Environmental contamination

Focus on microbiological aspects in sugar products

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Agenda

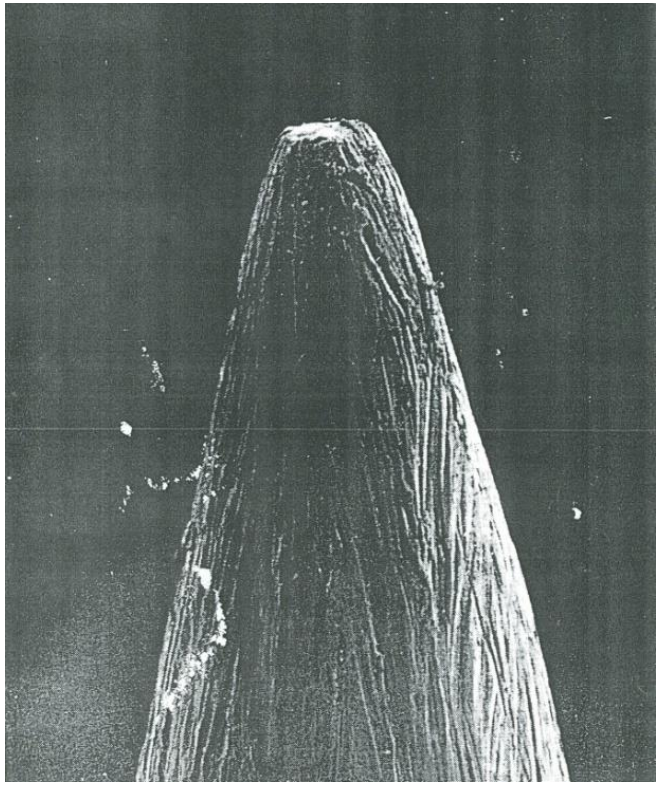
1. Microorganisms responsible for environmental contamination
2. Basic properties of sugar products in relation to contamination
3. Environmental contamination in a sugar factory
4. Typical sugar related microbial contaminants from environmental sources

What are we dealing with?

We are dealing with something we cannot see, smell, hear or touch!

Pin (needle)

1000x magnification



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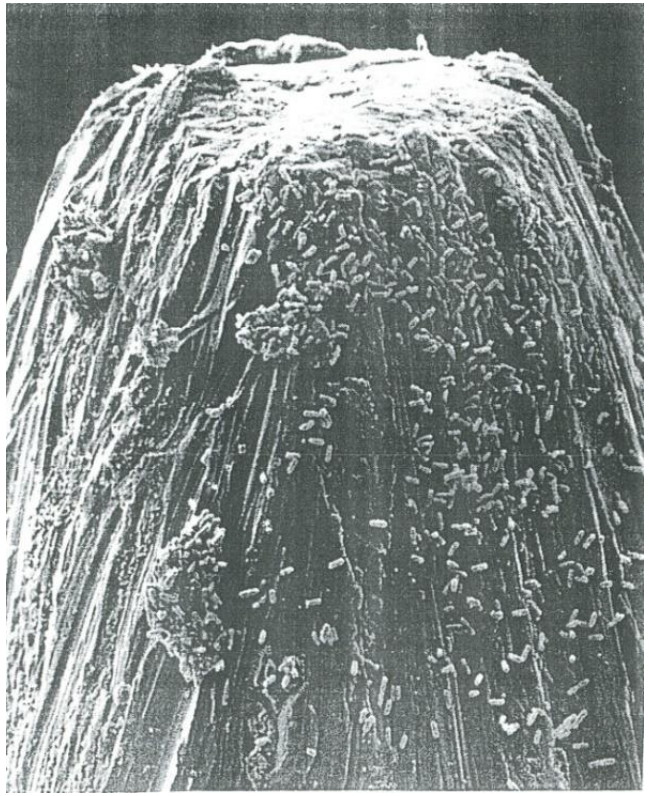

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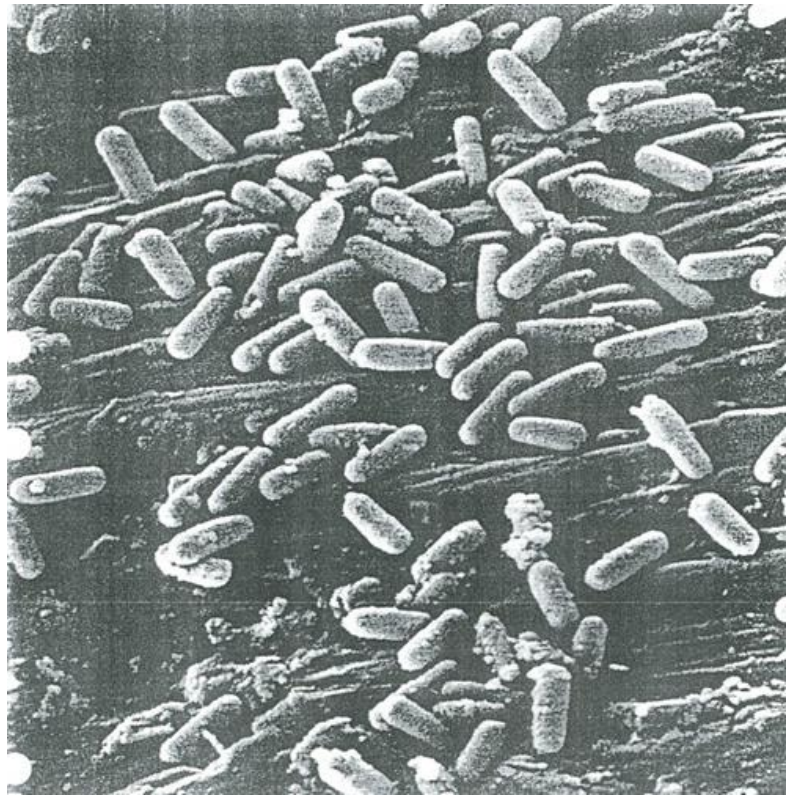
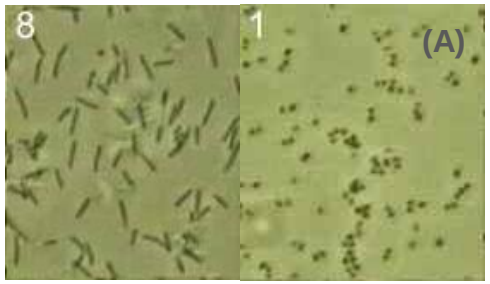
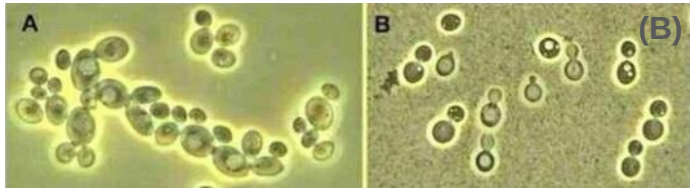


