Occurrence of Heat-resistant Mold Ascospores in the Beverage Processing Environment: Assessment, Prevention and Elimination

Emilia Rico, Ph.D.
BCN Research Laboratories, Inc.
Rockford, TN
Emilia.Rico@bcnlabs.com

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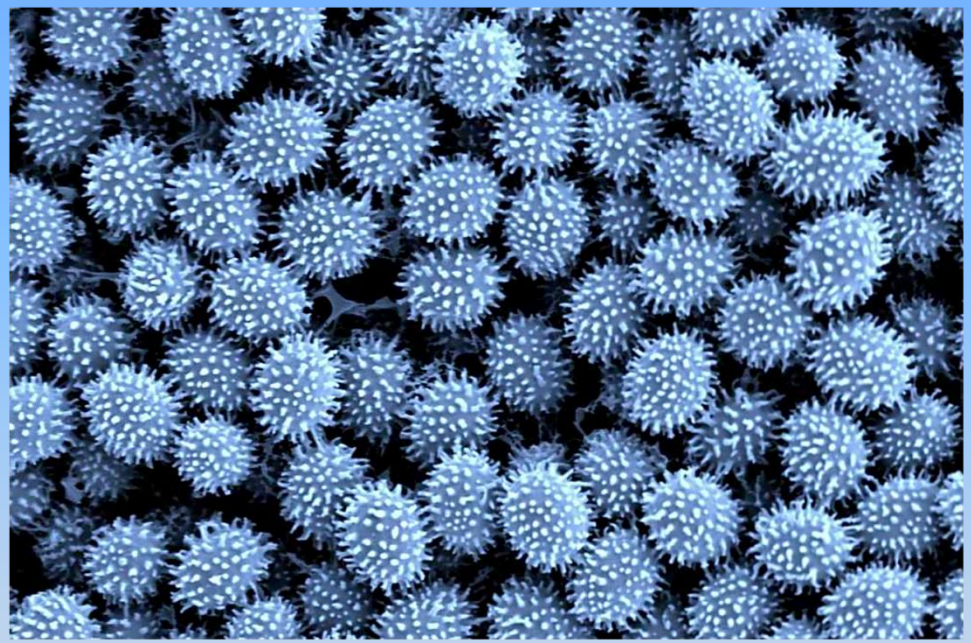
Agenda

- HRM life cycle
- Ascospore activation
- Sources of HRM ascospores
- Objectives – Part I and Part II
- Part I
  - Methodology
  - Results
- Part II
  - Methodology
  - Results
- Prevention of contamination
- Conclusions



Talaromyces macrosporus ascospores (courtesy of Rob Samson, CBS, the Netherlands)

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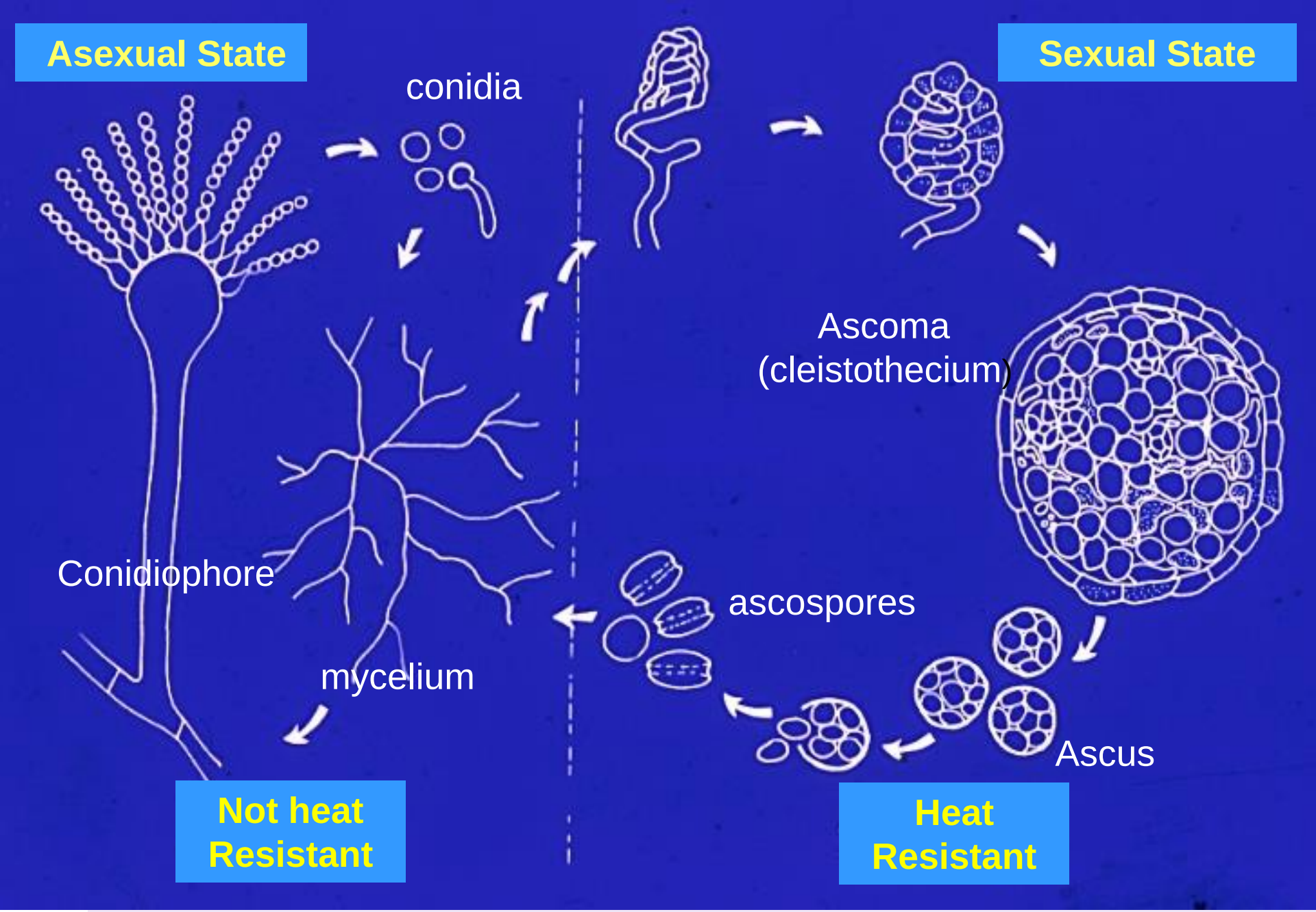


Life Cycle of HRM

Courtesy of Rob Samson, CBS, Utrecht, NH

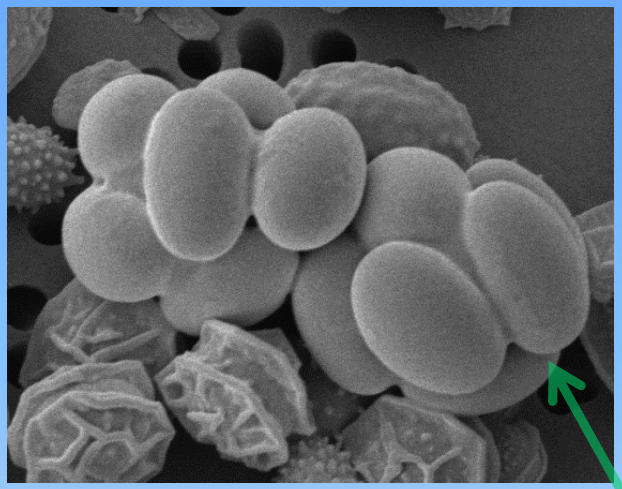
Ascospore Activation

Ascospores need to be activated to be able to germinate

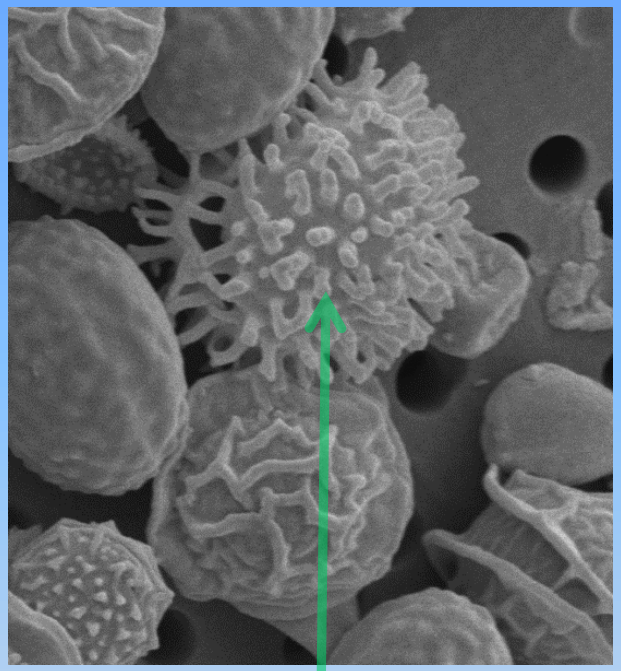
Ascospores can be activated by the heating during pasteurization, hot filling or baking

