Control of mold contamination in food manufacturing facilities

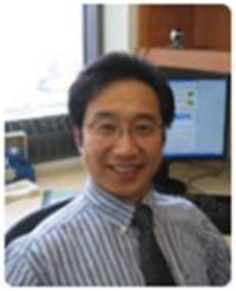
October 24, 2018 WAFP Fall Workshop

Jae-Hyuk Yu

Food Research Institute

Depts. of Bacteriology and Genetics

& Molecular Environmental Toxicology Center



Jae-Hyuk Yu

Professor of Bacteriology and Genetics

3155 Microbial Sciences Building
Office: (608) 262-4696
Lab: (608) 263-6830
jyu1@wisc.edu

Google

Start and Promotion Dates

- Assistant Professor: 2000
- Associate Professor: 2006
- Full Professor: 2011

Education

B.S., Microbiology, Seoul National University, Seoul, Korea 1986
M.S., Food Science, University of Wisconsin-Madison 1991
Ph.D., Genetics, University of Wisconsin-Madison 1995
Postdoctoral Research: Genetics, Texas A&M University

Affiliations

Food Research Institute
Professor, Department of Genetics
Editorial Board Member of Scientific Reports
Graduate Trainer, Genetics, Molecular and Environmental Toxicology Center, Plant Pathology, Food Science.
Faculty of 1000 Biology, Faculty Member for Microbiology, Microbial Growth & Development Section
Microbiology Teaching Fellows Program

Total Google Scholar Articles 155

Cited by		VIEW ALL
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Citations	10739	5111
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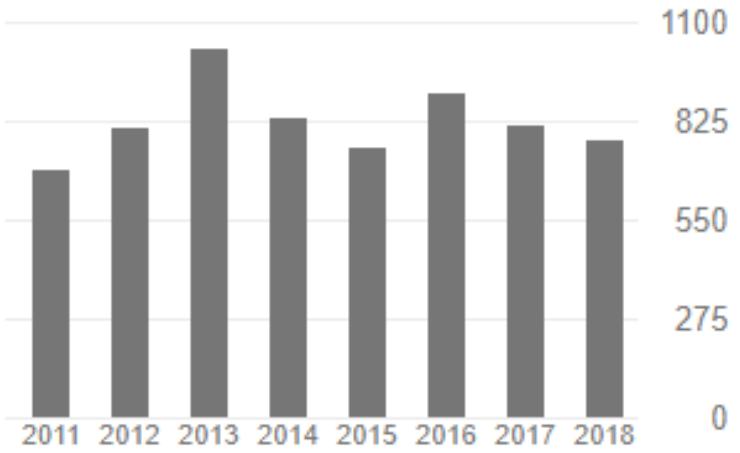
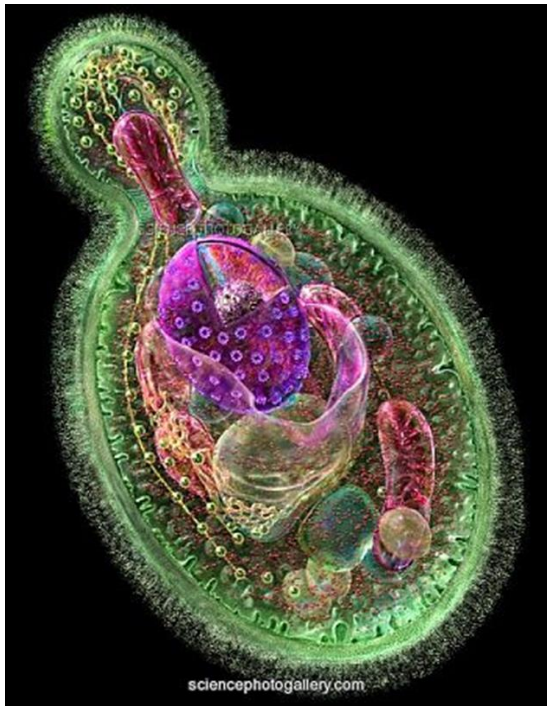
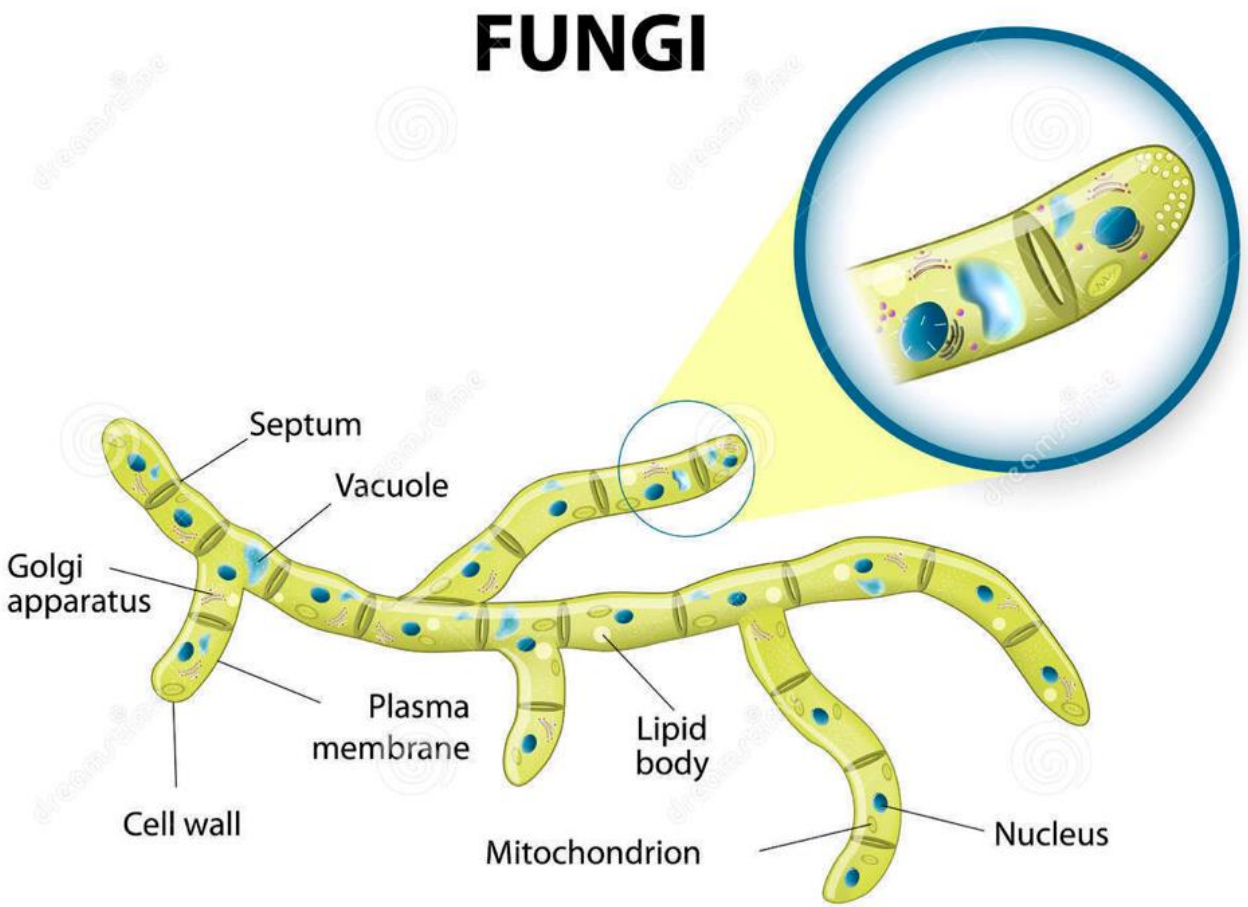