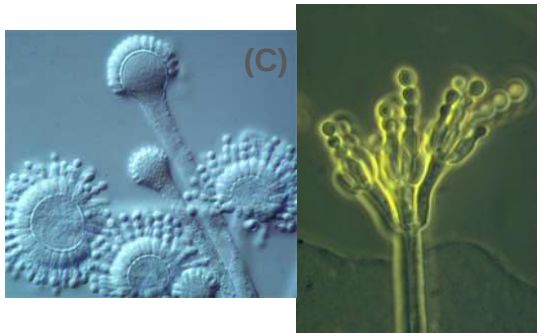
Introduction cont.

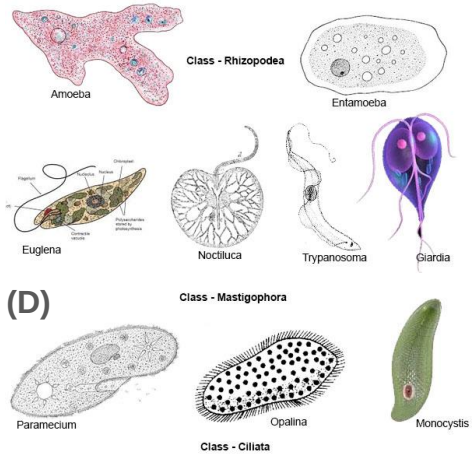
Environmental microorganisms

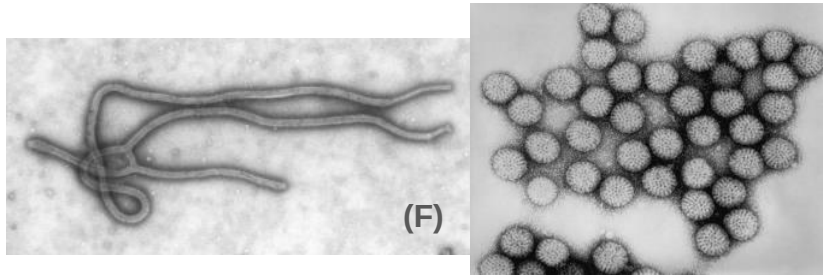
- Microorganisms are living organisms that are so small they must be viewed with a microscope
- Bacteria (A)
- Yeasts (B)
- Moulds (C)
- Protozoa (D)
- Algae (E)
- Virus (F)







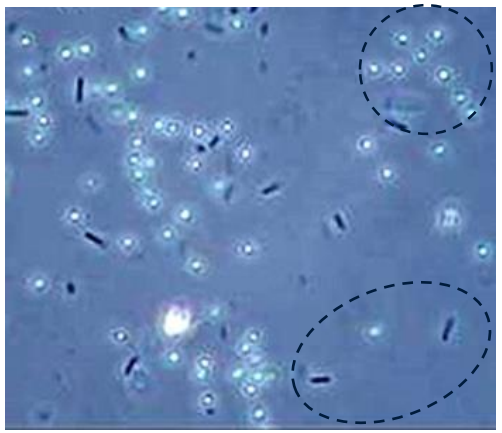




Inactive spores are the main enemy in sugar

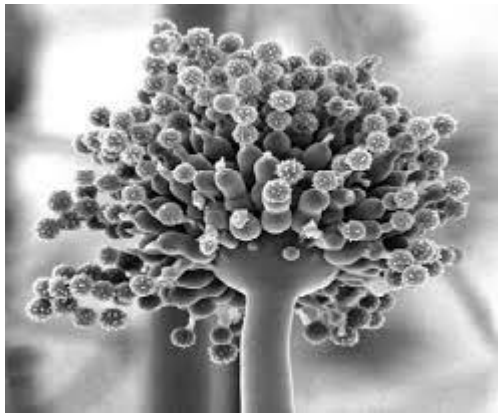
Environmental contamination of inactive spores (compare to plant seeds)

- **Bacterial spores**
  - Can survive extreme environmental conditions (bacterial spores)
  - Drying, freezing, boiling, cleaning chemicals, extreme pH, UV-light
  - *Bacillus subtilis* in bread (ropy bread) and *Bacillus cereus* in milk (sour milk)
  - *Clostridium butyricum/tyrobutyricum* in cheese (exploding cheese)
- **Mould spores**
  - Not so tolerant to environmental conditions
  - Will survive for long periods in granulated sugar products
  - Heat Resistant Moulds (HRM) is a new problem (more below)