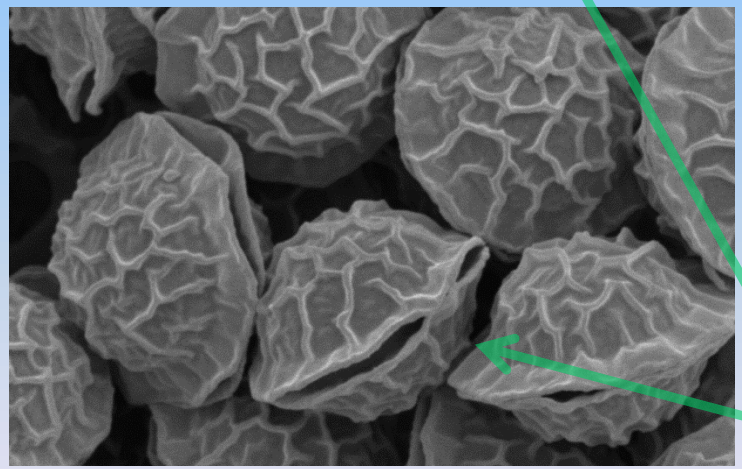
After germination, they can grow and spoil the product during storage at room temperature or somewhat higher

ASCOSPORES



Ascospores are produced by different members of the order **Eurotiales**

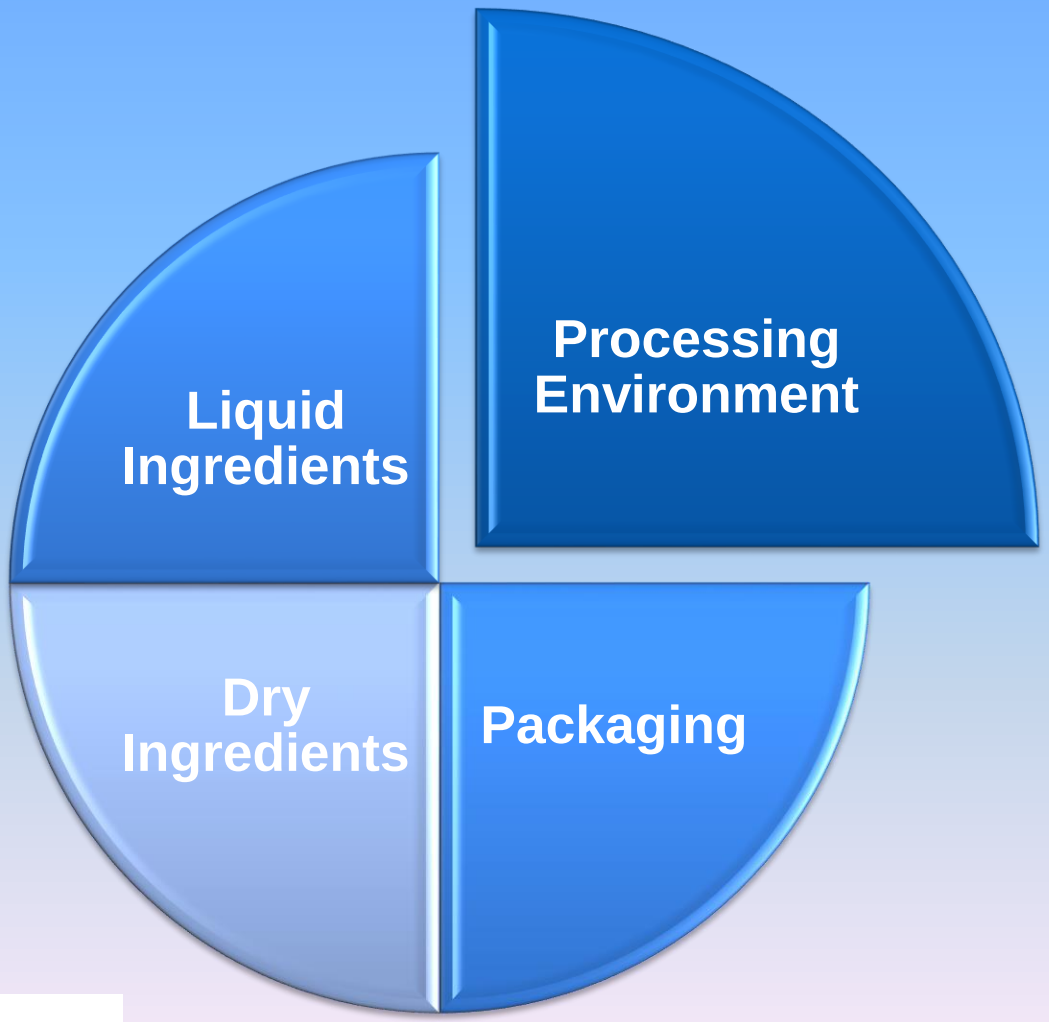




Talaromyces macrosporus
Byssochlamys nivea and *spectabilis*
Neosartorya fischeri

