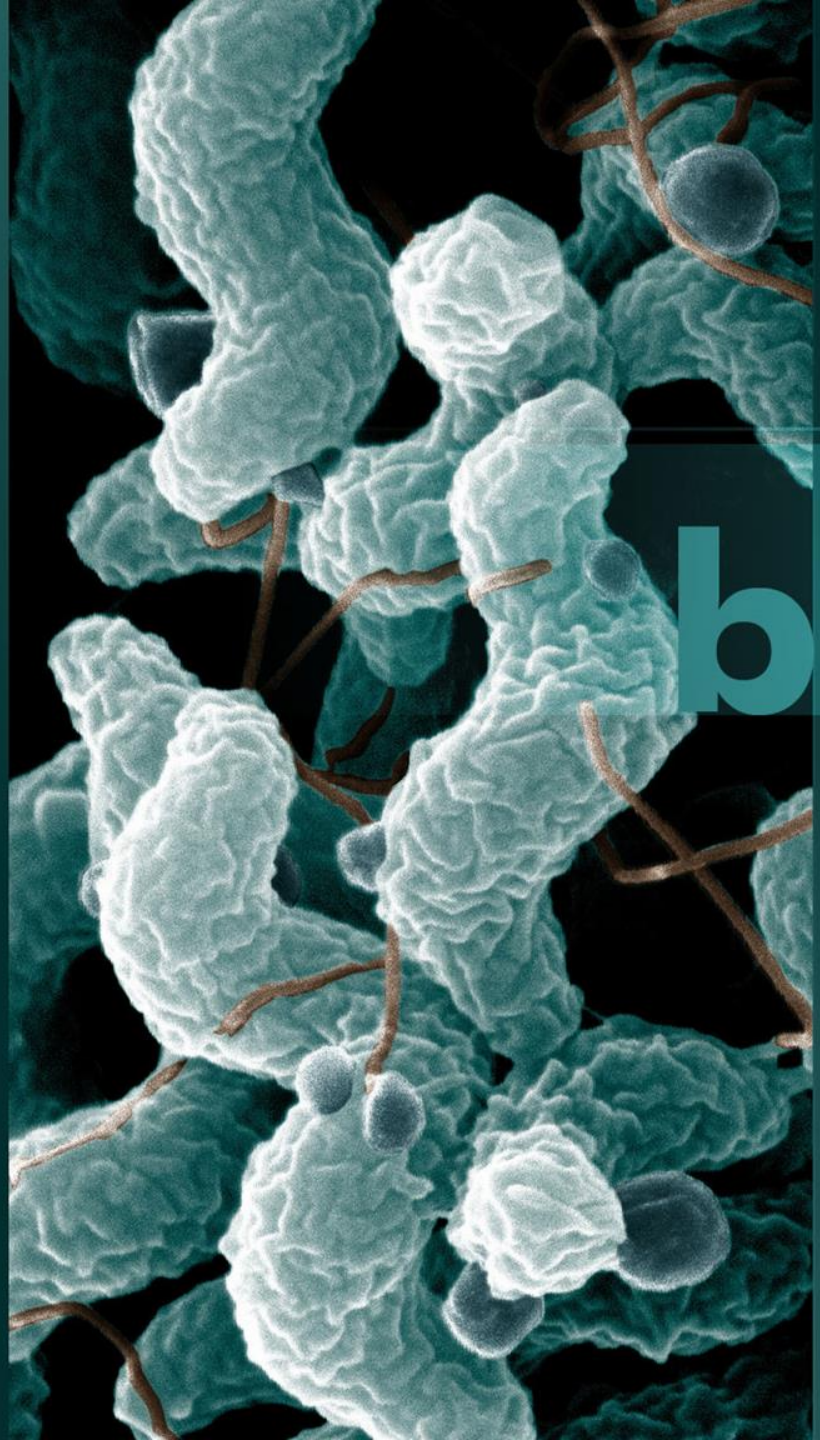


2nd Seminar of Microbiology

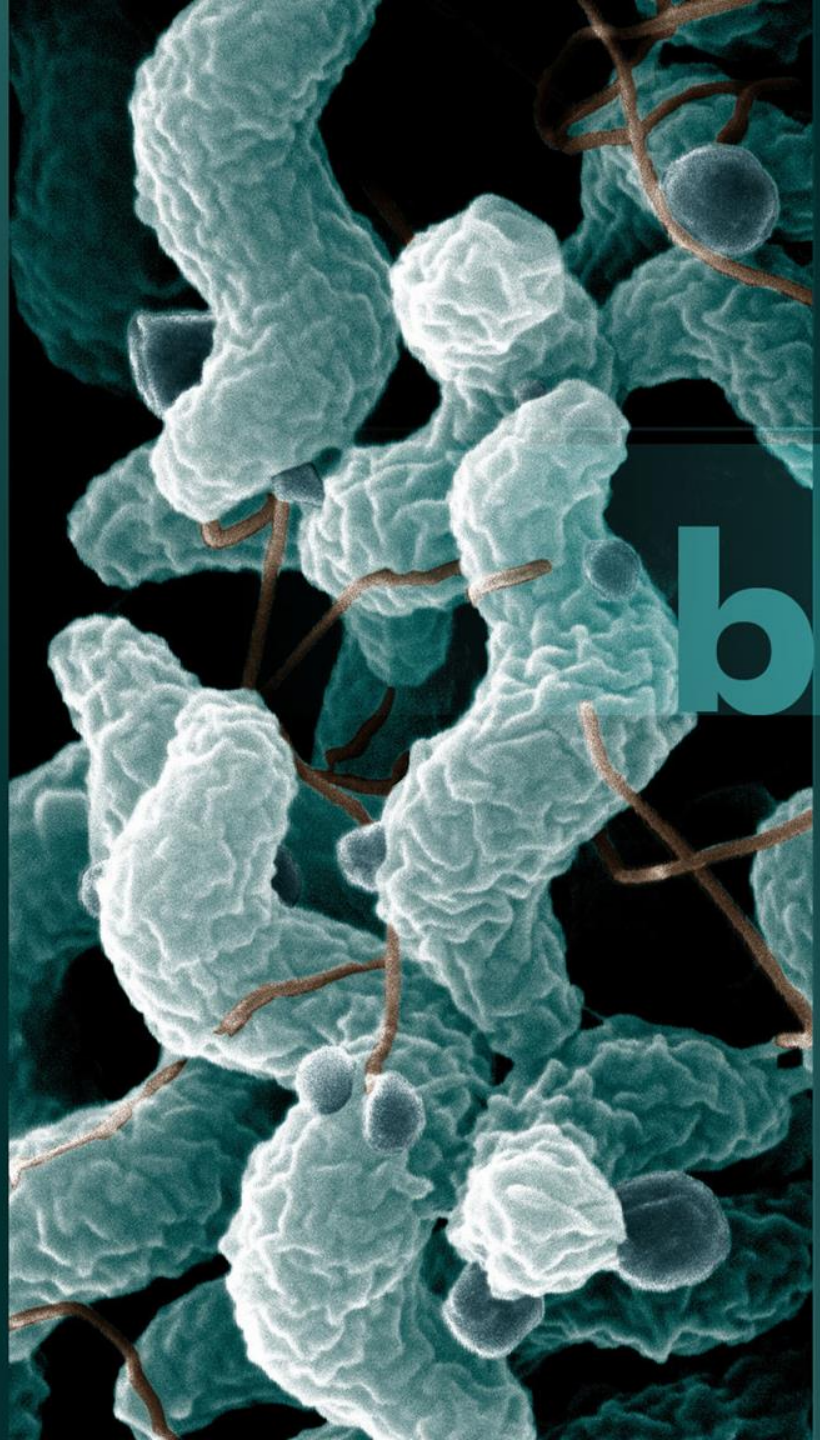
FaF VFU BRNO (theory to lab 1)

bacteria

Disinfectants and antiseptics

Nutrient media

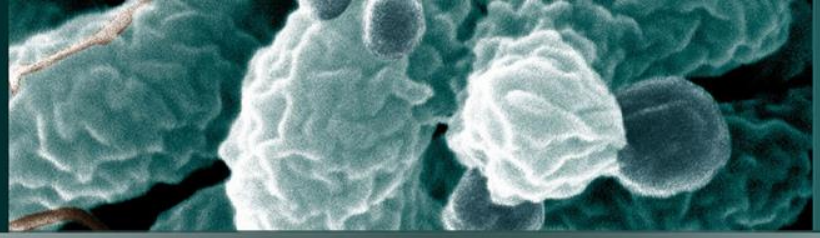
Normal microbiota of human body



2nd Seminar of Microbiology
FaF VFU BRNO

bacteria

Disinfectants and antiseptics



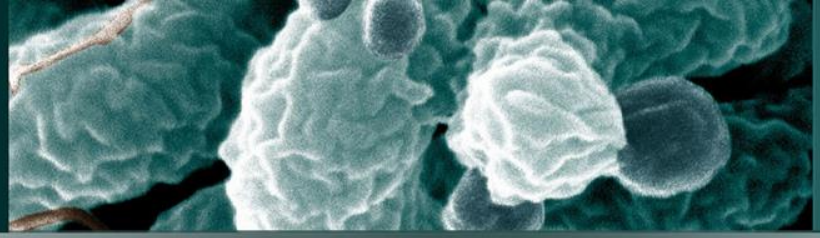
Disinfectants and antiseptics

Disinfection = **destruction** of all pathogenic microorganisms in a given place

Decontamination = substantial **reduction in the number** of microbes in a given place

Sterilisation = **killing** of all forms of microorganisms in a given place

- *Disinfectants* – used on inanimate surfaces
- *Antiseptics*– disinfectants for application on living organisms in safe concentrations



Principles for the use of chemical disinfectants

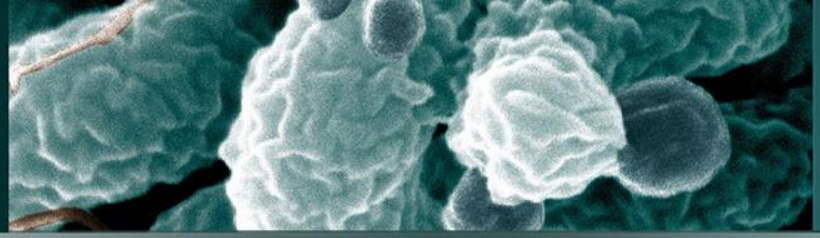
1) Use of recommended concentration, temperature and exposition time of disinfectant solution.

