues of higher organisms. **usually contribute to its formation**.

Biofilm **increases the resistance of bacteria** (to adverse environmental conditions or immune mechanisms) and allows for their rich communication, which can greatly complicate treatment.

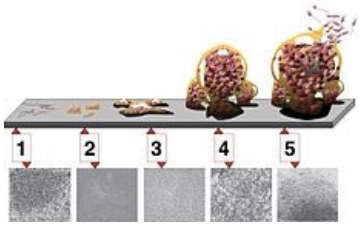
Biofilm formation



Staphylococcus_aureus_biofilm

The adhesion of bacteria to the surface (using fimbriae, glycocalyx, surface proteins, etc.) conditions the triggering of genes for the formation of extracellular polymers. Additional cells form in the **extracellular Polysaccharide mass**. Dividing creates so-called **microcolonies**which grow rapidly. The microcolony is enveloped in mucus and differentiates into a biofilm. They can be formed not only by bacteria, but also by higher organisms (e.g.*Candida albicans* in the body). Free cells **(planktonic cells)**can be released from the biofilm and colonize other sites.

Communication of bacteria through biofilm consists mainly in the exchange of genetic information (e.g. plasmids) between bacteria. In this way, bacteria can, for example, acquire resistance to antibiotics.



5 stages of biofilm development. Stage 1, initial attachment; stage 2, irreversible attachment; stage 3, maturation I; stage 4, maturation II; stage 5, dispersion.

Quorum sensing
The mechanisms by which bacteria can sense and adapt to the presence of other bacteria in their environment. During their growth, bacteria produce so-called **autoinductors**. When the concentration of autoinductors reaches the threshold concentration, the transcription of the genes of the multiplying bacteria is affected. By this change in gene expression, they can condition **growth arrest** bacteria or their further growth. By this mechanism, the colony maintains an optimal population density.

Another factor that autoinductors may affect is **the production of some metabolites** (e.g. the production of proteases in *Pseudomonas aeruginosa*).

The biofilm can be observed with a confocal laser microscope in optical sections and the spatial structure can be composed of them. Biofilm thickness varies from a few to hundreds of micrometers, depending on nutrient availability.

Examples of biofilm formation sites

- **Dental plaque** – viridizing streptococci.
- Periodontitis – in the pockets under the gums (formation of a polymicrobial biofilm where oxygen does not penetrate), accumulated bacterial mass, dead cells and purulent cells worsen the situation.
- **Inflammation of the middle ear** – Haemophilus.
- Osteomyelitis – *Staphylococcus aureus*;
- Cystic fibrosis chronic or recurrent infections are added to primary respiratory infections. Bronchial congestion and permanent epithelial damage occur. *Pseudomonas aeruginosa* and the like settle on this damaged epithelium with a biofilm.
- Inflammation of the bile ducts – G− intestinal stick.
- With chronic prostatitis - bacteria enter the prostate upstream, acute prostatitis can turn chronic. Initially, biofilm is just a complication, later the cause of the disease.
- Poorly nourished skin and subcutaneous tissue in shin ulcers, on the surface of burned areas.
- The intrauterine device can cause inflammation of the pelvic cavity and sepsis.
- Assisted breathing - formation on the wall of tubes. In case of lack of treatment, bacteria can penetrate into the bronchi and lungs.
- Intravenous **catheters** – coagulase negative staphylococci.
- Artificial heart valves.
- Joint replacements.
- Contact lenses − if used incorrectly *Pseudomonas aeruginosa* settles.
- In urology, biofilm is the most common cause of infection with a **urinary catheter** inserted.

Biofilm resistance to antibiotics

Biofilm cells are **very resistant to antimicrobials and disinfectants** (up to a thousand times more than planktonic cells). In medical practice, this means that even high doses of antibioticsare not enough for treatment . Resistance is expressed phenotypically - it is not genetically determined resistance.

Laboratory tests for antibiotic susceptibility give incorrect results. Biofilm bacteria are resistant, but compared to planktonic bacteria, they appear sensitive under optimal laboratory conditions.

The resistant cells that tolerate the antibiotic and remain in the body are generally called **persists**. In a biofilm, genes are up to a thousand times more successful between cells than between planktonic cells (promoting resistance gene transfer in the population). The mucus mass also physically **protects the cells in the biofilm from antibodies**.

Factors affecting bacterial proliferation

- - **oxygen**

    - aerobic - Pseudomonas, oxygen electron acceptor
    - facultatively anaerobic - Enterobacteriaceae
    - anaerobic - clostridia
    - microaerophilic - *Neisseria*
    - capnophilic - meningococci, gonococci
- **water**
    - most hygrophilic (x lyophilization - drying of frozen bacteria in vacuum), more resistant to drying G+ and acid resistant (staphylococcus and corynebacteria on skin)
    - xerophilic - water on particle surface (soil), Nocardia, actinomycetes, fungi
- **temperature** - minimum, optimum and maximum growth temperature - temperature range
    - psychrophiles - 0-20°C
    - psychrotolerant - yersenia, listeria, salmonella, S. aureus
    - mesophiles - 20-40 °C
    - thermophiles - above 40 °C
    - hyperthermophiles - above 80 °C
- **hydrostatic pressure** - deep-sea
- **osmotic pressure** - mostly hypotonic environment, protection by wall
    - hypertonic - plasmolysis (food preservation)
    - halophiles - halotolerant and obligate (enterococci, staphylococcus genus, **Vibrio genus**) or extreme halophiles
- **pH**
    - neutrophils - most
    - alkaliphiles - *Vibrio cholerae*, alkali-tolerant - *Proteus*, enterococci
    - acidophiles - lactobacilli
- **Oxido-reduction potential**
    - aerobes - oxidized environment
    - anaerobes - low potential required
- **Radiation** - ultraviolet and ionizing radiation damage

References

Related articles

- Cultivation of cells and tissues in vitro, importance in medicine
- Cell culture

Source

- JANSKÝ, Petr. *Zpracované otázky z mikrobiologie* [online]. [cit. 2022-02-18]. <https://www.yammer.com/wikiskripta.eu/uploaded_files/3804405>.