Courtesy of Jan Dijksterhuis, CBS, Utrecht, NH

Sources of HRM Ascospores



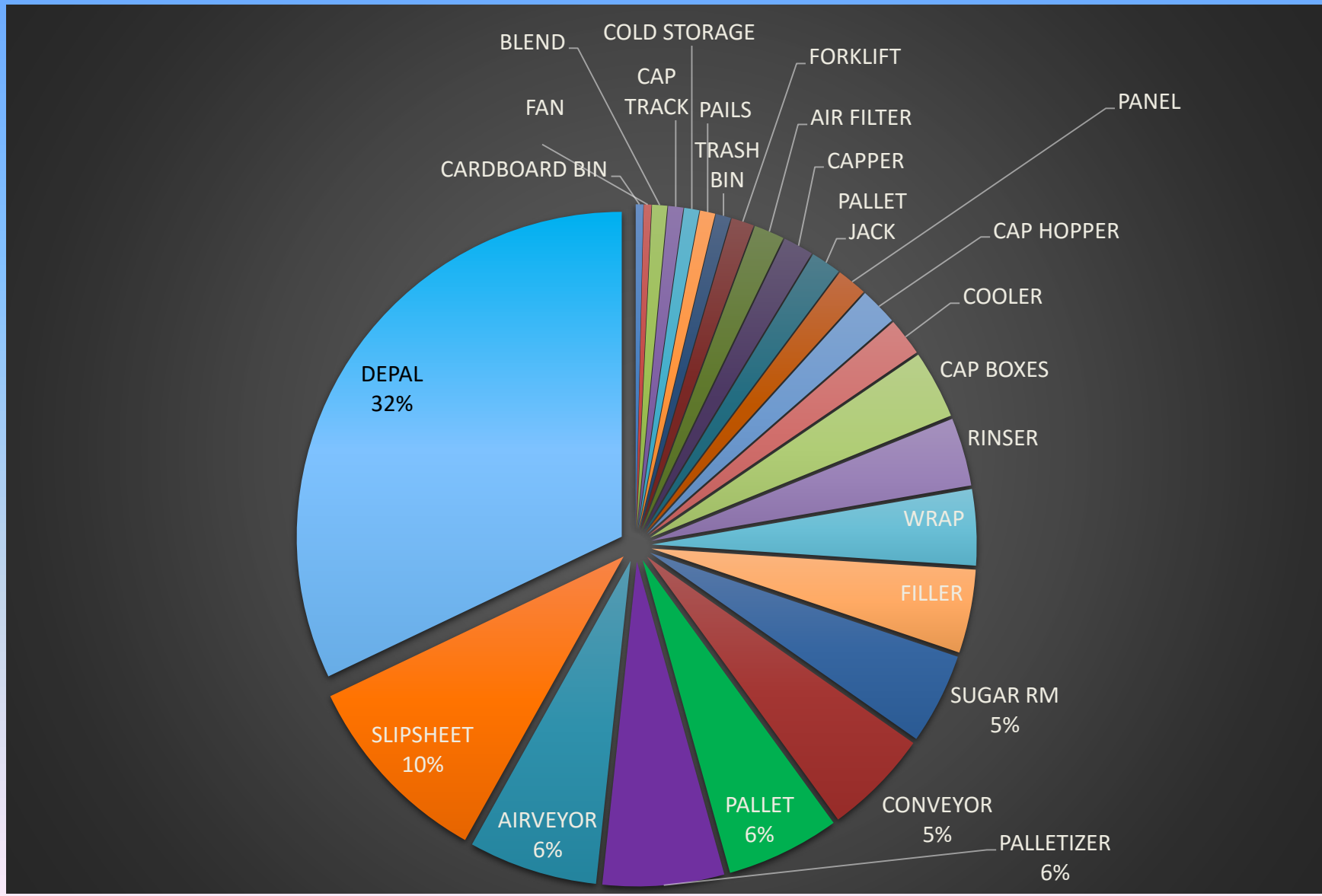
Objectives

- Part I: to determine the occurrence of HRM ascospores in the beverage processing environment
- Part II: to determine which sanitizer is most effective against HRM ascospores for their elimination from the processing environment:
  - ✓ Phase 1: determine the best methodology and sanitizer – CBS-KNAW Fungal Biodiversity Centre, Utrecht, the Netherlands
  - ✓ Phase 2: expose all HRM isolated from Part I to the best sanitizer – BCN Labs

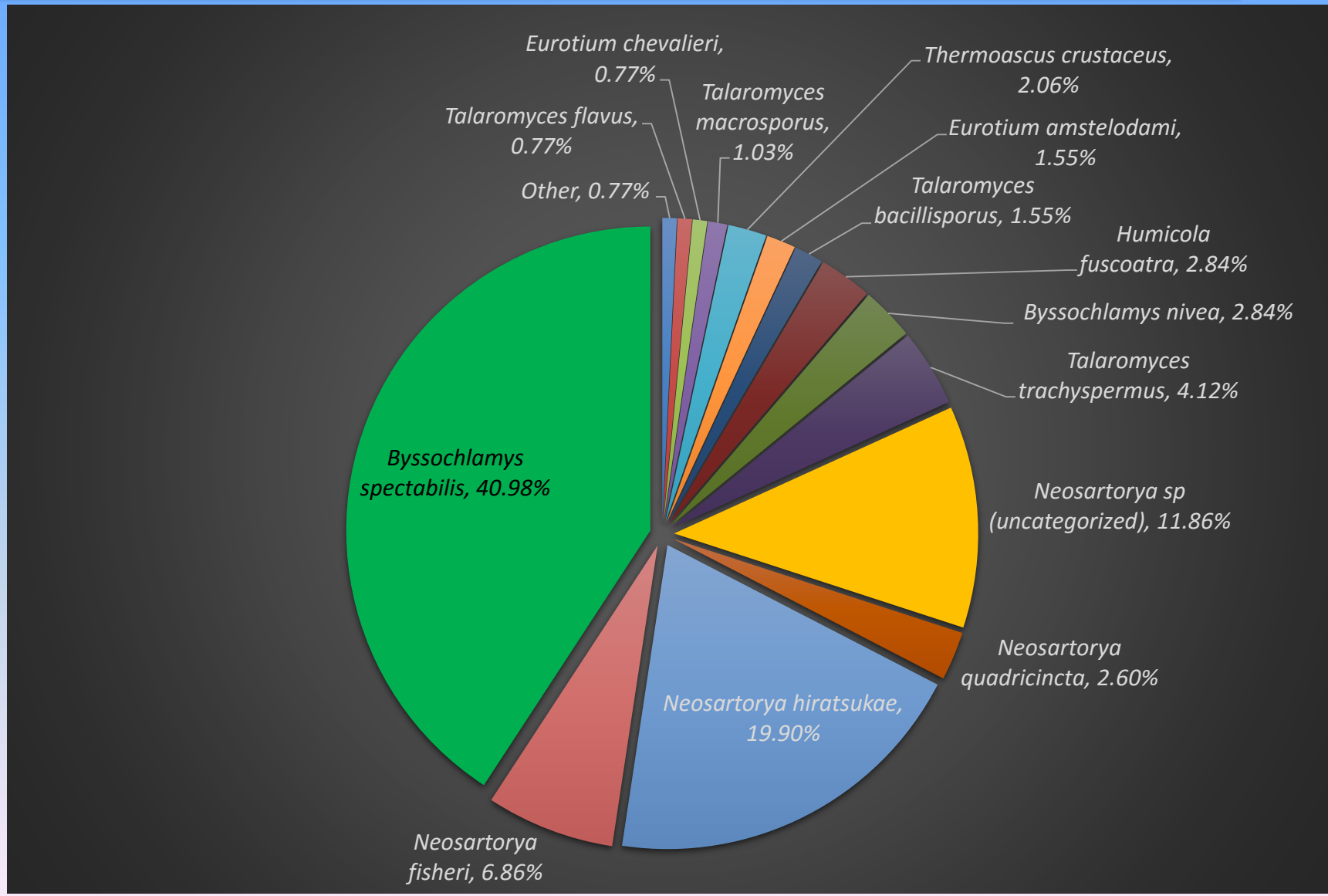
Part I - Methodology

- Large processing environment samples were collected using sterile sponges moistened with Neutralizing buffer
- Over 2,500 samples were collected in 2013-2015 in 15 different beverage processing facilities
- Sponges were tested by the heat-shock HRM method (CMMEF, 2014)