2) Before disinfection perform mechanical cleaning.

3) Prepare the disinfectant solution right before usage.

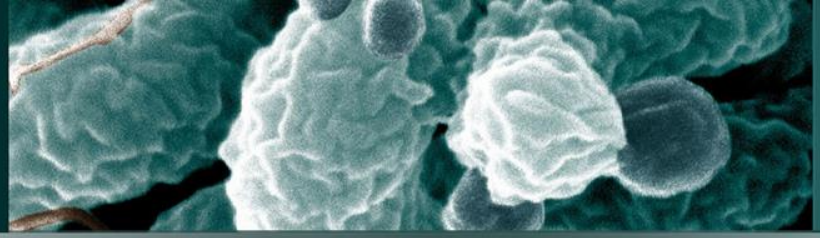
4) Change the types of disinfectant solutions to prevent development of resistance.

5) Observe the principles of occupational safety and health protection when working with disinfectants (most disinfectants are more or less toxic).



Appropriate disinfectant according to:

- Spectrum of action (A - germicidal, B – fully virucidal, C - sporicidal, T - tuberculocidal, M - mycobactericidal, V - fungicidal)
- Exposure time
- Influence on the material under disinfection and environment
- Method of use
- Toxicity and irritability for animals and people
- Leaving residues and biodegradability
- Stability
- Economic demands



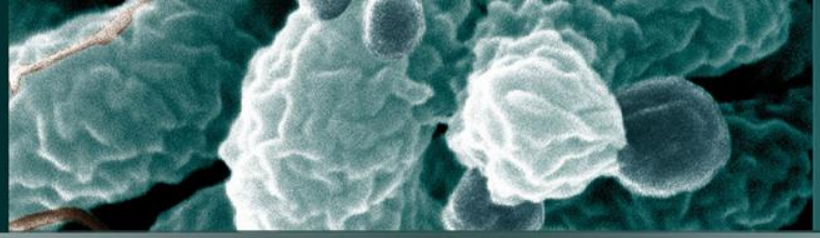
Spectrum of action

----- **cidal**

----- **static**

on:

- bacteria (vegetative forms or spores) + mycobacteria
- viruses
- fungi (molds, yeasts)

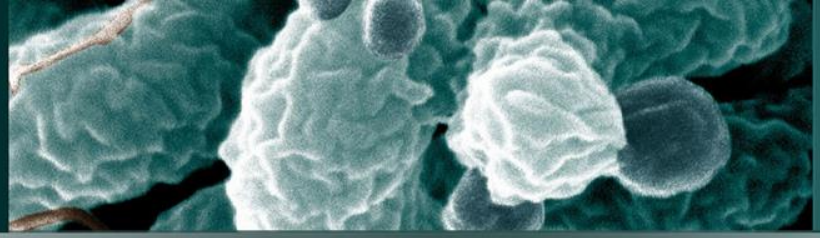


Method of use

- Wiping
- Spray
- Immersion
- Gassing

Forms of disinfectants

Liquid	Gas	Solid
Foam	Aerosol	Gel
Solution		Soap
		Cream



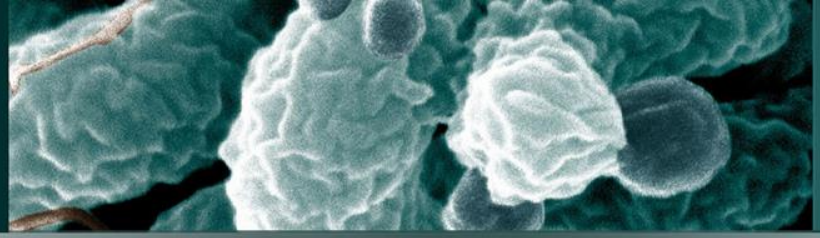
Disinfectants - Oxidants

- in touch with tissues where peroxidases are present release molecular oxygen, which kills microorganisms.
- release of oxygen in the wound leads to formation of the foam, which helps remove dirt and germs.

Potassium permanganate ($KMnO_4$)

– acts also on viruses, has adstringent effect (higher concentrations cause irritation)

- 0,1 – 1% solution…. Wounds, mucosa
- 0,5 – 2% solution…. Disinfection of hands
- 2 – 4 % solution…. Objects

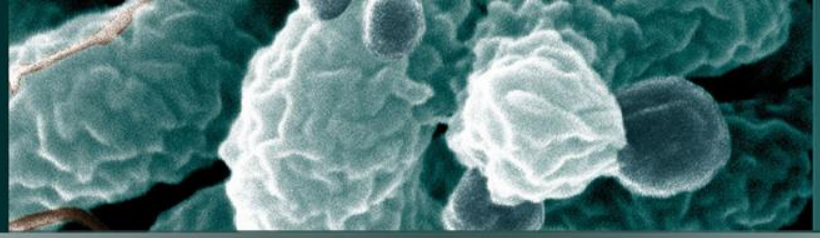


Disinfectants - Oxidants

Peracetic acid – CH_3COOOH

- most common is 36 – 40% solution (PERSTERIL)
- acts on spores, fungi and mycobacteria, but has corrosive effects (discolouration of textiles, instability of solutions)
- even fumes are effective

- 0,5 – 1% solution …. Surfaces, objects



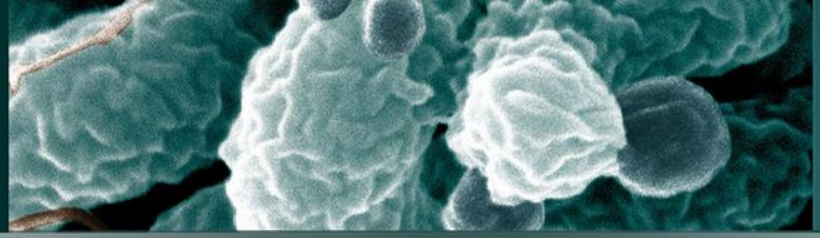
Disinfectants - Oxidants

Hydrogen peroxide – H_2O_2

- usually as 3% solution
- wound disinfection, but does not penetrate deep into tissues
- not effective on spores and viruses

Benzoylperoxide – $(C_6H_5CO)_2O_2$

- therapy of acne



Disinfectants - Halogens

- Mainly compounds containing chlorine or iodine

Chlorine

– mechanism of action: oxidation and chloration (release of these atoms)

– chlorine compounds have strong effetc on bacteria, virues, fungi and protozoa

– chlorine gas itself is strong irritant (do not forget ventilation!) and also corrosive

– use: disinfection of water, hands, wounds, surfaces…

Disinfectants - Halogens

Sodium hypochlorite – NaClO

– Savo

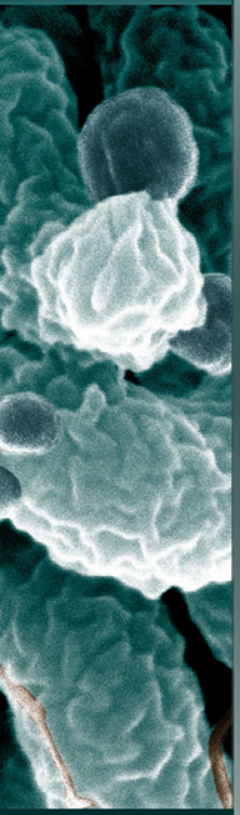
Calcium hypochlorite – $Ca(OCl)_2$

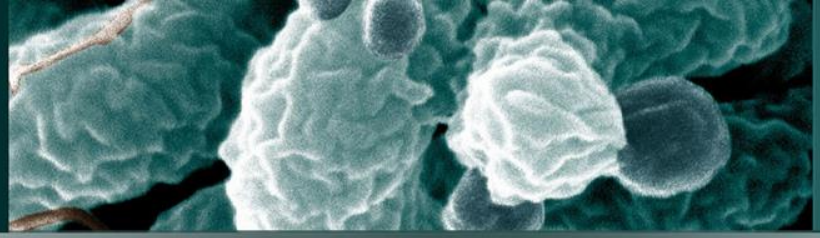
– together with calcium chloride = chlorinated lime

Chloramin – NH_2Cl

– Chloramin B

Organic compounds with chlorine (benzenchloramins)





Disinfectants - Halogens

Iodine

– mechanism of action: oxidation and iodation (release of these atoms)

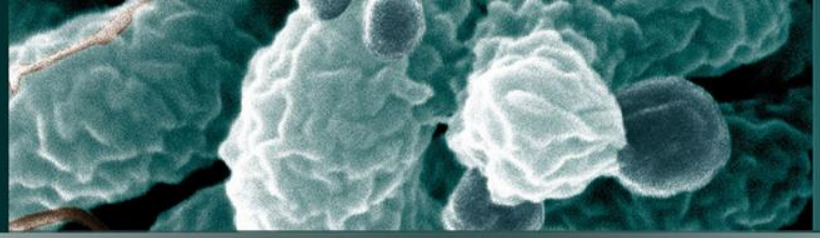
– acts on bacteria (including spores and mycobacteria) and viruses

– rapid absorption, but can be irritant and toxic

Tincture of iodine (2% I_2 + 2,4% KI – solved in 50% ethanol)

– ethanolic iodine solution

– used for skin disinfection before surgery and for small wounds

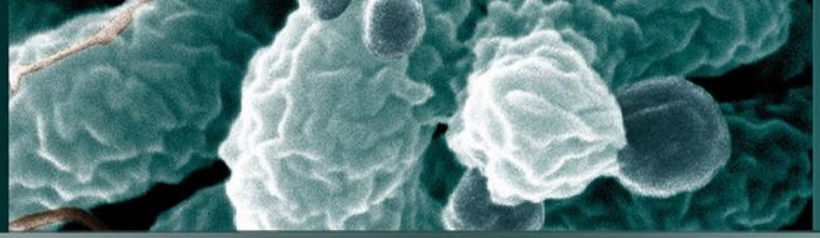


Disinfectants - Halogens

Iodophores

- e.g. povidone-iodine – iodine bound to a complex – continually released in water solution
- less corrosive, odorless, no discolouration
- Jodisol, Betadine
- Jodonal A, B
- for non-alergic patients should be used Jodonal B in stead of Ajatin (Benzododecinii bromidum)