Spores
Bacteria

Growing/Active
Bacteria

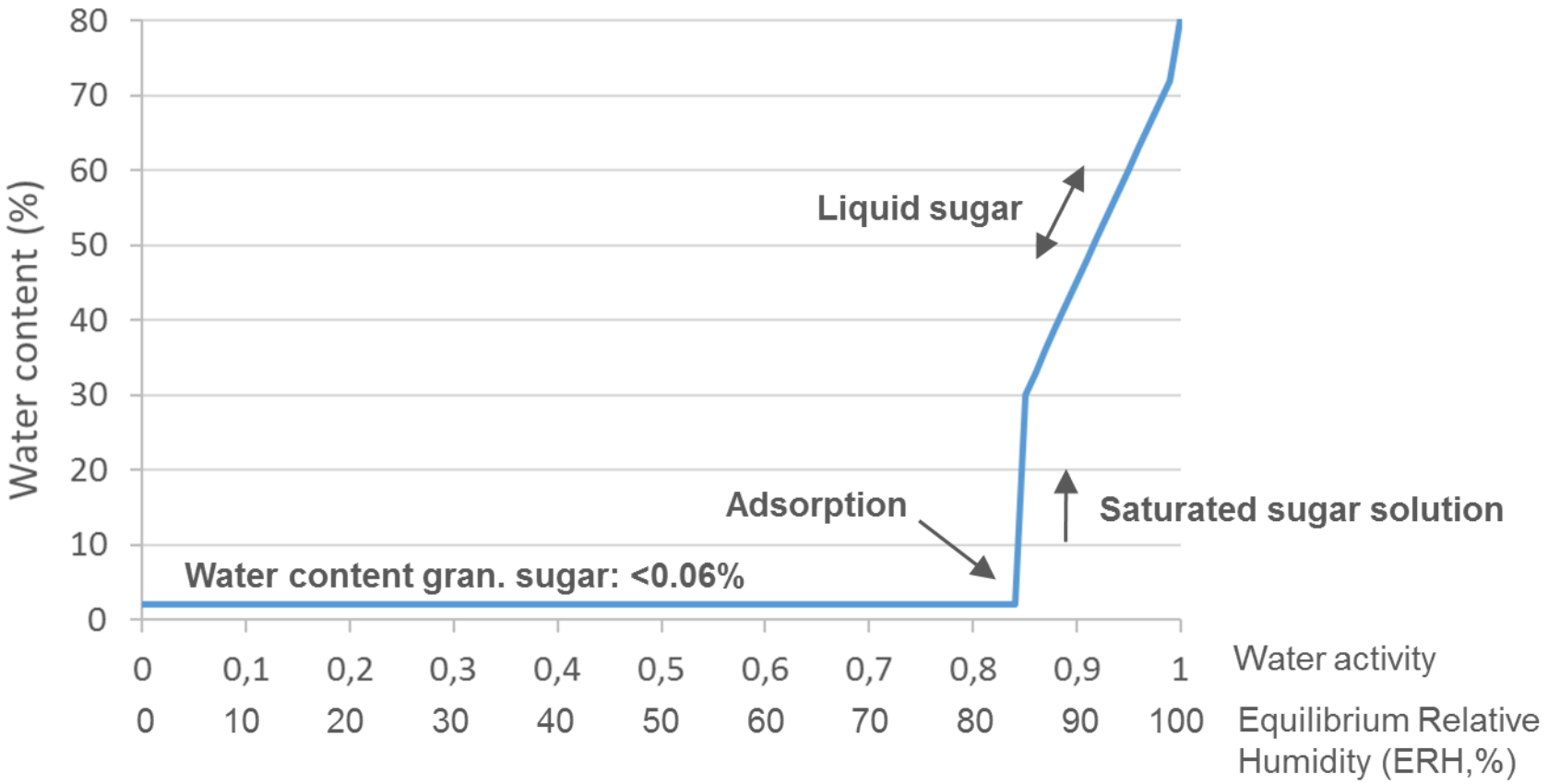


Mould spores

Basic properties

Sorption isotherm (uptake) of granulated sugar

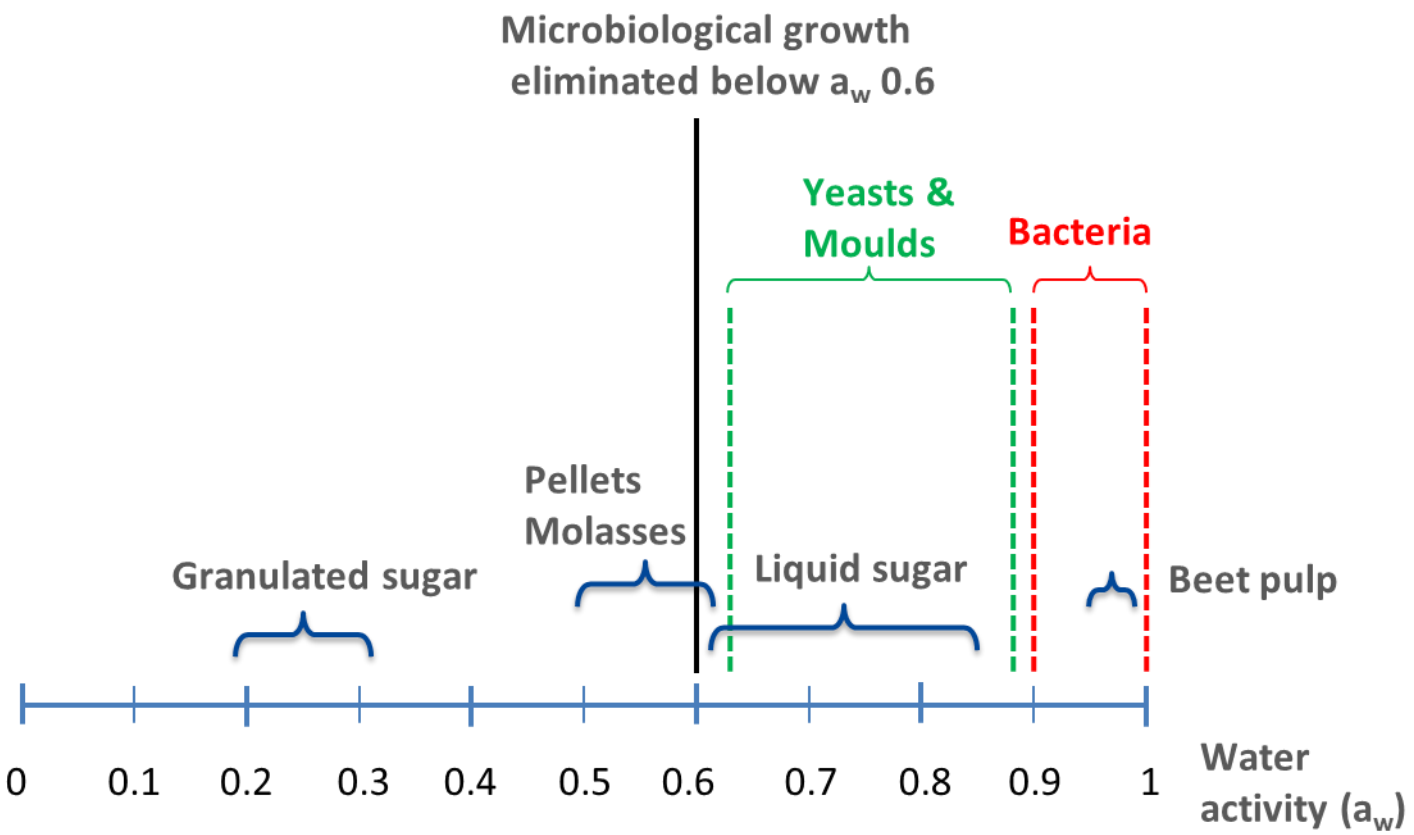
- Sorption isotherm describes water uptake profile of sugar
- Critical point at approx. 85% ERH
- Granulated sugar products most vulnerable at this point and above
- Environmental contamination can give uncontrollable conditions