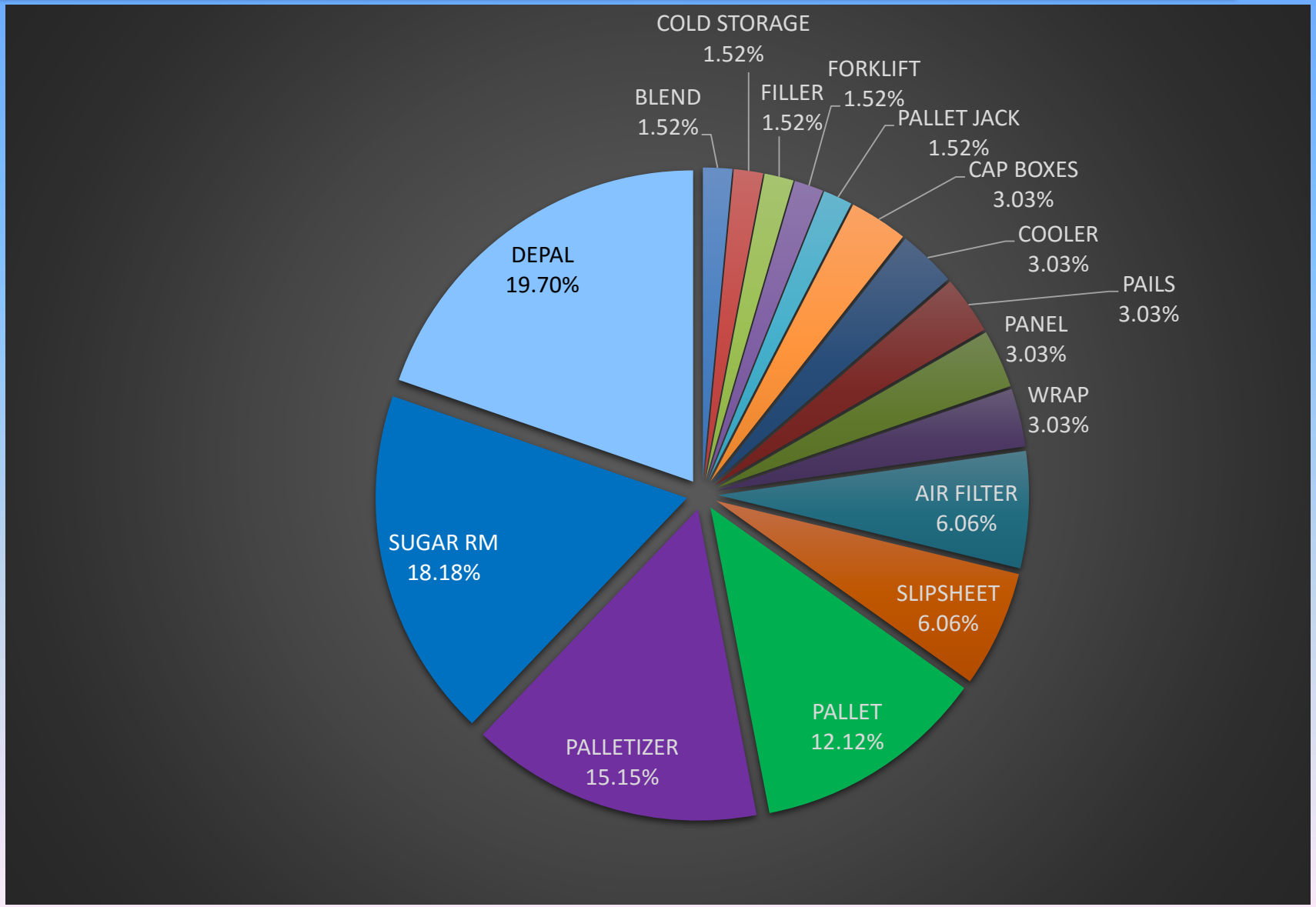
% Positive Samples per Area



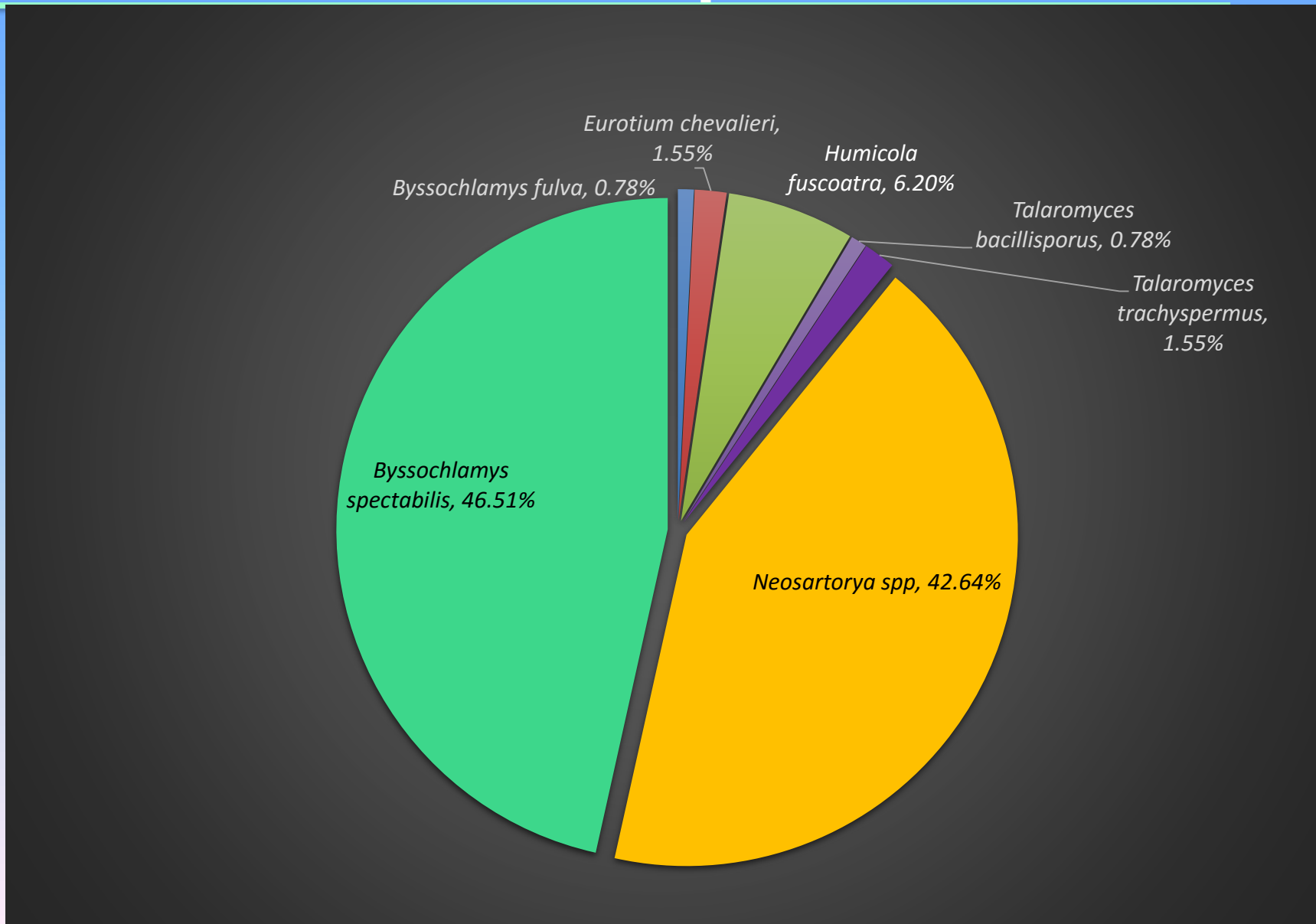
HRM Isolated from the Processing Environment: % Occurrence per mold



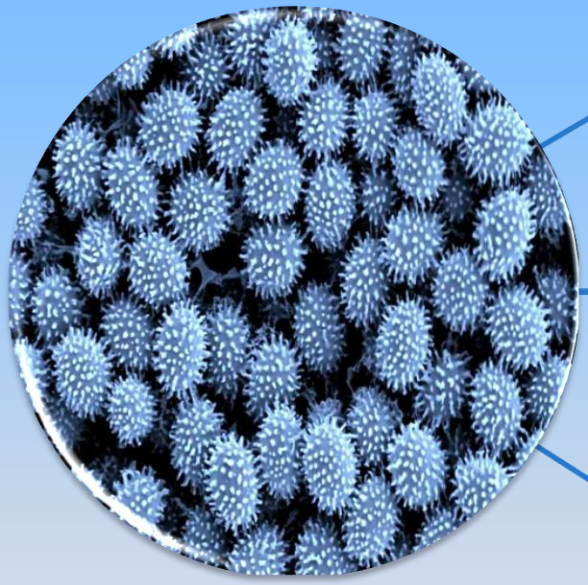
Spoilage Investigation Case % Positive Areas



Spoilage Investigation Case % Occurrence per mold



Contamination of Processing Environment of Pasteurized Hot-filled Juices and Beverages by HRM



Ingredients

- Ascospores in ingredients
- Packaging of ingredients

Packaging

- Ascospores inside bottles (Airveyor, Rinser)
- Bottle and cap packaging (Depal, Hopper)
- Palletizer

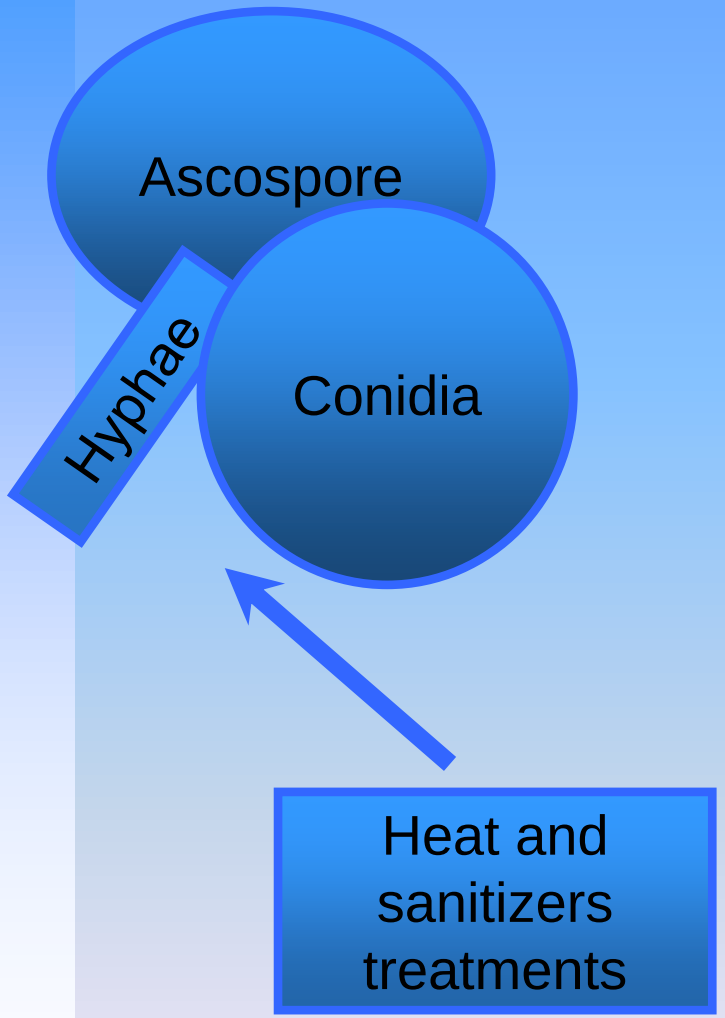
Transport. Equipment

- Forklifts
- Pallet Jacks
- High lifts

PART II - Sanitizer Study

- Preliminary study: best sanitizers at “no-rinse” concentration against conidia were chlorine dioxide (ClO2; acidified sodium chlorite) and iodine.
- Sanitizers tested in this study:
  - Product A – ClO2 3,000 ppm concentrate -- no activation needed
  - Product B – 2% sodium chlorite concentrate – needs to be activated with an acid
  - Product C – 2% stabilized chlorine dioxide concentrate – needs to be activated with an acid
  - Product D – iodophor, 3.5% concentrate (not the 1.75% concentrate)

PART II -Trial 1 Methodology



Two ascospore solutions were tested:

1. Dormant ascospores and other (living) cells directly with sanitizer.
2. Ascospores activated (5 min, 80 ºC), sanitizer treatment afterwards.