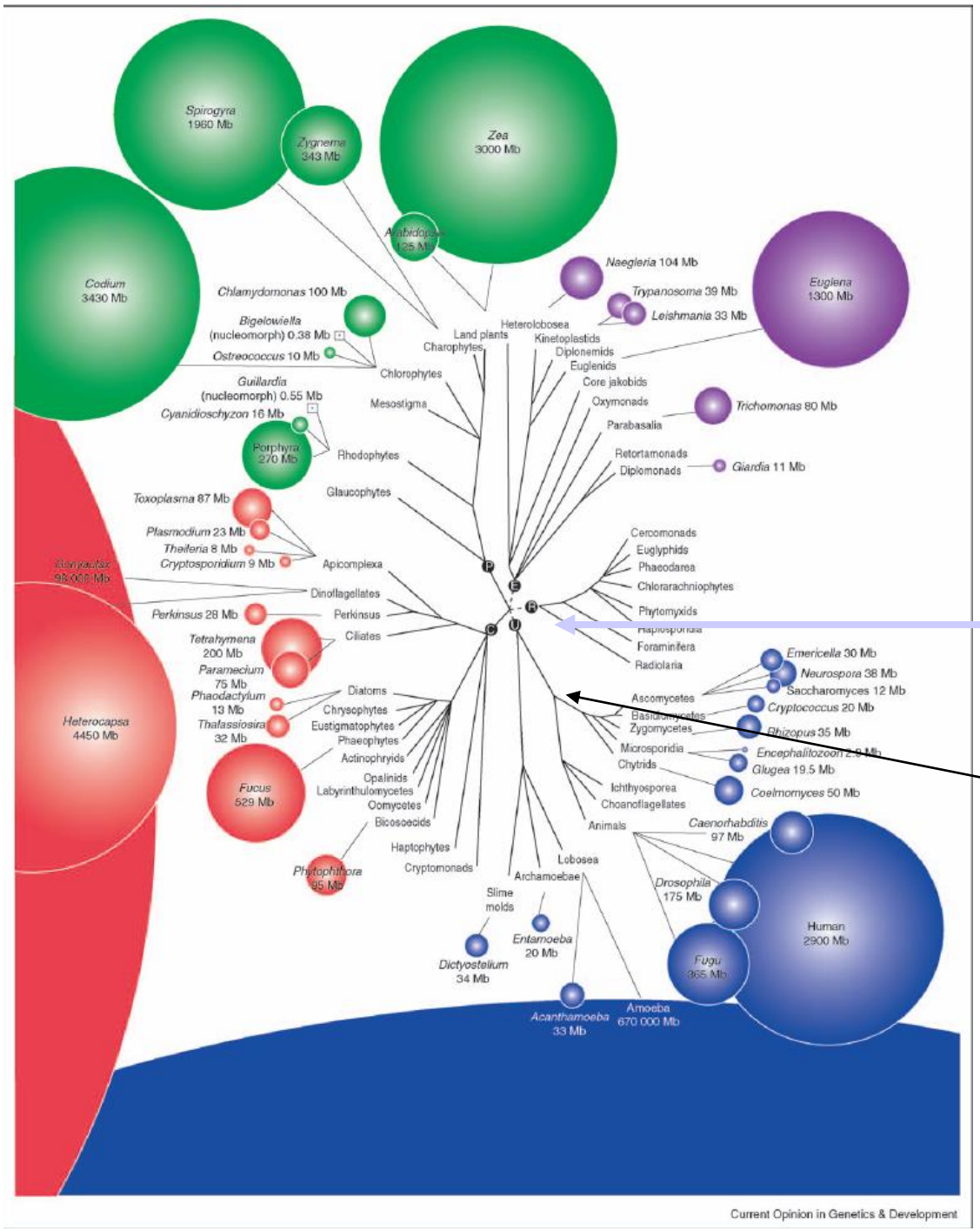
Outline

1. Fungi Basics
2. Food Spoilage and Fungi
3. Fungi and Mycotoxins
4. **How to control molds/yeasts?**

Fungi are eukaryotic microbes.







Tree of eukaryotes with variations in genome size.

From Keeling and Slamovits (2005). Current Opinion in Genetics and Development 15: 601-608

“Unikont”—eukaryotic cell with one flagellum ~ 3 billion years ago

~ 1 billion years ago

animals and fungi had a common ancestor

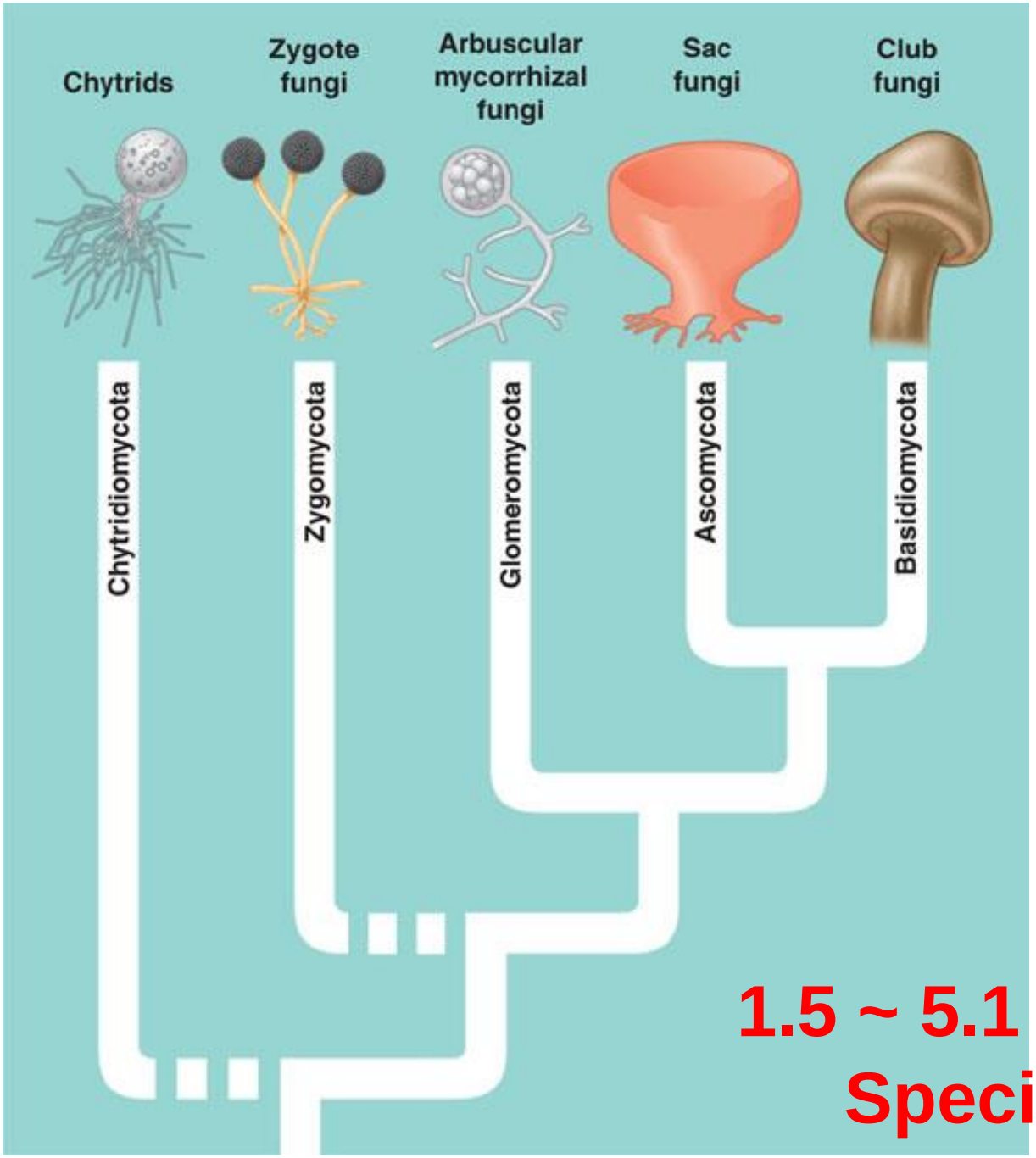
Fungi are a way more closely related to Adam than to plants