Basic properties cont.

Water activity (a_w) of sugar and feed products

- **Water activity**
  - Free water available for survival and growth of microorganisms
- **Water content (%)**
  - Total amount of chemically bound and free water
- **Normal factory conditions**
  - Gran. sugar are safe/stable products
  - Feed products are safe/stable products
  - Liquid sugar: Sensitive to yeasts and moulds
  - Beet pulp: Unstable products

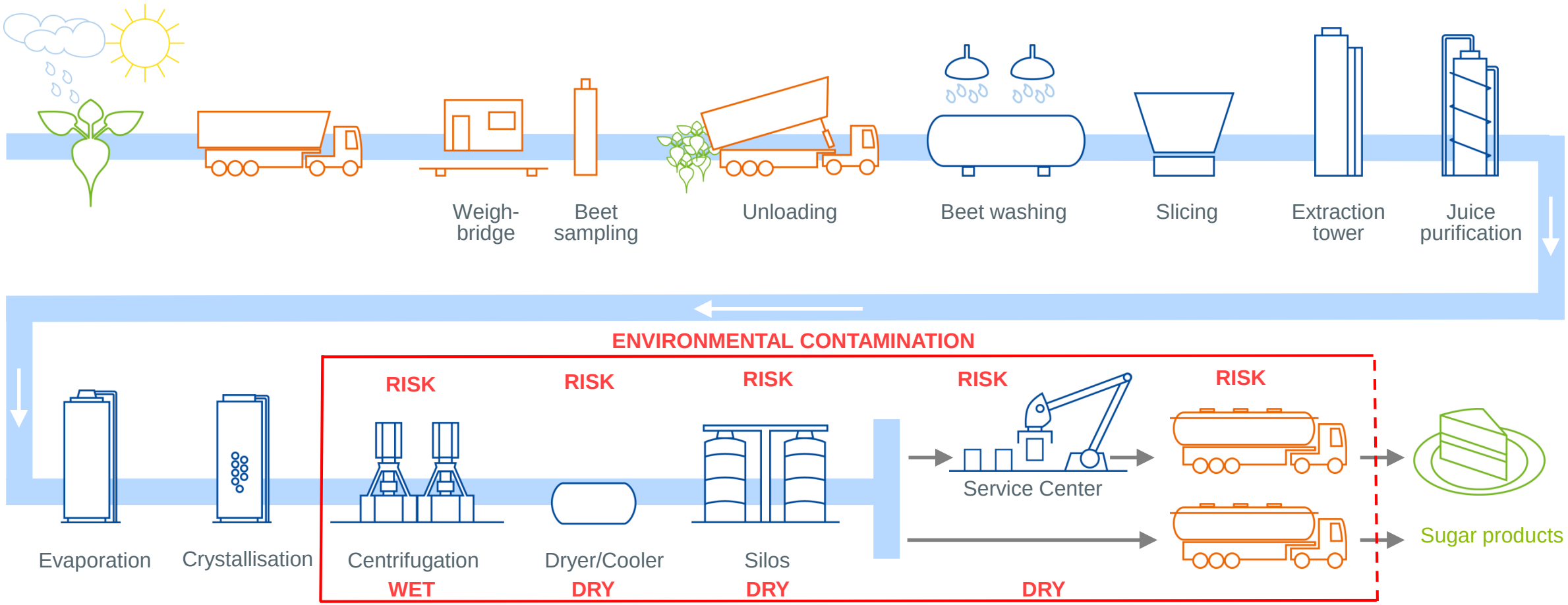


What is environmental contamination?

- Environmental contamination is the introduction of water, air, microorganisms, chemicals, toxic substances, wastes or waste water in a concentration that makes the product unfit for its next intended use.
- Unplanned introduction of microorganisms into food raw materials or food products
- Main environmental contamination routes are air, surfaces and human contact (skin and clothing)

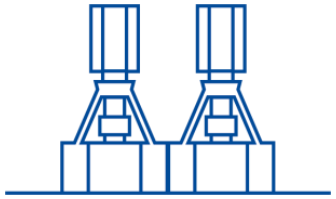
Environmental contamination at sugar factory

Where do we have high contamination risks?



Centrifugation and wet sugar transport

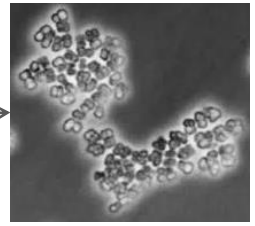
Important things to keep in mind!



