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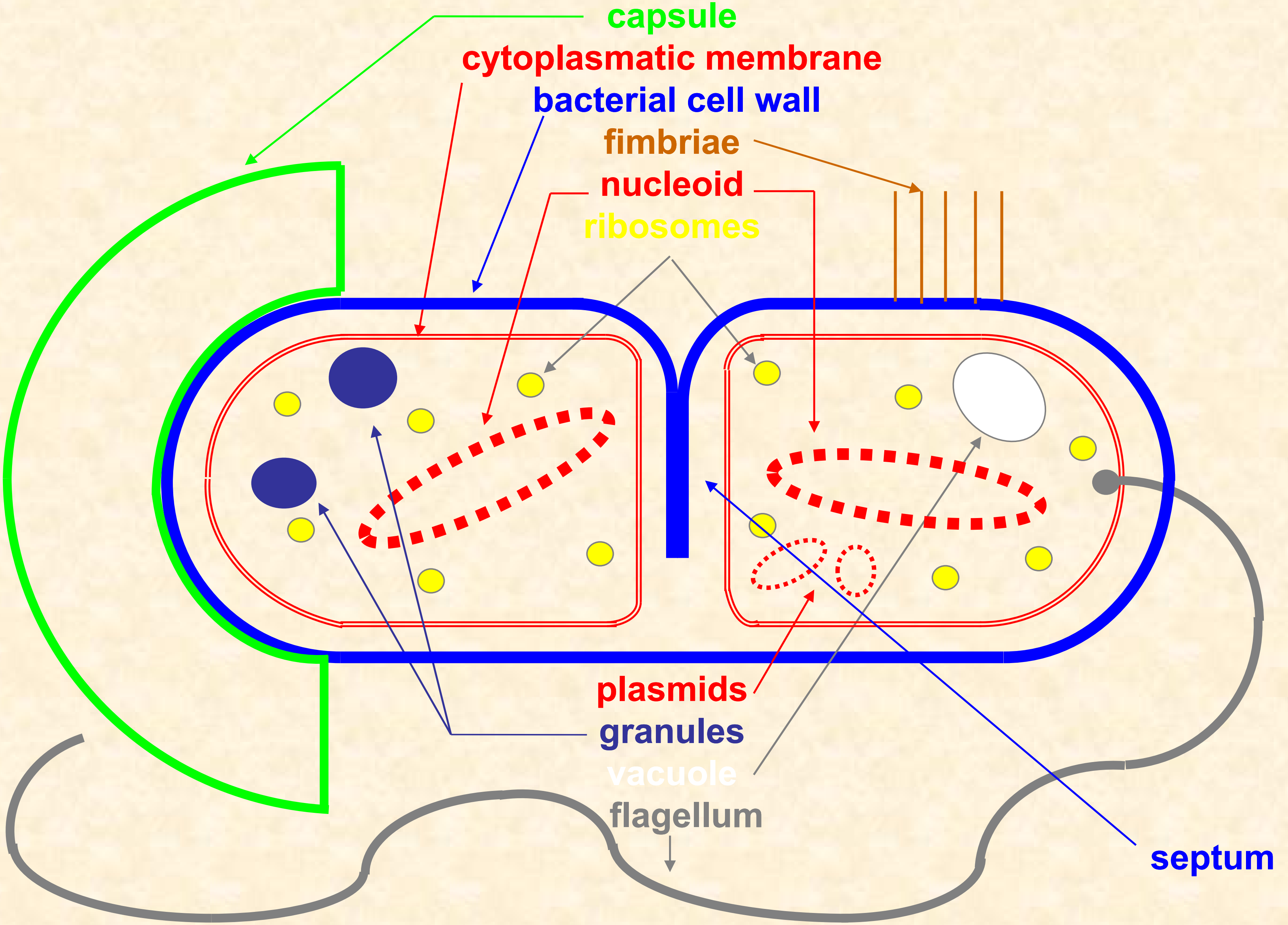
RESISTANCE OF MICROBES TO THEIR ENVIRONMENT (TENACITY)