Animalia

Fungi

Planta

Common ancestor

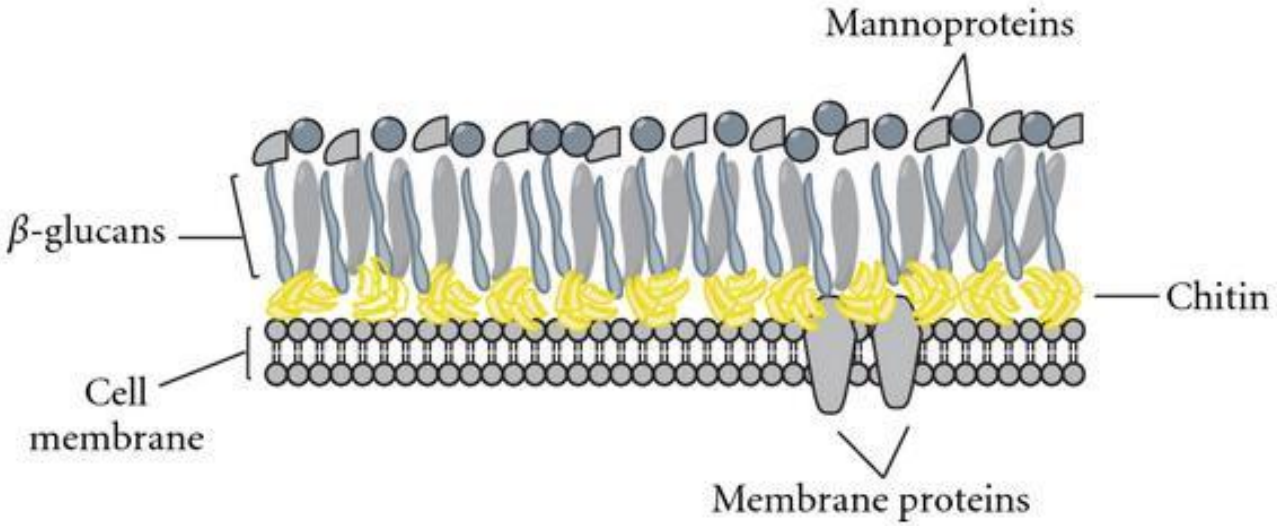


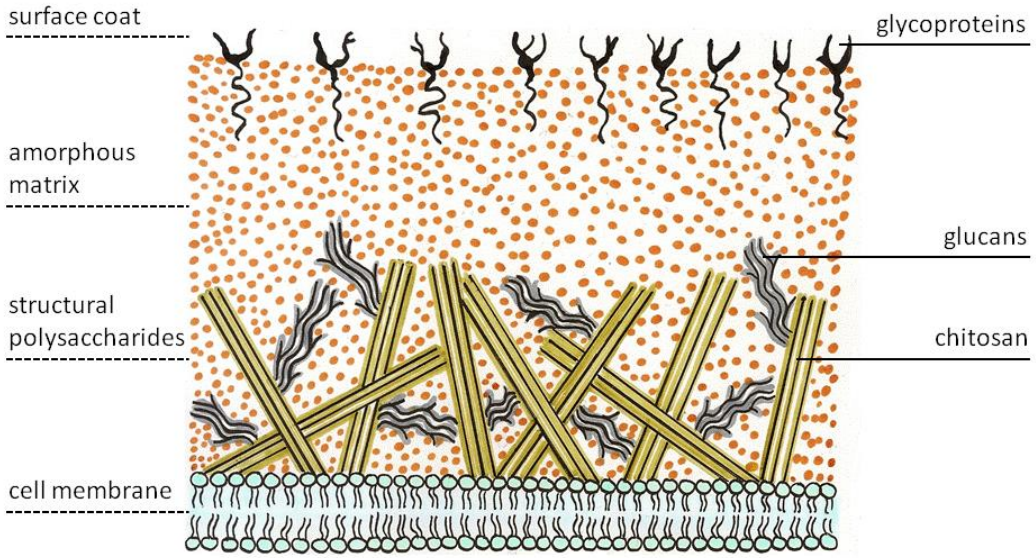
1.5 ~ 5.1 Million Species?

General features of Fungi

- unicellular (**yeasts**) and multicellular (**molds/moulds**)
- Non-photosynthetic organisms with cell wall
- Multicellular, filamentous organisms
- Normal inhabitants of the soil, rhizosphere and water
- Can tolerate acidic and dry conditions

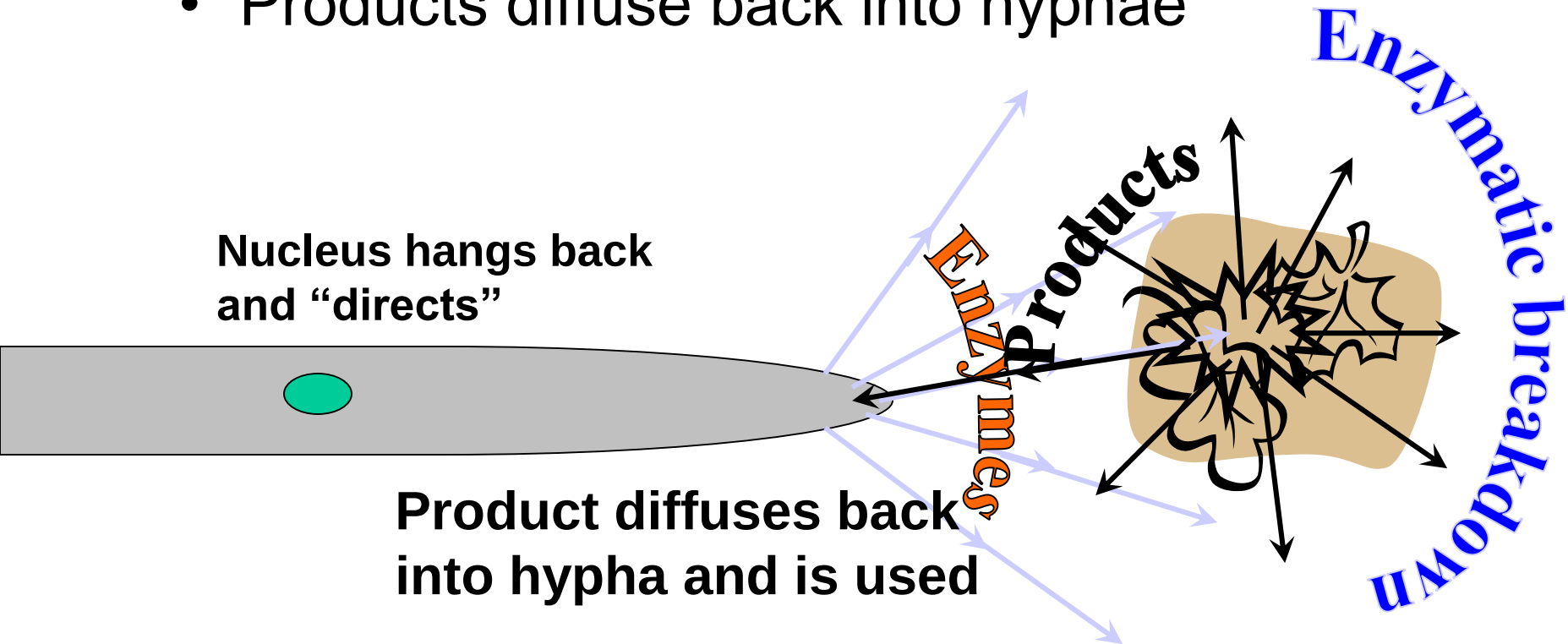
Fungal Cell Wall



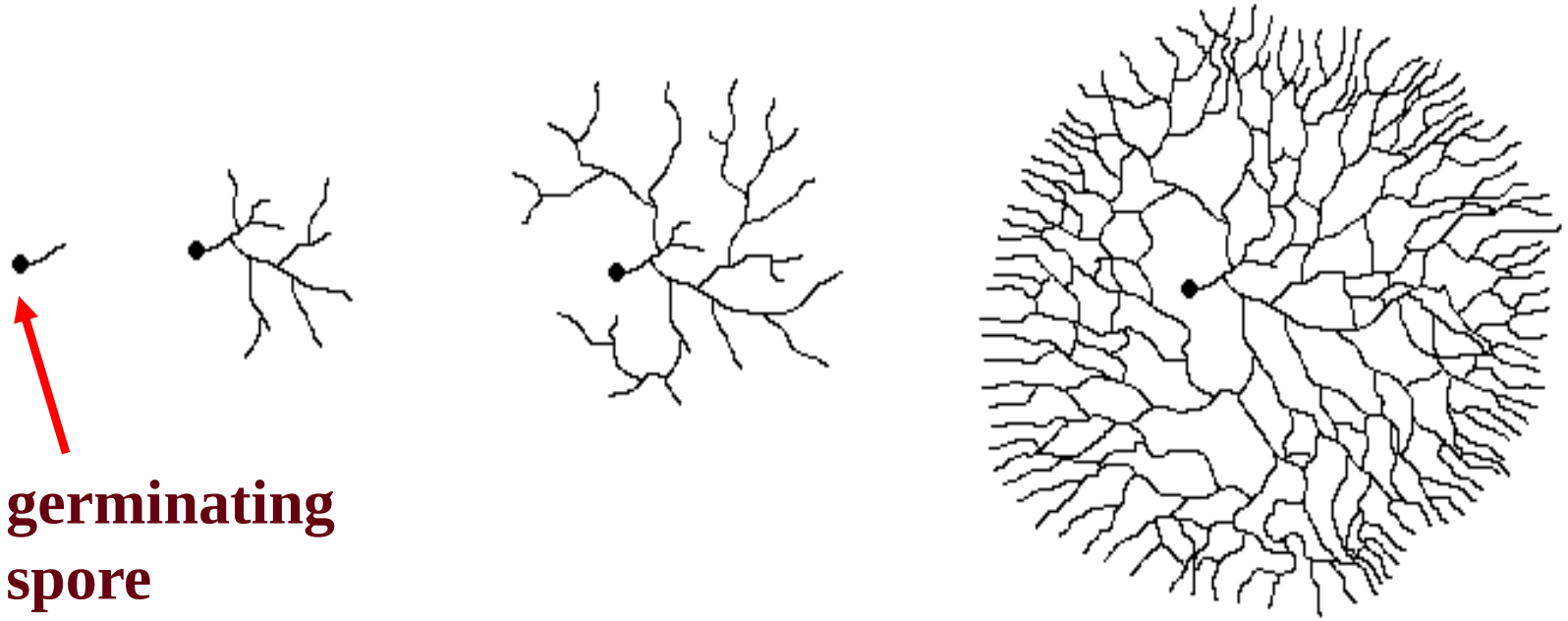


Heterotrophic by Absorption

- Fungi get carbon from organic sources
- Hyphal tips release enzymes
- Enzymatic breakdown of substrate
- Products diffuse back into hyphae



