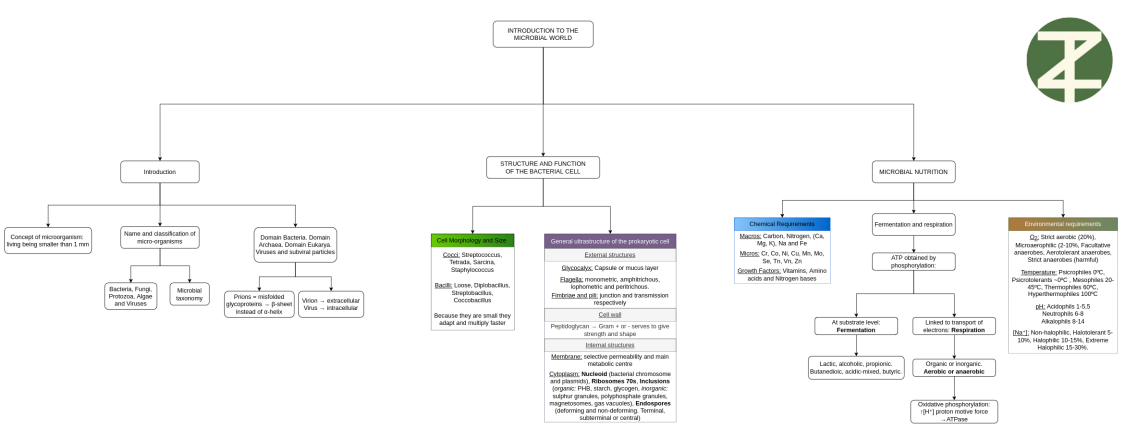

INTRODUCTION TO THE MICROBIAL WORLD
Introduction
Concept of microorganism: living being smaller than 1 mm
Name and classification of micro-organisms
Bacteria, Fungi, Protozoa, Algae and Viruses
Microbial taxonomy
Domain Bacteria, Domain Archaea, Domain Eukarya, Viruses and subviral particles
Prions = misfolded glycoproteins → β-sheet instead of α-helix
Virion → extracellular Virus → intracellular
STRUCTURE AND FUNCTION OF THE BACTERIAL CELL
Cell Morphology and Size
Cocci: Streptococcus, Tetrada, Sarcina, Staphylococcus
Bacilli: Loose, Diplobacillus, Streptobacillus, Coccobacillus
Because they are small they adapt and multiply faster
General ultrastructure of the prokaryotic cell
External structures
Glycocalyx: Capsule or mucus layer
Flagella: monomeric, amphitrichous, lophotrichic and peritrichous.
Fimbriae and pili: junction and transmission respectively
Cell wall
Peptidoglycan → Gram + or - serves to give strength and shape
Internal structures
Membrane: selective permeability and main metabolic centre
Cytoplasm: Nucleoid (bacterial chromosome and plasmids), Ribosomes 70s, Inclusions (organic: PHB, starch, glycogen, inorganic: sulphur granules, polyphosphate granules, magnetosomes, gas vacuoles), Endospores (deforming and non-deforming. Terminal, subterminal or central)
MICROBIAL NUTRITION
Chemical Requirements
Macros: Carbon, Nitrogen, (Ca, Mg, K), Na and Fe
Micros: Cr, Co, Ni, Cu, Mn, Mo, Se, Tn, Vn, Zn
Growth Factors: Vitamins, Amino acids and Nitrogen bases
Fermentation and respiration
ATP obtained by phosphorylation.
At substrate level: Fermentation
Linked to transport of electrons: Respiration
Lactic, alcoholic, propionic, Butanediolic, acidic-mixed, butyric
Organic or inorganic. Aerobic or anaerobic
Oxidative phosphorylation: ↑[H+] proton motive force → ATPase
Environmental requirements
O2: Strict aerobic (20%), Microaerophilic (2-10%, Facultative anaerobes, Aerotolerant anaerobes, Strict anaerobes (harmful)
Temperature: Psicrophiles 0ºC, Psicotolerants <0ºC , Mesophiles 20-45ºC, Thermophiles 60ºC, Hyperthermophiles 100ºC
pH: Acidophils 1-5.5 Neutrophils 6-8 Alkalophils 8-14
[Na+]: Non-halophilic, Halotolerant 5-10%, Halophilic 10-15%, Extreme Halophilic 15-30%.