The 4th lecture for 2nd-year students of General Medicine

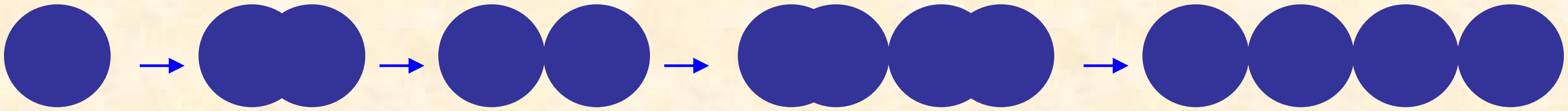
March 9th, 2015

Division of bacterial cell – revision



Div. & arrrangem. of cocci – revision

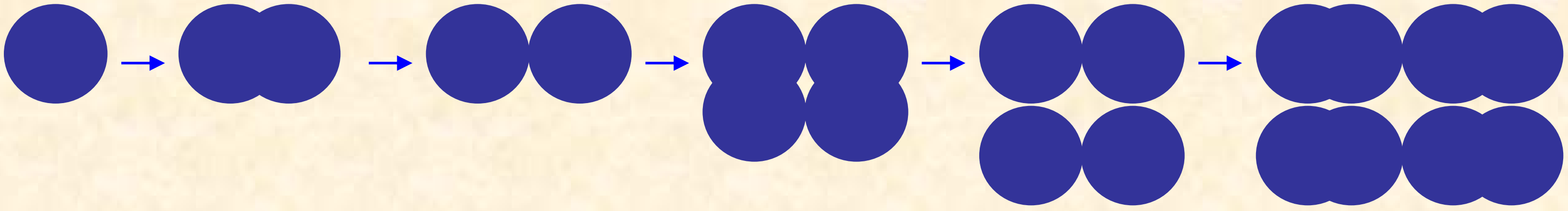
Cocci, dividing in one plane: streptococci ➡ chains



Cocci, in different planes: staphylococci ➡ clumps

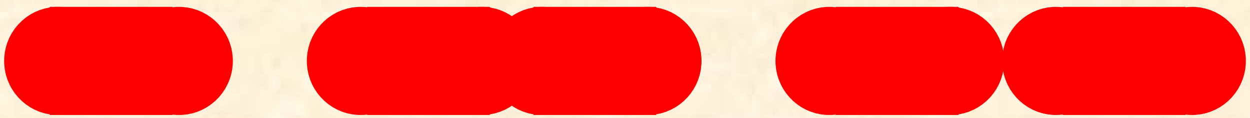
Cocci, in two perpendicular planes:

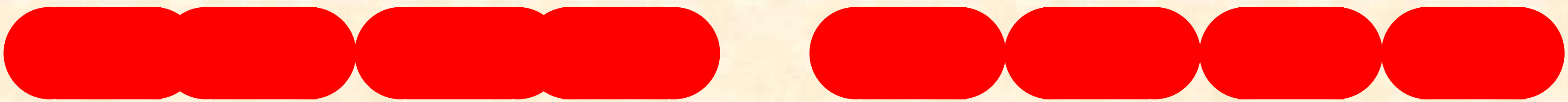
micrococci ➡ tetrads



Division and arrangement of rods – revision

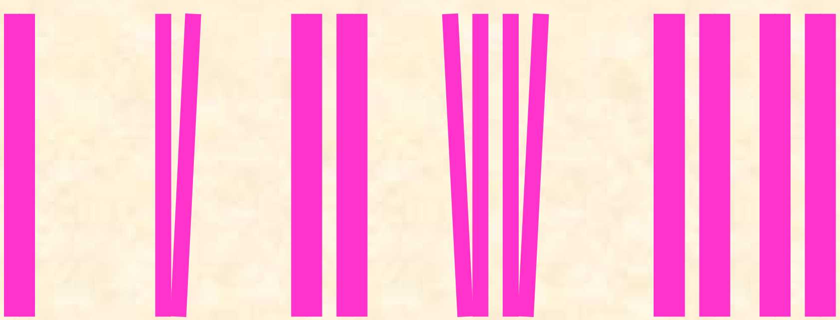
Rods, transverse division: **majority (chains of rods)**





Rods, lengthwise division: **mycobacteria**

corynebacteria



(arrangement in palisades)

Generation time – revision

Generation time = duration of the growth cycle =
= duplication time = duration of doubling the number of bacteria

Generation time of bacteria: on average cca 30 min

Mycobacterium tuberculosis

approximately 12 hrs

Since during each generation time the number of bacteria doubles, bacteria multiply by geometric progression

Geometric progression – revision

If the generation time is 30 min, after 24 hrs theoretically one cell gives origin to 2^{48} = 2.8 10^{14} cells,
actually it is by approximately 5 orders less (i.e. around 10^9 cells)