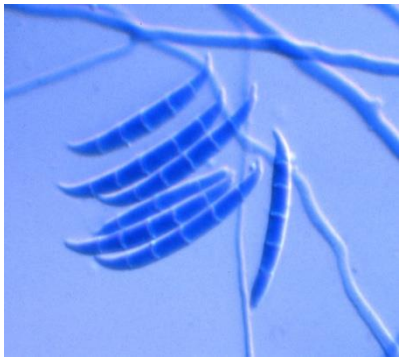
Hyphal growth from spore

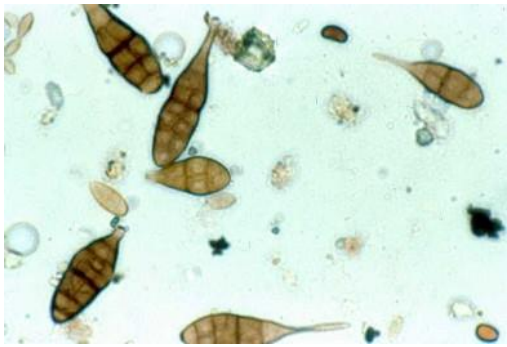


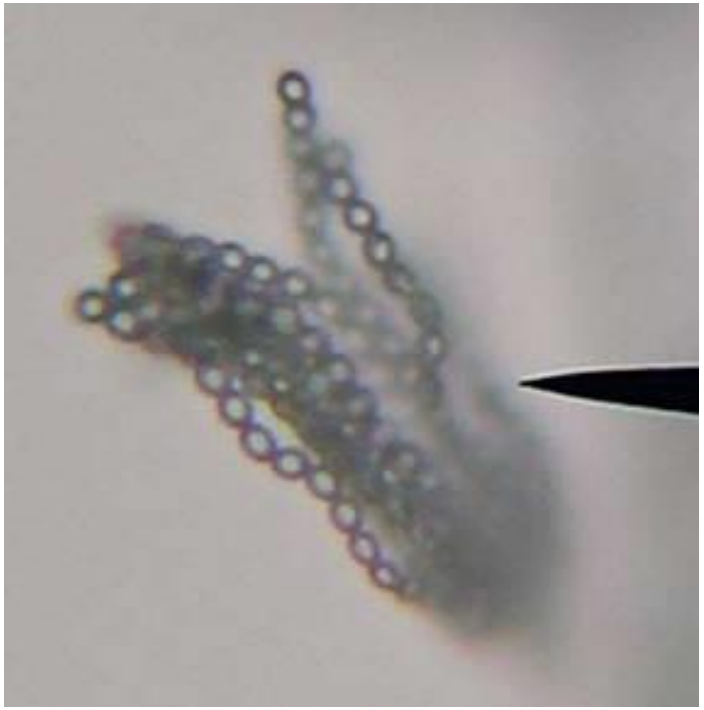
- Mycelia have a huge surface area

Fungi are Spore-ific!!!

- Spores - asexual (product of mitosis) or sexual (product of meiosis) in origin.
- Purpose of Spores
  - Allows the fungus to move to new food source.
  - Resistant stage - allows fungus to survive periods of adversity.
  - Means of introducing new genetic combinations into a population





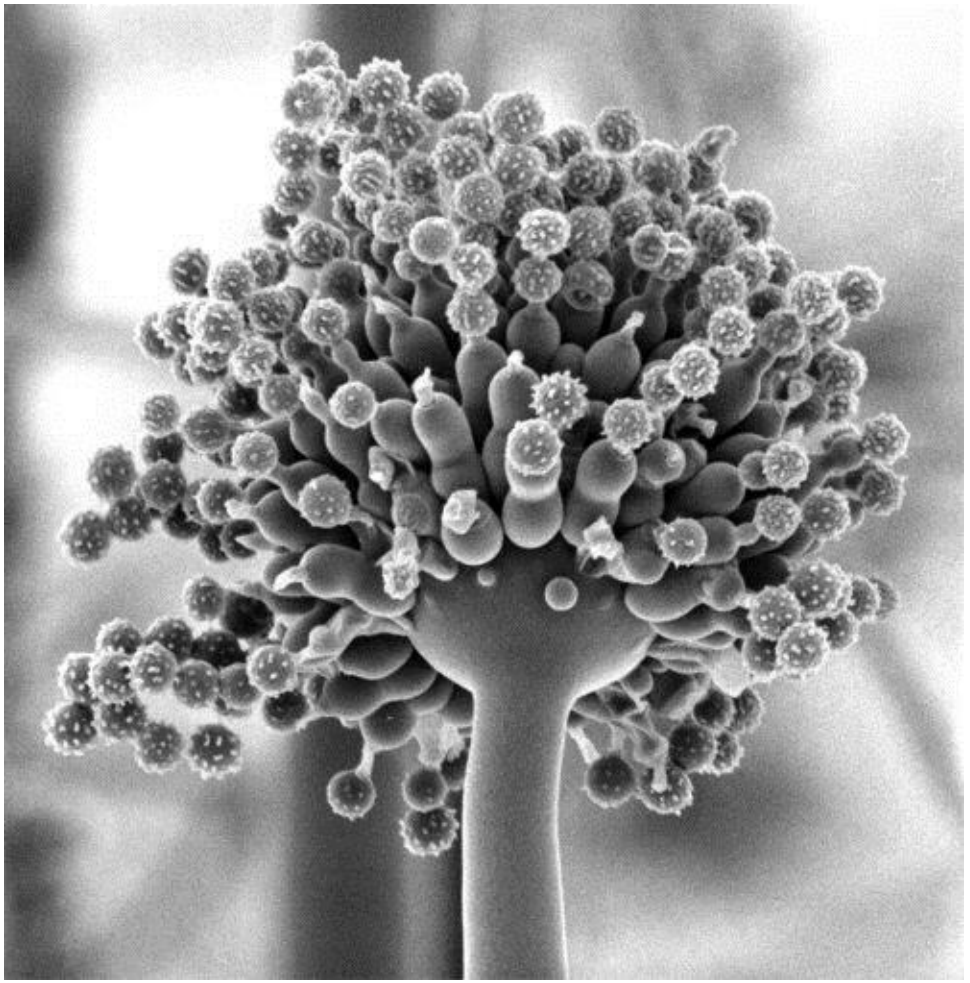


Some fungi have more than one scientific name – Why?

- **Teleomorph**: the sexual reproductive stage (morph), typically a fruiting body (e.g., *Morchella esculenta*, *Agaricus brunescens*).
- **Anamorph**: an asexual reproductive stage (morph), often mold-like (e.g. *Aspergillus flavus*, *Fusarium solani*). When a single fungus produces multiple morphologically distinct anamorphs, they are called **synanamorphs**.
- **Holomorph**: the whole fungus, including all anamorphs and the teleomorph.