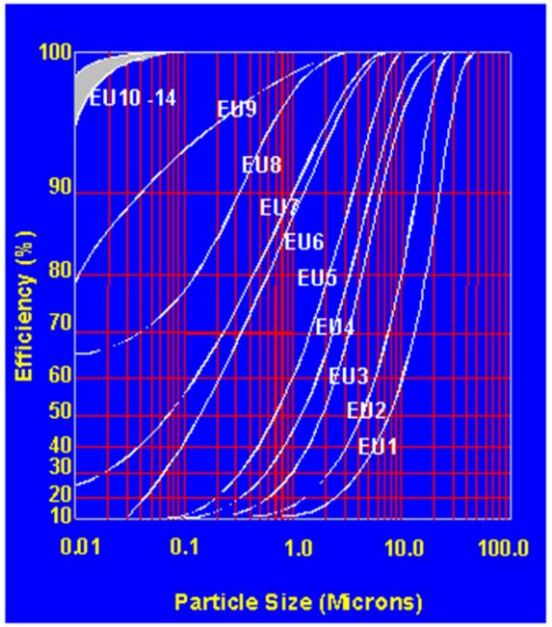
- **Centrifugation step**
  - Dry outside centrifuge environment = no dirty water on the outside centrifuge surfaces
  - Optimal cleaning of inside centrifuge basket at a regular frequency
  - No massecuite leakage through the centrifuges into the white sugar
  - Repair leaking centrifuges, dirty water must not drip into the white sugar
  - Drinking water or condensates for final sugar wash
  - Avoid opening centrifuges just to look, only when doing maintenance and repairs!
- **Transport (elevators and conveyor belts)**
  - Enclosed transport belts from centrifuges to sugar drier are the best solution
  - High possibility for environmental contamination of inactive bacterial spores in an open conveyor belt
  - Elevators for wet sugar are a high risk zone
  - Sugar will stick to the walls and the bottom part of the elevator
  - Growth of microorganisms are common and will give a negative effect in silo sugar
  - Regular campaign cleaning of elevators is an advantage

Sugar drying/cooling and silo storage

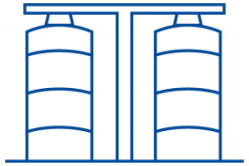
- **Air filtering**
  - Significant air volumes in drying/cooling and in silos → Air filtering is critical
  - High risk of environmental (microbiological) contamination
  - Air filter grade should be assessed carefully (see blue image)
  - Typical size of bacterial spore is approx. 1 µm
  - In most environments microorganisms are aggregated
  - Typical size fraction target is 10 µm and above
  - Air filter selected to filter out a size fraction in the range of 1 to 10 µm
  - Air filters must used in sugar drying/cooling and in silo conditioning system