10^9 bacteria is such an amount that it is visible even by the naked eye:

Liquid medium (broth) becomes 1. cloudy or 2. a sediment appears at the bottom or 3. a pellicle is seen at the top

On a solid medium (agar) a bacterial colony forms

What is a bacterial colony – revision

- Bacterial colony = a form on the surface of the agar, containing mutually touching cells, cca 10^9 living and cca 10^5 already dead

- Appearance of the colony depends apart from other things on the
  - microbial species (e.g. on the size of its cells)
  - sort of culture medium (e.g. on the amount of its nutrients)
  - distance among colonies (the higher distance, the larger and more typical the colony)

- By appearances of the colonies microbiologists recognize different microbes

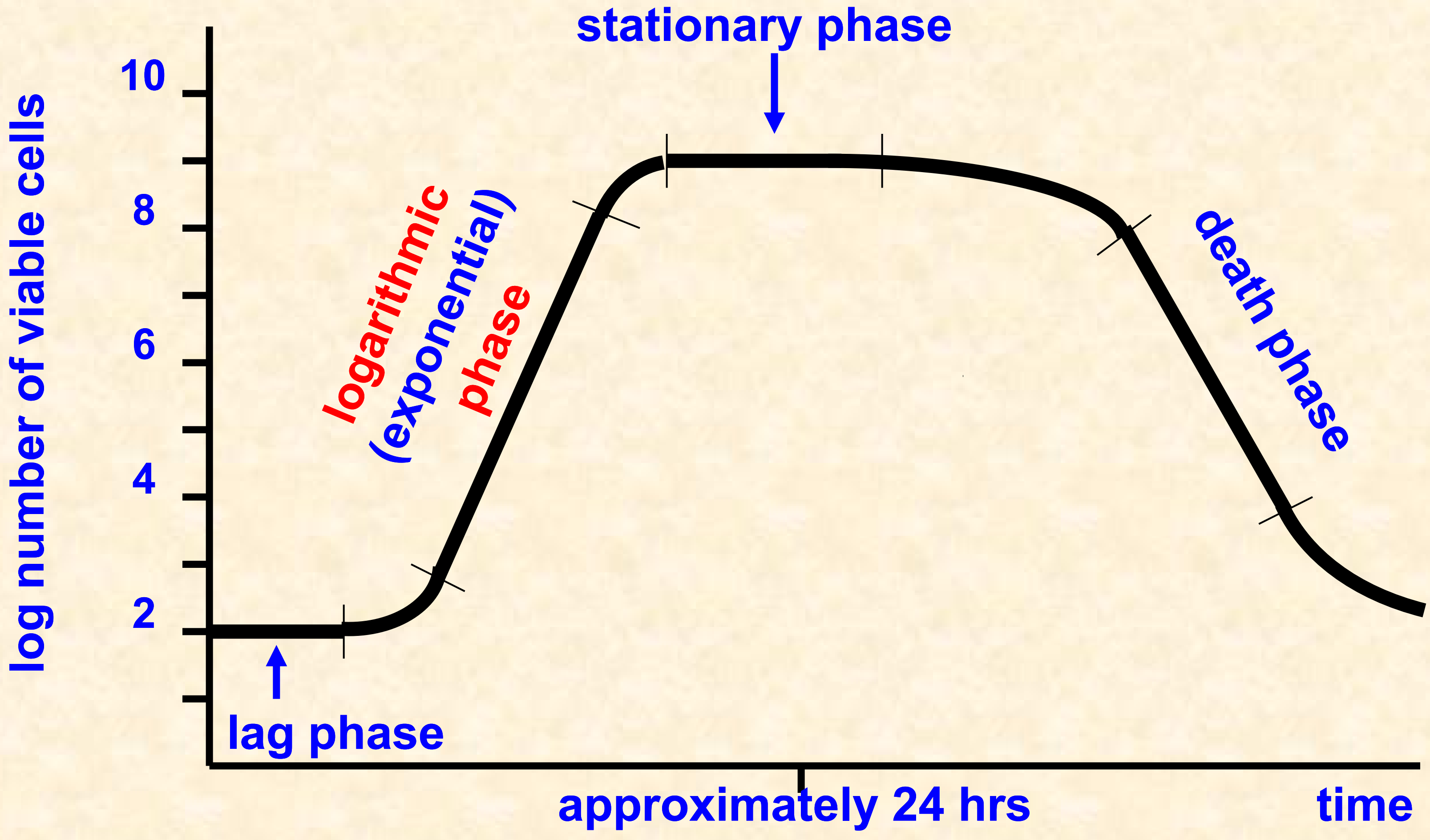
Features of a bacterial colony – revision