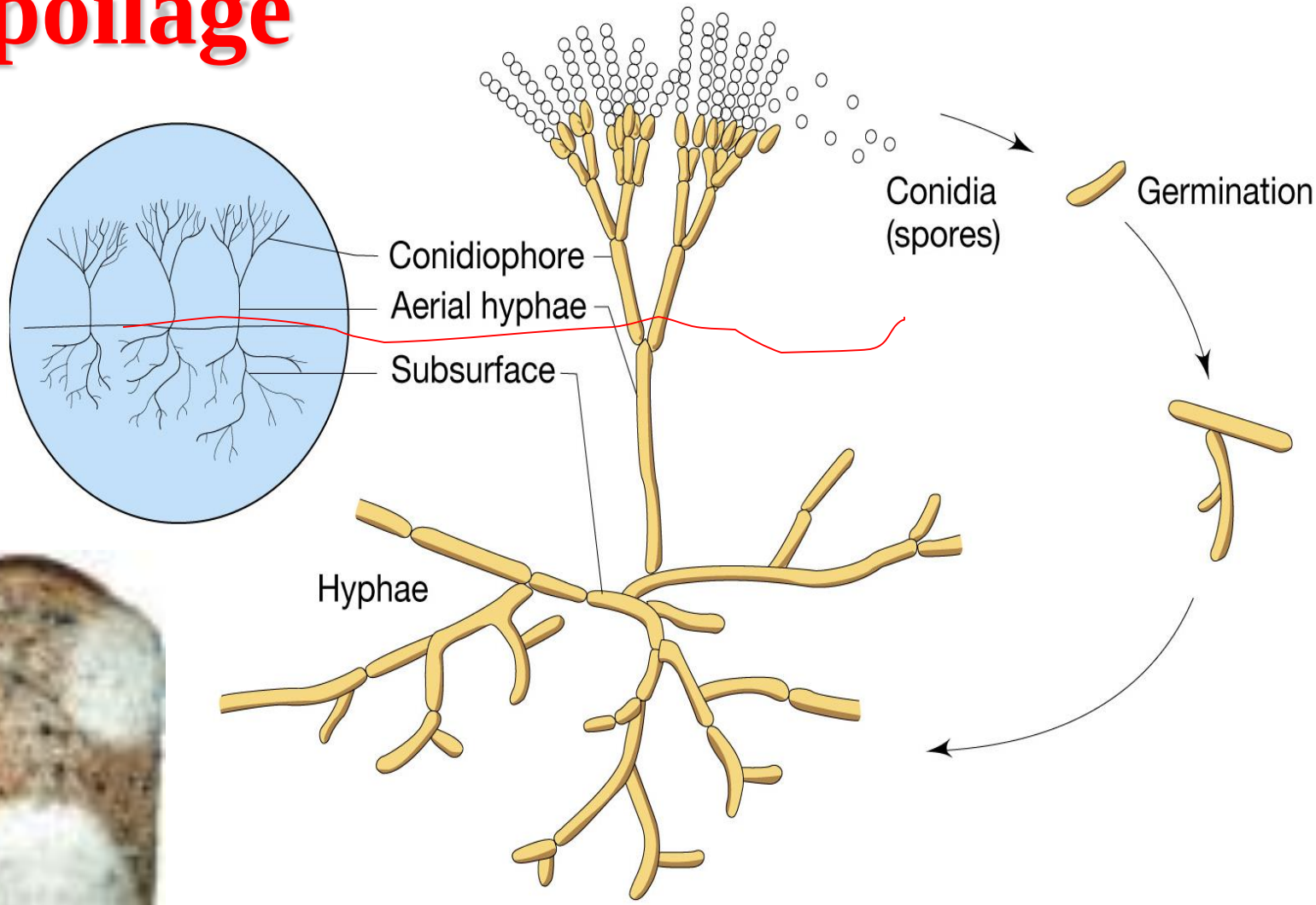
Molds Key Points

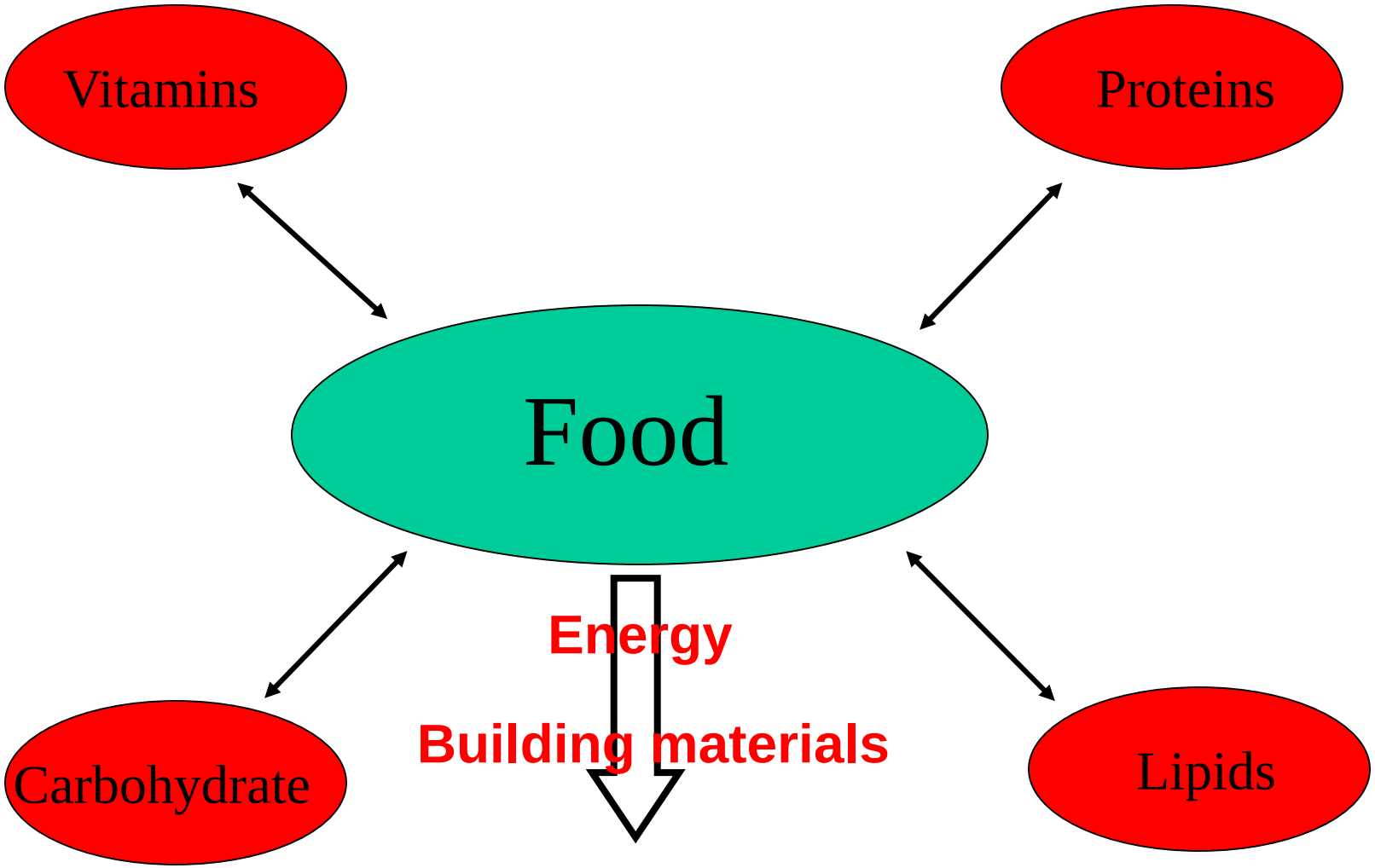
- Rapid growth
- Hyphae
- Produce Spores
  - For Reproduction
  - Easily Spread
  - NOT so Resistant like bacterial Spores