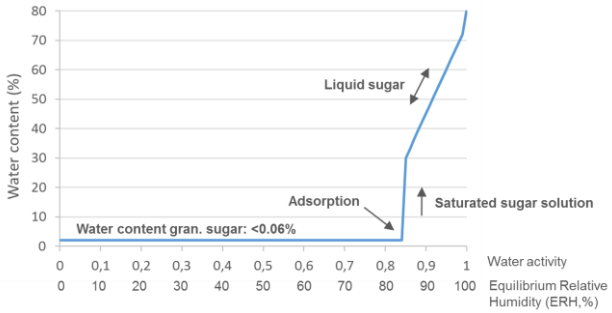
Aggregate of bacteria in air



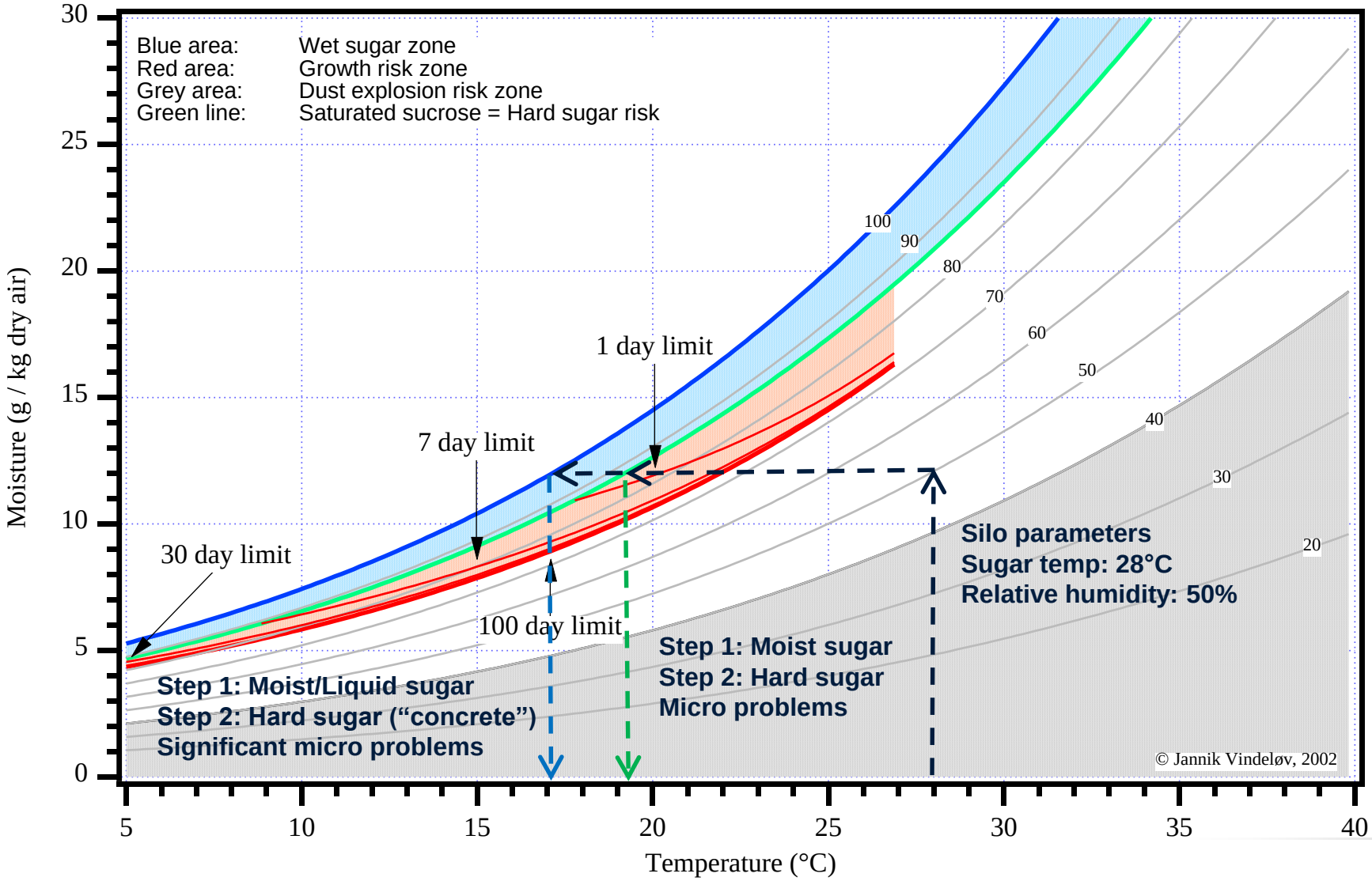
Silo storage



- **Silo conditioning system**
  - Silo conditioning is critical for long term storage of sugar
  - Moulds and yeasts can cause significant contamination
  - Inactive spores will contaminate sugar from air if air filter not correct/broken/dirty
  - Water condensation can in cold winters cause micro problems and hard sugar formation
  - Approx. 85% relative humidity → moist sugar → micro problem
  - At 100% relative humidity → water condensation → significant micro problems
  - Water condensation on silo walls due to large temperature difference
  - Silo wall temperature is an important parameter
  - Correct temperature of the silo wall will reduce problems significantly



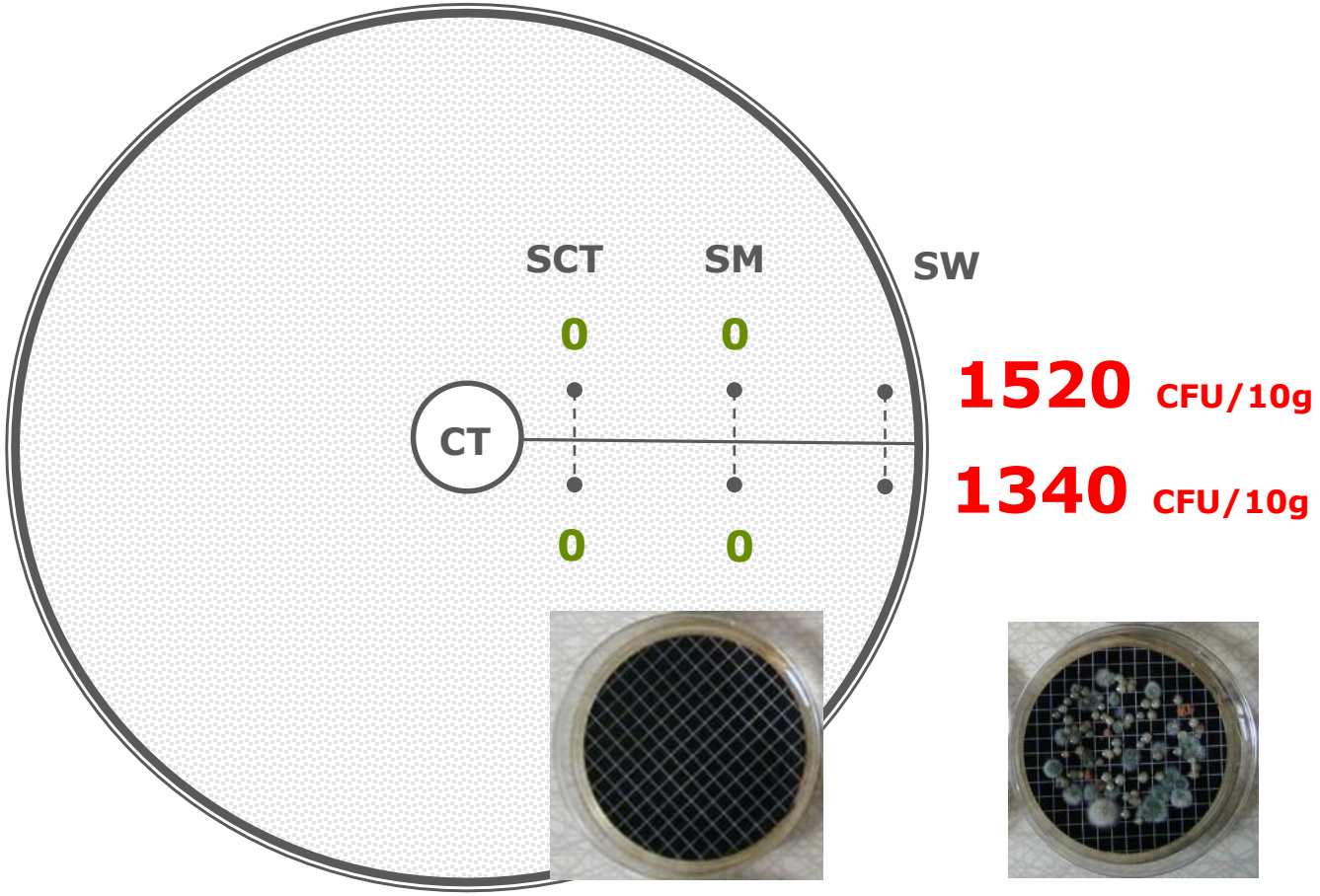
Silo chart to assess risk for micro contamination



Results of environmental contamination

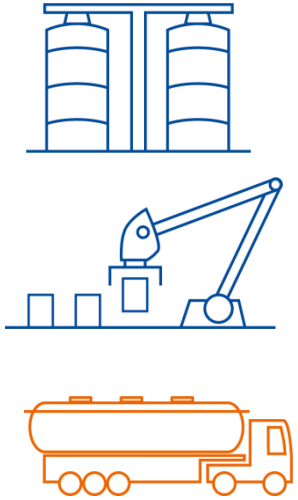
Moulds growing in silo sugar!