Iodglycerin, Jodbenzine



Disinfectants - Phenols

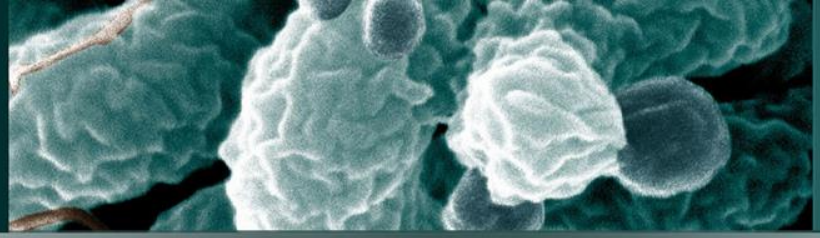
- mechanism of action: denaturation of proetins
- destroys only vegetative forms of microorganisms

Phenol (carbol acid) – C_6H_6O

– cancerogenic, toxic, strong odor → not used on skin, usually for disinfection of equipment or contaminated organic materials

Cresol(hydroxytoluene) – C_7H_8O

– usually as 2% solution = lysol – disinfection of environment, equipment (less toxic than phenol)



Disinfectants - Alcohols and glycols

- mechanism of action: destruction of cellular membrane and protein denaturation
- lacks sporicidal effects, used mainly in skin disinfectants

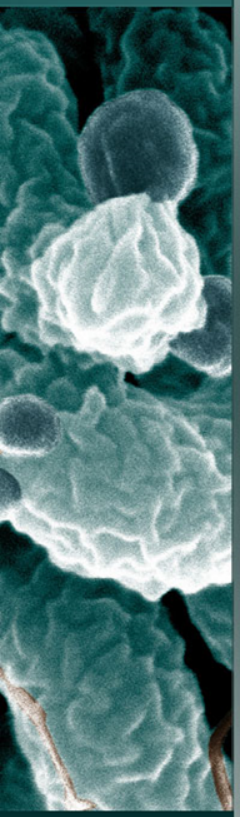
Ethanol (60 – 70%) – C_2H_5OH

Isopropanol (50%) – $(CH_3)_2CHOH$

Propylenglycol – $C_3H_8O_2$

– effective also againts mycobacteria

Disinfectants - Aldehydes



Formaldehyde

- gas, but used as 40% solution (formalin), mainly as preservative for biological materials
- strong effect on bacteria (also mycobacteria and spores), viruses, fungi
- irritant for skin and mucosa

Glutaraldehyde

– 0,1 – 1% for disinfection

Disinfectants - Surfactants

- mechanism of action: surfactants reduce surface tension of solutions and through it they disrupt energetic ratios on membranes

Anionic = soaps (RCOONa/K)

– basic solutions – often in combination with other disinfectants

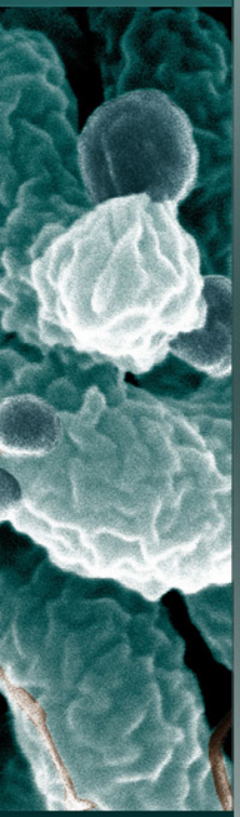
Cationic = quaternary ammonium compounds

Benzalkonium chloride, Cetylpyridinium chloride,…

– does not destroy viruses and spores

– it is not possible to combine them with anionic

– e.g. AJATIN, SEPTONEX



Disinfectants - Dyes

– active mainly against G+ cocci

Aniline dyes

Methylene blue, Gentian violet, Brilliant green

Akridine dyes

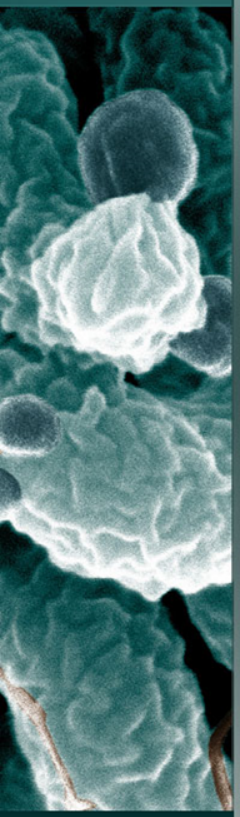
– non-irritant; decomposition under light

Akriflavine

– reduces wound and mucosa secretion

– used as 0,1% solution

Proflavine, Aminakrine, Rivanol



Disinfectants – Acids and bases

– by change in pH or by disrupting some enzymatic reactions

Boric acid – H_3BO_3

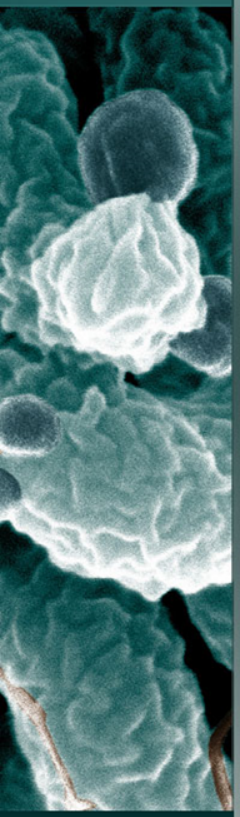
– 3% solution, ointment (ophthalmology, dermatology)

Salicylic acid – C_6H_4COOH

– antiseptic, antiinflamatory, antihydrotic, keratolytic

Sodium tetraborate – $Na_2B_4O_7 \cdot 10\ H_2O$

Sodium bicarbonate – $NaHCO_3$



Disinfectants – Heavy metals

– coagulation of proteins of microorganisms

Compounds of mercury

Mercuric amidochloride

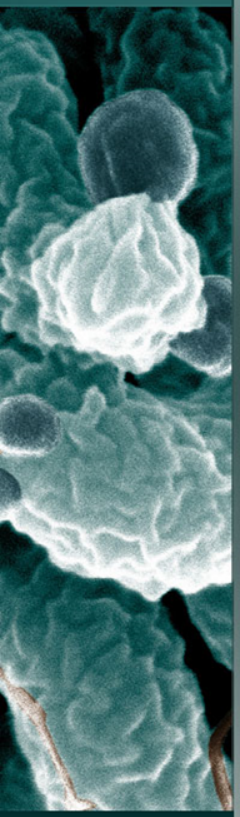
– bacteriostatic/fungistatic effect, not on mycobacteria and spores
– adjuvant drug in treatment of seborrhoeic dermatitis and psoriasis
– 1x daily max on ¼ body surface (nefrotoxic)