Bacterial colony can have up to 10 features:

1. Size – usually around 1-2 mm
2. Shape – round, oval, irregular, lobular etc.
3. Profile – flat, convex, dish-shaped etc.
4. Margins – straight, fibrous, with projections etc.
5. Surface – smooth & glossy, matt, rough, wrinkled
6. Transparency – transparent, nontransparent
7. Colour – colourless, pigmented (yellowish etc.)
8. Changes in vicinity – pigmentation, haemolysis
9. Consistency – sticky, mucous, crumbly, rooted
10. Smell – foul, pungent, of jasmin, sperm, fruit etc.

Microbial growth curve – revision

Growth Curve in a Closed System



Factors of the outer environment

- water
- nutrients
- temperature
- osmotic pressure
- pH
- redox potential
- radiation
- toxic substances

Water shortage

Water = 80 % live weight of the bacterial cell

(only 15 % live weight of the bacterial spore)

Hygrophile organisms (most of bacteria) need freely accessible water

For xerophiles (actinomycetes, nocardiae, moulds) water bound to the surface of environmental particles (e.g. in soil) suffices

Water availability

Degree of water availability = water activity of the environment (a_w)

a_w of pure water = 1.0

a_w is inversely related to osmotic pressure (the higher the osmotic pressure, the lower a_w)

The water activity (a_w) tolerated by different microbes:

G– bacteria	$a_w \geq 0.95$ (meat)
G+ bacteria and most yeasts	$a_w \geq 0.9$ (ham)
staphylococci	$a_w \geq 0.85$ (salami)
moulds and some yeasts	$a_w \geq 0.6$ (chocolate, honey)

Resistance to drying up

Very sensitive: agents of STD – gonococci,
treponemes

Less sensitive: all Gram-negative bacteria

A bit more resistant: skin flora – staphylococci,
corynebacteria
acidoresistant rods –
mycobacteria

Rather resistant: xerophiles – actinomycetes,
nocardiae, moulds
parasite cysts, helminth eggs

Highly resistant: bacterial spores

Practical application of water shortage

Lowering water activity stops action of most microbes → we use it for food preservation

- drying – meat, mushroom, fruit (prunes)
- concentration – plum jam
- salting – meat, fish, butter
- sugaring – sirups, jams, candied fruit

Nutrient deficiency

Microorganisms do not multiply in clean water

The problem lies in keeping water pure

After some time, even in distilled water e.g. *Pseudomonas aeruginosa* or *Pseudomonas fluorescens* start to multiply

In shower sprinklers: *Legionella pneumophila* grows (and can cause pneumonia)

However, *Salmonella* Typhi lives longer in well water than in waste water – why?

Temperature

Cardinal growth temperatures:

Minimum – sometimes <0 C (in sea water)

Optimum – psychrophiles: 0 – 20 °C

mesophiles: 20 – 45 °C (medically important microbes)

thermophiles: 45 – 80 C

hyperthermophiles: >80 C

Maximum – sometimes >110 C (in geysers)

Growth temperature range:

narrow (gonococci 30 – 38.5 °C)

wide (salmonellae 8 – 42 °C)

The influence of cold

Cold shock: gonococci will die if inoculated at cold agar media freshly taken out of the fridge

Growth temperature minimum:

at 5 °C: salmonellae & campylobacters survive,
yersiniae & listeriae even multiply!

Lyophilization, used for the conservation of microbial cultures × common freezing!

Slow freezing and repeated defrosting is somewhat harmful, but most microbes survive it