- Sugar at silo wall contaminated with moulds
- Not the entire silo bulk
  - CT = Central Tower
  - SCT = Silo Central Tower
  - SM = Silo Middle
  - SW = Silo Wall



Service centers and packing/loading

- **Sevice center and packing/loading**
  - Significant probability for environmental/microbiological contamination (air, surface and human)
  - Advantage is dry sugar with low water activity, which reduce the problems
  - Avoid open transport of sugar
  - Consider filtration of service center air (outside air into the plant)
  - Wet cleaning only on a need to do basis, must dry completely before start
  - Clean clothing and equipment
  - Maintenance staff must wear disposable protective clothing
  - Minimise or eliminate human contact to sugar
  - Correct sampling procedures is critical due potential human contamination
  - Transport vehicle hygiene is critical especially for long distance transport in winter time
  - Specific problem is filling of containers such as bag in box or direct filling



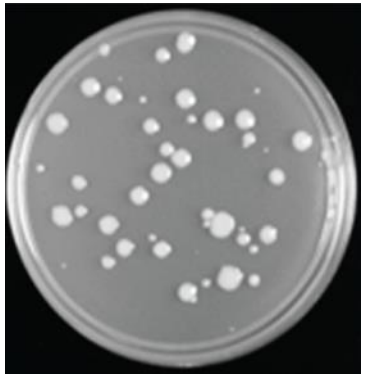
Pathogenic (disease causing) bacteria

- **Not a problem in sugar products**
  - Sugar products do not contain pathogenic non spore forming pathogenic microorganisms such *Salmonella* sp and *E. coli*
  - Pathogenic bacteria have a water activity requirement in the range 0.92 to 0.95.
  - Water activity of sugar product generally below 0.9
  - The factory processing conditions (high temperatures and variable pH) in combination with low water activity of sugar products.
  - Low water activity of sugar products will cause osmotic chock
  - If we have accidental contamination pathogenic bacteria will eliminated completely within in hours or days (weeks if high contamination and a moisture problem)
  - Annual monitoring either internally or by external microbiological laboratory is sufficient

Thermophilic Acidophilic Bacteria

Alicyclobacillus bacteria

- **TAB characteristics**
  - Soil bacteria
  - Fruit and berry and concentrates contamination
  - Sugar products suspected to a source
  - Temperature range: 20 to 65°C
  - Optimum temperature: 35 to 55°C
  - pH range: 1.5 to 6.5
  - Optimum pH: 3.0 to 5.0
  - Water activity minimum: 0.97 (ca 20% sugar)
  - Heat resistant spores
  - $D_{90°C}$ 10 to 60 min
  - *Alicyclobacillus acidoterrestris*

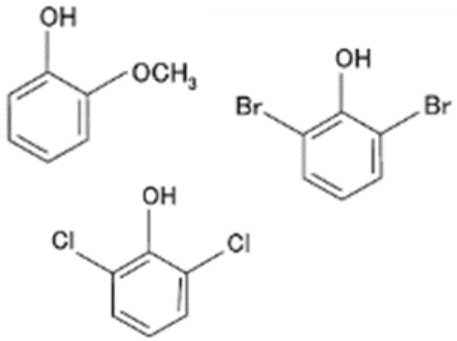


A. acidiphilus *(spoilage)*
A. acidocaldarius *(spoilage)*
A. acidoterrestris *(spoilage)*
A. aeris
A. cellulosilyticus
A. consociatus
A. contaminans *(spoilage)*
A. cycloheptanicus *(spoilage)*
A. dauci
A. disulfidooxidans
A. fastidiosus
A. ferrooxydans
A. herbarius *(spoilage)*
A. hesperidum
A. kakegawensis
A. macrosporangiidus
A. pohliae
A. pomorum *(spoilage)*
A. sacchari *(liquid sugar)*
A. sendaiensis
A. shizuokaensis
A. tengchongensis
A. tolerans
A. vulcanalis

Thermophilic Acidophilic Bacteria cont.

Spoilage problems in beverage products

- **Spoilage**
  - Characterized by production of potent taint compounds
  - Guaiacol (methoxyphenol), bromophenols and chlorophenols
  - Flavor profile: “phenolic”, “medicinal”, “disinfectant-like” and “smoky”
  - Guaiacol is one main component of smoky/peaty single malt whisky
  - TAB are now recorded as Guaiacol Producing TAB (GP-TAB)
- **Characteristics of spoilage**
  - No gas production i.e. no swelling of bottles
  - Acid can be produced, but low pH normal in beverages
  - Light sediment/cloudiness can be observed occasionally
  - Spoilage often apparent only at the time of consumption
  - Can survive beverage pasteurization (30-60 s for 80-95°C)
  - Identified as a critical microbiological hazard for the beverage industry



Guaiacol, main flavor component of smoky/ peaty single malt whisky

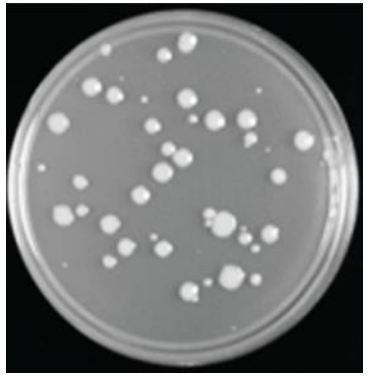
Thermophilic Acidophilic Bacteria cont.