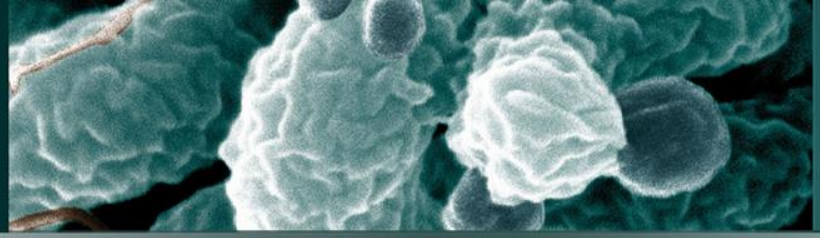
Compounds of silver

Silver nitrate

Targesin – in ophthalmology, ENT

Zinc sulphate – ophthalmology





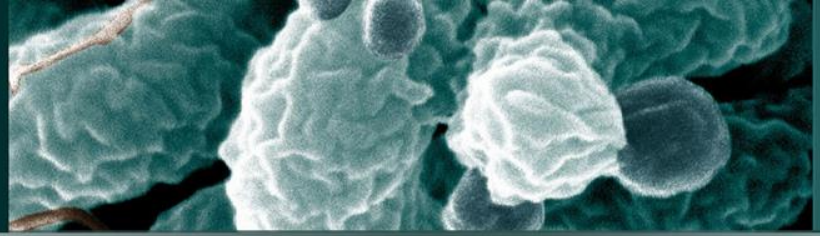
Disinfectants

Chlorhexidine

– broad spectrum of activity

– bacteria, yeasts, spores

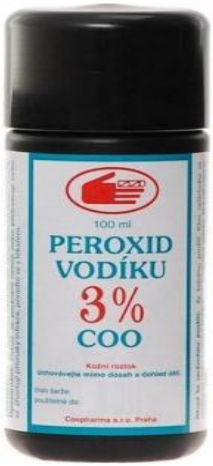
– surgical disinfection of hands



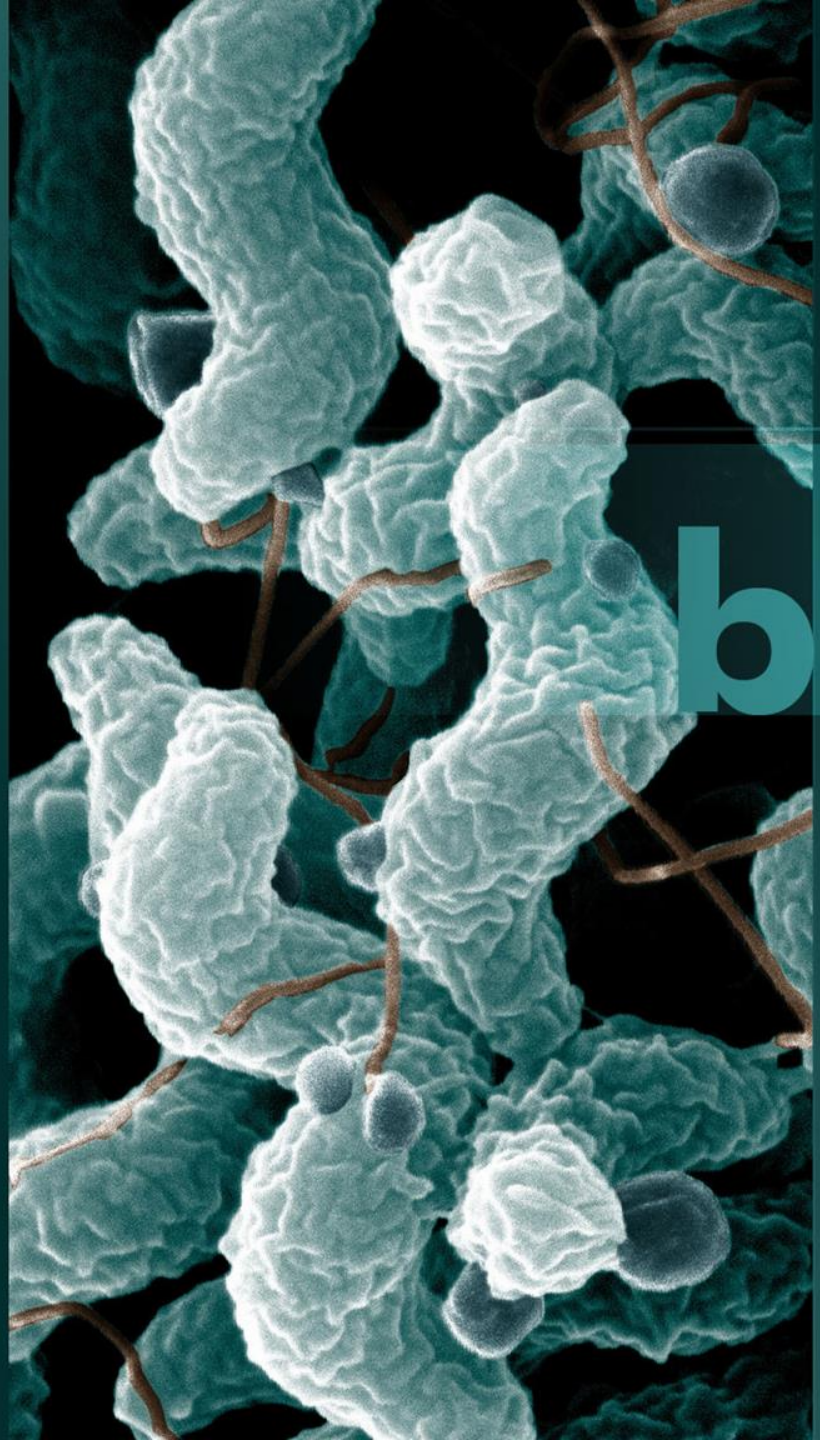

profarma
AJATIN®
PROFARMA
kožní roztok
50 ml tinktury
AJATIN® PROFARMA tinktura


30 g Gel
Duac®
Once Daily
10 mg/g + 50 mg/g Gel
clindamycin + anhydrous
benzoyl peroxide
Stiefel


Betadine®
roztok
povidonum iodatum
na dezinfekciu kože a slizníc
LEN NA VONKAJŠIE POUŽITIE
1000 ml
EGIS


100 ml
PEROXID
VODÍKU
3%
COO

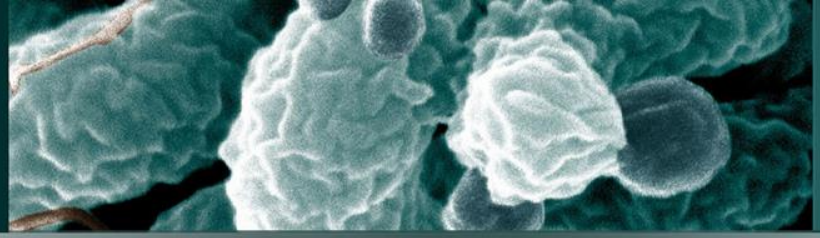

OČNÍ VODA S KYSELINOU BORITOU



2nd Seminar of Microbiology
FaF VFU BRNO

bacteria

Nutrient media

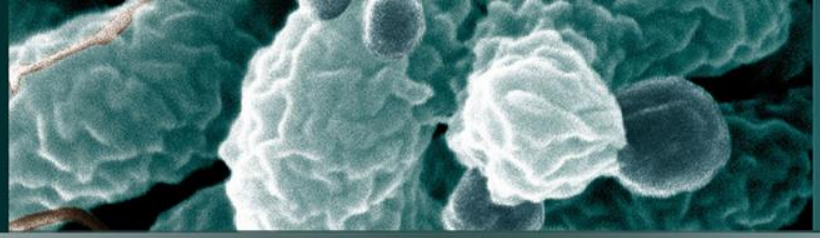


Cultivation media

- there is no universal medium or broth for all kind of bacteria

* CONSISTENCY
- solid (agar plates; for isolation of strains)
- liquid (for multiplication)

* USAGE
- basic (blood agar, broth…)
- selective (D.C., Slanetz-Bartley agar…)
- diagnostic (Endo agar, Tergitol 7…)



Cultivation media

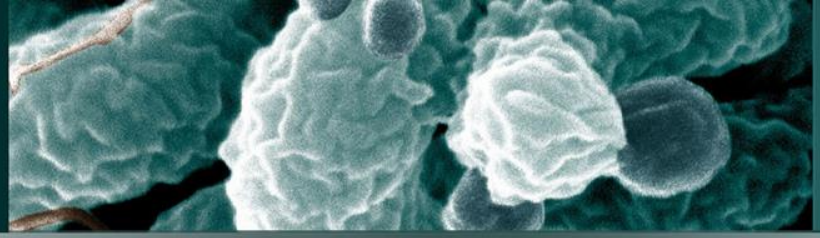
- USAGE

- Basic

Generally applicable culture medium. After the addition of blood or other suitable supplement can be used to cultivate nutritionally demanding microbes

Nutrient Broth w/ 1% Peptone (Nutrient medium)

	Constituent	Amount (grams/liter)
	meat peptone	10 g
	beef extract	10 g
pH: 7,4 ± 0,2	sodium chloride	5 g



Cultivation media

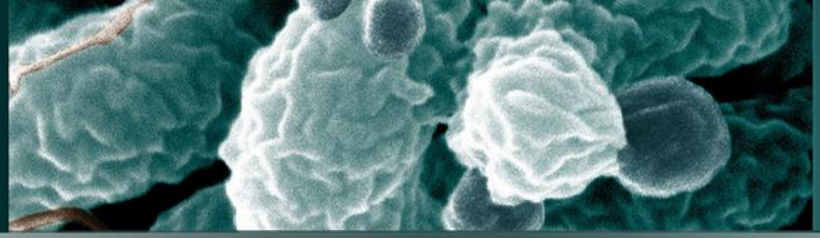
- USAGE

- Selective

For isolation and cultivation of yeast, moulds and acidotolerant microorganisms.

Malt Extract Broth Base (Malt medium)

	Constituent	Amount (grams/liter)
pH 5.4±0.2	malt extract	17 g
	mycologic peptone	3 g



Cultivation media

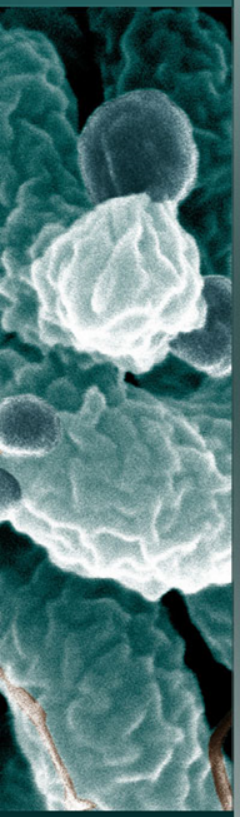
- PREPARATION

- **natural**
  (from milk, rice, potatoes, meat or soil extract)
- **synthetic**
  (water, minerals, source of C, vitamins, growth factors, AA, trace elements)
- **semisynthetic**
  (synthetic + peptone, casein…)

Basic components of cultivation media

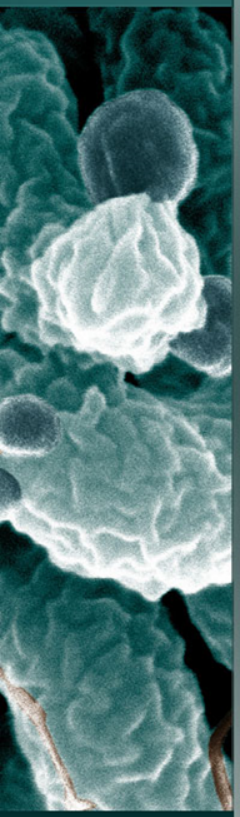
- water (distiled)
- trace elements (Zn, B, Mn, Mo, Ni,…)
- chemicals (pure)
- peptone = products of hydrolysis of meat proteins, blood serum, fibrin, casein, gelatin

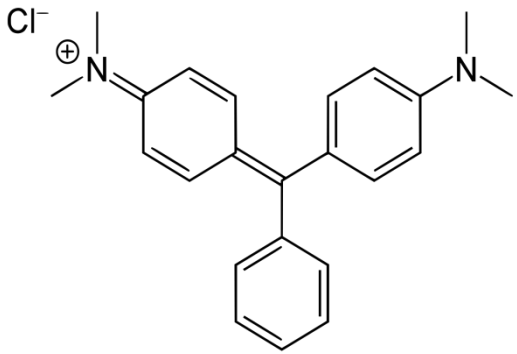
- BUFFERS



Basic components of cultivation media

- DYES AND INDICATORS

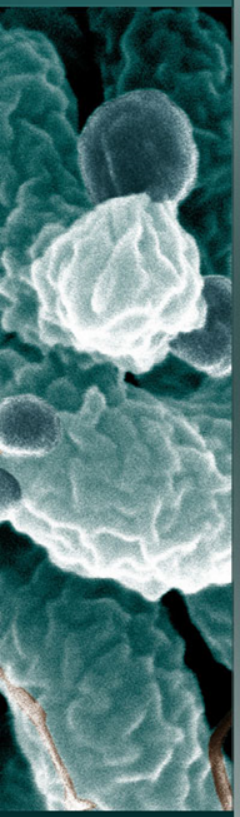


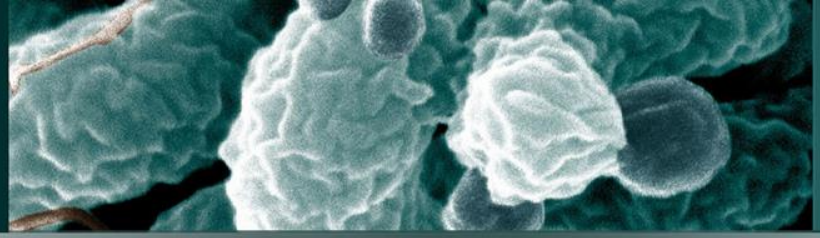


- bacteriostatic effect
- inhibitors of growth
- indicators of metabolism
- color changes due to change in pH
- triphenylmethan dyes (malachite green, fuchsine, crystal violet, methylene blue), acridine dyes