# CONTROL OF MICROBIAL GROWTH IN FOOD

## MICRO-ORGANISMS PRESENT IN FOOD

### Types

Beneficial: Fermented

Spoilage: decomposition

Pathogenic: Diseases - infection or poisoning

Indicators: Mesophiles, Enteric (E.coli, Coliforms, Enterococci), Yeasts and Moulds, Staphylococcus aureus

### Food interest groups

Bacteria: **Lactic** (beneficial, spoilage and indicator), **Coliforms** (indicator, pathogenic), **Acetic** (beneficial), **Butyric** (spoilage and pathogenic), **Putrefaction** (spoilage)

Yeasts (beneficial, spoilage and indicator)

Moulds (beneficial, spoilage, pathogenic and indicator)

Viruses and Prions (pathogens for bacteriophages and human)

### Origin of the contamination

From water, soil, air and/or dust

From micro-organisms naturally present in the food

From manufacturing, storage and transport

From marketing and consumption

## FACTORS AFFECTING MICROBIAL GROWTH IN FOOD

### Duplication, binary fission. Growth = population increase

Microbial growth curve: Dormant, exponential, stationary and death phase.

### Types of factors

#### Intrinsic factors

Food Composition: ↓WB = ↓it B-dependent non-bacteria, ↑Carbs = fungal

↑Fats&prot = putrefaction and rancidity

pH: bacteria 4.5-9, moulds 2-11, yeasts 2-9

↓Water activity = microbial growth

ORP: indicates amount of air

Physical structure of the food: cutting them destroys the natural protection and increases the contact surface.

Presence of natural antimicrobial substances: coumarins, lysozyme, tabasco, polyphenols, allicin and catechol, acetic acid, lactic acid, bacteriocins.

#### Extrinsic factors

↑Temperature = ↑Growth

↓Humidity = ↑Growth

Storage atmosphere: ↑O2 = ↓Aerobic growth, ↓O2 = ↑Anaerobic growth ↑CO2 = ↓Microbial growth

## CONTROL OF MICROBIAL GROWTH IN FOOD

### Work with populations, not individuals. Decimal reductions -90%

Cleaning: separation of microorganisms, D1, with water at 30°C D4.5

Disinfection: destruction of pathogens D6-13

Sterilisation: destruction of all microorganisms

Preservation: ensure quality and safety. Microbiostatic (inactivates) or Microbicidal (kills). Efficacy depends on: size, intensity, exposure and environmental conditions.

### Physical Methods

Filtration: depth or membrane filtration

High Temperatures: UHT, Pasteurisation

Low Temperatures: Refrigeration or freezing

Lower AW: dehydration or addition of solutes

Modified atmosphere

Radiation: Radapertisation, Radicisation and Radurisation or ionisation.

High pressures: 400mPa D1

### Chemical Methods

Smoking: acids, aldehydes and phenols

Nitrites and Nitrates

SO2, Sulphites and Bisulphites: break disulphide bridges and degrade sugars

Saturated FA and their Ca, Na and K salts

Sorbic Acid: inhibits moulds

Benzoic acid: inhibits moulds and yeasts

Parabens: not pH-dependent

Organic acids: acidify cytoplasm and denature proteins.

Phenolic antioxidants: act on membrane

Antibiotics: damage microbiota

Bacteriocins: peptides with antimicrobial action, added to foodstuffs

SPOILING MICROORGANISMS

Introduction

Alteration: organoleptic, chemical & physical changes

Useful life: safe, maintains its properties and nutrition

Physical & chemical and microbiological alteration 10ª

Perishable, Semi-perishable and Non-perishable food

Most changes in the surface area of food

Microorganisms

Moulds and yeasts: they tolerate ↓Aw and/or ↓pH
better than bacteria.

Gram-negative bacteria: Pseudomonadaceae, Neisseriaceae

Gram-positive bacteria: Micrococcaceae, LAB, Brochotrix thermophacta.