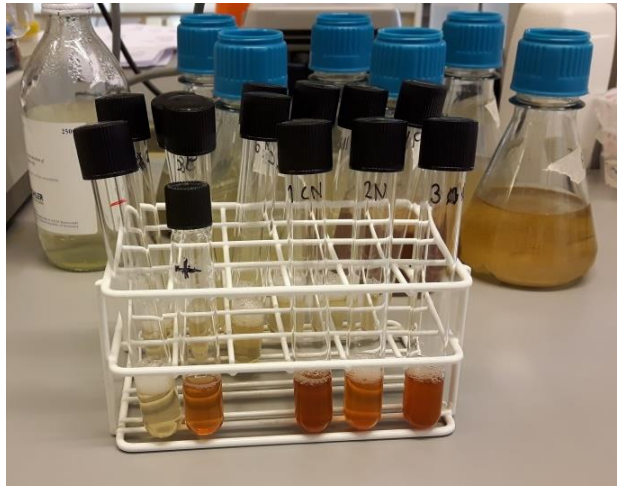
Methods and proficiency testing (ring test)

- Microbiological method
  - Published as ICUMSA GS2/3-50 (2017)
  - Isolation of TAB on BAT-agar
  - Biochemical verification of GP-TAB
- Proficiency testing (ring testing)
  - Available from UK based LGC Standards in collaboration with ICUMSA
  - Micro SUPS program
  - Recommended for all factories supplying beverage customers

ICUMSA

Method GS2/3-50 (2017)
The Determination of Thermophilic Acidophilic Bacteria (TAB) and Guaiacol Producing TAB (GP-TAB) in Sugar Products – Accepted

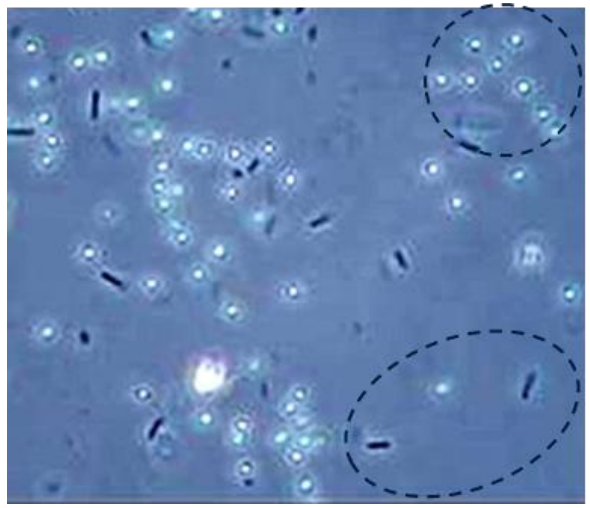




Sporolactobacillus sp

The Perfect Enemy!

- ***Sporolactobacillus* sp**
  - Spore forming bacteria that produces lactic acid
  - Considered as a spoilage organism in soft drinks and fruit juices.
  - Spoilage is based on production of D-lactic acid from sugars
  - High resistance to preservatives such as sorbate and nitrite
  - Grows 15 to 20°C up to 40°C, optimum 30°C
  - Growth between pH 3.5 and 5,5, optimum 4.5
  - Spore heat resistance $D_{90°C}$ in the range of 1 to 8 minutes
  - Can survive beverage pasteurization (30-60 s for 80-95°C)
  - Raw material control is essential to prevent spoilage in foods and beverages
  - Currently not a focus area for the food and beverage industry, but can change quickly

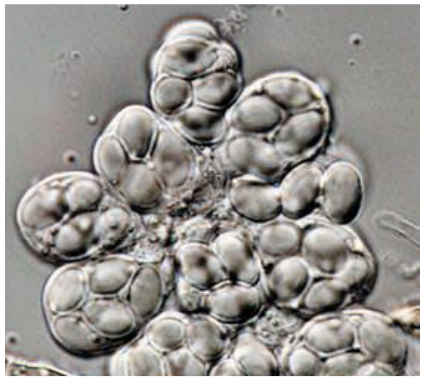


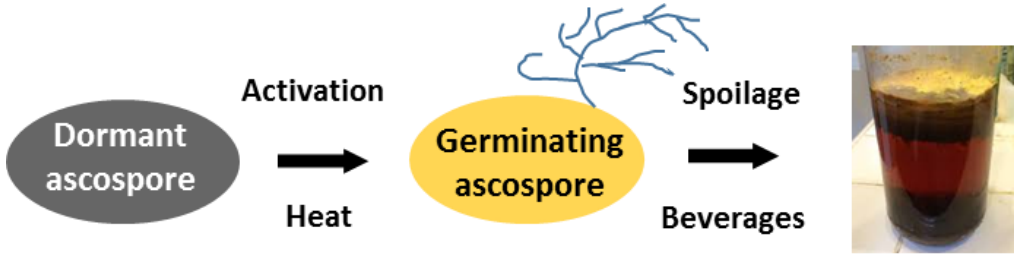
Sporolactobacillus sp cont.

- **Microbiological method**
  - Can be isolated on PDA, GYP, MRS agar medium
  - Modified specific media has been published
  - Agar pH 4.5 and 5.5 with acetic acid
  - Heat treatment of sample 80°C for 5 to 10 min
  - Incubated microaerophilic (ca 5% CO_2) or anaerobic
  - Incubation temperature 35 to 37°C
  - Results as presumptive *Sporolactobacillus*
  - Need biochemical characterization
    - Gram staining, oxidase, catalase, nitrate reduction spore check, cell mobility

Heat Resistant Moulds (HRM)

- **Heat Resistant Moulds (HRM)**
  - HRM can be isolated from all types of cultivated soils.
  - Food sources: cereals, starch, guar, fruit raw material, fruit concentrates, sugar products, liquid sweeteners
  - Currently focus on HRM by beverage customers
  - Customer specifications generally <1 CFU per 50 or 100 gram
  - Ascospores are most heat resistant spores of filamentous fungi
  - Can survive beverage pasteurization (30-60 s for 80-95°C)
  - *Byssochlamys, Neosartorya, Eupenicillium, Talaromyces, Eurotium* and *Paecilomyces*
  - Heat activation leads to active growth and to spoilage in products
  - Grows at low oxygen pressure (low oxygen content)
  - Probable HRM contamination level is 1 to 2% in sugar products





Heat Resistant Moulds (HRM) cont.

- **Microbiological methods**
  - Can be isolated on MEA or PDA
  - Chloramphenicol 100 µg/mL to eliminate bacteria
  - Agar pH 4.5 or 5.5
  - Sample heat treatment 75°C for 30 min
  - Aerobic incubation
  - Incubation temperature 30°C
  - Incubation time: 30 days (not a fast method).
  - Can be identified to genus and species using phase contrast microscope and HRM key document.

Typical heat resistant moulds

Thank you for your attention

Nordzucker