<u>Two levels of inoculation</u>: 10,000 and 100,000.

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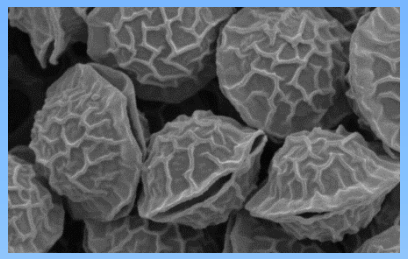
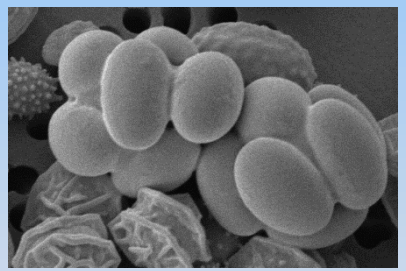
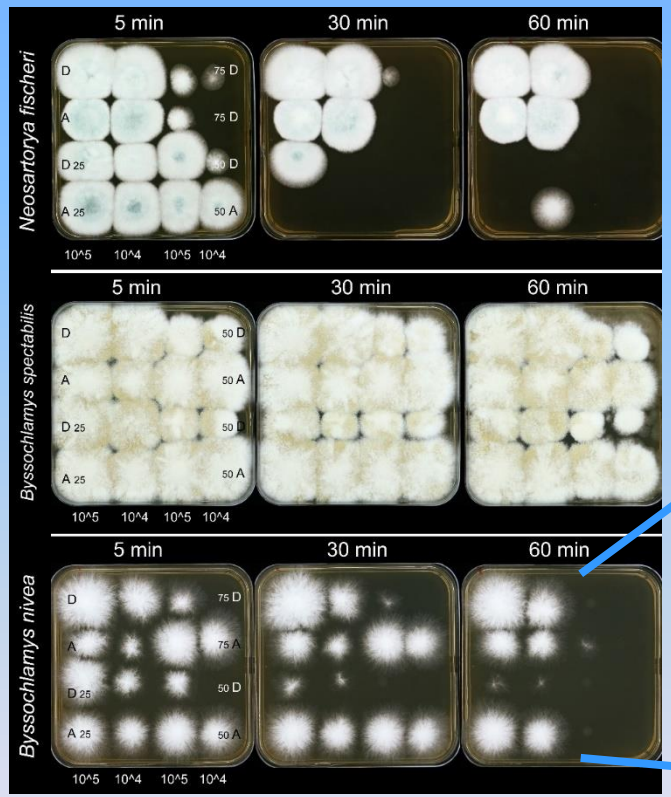
Courtesy of Jan Dijksterhuis, CBS, Utrecht, NH

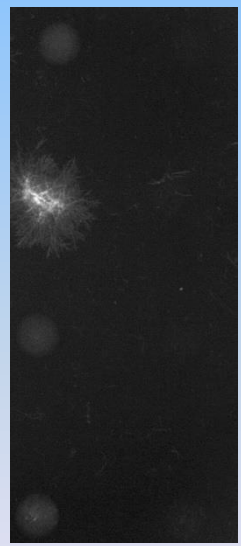
Product D -- iodophor (3.5% concentrate) at 0, 25, 50 and 75 ppm for 5, 30 and 60 min at two levels of inoculation







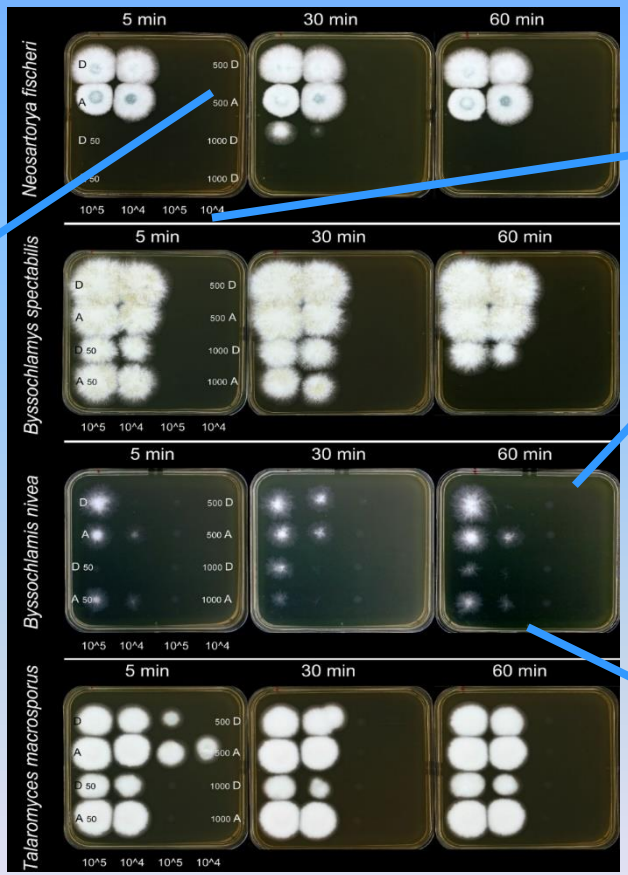
75 ppm for 60 min inactivates *N. fischeri* and partly *B. nivea*



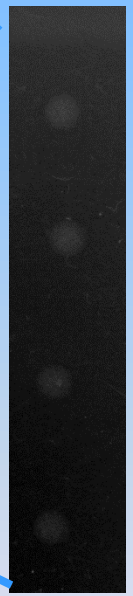
Courtesy of Jan Dijksterhuis, CBS, Utrecht, NH

Product A -- Chlorine dioxide (3,000 ppm concentrate) at 0, 50, 500 and 1000 ppm for 5, 30 and 60 min and two levels of inoculation

Ascospores of all species are inactivated at 500 ppm after 30 and 60 min in the case of either dormant or activated spores.



Droplets contain 100,000 and 10,000 ascospores

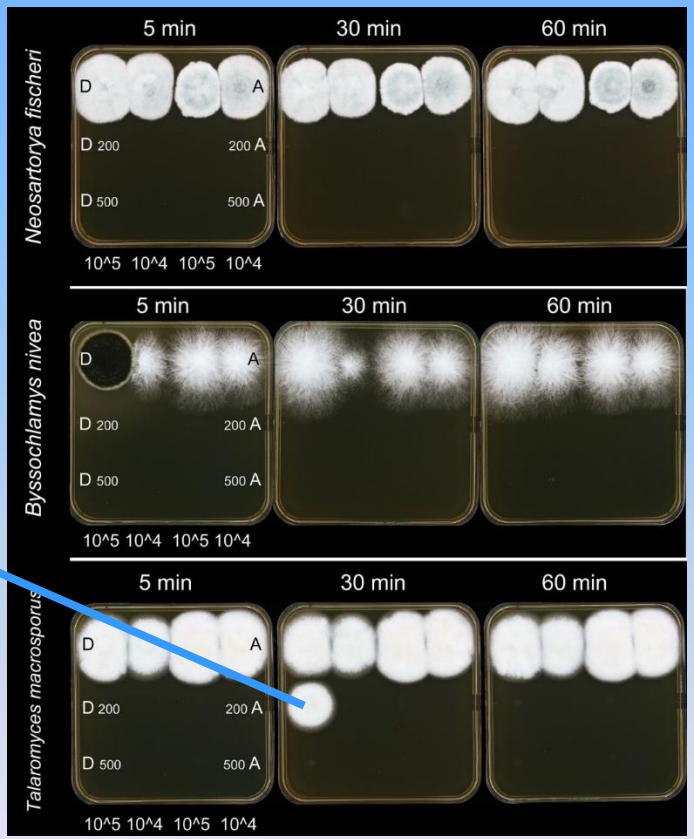


These are no cultures but remnants of inoculation

Courtesy of Jan Dijksterhuis, CBS, Utrecht, NH

Chlorine dioxide -- Product B (acidified sodium chlorite) and C (acidified stabilized chlorine dioxide) at 200 and 500 ppm for 5, 30 and 60 min and two levels of inoculation