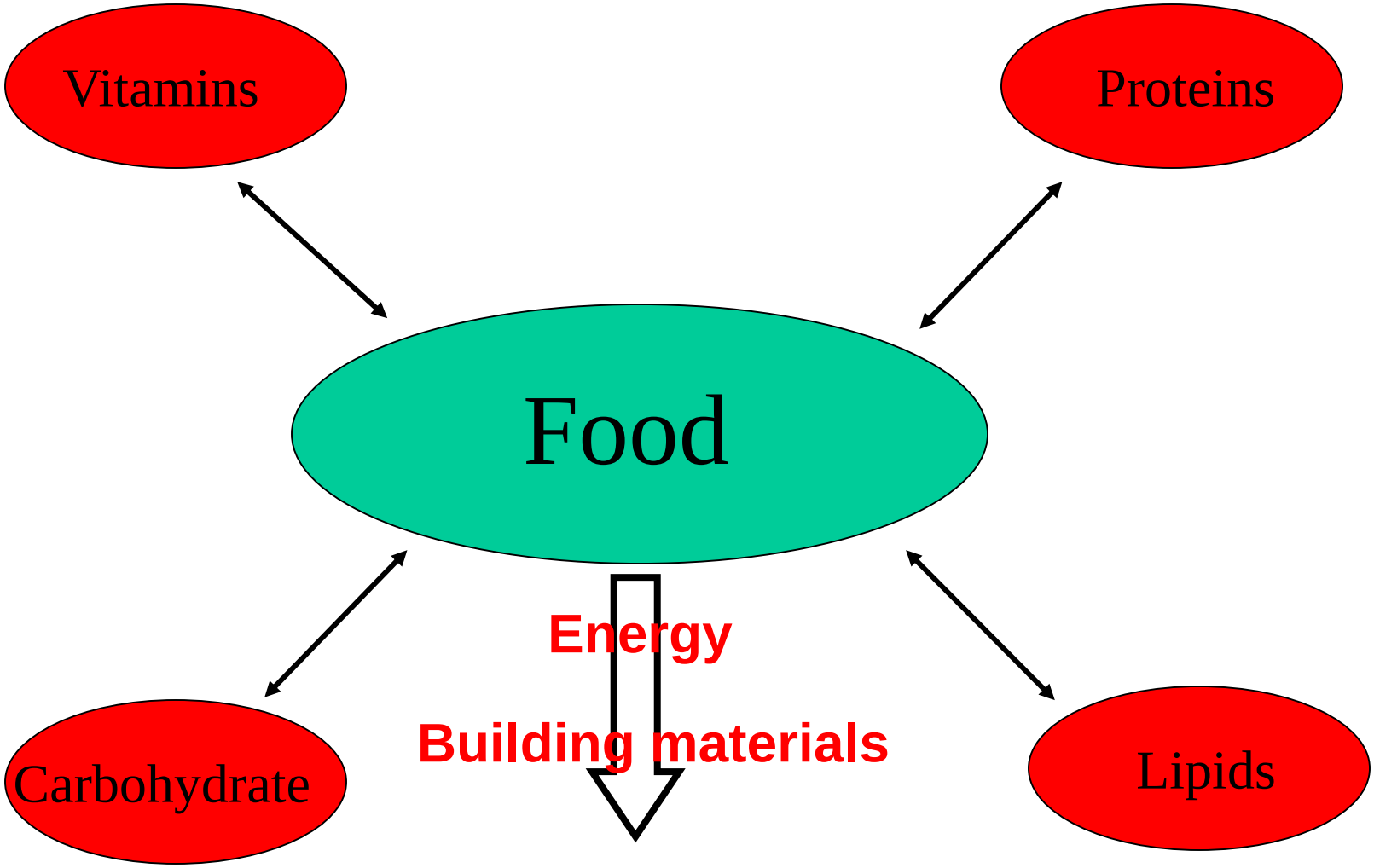
http://www.fungalcell.org/asexual-development-image-gallery

Molds and Food Spoilage





Human Growth



Microbial Growth

(uncontrolled/undesirable)


SCIENCEPHOTOLIBRARY


25% of the
world's food supply
is lost to spoilage.

Signs of Fungal Food Spoilage

- **Yeast spoilage**
  - Bubbles or foam
  - Slime
- **Mold spoilage**
  - Various colors of fuzzy growth
  - Circular pattern of growth

- Other signs
  - Off odor
  - Discoloration
  - Mushy texture
  - Bulging or corroded can
  - Cloudy appearance
  - Soft spots or breaks in the skin on fruits & veggies

- If you suspect a food is spoiled, DO NOT TASTE IT.

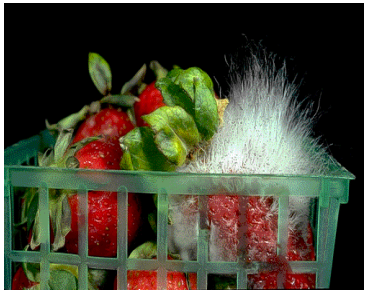
Molds

- ***Aspergillus***
- ***Penicillium***
- ***Fusarium***
- ***Mucor***
- ***Rhizopus***
- *Geotrichum*
- *Neurospora*
- *Botrytis*
- *Sporotrichum*
- *Alternaria*

- **Field Fungi:**
  - ***Fusarium***
  - ***Cladosporium***
  - ***Alternaria***