Tissue cysts of *Toxoplasma gondii* in meat do not survive common freezing

The influence of heat

The temperature higher than optimum → heat shock and gradual dying of cells

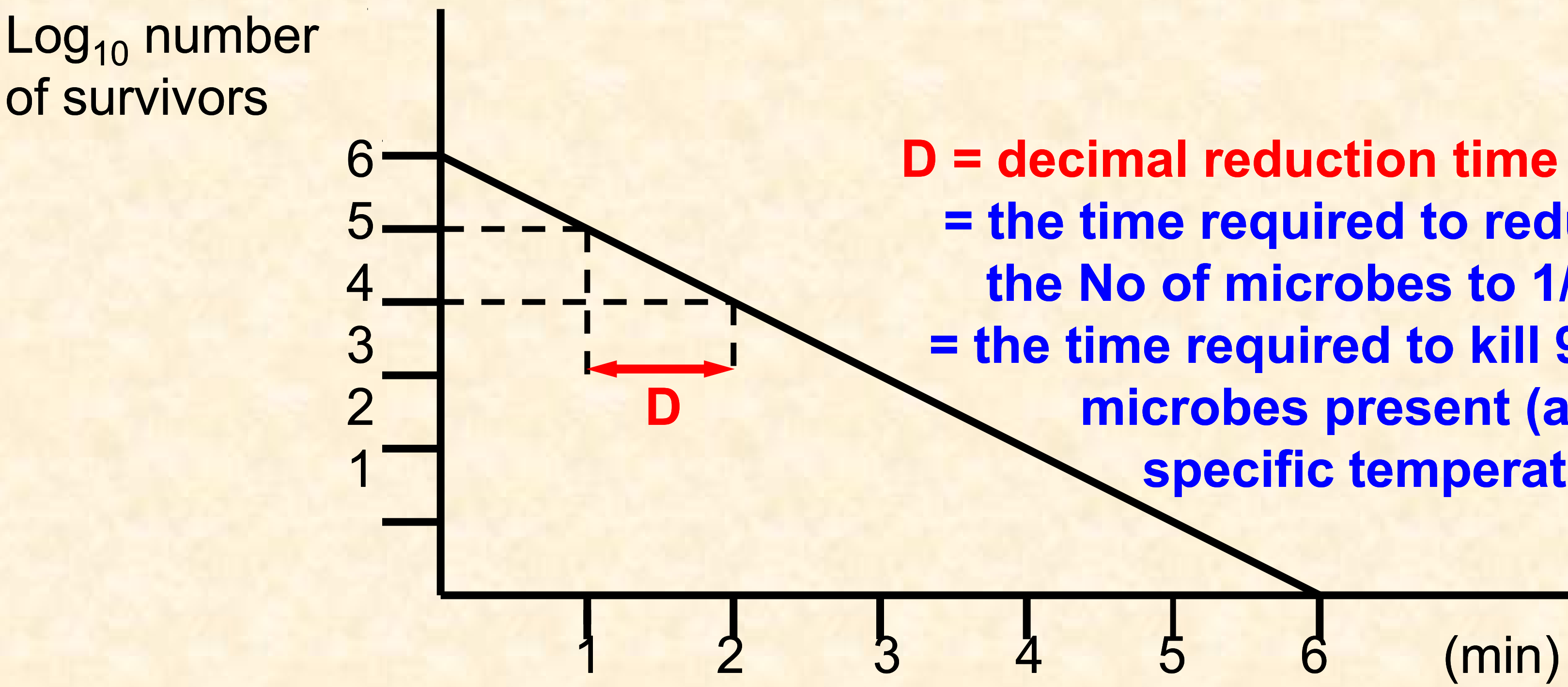
The number of killed cells depends on the duration of the exposure to higher temperature

The relation between the number of surviving cells and the duration of heating is logarithmic one

The time needed for exterminating (killing) the whole population depends on its size (on the initial number of microbes)

Temperature – important parameters I

The relation between the duration of heating and the number of surviving microbes



D = decimal reduction time =
= the time required to reduce the No of microbes to 1/10 =
= the time required to kill 90 % of microbes present (at the specific temperature)

Temperature – important parameters II

Thermal death point (TDP) = the lowest temperature at which a microbial suspension is killed in a specific time (usually in 10 minutes)

TDP depends not only on the nature of the microbial species but also on its stage, number and on the local environment

Thermal death time (TDT) = the shortest time needed to kill all microbes in a suspension For most bacteria it averages 10-15 minutes at 60-65 °C

Osmotic pressure

Hypotony – the damage is prevented by the cell wall

Hypertony mostly hinders microbes in multiplying (therefore fruit is candied, meat salted)

Higher osmotic pressure is endured by:

halophiles – halotolerant: enterococci (6.5% NaCl)
staphyloccoci (10% NaCl)
– obligate: halophilic vibria (in sea water)

moulds – tolerate higher content of saccharose (in jams)

pH

Neutrophiles: growth optimum at pH 6 až 8 – most

Alkalophiles: e.g. *Vibrio cholerae* (pH 7.4-9.6)
alkalotolerant: *Proteus* (it splits urea), *Enterococcus* (broad range of pH 4.8-11)