Product C -- Ascospores of three species are inactivated at 200 and 500 ppm after 5, 30 and 60 min in the case of either dormant or activated spores except in one case of *T. macrosporus*



Also, not shown: Product B inactivates *N. fischeri* and *B. nivea* at 200 and 500 ppm at all treatment times. *T. macrosporus* after 30 and 60 min. *B.spectabilis* is inactivated at 500 ppm

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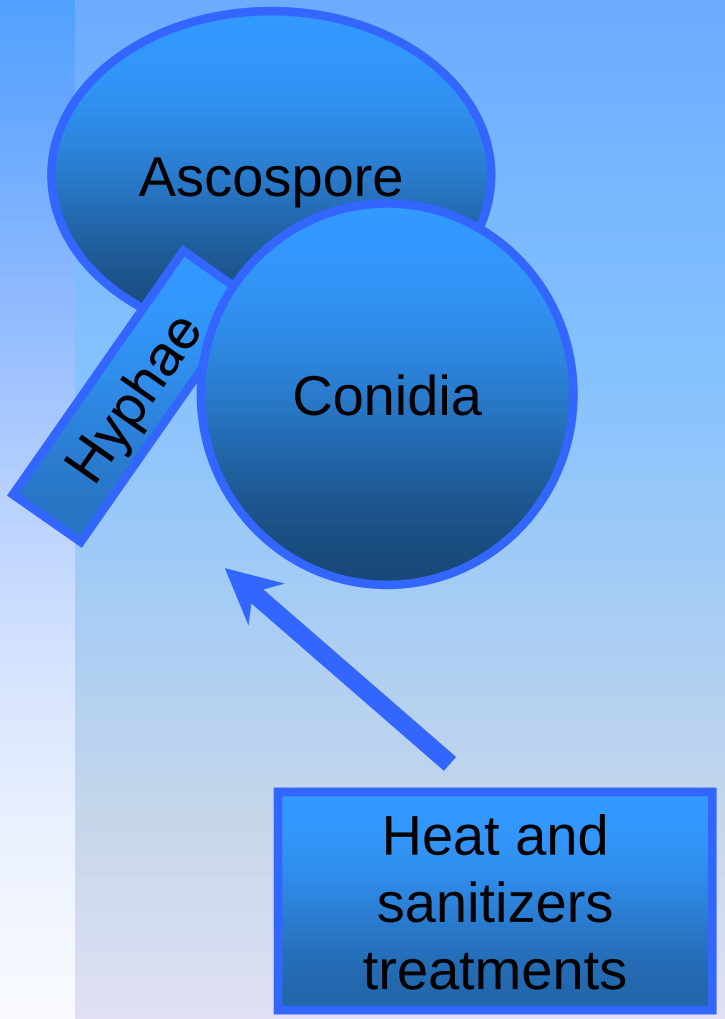
Courtesy of Jan Dijksterhuis, CBS, Utrecht, NH

Part II -Trial 2 Methodology



Four solutions of ascospores tested were:

1. Dormant ascospores and other (living) cells directly with sanitizer.
2. Heated solutions (60 ºC), conidia and hyphae killed, ascospores dormant, sanitizer afterwards
3. Ascospores activated (5 min, 80 ºC), sanitizer treatment afterwards.
4. Ascospores activated after sanitizer treatment.

<u>Four levels of inoculation were used</u>: 100, 1,000, 10,000 and 100,000.

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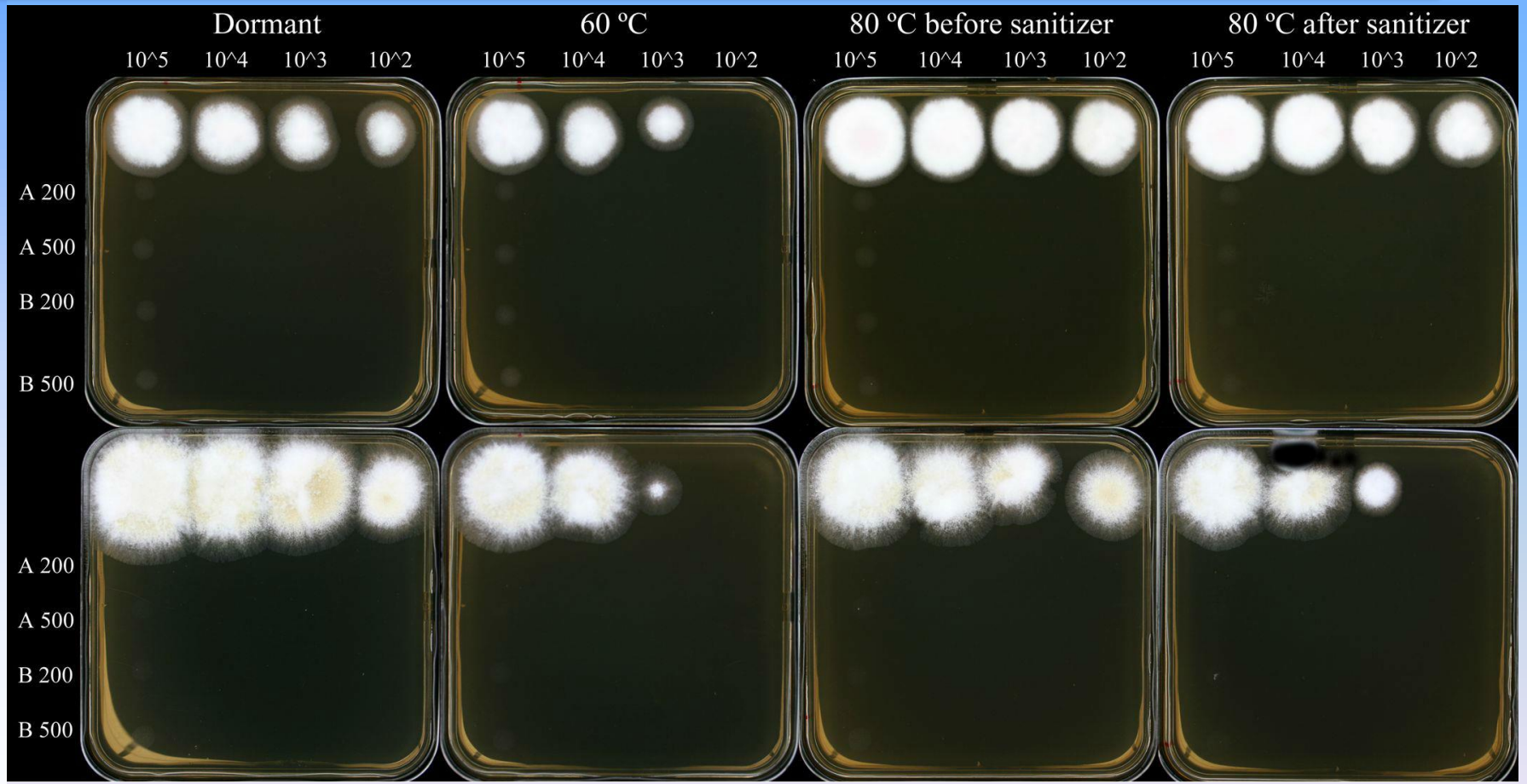
Courtesy of Jan Dijksterhuis, CBS, Utrecht, NH