- **Storage Fungi:**
  - ***Penicillium***
  - ***Aspergillus***

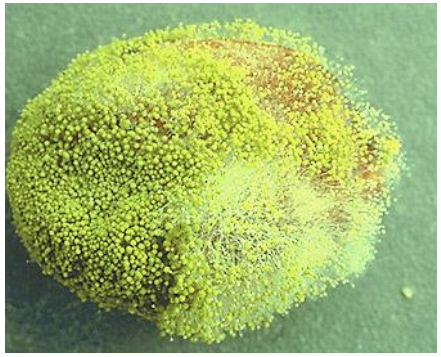
Common Spoilage Molds



Mucor

Rhizopus

Penicillium



Aspergillus

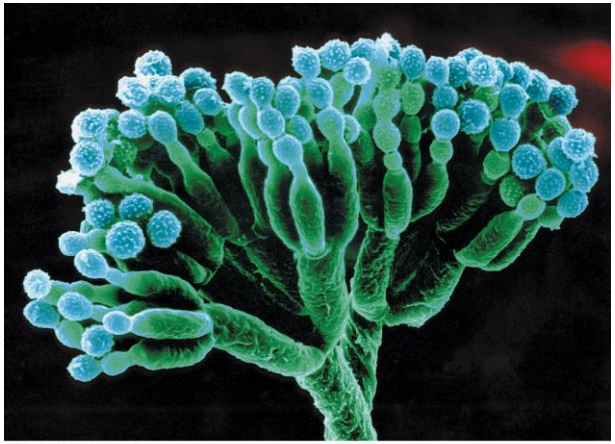
Fusarium

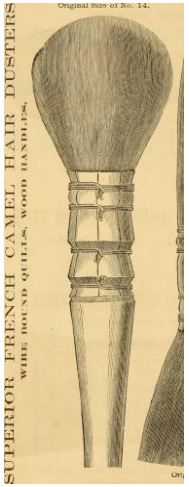
Neurospora

Botrytis

Alternaria

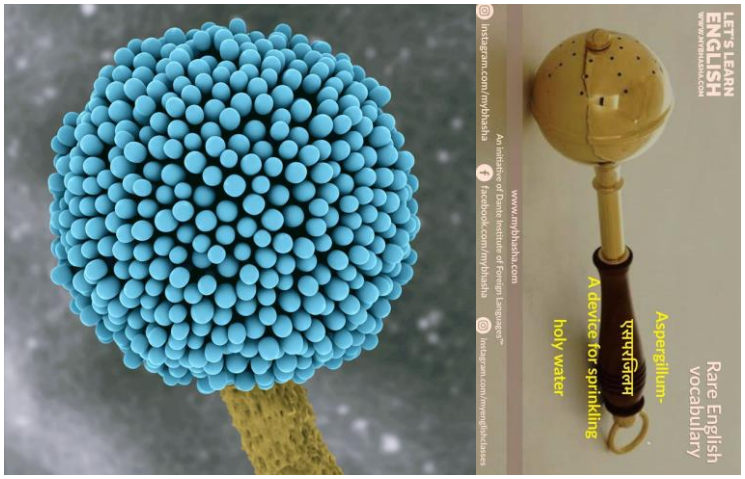
Penicillium = Little Brush

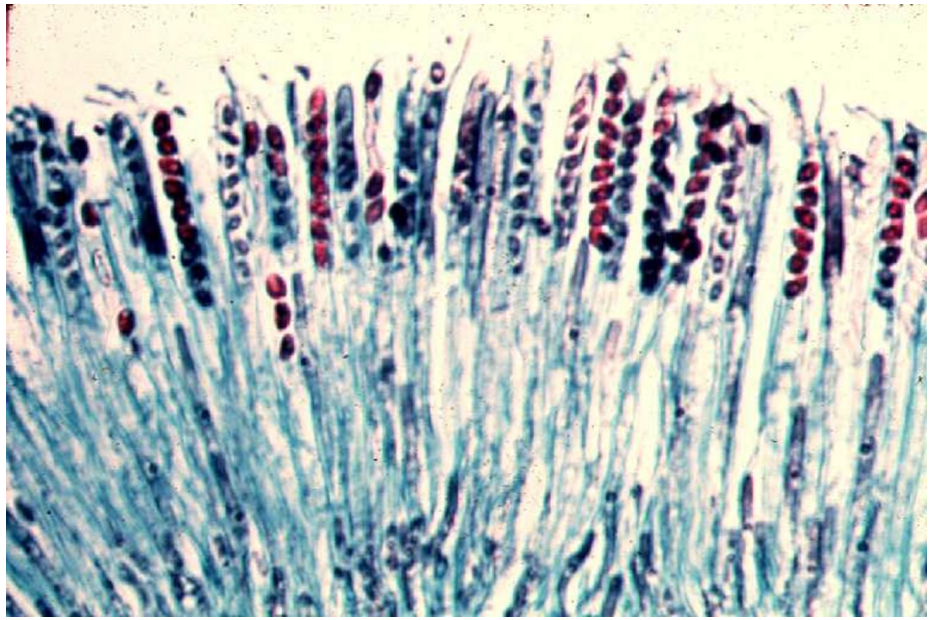


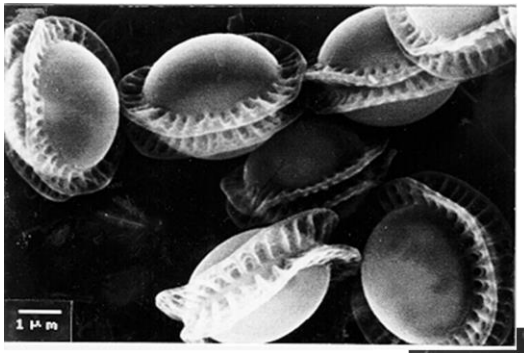


Produce penicillin

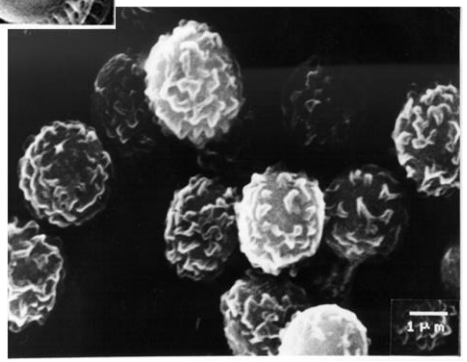
Aspergillus = aspergillum, a holy water sprinkler







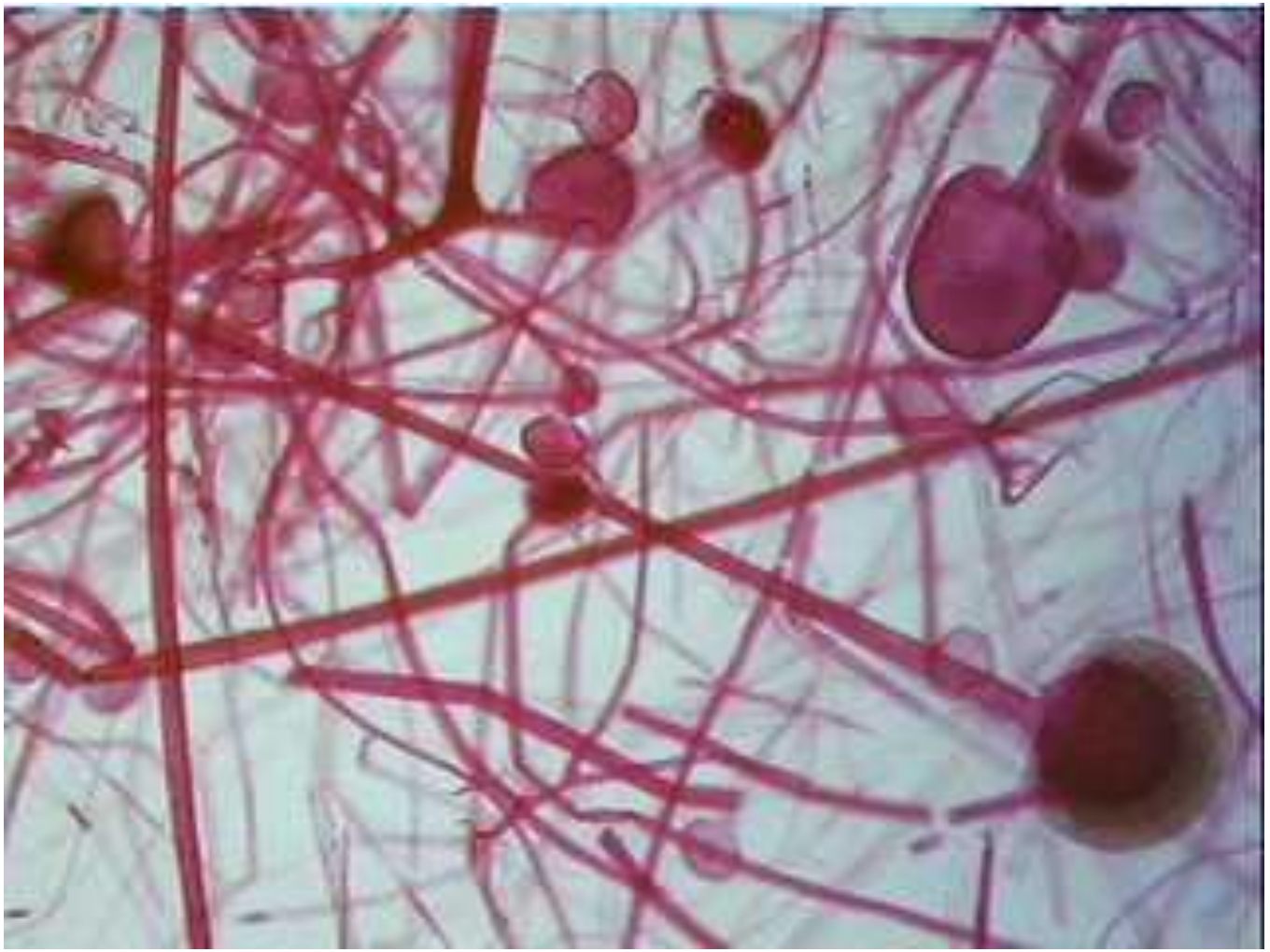
SEM Image of *Aspergillus nidulans* Ascospore (Sexual Spores)



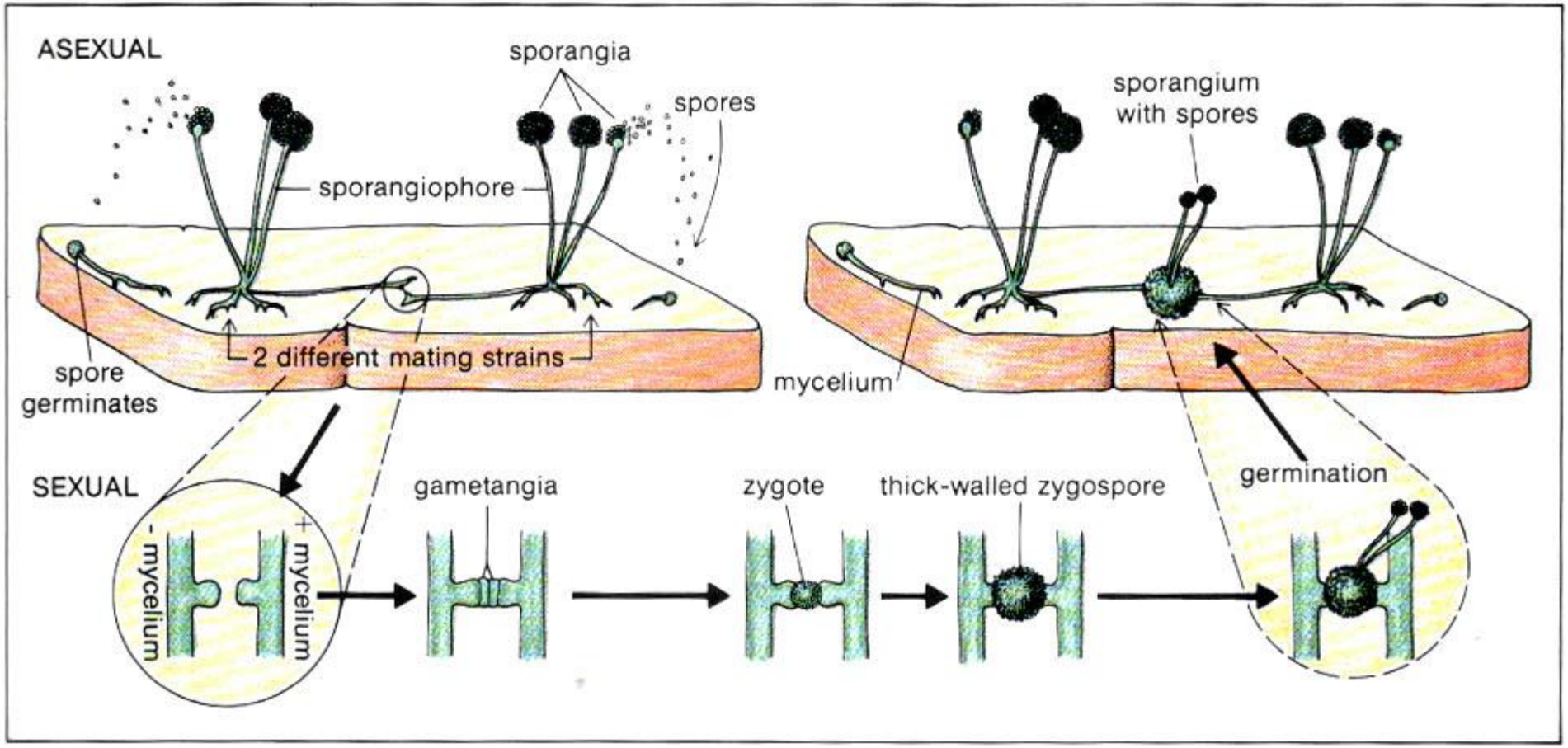
SEM image of *Aspergillus nidulans* Conidia (Asexual Spores)