Sporulated bacilli: Bacillus (anaerobic facultative mesophilic), Clostridium (obligate anarobes, ↓redox)

Metabolism

Carbs: fermentation, pectin degradation (galacturonic acid), amylolytic enzymes, exopolysaccharides

Proteins: hydrolysis. aa's degradation → putrefaction (smell)
$H_2S$ + oxihaemoglobin → sulphohaemoglobin (green)

Fats: hydrolysis. Glycerol + Fatty Acids → ketones (smell)

FOODBORNE PATHOGENIC BACTERIA

GRAM-NEGATIVE FOODBORNE PATHOGENIC BACTERIA

Vibrio spp.

bacilli, motile, flagellate acuatic

facultative anaerobes

**catalase and oxidase +**

Vibrio cholerae

**Gastroenteritis and cholera**

Choleric toxin: 2 parts A & B
A enters enterocyte → Gprotein → cAMP ↑ P → membrane channels → $HCO_3^-$ & $Cl^-$ out, $Na^+$ blocked

ZOT (relaxes tight junctions), ACE (cholera toxin), adhesins, biofilms...

oral faecal transmission, >10ª bacteria

Rehydratation, antibiotics.
Hygiene and cooking to prevent

Vibrio parahaemolyticus

**Gastroenteritis during summer**

Adhesins and enterotoxins
It's not invasive

Associated with seafood and fish consumption

Treatment with antibiotics

Vibrio vulnificus

**Gastroenteritis, wound infections and sepsis**

It has a polysaccharide capsule as a protection. It is invasive (bloodstream). It's a flesh-eating bacteria

Causes more deaths than any other vibri
Treatment with antibiotics

Enterobacteria family

bacilli, flagellate (peritrtic), ubiquitous

facultative anaerobes

**catalase + and oxidase -**

Escherichia coli

General characteristics + motile and lactose fermentation capacity

Gastroenteritis, urinary tract infection, systemic infection and meningitis

Enterotoxygenic: **traveler's diarrhea**, no h-h transmission, high inoculum

Enteropathogenic: **childhood diarrhoea**, ↓h-h transmission, low inoculum

Enteronegative: **childhood diarrhoea** → microbiota damage, growth alteration

Enterohaemorrhagic: **Bleeding diarrhoea**, h-h transmission, low inoculum. O:157, H:7 Shiga toxin: Haemolytic Uraemic Syndrome

Enteroinvasive: **watery diarrhoea,** colon infection, ulcerations and fever

Diffusely adherent: no invasives, no toxins, **↓Absorption**
affect only children, elderly and immunosuppressed
Rehydratation and antibiotics
Hygiene and food cooking

Salmonella spp.

General characteristics + enterotoxin + cytotoxin + antiphagocytic protein + complex IV protector

Salmonellosis Thyphimurium: **gastroenteritis, nausea and vomiting**, no h-h transmission, systemic diagnosis, self-limited treatment

Salmonellosis Thyphi: **typhoid fever**, h-h transmission, no animals, settles in the **gallbladder**. Gastroenteritis at intervals, treatment with antibiotics

Shigella spp.

immotile bacilli, no lactose fermentation no capsule protection. Human reservoir

Shigellosis: adhesion to M cells. Shiga toxin → Haemolytic Uraemic Syndrome

**Abdominal spasms, bleeding diarrhoea and fever**

h-h and sexual transmission

Yersinia spp.

short bacilli, psychotrof (15-30ºC), lactose -, urease +, immotile 37ºC, motile 25ºC

Attack M Cells in the ileum, settles in mesenteric lymph nodes. Survives inmunity

**Watery diarrhoea, bleeding mucosa and fever**

Withstands vacuum in foodstuffs for immediate consumption

GRAM-POSITIVE FOODBORNE PATHOGENIC BACTERIA

Staphylococcus spp.

immotile cocci, no spores, capsule, facultative anaerobes, **catalase +**

Aureus species: **coagulase +**, glucose & manitol fermentation, bacitlens, resists dessication and salinity

Diseases: suppurative with pus or toxin-caused, no suppurative

Transmission: h-h, h-food, animal-food

Pathogenesis: survives a lot in food, resistant to gastric acid, enzymes, 1ºC and is superantigenic
intoxication vs. toxinfection

**Gastroenteritis: diarrhoea & vomits**
**Enterocolitis: gastroenteritis + fever**

Rehydratation and maybe antibiotics

Isolation and culture of the bacteria in a laboratory → Toxin study

Bacillus spp.