Conditions of cultivations

- temperature
- nutrition resources
- humidity
- atmosphere
- pH
- light conditions





Nutrition resources

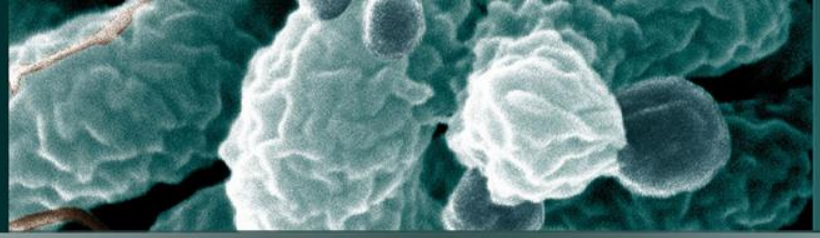
Carbon

- autotrophic organisms: anorganic
- heterotrophic organisms: organic

Nitrogen

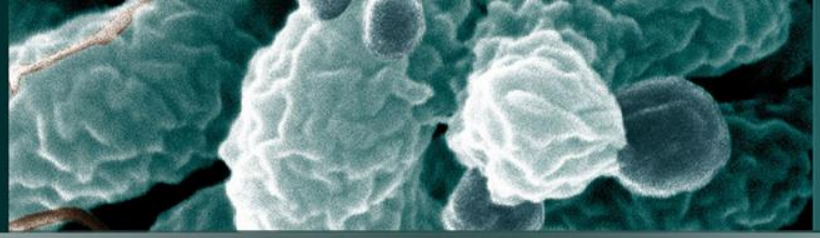
- from nitrates by reduction to ammonia

Vitamins, minerals, trace elements



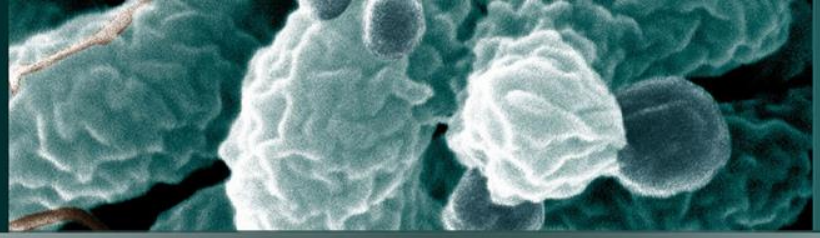
Carbon sources

- salts of organic acids
- sugars: pentoses (ara, xyl, rib, rha), hexoses (glu, man, gal, fru)
- disaccharides (sucrose, lactose)
- polysaccharides (starch, glycogen, pectin, celullose)
- lipids
- AA, proteins



Nitrogen sources

- molecular N: nitrogen-fixing organisms (*Clostridium, Azotobacter, Rhizobium*…)
- ammonium salts and ammonia – formed form AA – most bacteria
- nitrates - actinomycetes
- urea - ureolytic bacteria – production of NH_3 and CO_2 (urease), *H. pylori, Proteus, Pseudomonas*…
- AA

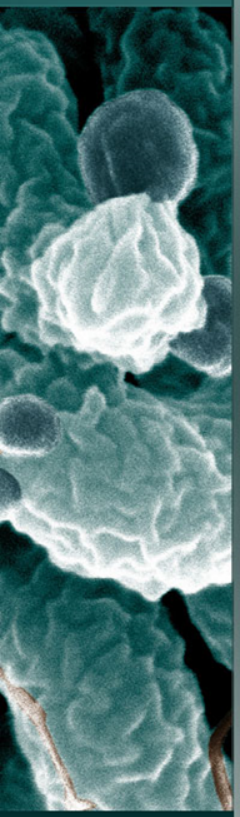


Temperature

- Psychrophilic b. - under 20 °C
- Mesophilic b. - 20-40 °C; 37 °C
- Thermophilic b. - 75-90 °C; 55 °C

Humidity

- Sensitive – gonococci, meningococci, *Vibrio cholerae*
- Resistant – *Mycobacterium tuberculosis*, *M. bovis*, spores

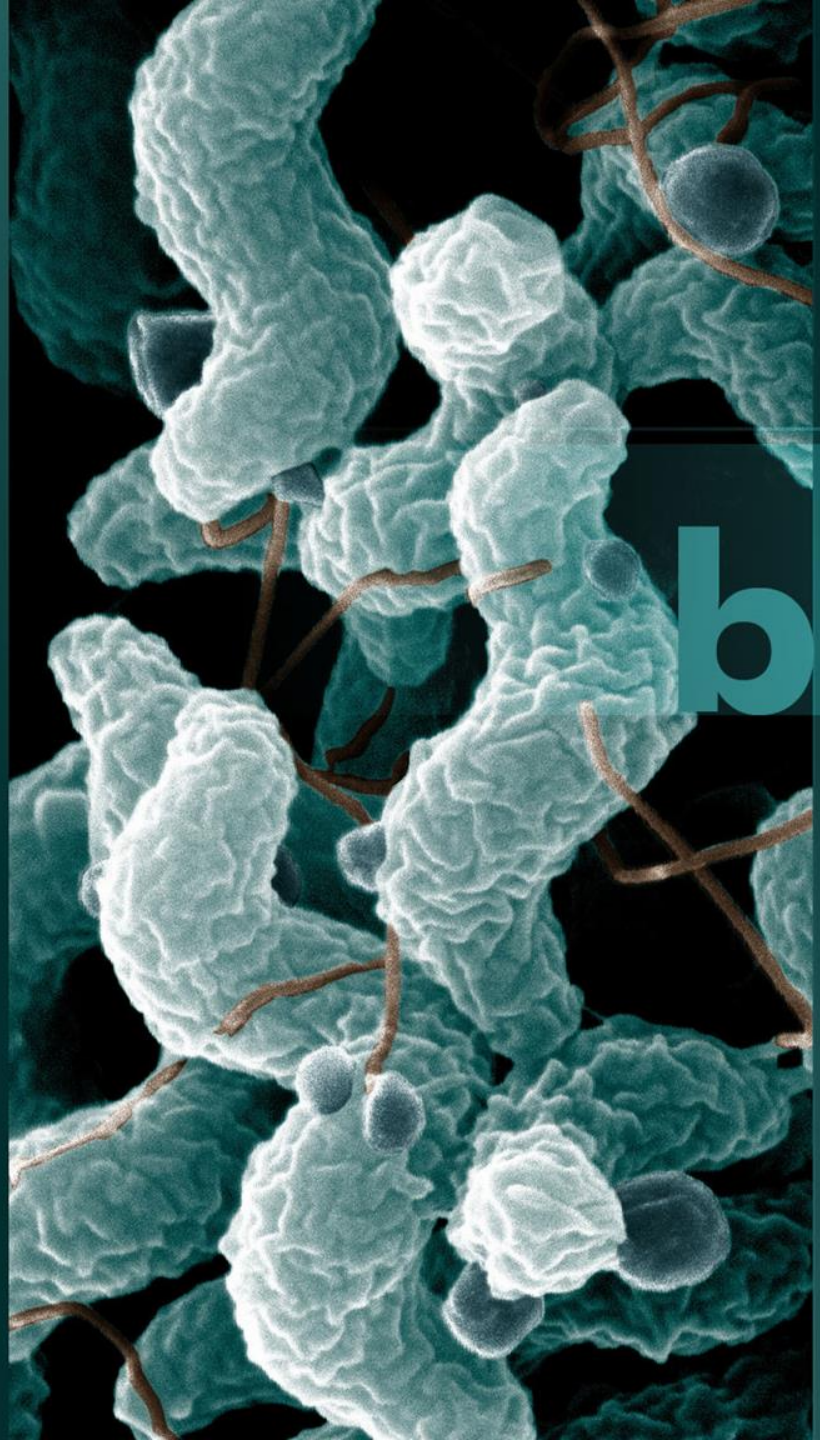


Atmosphere

- aerobic
- anaerobic: *Clostridium, E. coli* (optional)
- microaerophilic – more CO_2, less O_2
- capnophilic – more CO_2

pH

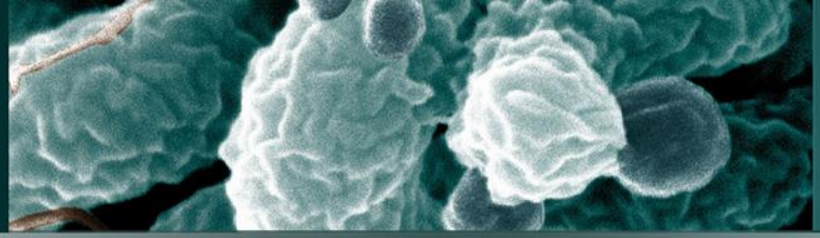
- 7.2 - 7.4 pathogenic bacteria
- 3.0 - 6.0 moulds, yeasts
- 8.0 - 8.5 *Vibrio cholerae*



2nd Seminar of Microbiology
FaF VFU BRNO

bacteria

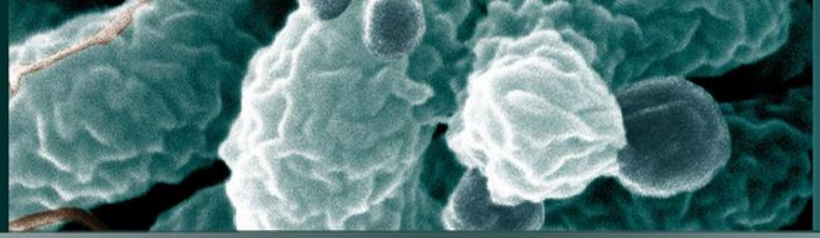
Normal microbiota of human body



Normal microbiota of human body

= *microbes, which we usually find on normal individual*

- composition vary according to part of the body and depends on: age, nutrition, genetics, hygiene, postnatal colonisation and environment
- total number of microbes exceeds approx. 10× number of somatic cells.
- microbes colonize all parts of macroorganism, which are in touch with external environment.
- normal microbiota prevents pathogenic microorganisms to settle and stimulate immune system.
- under certain circumstances, all of them can be pathogenic.