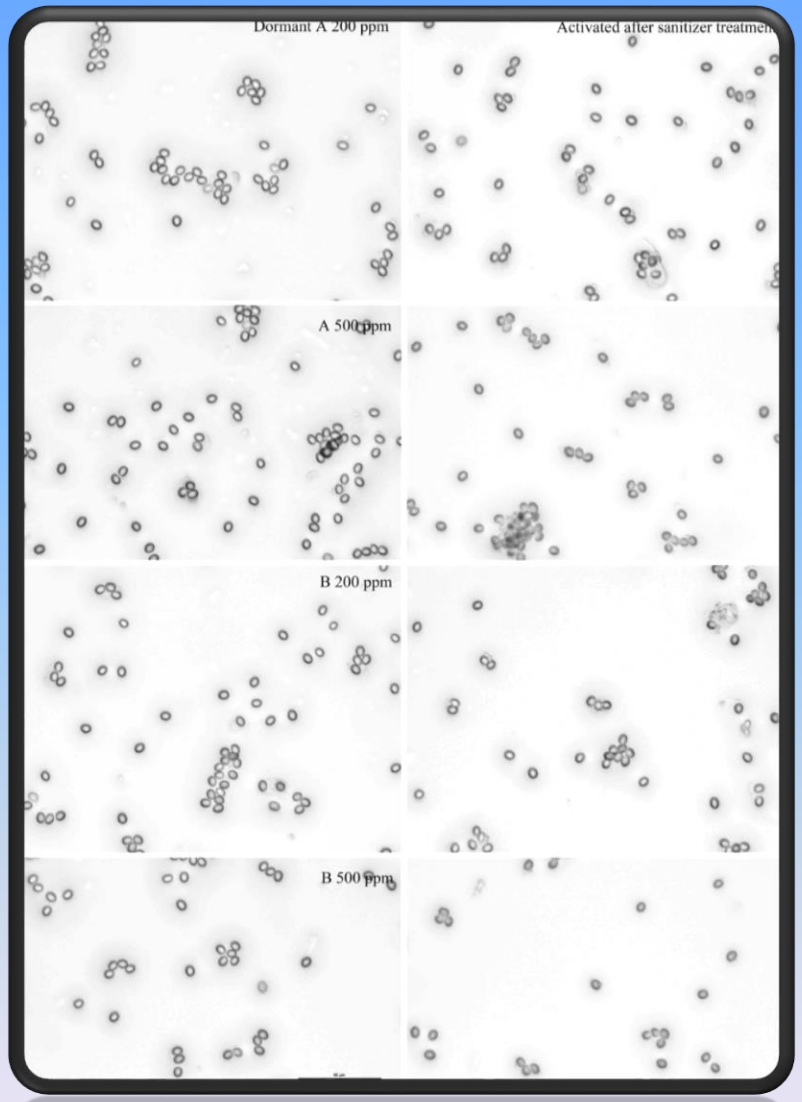
Chlorine dioxide -- Products A (3,000 ppm concentrate) and B (acidified sodium chlorite) inactivate the two most resilient species at 200 and 500 ppm for 60 min



Talaromyces macrosporus

T. macrosporus ascospores after different treatments with chlorine solutions A and B are present on the agar without germination. No germ tubes are observed.

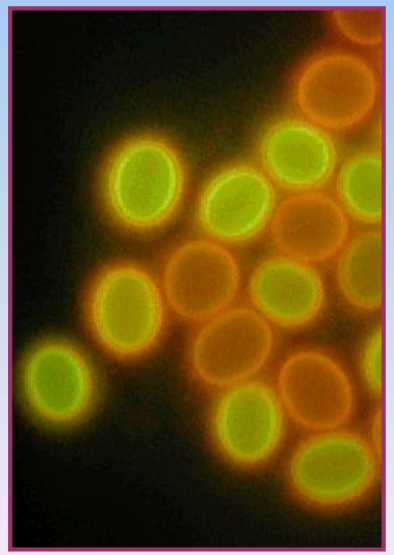
The asci are broken easily and the ascospores are isolated.

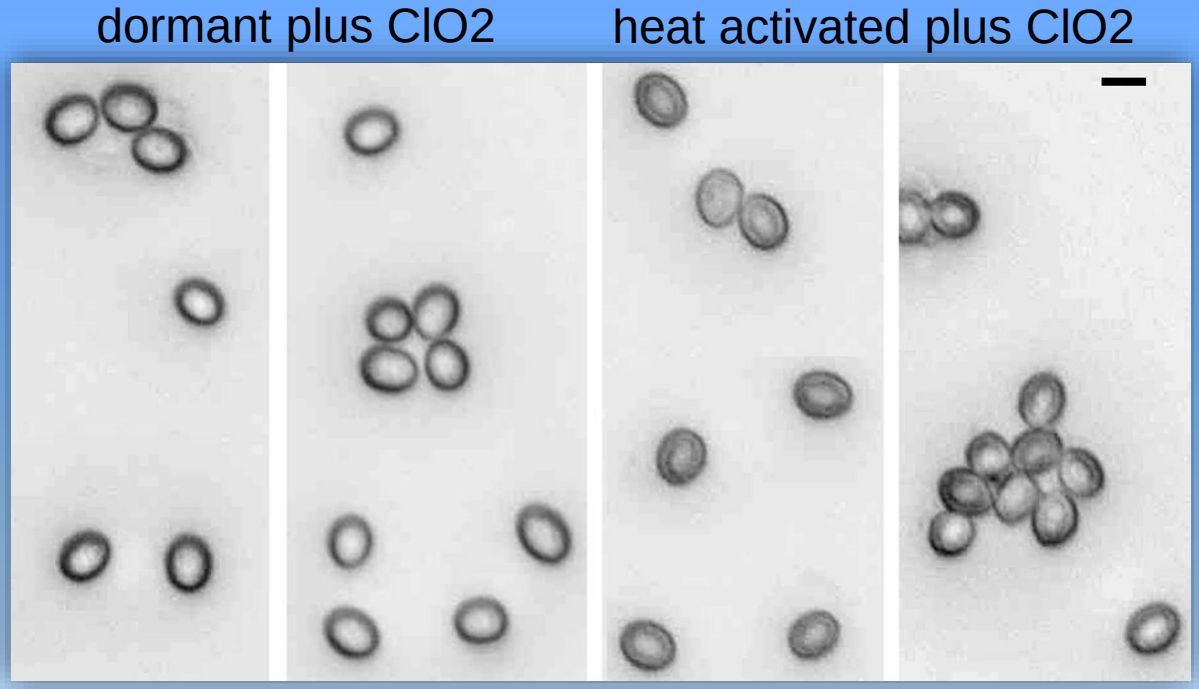


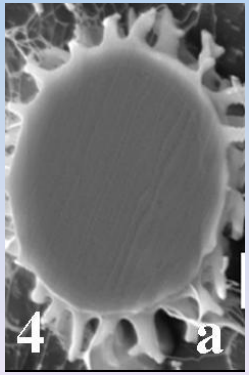
Courtesy of Jan Dijksterhuis, CBS, Utrecht, NH

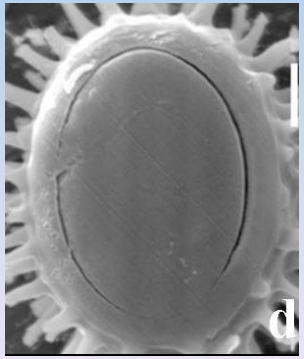
Talaromyces macrosporus

T. macrosporus ascospores show visibility of a thick cell wall before and after heat activation, but all spores are inactivated by chlorine dioxide and do not germinate.