Bacilli, facultative anaerobes, create endospores, **catalase +**

Cereus species: grows in starch rich or animal protein rich foods

**Emetic gastroenteritis:** nausea and vomits, no diarrhoea. Intoxication

**Diarrhoeic gastroenteritis:** diarrhoea and nausea, no vomits. Toxinfection

Treatment: self-limited. Lab isolation. Be careful with rice.

Clostridium spp

Bacilli, creates endospores, strict anaerobes, fermentation, **catalase and oxidase -**

Pathogenic capacities: Spores, speedy growth and powerful enterotoxins

Clostridium botulinum

big bacilli, peritrtic flagella, strict anaerobes, no capsule

Botulism → botulinum toxins (block muscle contraction)

Food-borne botulism: intoxication
Infant botulism: toxin+infection (ingestion of spores in honey)

Toxin: a monoprotease Zn dependent. It blocks the Acetylcholine signals in the neurons

**Weakness, vomits, blurred vision, constipation**

Ventilatory support, antitoxins, gastric lavages. Long recovery

Toxin detection in patient blood test

Don't eat swollen canned food; cook the food well, fridge, no honey to children

Clostridium perfringens

immotile Bacilli, B Haemolytic, histotoxic clostridia can cause myonecrosis

Toxinotypes: A, B, C, D, E
they all contain lethal toxins

Spore → germination → multiplication → ingestion → sporulation → enterotoxin

**Gastroenteritis or Necrotic enteritis: bleeding diarrhoea, vomits, ulcers and intestine perforations**

Clinical diagnosis,
rehydratation and antibiotics treatment

Hygiene. Cooking >60ºC / freezing -40ºC

# HYGIENE AND SANITARY ASPECTS OF FOOD

## OTHER FOODBORNE PATHOGENIC BACTERIA

### Campylobacter spp.

Gram-negative, highly motile, very prosaerophilic (microaerophiles)

jejuni species (birds, ↑frequency)
coli species (pigs, ↑frequency)

intest: **catalase and oxidase +**, mesophilic 42ºC>37ºC. Can cross membranes. Sensitive to salinity, freezing and ↓pH, can create biofilms.

Many virulence factors: flagella, capsule, enterotoxins, cytotoxic enzymes

**Gastroenteritis + bleeding diarrhoea**

Water contaminated by birds. More cases than Salmonella and Shigella together

Rehydratation and mineralisation, maybe antibiotics in serious cases

Guillain-Barré syndrome

### Plesiomonas shigelloides

Bacili gram –, no spores, motile, flagella, facultative anaerobe. **catalase and oxidase +**, and no lactose fermentation.

It's the exception in the enterobacteria family for the last 2 properties

**Gastroenteritis: watery diarrhoea (traveller's diarrhoea)**

High infective dose, transmission through raw seafood

### Listeria monocytogenes

bacili gram +, palisade aggregation, no spores, **catalase + and oxidase –**, facultative anaerobe, "umbrella" mobility, psychotrof and halophile. biofilms

Many virulence factors make it resistant, can enter cells if it wants and can survive the stomach acid (glutamate decarboxylase)

Low infective dose

**Febrile gastroenteritis (microvilli damage) invasive systemic dis. (brain and placenta) Abortion and neonatal listeriosis**

raw dairy, fish and meat and sprouts
Cooking 75ºC, hygiene, cross-contamination

### Aeromonas spp.

bacili gram –, psychrotrof, facultative anaerobe, motile with polar flagella no halophile, prot-, biofilms

Contaminated water or food, can enter the body orally or throug a wound

Many virulence factors but high infective dose

Cytotoxins: create pores in the plasmatic membrane
Cytotoxic enterotoxins: pull water out of the cell

**Gastroenteritis: nausea, vomits, bleeding diarrhoea. Septicaemia**

It's a flesh-eating. It will eat the wound down to reach the connective muscle tissue

### Brucella spp.

coccobacillus gram –, intercide, neutral pH, strict anaerobic. **catalase and oxidase +**

Only on high nutritious food: milk and meat. Zoonosis

low infective dose <500 cells.
Oral, contact, air and parenteral transmission

Macrophages carry the bacteria as a trojan horse to the lymph nodes.
Can generate **granulosomes**.