On the contrary, there are microbes sensitive to extremes of pH: e.g. gonococci

Acidophiles: facultative: yeasts, moulds, lactobacilli (>3), coxiellae (tolerate low pH of phagosome)
obligate: *Thiobacillus thiooxidans* (pH <1)

Microbes sensitive to low pH: mainly vibrios, streptococci, putrefactive bacteria; low pH hinders most bacteria

Why sparkling water lasts longer? Because its pH is lower

Low pH keeps spores from germinating → botulism can be obtained from oil-preserved mushrooms or preserved strawberries, not from pickled gherkins or mixed pickles

Redox potential (rH)

Level of rH depends both on the composition of the environment and of the atmosphere

Aerobes – need high rH levels (>200 mV)

Anaerobes – need low rH levels (≤0 mV)

Anaerobes are killed by O_2, aerobes without O_2 will live

Even so, anaerobes prosper both in nature and in our bodies – thanks to the cooperation with aerobes and facultative anaerobes (e.g. in biofilms)

Anaerobes in the body:

- large intestine (99 % of bowel microorganisms)
- vagina
- oral cavity (sulci gingivales)

Radiation

UV radiation (maximum effect around 260 nm)

In nature airborne bacteria protect themselves by pigments → they have coloured colonies

Artificially: UV radiation is used for disinfection of surfaces, water, air; in PCR laboratories for destroying residues of DNA

Ionizing radiation (X and gamma radiation)

For sterilizing disposable syringes, infusion sets, materials for dressing and sewing, tissue grafts, some drugs, even waste and food (not in EU)

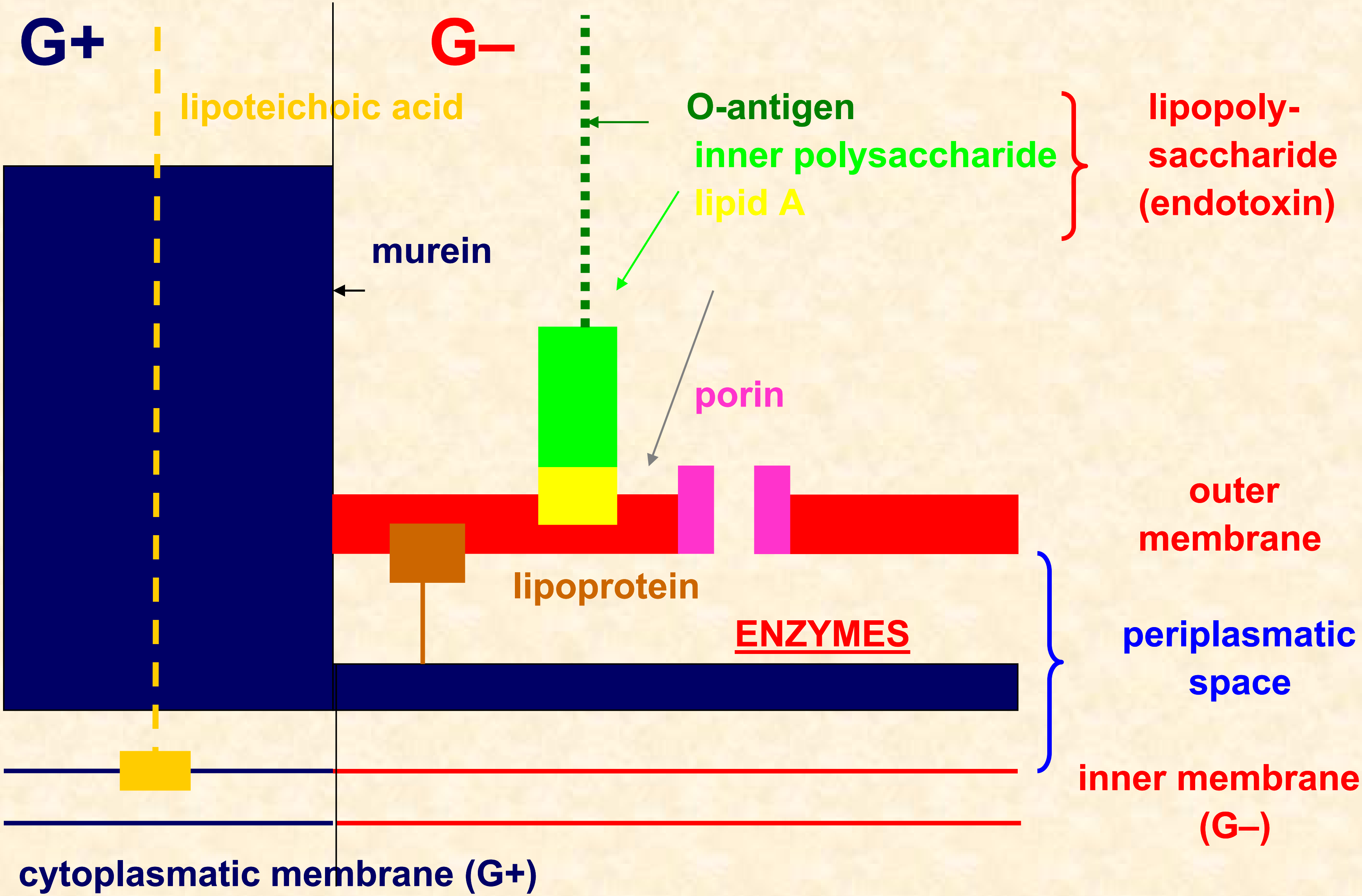
Record holders for radiation resistance: *Deinococcus radiodurans* and bacterial spores