Microbes and macroorganism

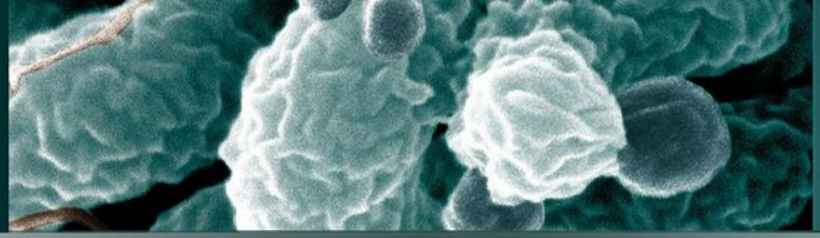
Symbiotic relations:

- **Commensalism** = to one of the partners is benefiting, to the other there is no benefit or harm
- **Mutualism** = relation is benefiting for both partners
- **Parasitism** = to one of the partners is benefiting, to the other the relation is harmful

- Dynamics of the relations:

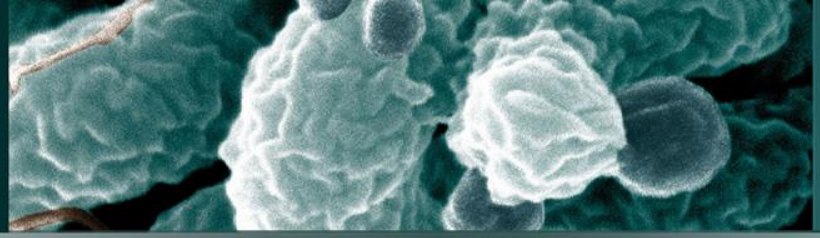
mutualism <-----> commensalism <-----> parasitism

(recovery <--) (--> infectious disease)



Relations between microbe and host

- **Microbes**:
  - **Species**:
    obligatory pathogenic --- facultative path. --- not pathogenic
  - **Strains**:
    virulent --- avirulent
- **Hosts**:
  - **Individual**:
    senstitive --- unspecifically resistant
  - **Species**:
    suspectible --- resistant

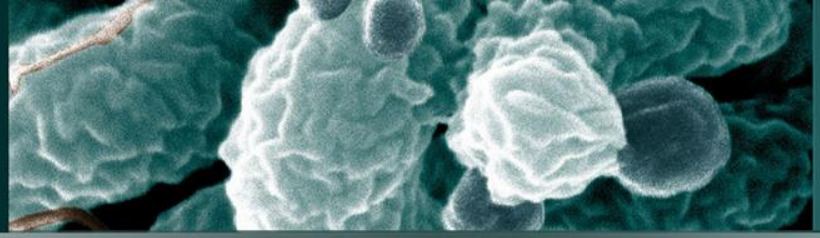


Pathogenicity (virulence)

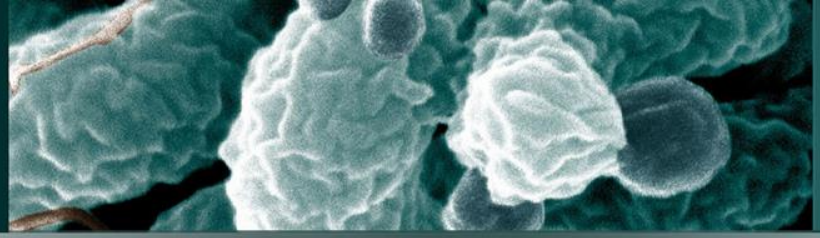
- **Portability** of diseases

- Ability of **overcome defense system** of host:
  - adherence + penetration

- **Toxicity**: direct or indirect

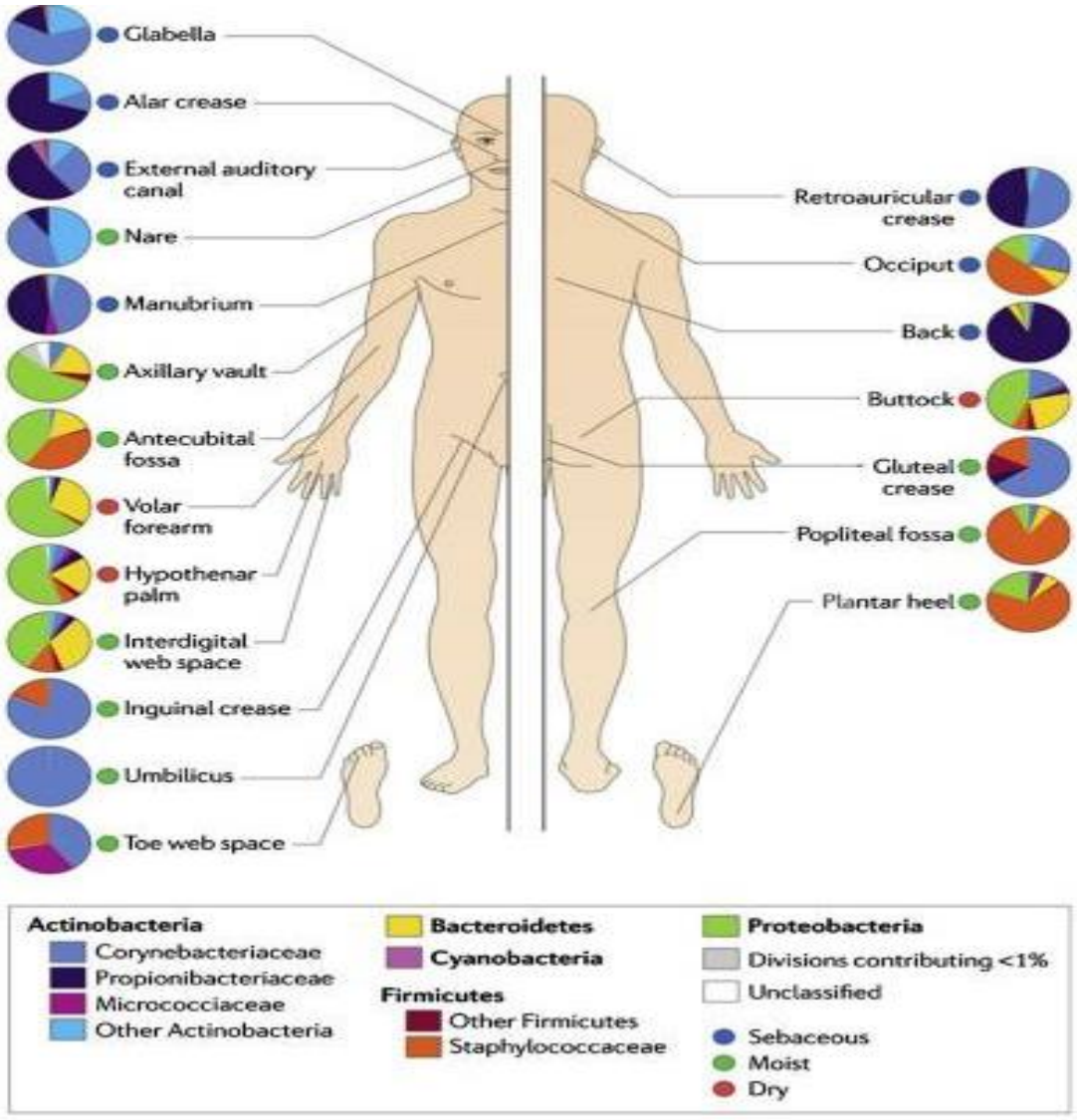


Skin	***Propionibacterium acnes, Staphylococcus epidermidis, Staphylococcus aureus Corynebacterium***
Respiratory tract	***Staphylococcus epidermidis,* non-pathogenic G+ bacilli – diphteroids, *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae, Neisseria***
Conjuctiva	**Coagulasa-negative staphylococci, Diphteroids, *Staphylococcus aureus, Streptococcus penaumoniae***
GIT - nasopharynx, oral cavity	**Non-pathogenic *Streptococcus, Staphylococcus, Haemophilus, (Lactobacillus, Actinomyces, Peptostreptococcus, Veillonella, Bacteriodes, Prevotella, Porphyromonas, Fusobacterium)***
GIT - colon	***Bifidobacterium, Eubacterium, Peptostreptococcus Escherichia coli,* enterococi, *Clostridium perfringens, Bacillus cereus, Candida* and harmless commensal protozoa**
Vaginal mucosa	***Lactobacillus acidophilus, Candida* and others**

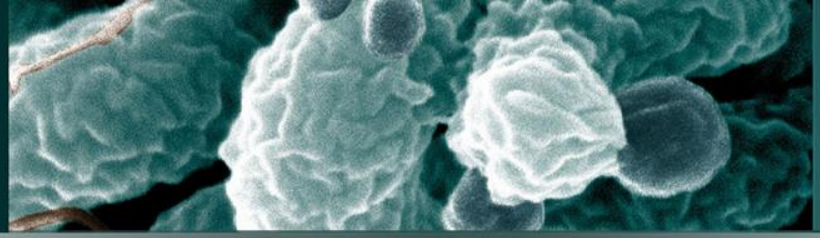


Microbiota of skin

Importance of disinfection of hands for paramedics!



Semin Immunol. 2013 Nov 30;25(5):370-7. doi: 10.1016/j.smim.2013.09.005. Epub 2013 Nov 20.
Functions of the skin microbiota in health and disease.
Sanford JA[1], Gallo RL.