Raw milk from sick cows or goats. Animal vaccination and milk pasteurisation

## VIRUSES AND PRIONS

### VIRUS

#### Hepatitis

Hepatitis A: picornavirus enteric transmission, very resistant (pH 1, 60ºC, salty water, months)

Intestine → enterocyte → blood (viraemia) → liver → hepatocytes (internal multiplication) → T cells attack hepatocytes to kill internal virus → bile sends virus back to gut → exit in faeces

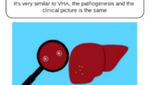
Fever, malaise, loss of appetite and nausea

Bivalve molluscs, contaminated water (veggies). Hygiene, cooking/freezing and vaccine

low infective dose <500 cells.
Oral, contact, air and parenteral transmission

Hepatitis E: hepevirus enteric transmission. can be dangerous for pregnant women, leads to hepatic failure due to less active immune system

It's very similar to VHA, the pathogenesis and the clinical picture is the same



#### Viral gastroenteritis

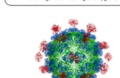
Rotavirus, Norovirus, Sapovirus, Enteric Adenovirus (DNA virus) Astrovirus. All enteric transmitted

PCR with reverse transcriptase to detect RNA viruses

Enterocyte invasion and destruction → malabsorption → accumulation of carbs and Na+ in the lumen → water exits to equal concentrations → diarrhoea

Neurotransmitters also are affected → signal to the brain → vagus nerve → vomiting

Raw molluscs, fruits and veggies. Very resistant: well cooking, hand washing and hygiene

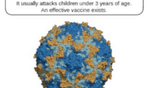


#### Polio

Human the only natural host of poliovirus.
h-h transmission nasopharyngeal or enteric.

It grow in the nasopharyngeal mucosa and then attacks the Central Nervous System, motor neurons of the spinal cord → irreversible paralysis

It usually attacks children under 3 years of age. An effective vaccine exists



### Prions

Misfolded proteins, incubation period between 20 and 30 years

They create damage to the CNS, developing subacute spongiform encephalopathy (fatal)

Transmission through contaminated food, hereditay or by a spontaneous mutation

Mad Cow disease is caused by a prion.
Prions are rich in β-sheets, they're insoluble and they aggregate themselves

Intestine → peyer plates → lymphatic system → brain → union to normal proteins → conversion to prions → aggregation creating fibrils → fibrils inactivate neurons

Gradually loss of muscle control, coordination, increased depression, insomnia → death after 1 year

Prions can be found in meat products with a lot of nerves such as the brain, eyes, spinal cord of infected animals.

Prevention: control on the farm, in the slaughterhouse and in the meat industry. Resistant to formaldehide, UV, 80ºC - Autoclave 134ºC

## MICROBIOLOGICAL MONITORING OF FOODSTUFFS

### Microbiological criteria

#### Safety: bacteria and toxins

If it fails this criteria → remove from the market (not edible, not safe)

#### Contents

Found Microorganism

Method used

n² of samples

m.b. limit on that food

n² of analytical units

Analysed food

In the market / factory

Actions if not complies

#### Quality (hygiene of the processes): applies to the product at the factory

If it fails this criteria → check production processes and improve them

#### How to take samples

Representative of the whole lot, identifiable.

Protected from contamination, appropriate temperature

Analysis within 36 hours

Finished product: original sealed container or in bulk → sterile container

Production line: a little bit all over the place

Storage: non-perishable → T ambient, refrigerated → 0-4ºC and frozen → -20ºC

## CONVENTIONAL METHODS OF MICROBIAL IDENTIFICATION

### Immunological methods

### Conventional or classical methods

1. Obtain pure culture of the micro-organism
2. Identify colonies
3. Observe morphology using Gram stain
4. Indentification tests

Counting → Direct Isolation
Detect → Enrichment: primary (reactivation) and secondary (selection)

Primary tests detect taxonomic gender: **catalase** (H2O2 → bubbles), **oxidase** (reactive → oxidised=purple, reduced=clear), **coagulase** (plasma → coagulate+white dots)

Secondary and tertiary tests detect species: lactose fermentation, Methyl Red and Voges Proskauer, decarboxylation of aa's, citrate as carbon source.