Toxic substances

- Their influence depends on the concentration and duration of exposure
- Various microbes markedly differ in relative resistance to different types of toxic substances
- In general (and contrary to drying): G– bacteria are more resistant to toxic substances than G+ bacteria (because of different structure of bacterial cell wall → presence of enzymes in periplasmatic space of G– bacteria)

- For application it is vital to know the effects of the particular substances used for disinfection

Bacterial cell wall



Sterilization versus disinfection

Sterilization = removal of all microorganisms from objects or environment

Disinfection = removal of infectious agents from objects and environment or from the body surface

Disinfection aims at breaking the chain of infection transmission

Biocides = a new general term including also disinfectants

Types of disinfectants

1. Oxidizing agents (peracetic acid, H_2O_2, O_3)
2. Halogens (hypochlorite, sol. iodi)
3. Alkylating agents (aldehydes)
4. Cyclic compounds (cresol, chlorophenols)
5. Biguanides (chlorhexidine)
6. Strong acids and alkali (e.g. slaked lime)
7. Heavy metal compounds (Hg, Ag, Cu, Sn)
8. Alcohols (ethanol, propanols)
9. Surface active agents (QAS; e.g. cetrimid)
10. Others (e.g. crystal violet & other dyes)

Relative resistance of different agents to biocides

Agents	Susceptibility	Examples
Enveloped viruses	very susceptible	herpesviruses
Some protozoa		*Trichomonas*
Gram-positive bacteria		*Streptococcus*
Gram-negative bacteria	susceptible	*Salmonella*
Yeasts		*Candida*
Moulds		*Trichophyton*
Naked viruses	relatively resistant	enteroviruses
Protozoal cysts		*Giardia*
Acidoresistant rods		*Mycobacterium*
Helminth eggs	very resistant	*Ascaris*
Bacterial spores		*Clostridium*
Coccidia		*Cryptosporidium*
Prions	extremely resistant	agent of CJD

Universally effective biocides

On small, naked viruses: oxidizing agents
halogens
aldehydes
strong acids and alkali

On mycobacteria: oxidizing agents
aldehydes
lysol
strong acids and alkali

On bacterial spores: (oxidizing agents)
aldehydes
strong acids and alkali
(not alcohols!)

Curriculum of lectures, 2014/15, spring term

1.	Microbiology and medicine
2.	Morphology and structure of bacteria
3.	Bacterial growth, growth curve
4.	Tenacity of microbes (their resistance to the environtment)
5.	Microbial biofilm
6. – 7.	Pathogenicity and virulence
8. – 9.	Pathogenesis of infection
10. – 11.	Course and forms of infection
12. – 13.	Active and passive immunization
14. – 15.	Antimicrobial therapy

Recommended reading material

Paul de Kruif: Microbe Hunters

Paul de Kruif: Men against Death

Axel Munthe: The Story of San Michele

Sinclair Lewis: Arrowsmith

Could you kindly supply me with another work in connection with microbes or at least medicine?

Please mail me your suggestions at:

mvotava@med.muni.cz

Thank you for your attention