Microbiota of respiratory tract

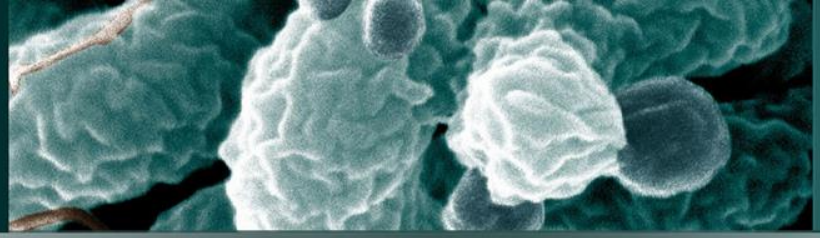
- Total surface of lungs is 40–100 m^2 → there is stable (resident) and temporary (transient) microbiom.
- Natural elimination of microorganisms:

  mechanically (exhalation, cough, sneezing)

  transport (cilia)

  surfactant (= bacteriostatic)

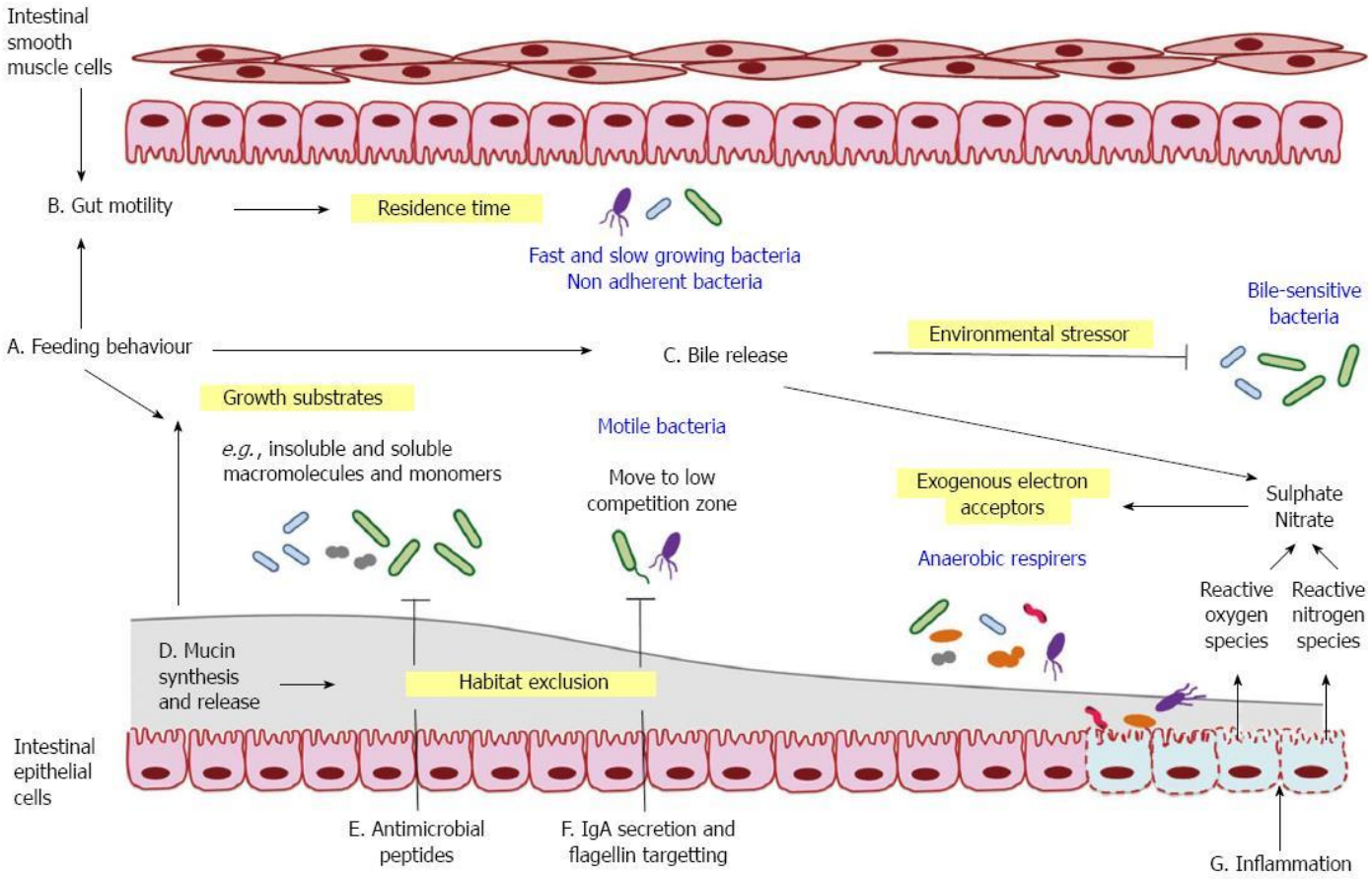
  macrophage
- *Staphylococcus epidermidis,* nepatogenní G+ tyčinky – difteroidy, *Staphylococcus aureus, Haemophilus influenzae, Streptococcus pneumoniae, Neisseria*



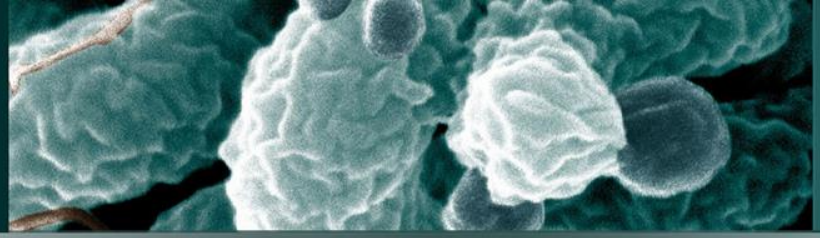
Microbiota of GIT – in relation to host

- digesting some otherwise ingestible food components
- biosynthesis of vitamins (group of vitamins B, vitamin K)
- contribution on absorption of nutrients (calcium)
- drug metabolization
- barrier against colonisation by pathogens
- regulation of immune system (innate as well as acquired)
- maintaining the integrity of an epithelial surface
- transformation of bile acids

Regulation of bacterial growth in GIT



World J Gastroenterol. 2014 Nov 28;20(44):16498-517. doi: 10.3748/wjg.v20.i44.16498.
Mechanistic links between gut microbial community dynamics, microbial functions and metabolic health.
Ha CW[1], Lam YY[1], Holmes AJ[1].



Microbiota of GIT

Oral cavity

- biofilms on teeths (*Streptococcus mutans*) – dental caries, parodontitis

Stomach

- Acid pH destroys most microorganisms
- *Helicobacter pylori*, yeast, lactobacilli

Intestine

Bacteroides – gain energy mostly from saccharides fermentation

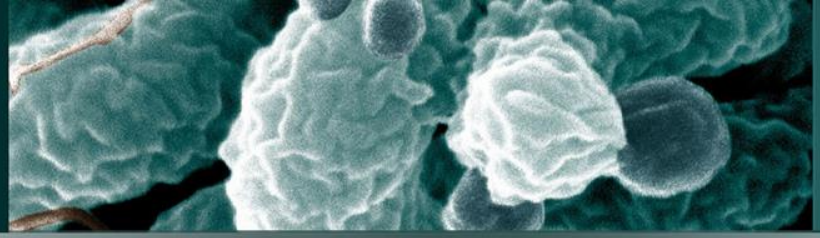
Prevotella - degrade glykoproteins of intestine mucosa

Ruminococcus – bind mucins, transport and degrade saccharids

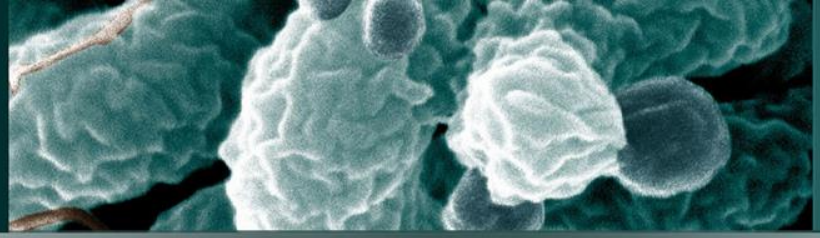
Microbiota URO-GEN tract

- mostly lower urinary tract (anaerobs)
- men: in genitals also mycoplasmata and ureoplasmata
- vaginal microbiota is distinctly influenced by hormonal dysbalance

→ lactobacilli (balance pH under 4,5 by production of lactate)



BACTERIA		
Actinomyces	G+ strains anaerobic, microaerofilic	mouth, GIT, vagina
Bacillus	G+ bacilli facultative aerobic, spores	intestine, spores
Bacteroides	G- pleomorphy (cocci, bacilli, strains), anaerobic	mouth, GIT
Bifidobacterium	G+ bacilli anaerobic	mouth, GIT, vagina
Capnocytophaga	G- bacilli facultative anaerobic	oral cavity
Clostridium	G+ bacilli anaerobic or aerotolerant, spores	intestine, spores
Corynebacterium	G+ bacilli facultative aerobic	skin
Enterococcus	G+ cocci facultative anaerobic	intestine
Escherichia	G- bacilli facultative anaerobic	intestine
Fusobacterium	G- bacilli or strains anaerobic	resp. tract, GIT, vagina
Gardnerella	G- bacilli microaerofilic	vagina
Haemophilus	G- bacilli facultative anaerobic	nosopharynx
Lactobacillus	G+ bacilli anaerobic or microaerofilic	mouth, GIT, vagina
Micrococcus	G+ cocci aerobic	skin, environment
Moraxella	G- bacilli microaerofilic	mammal musoca
Neisseria	G- cocci aerobic or microaerofilic	oral cavity
Prevotella	G- pleomorphy (cocci, bacilli, strains), anaerobic	mouth, GIT
Propionibacterium	G+ bacilli anaerobic	skin
Proteus	G- bacilli facultative anaerobic	intestine
Staphylococcus	G+ cocci facultative anaerobic	nose, skin, mouth
Streptococcus	G+ cocci facultative anaerobic	mouth, nosopharynx
YEASTS		
Candida		mouth, GIT, vagina
Saccharomyces		vagina, environment



Lab class no. 1

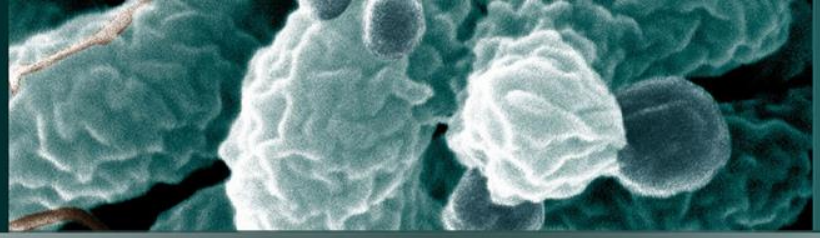
Cultivation of microorganisms and control of efficiency of disinfectants and antiseptics