Short time

Long time 24 hours → API multi-test systems

### Genetic methods and others

FERMENTED FOODS

INTRO

Microorganisms are used for creating fermented food with or without jurrganisms

Affected by: T, pH, inhibitors, food composition, precursors, [$O_2$ & $CO_2$], system operation, mixing

Fermentation: improves, preserves, adds nutrients, detox from antinutrients, less cooking time

Starter cultures: natural, mixed or from defined regions

types: alcoholic, lactic, acetic or alkaline

Probiotics: living jorganisms that improve host's health

Prebiotics: non-digestible component that is "food" for the microbiota

Increase epithelial barrier and the union to mucosa, block pathogens, produce antimicrobial substances and improve immune system

FOS and GOS, Inulin and Resistant starches, Microbiota growth and improve immune system

FERMENTED MEATS

Used to preserve and to add aromas

Little carbs in meat are fermentad → Problems: no fermentation or inconsistent

Microbiota

Impact on the meat

Ham: ¡¡coccaceae, staphylococcus, rmoulds & yeasts

Sausages: LAB (L.sakei), staphylococci (ievm inside)

Starter Cultures

Proteases → sweet and umami taste
LAB → acid, bacteriocins and peptidases
Staphylococci → nitratoreductase, catalase, aa's
Superficial microbiota → lipases, proteases and mitotoxins

Flavours: proteases and lipases
Odors, lipases, lipoxidases, volatile aa's

FERMENTED FISH

Fish has higher pH than meats and deepest fishes have a lot of Urea and Trimethylamide

Union of fish with cooked rice or maize, sucrose, salt and garlic
→
Fermentation at 24ºC or 30ºC

Solid, semi-solid and liquid fermentation.
Based on the final look, methods, substrate, quantity of salt

Hákarl (Iceland): Shark → LAB
Rastrisk (Norway): Salmon → L.sakei
Surströmming (Sweden): Harangue → Haloanerobium
Fish sauce: tetragenococcus
Narezushi: typical fermentation

FERMENTED DAIRY

Starter cultures in LAB

Sugars: homolactic, heterolactic fermentation or heterofermentation

Proteins: proteolysis to create aromas and flavours

Lipids: create organoleptic properties

Production of bacteriocins, Bacteriophage resistance, Autolysis

Types of fermentation

Mesophilic (Buttermilk): aroma/acid balance

Thermophilic (Yoghurt): grows fast, lactose fermentation, pH 5, anaerobic conditions

Therapeutic (acidified milk): with sterilized milk + L.acidophilus

With Yeast (Kefir): kefir grains with a lot of jorganisms variety. Create ethanol and polisaccharides

With Moulds (Vlli): ↑Viscosity, texture and characteristic flavour. Geotrichum candidum + LAB

Cheese production

High varieties, different jurganisms

1. Selection of the milk

2. Selection of the Starter culture

3. Milk to Cheese: Acidification (4 methods) → Coagulation (rennet) → Aromas and flavours (maturation)

FERMENTED DRINKS

Wine

Starter cultures with *Saccharomyces ellipsoideus* Alcoholic Fermentation

Brettanomyces bruxellensis → Brett aroma

Malolactic fermentation for Crianza wines

Oenococcus oeni, decarboxylation, less acidity, ↑pH → Stability

Beer

1. Malting of Barley
2. Mashing

3. Boil the wort and add the hops

4. Fermentation with *Saccharomyces cerevisiae* (Ale) or *S.uvarum* (Lager)

5. Treatment post-fermentation

Vinegar

2 ways: **with an Aerobic part** (alcoholic and then acetic) and **completely Anaerobic** (*Clostridium thermoaceticum*: carbs → acetic acid)

Orleans Method (let alone). Spontaneous wine acetic fermentation

Drip Method (Fast German method) Vine branches + acetic bacteria

Frings Actuator, tank with agitator

FERMENTED VEGETABLES

Grains fermentation is the most common fermented product on the market

Bread → Long or Short alcoholic fermentation → oven to cook and ethanol evaporates

Fermented veggies: sauerkraut with LAB, olives, kimichi

Fermented Soy: tempeh, miso and soy sauce. Natto and Pidan are the only alkaline fermentations

Fermented Coffee: wet or dry. Cocoa: directly below the leaves after recollection