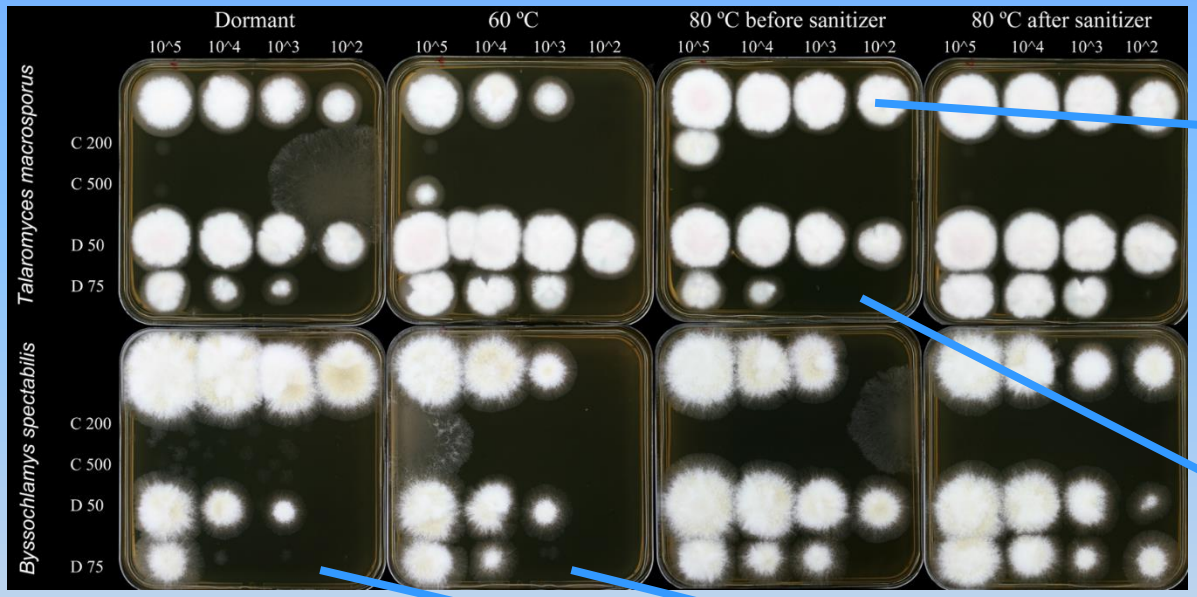






Courtesy of Jan Dijksterhuis, CBS, Utrecht, NH

Product C (stabilized ClO2) and D (Iodine) after 60 min

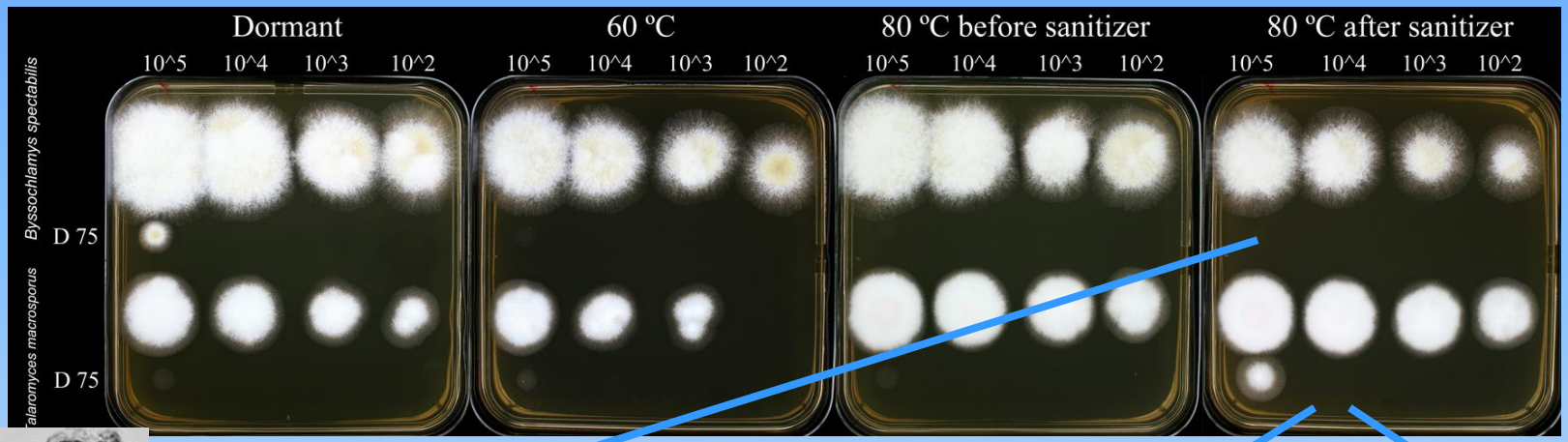


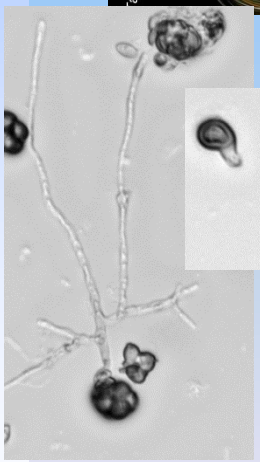
Increase of germinating cells after heat activation is clear with *T. macrosporus.*

Courtesy of Jan Dijksterhuis, CBS, Utrecht, NH

Iodine shows some effects at 75 ppm and in 7 out of 8 cases inactivates 100 ascospores (log 2 inactivation). Three cases of log 3 inactivation.

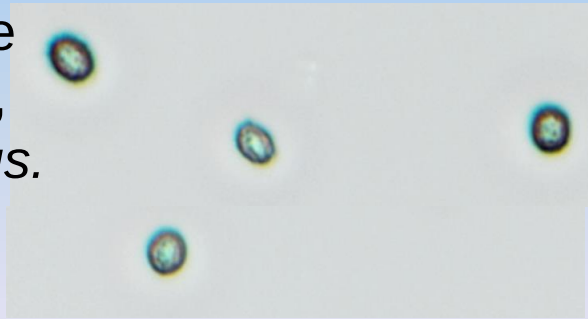
Product D (Iodine) inactivates 10,000 ascospores after a 16 hour treatment at 75 ppm





Ascospores of *B. spectabilis* seem to have germinated.

Iodine treatment results in a visible damage of ascospores, *T. macrosporus.*



Different mode of action of iodine?

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Prevention of Contamination

- Monitor processing environment – Establish an Environmental Monitoring Program (EMP) for HRM
- Accumulation of dust must be avoided – overhead surfaces cleaning & sanitizing
- Implement a strong sanitation program -- Challenge: dry cleaning
- Use the right sanitizer – chlorine dioxide (ClO_2)

Conclusions

One

- Ascospores of the HRM are present in the beverage processing environment – sometimes in high numbers

Two

- Contamination by HRM from the environment can cause a low spoilage rate under normal conditions

Three

- Depalletizers and palletizers have to be cleaned in depth and on a regular basis
- The use of wooden pallets should be minimized

Four

- Packaging wrap (bottle pallets, cap boxes, etc.) should be handled outside the processing area
- Transportation vehicles have to be sanitized

Conclusions

Five

- The sanitizers used in this study can inactivate ascospores of the HRM

Six

- Product A (3,000 ppm ClO2 concentrate) and Product B (acidified sodium chlorite) worked well at 200 ppm

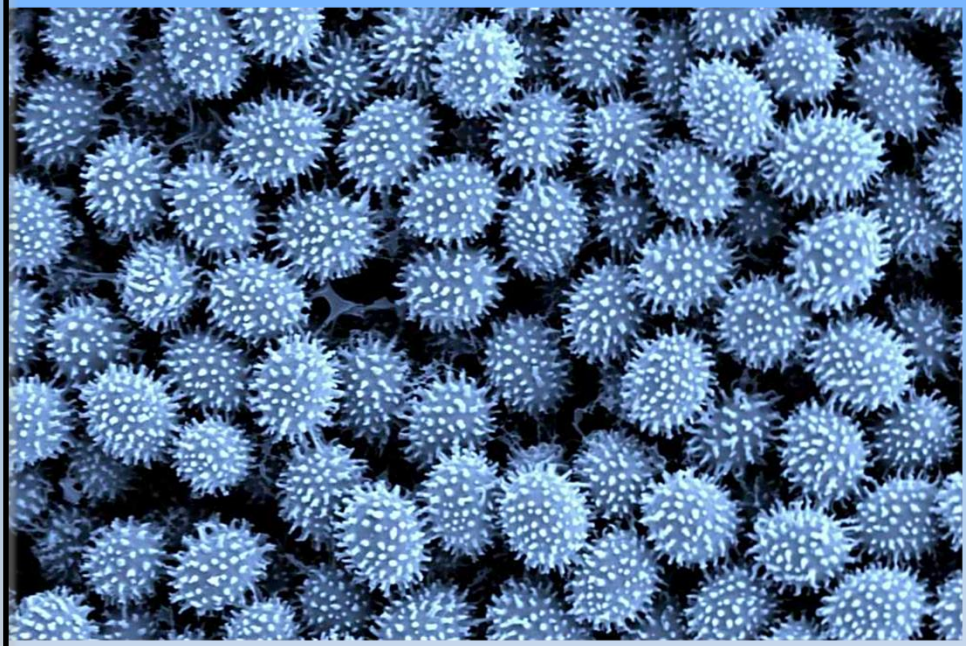
Seven

- Product C (stabilized chlorine dioxide) seemed to be less potent than Product A and B.

Eight

- Iodine inactivation needs longer time at 75 ppm -- different mode of action?
- It did not work at the “no-rinse” concentration

Thank you!
Questions?



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