Aims:

- ***Prepare nutient media for consequent cultivation of microorganisms.***
- ***Control of efficiency of disinfectants and antiseptics.***

Microrganisms from group 1 used in lab classes:

Micrococcus luteus CCM 732
Escherichia coli CCM 7929
Saccharomyces cerevisiae CCM 8191



Domovská stránka

Aktuality

O sbírce
Adresa
Jak najdete CCM?
Zaměstnanci

CCM Nabízí
Katalog kultur
Kontrolní kmeny
Ke stažení

Publikace
Výzkum
Odkazy

Vaše připomínky

CCM Czech Collection of Microorganisms

Vítejte na domovské stránce

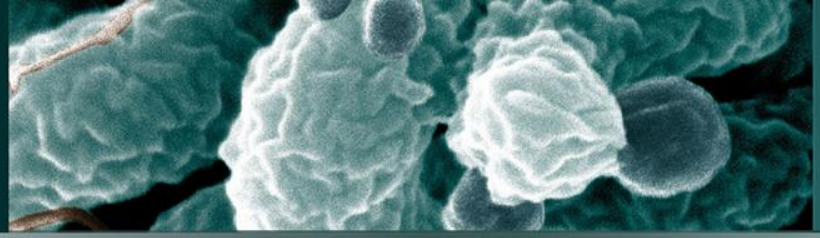
České sbírky mikroorganismů (CCM)

Masarykova univerzita, Přírodovědecká fakulta

Zvolte jazyk:

Česky English

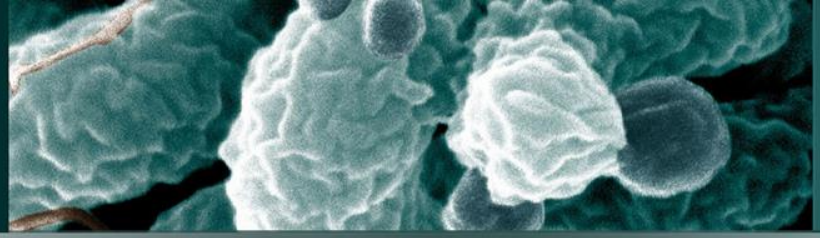
Datum aktualizace: 09.06.2017



Non-pathogenic strain *Escherichia coli* CCM 7929
= CECT 433 = CIP 54.117 = CNCTC 7388 = DSM 11250 = IFO 3301 = NBRC 3301 = NCDO 1984 = NCIMB 10083 = NCTC 10538 = PCM 2560 = strain K 12
< CNCTC. Human faeces. Medium 71, 30°C.

Pathogenic strain *Escherichia coli* **CCM 4724**
= H. Lhotová 27750
< H. Lhotová, NRL CEM < M. Bielaszewská. Serovar O:157. Stool; Czech Republic. Production of Vero cytotoxins VT1 and VT2. Sorbitol and ß-glucuronidase negative. Biohazard group 2. Medium 71, 37°C.

VZTAH MEZI PATOGENITOU MIKROBA A ODOLNOSTÍ HOSTITELE			
	MIKROB		
DRUH	obligátně (primárně) patogenní	⟷ oportunně (fakultativně) patogenní ⟷	nepatogenní
KMEN	virulentní	⟷	avirulentní



Annex no. 7 to Govt. Regulation No. 361/2007 Coll.
Biological agens, their groups, designation and workplace requirements

Examples:

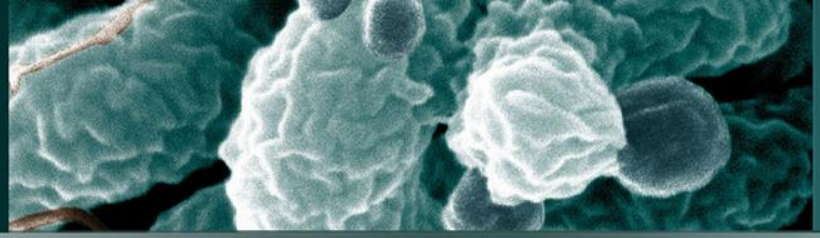
Normal microbiota and common pathogens belong to gr. 2

Bacillus anthracis gr. 3
Mycobacterium tuberculosis gr. 3

Virus Ebola gr. 4

PART A
Biological agens in groups 2, 3 or 4

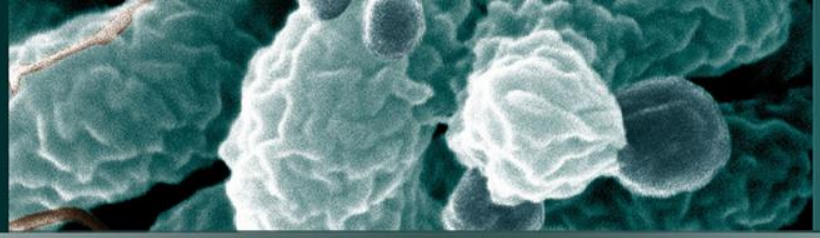
Biologický činitel	Skupina	Poznámka
Corynebacterium diphtheriae	2	T,V
Corynebacterium minutissimum	2	
Corynebacterium pseudotuberculosis	2	
Corynebacterium spp.	2	
Edwardsiella tarda	2	
Ehrlichia sennetsu (Rickettsia sennetsu)	2	
Ehrlichia spp.	2	
Eikenella corrodens	2	
Enterobacter aerogenes/cloacae	2	
Enterobacter spp.	2	
Enterococcus spp.	2	
Erysipelothrix rhusiopathiae	2	
Escherichia coli (s výjimkou nepatogenních kmenů)	2	
Flavobacterium meningosepticum (Chryseobacteriummeningosepticum)	2	
Fluoribacter bozemanae (Legionella)	2	
Francisella tularensis (typ B)	2	
Fusobacterium necrophorum	2	



***Micrococcus luteus* CCM 732**

= ATCC 10240 = CCTM La 2973 = CCUG 21988 = CIP 53.160 = CNCTC M 8/58 = DSM 1790 = FDA 16 = IFO 13867 = LMG 3293 = NBRC 13867 = NCDO 982 = NCIMB 8166 = NCIMB 8640 = NCTC 7743 = OUT 8276 = PCI 1216 = Commercial Solvents Corp.130.21

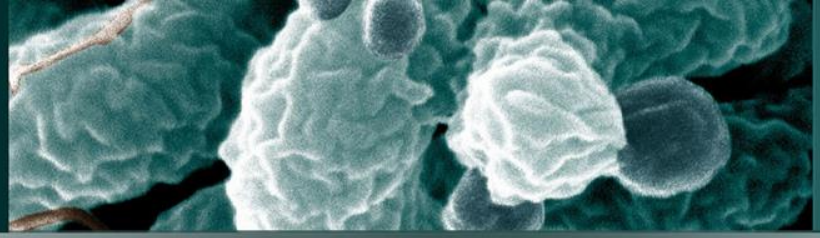
< ATCC (*Micrococcus flavus*) < Commercial Solvents Corporation. Cylinder-plate assay of bacitracin in body fluids, feeds, milk and pharmaceutic preparations (1451,2651,5738,5754,6287). Medium 71, 30°C.



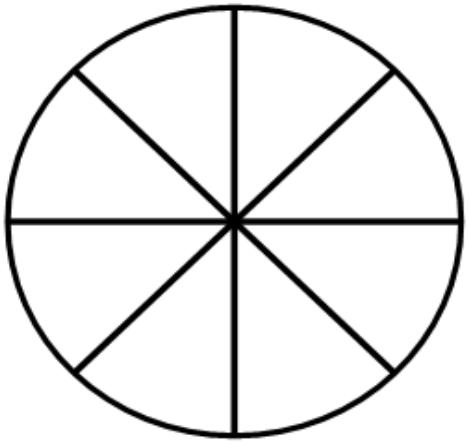
***Saccharomyces cerevisiae* CCM 8191**

Medium 23, 25°C. = ATCC 9763 = CBS 5900 = CCUG 1836 = CCUG 32994 = CCY 21-4-48 = CNCTC 51/65 = CNCTC 52/67 = DSM 1333 = IHEM 3961 = MUCL 30115 = NCTC 10716 = NCYC 87 = NRRL Y-567 = WDCM 00058

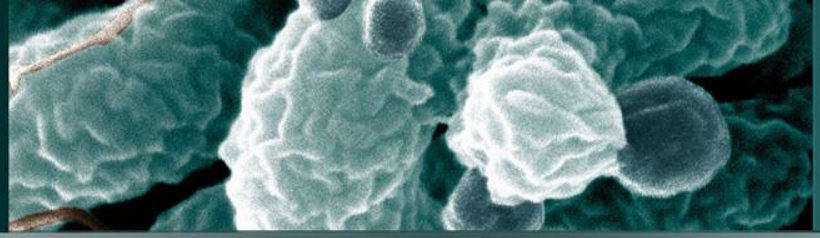
< E. Sláviková. Media testing. Assay of andicidin, amphothericin B, anisomycin, natamycin and nystatin.. Production of arginase. Production of nicotinic acid.



1. *Preparation of nutrient media*
2. *Pouring into Petri dishes*
3. *Inoculation with microorganisms gr. 1 – control of efficiency of disinfectants and antiseptics*



bivide dish on bottom side into 6 quadrants - Betadine, 70% ethanol, 0,1% $KMnO_4$, 2% chlorhexidine, Septonex and sterile water



Question for lab class test:

- Name main groups on disinfectants and antispetics.
- What is the difference between disinfectant and antiseptic?
- What does the basic medium contain?
- What does the medium for cultivation of yeast and moulds contain?
- Which is the optimal pH for bacteria and which is optimal for yeast and moulds?
- Name at least 5 genuses of G+ and 5 genuses of G- bacteria.
- Which genuses do populate GIT (at least 5)?
- Which genuses can be found on skin?
- Name main representative of vaginal microbiota.
- Describe the relation between pathogenicity of microbe and resistance of host.