Zygomycota = The Conjugation Fungi

Rhizopus sporangia



Rhizopus and *Mucor* life cycle

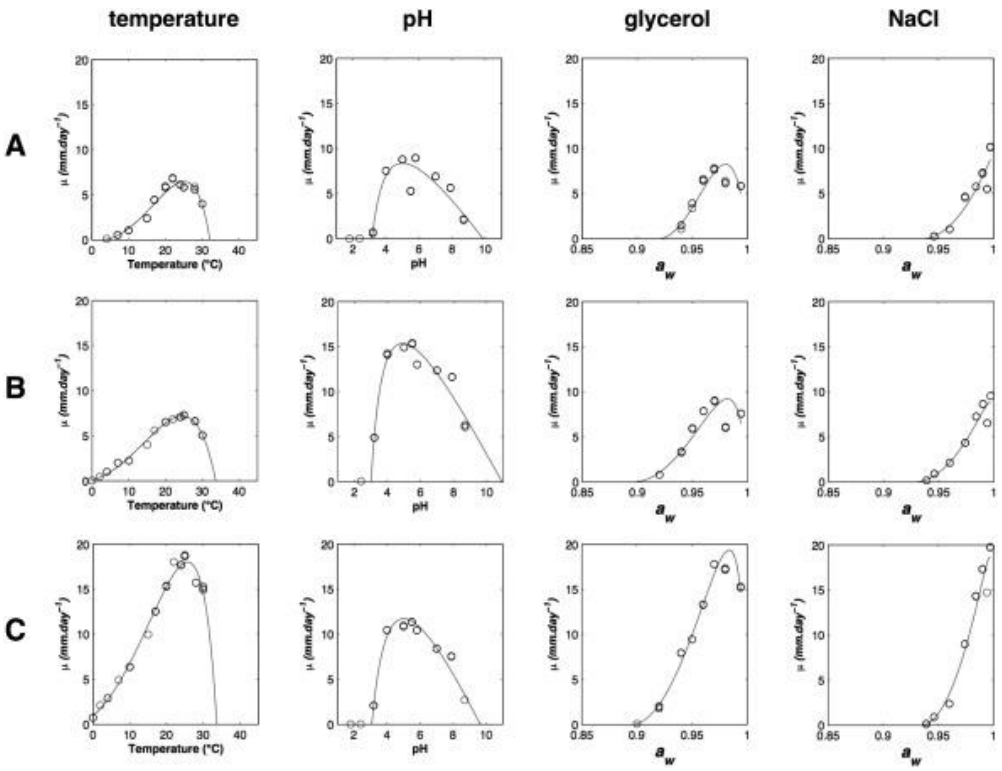


Rhizopus


gettyimages
E R DEGGINGER
128610762

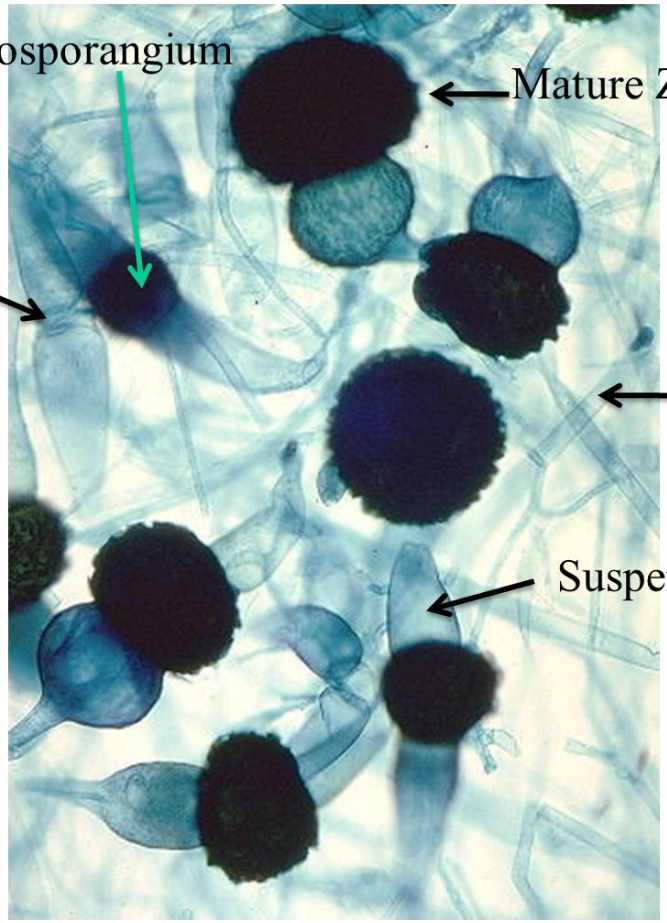

Rhizopus arrhizus
© A.J. Silverside

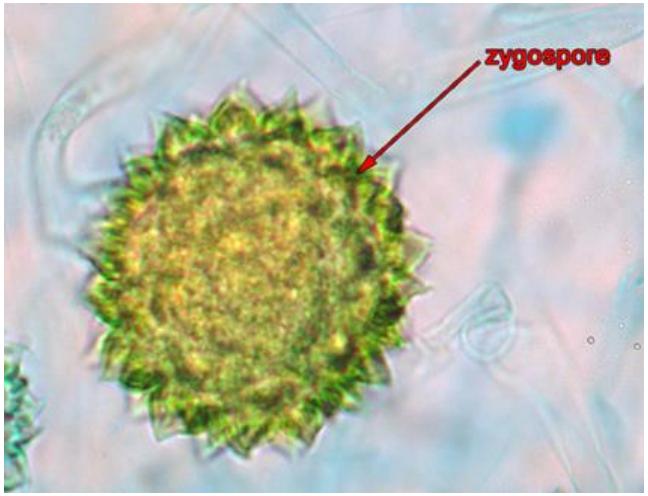
Mucor – soft rot



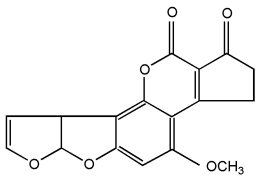
https://doi.org/10.1016/j.fm.2015.11.019

Zygosporangia and zygospore

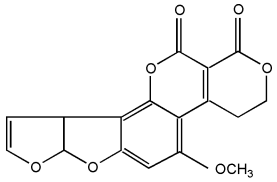




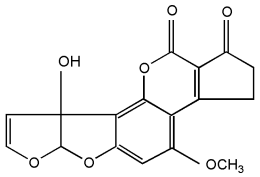
Major Mycotoxins



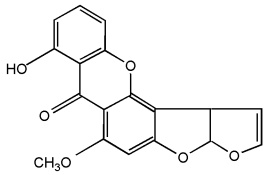
Aflatoxin B_1



Aflatoxin G_1

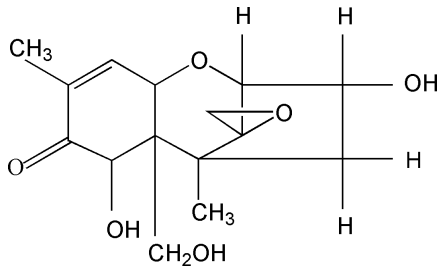


Aflatoxin M_1

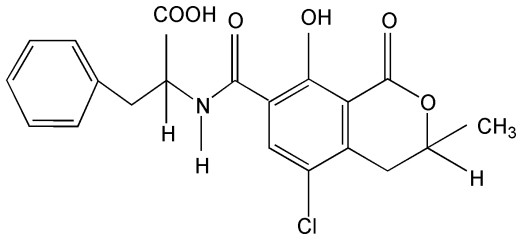


Sterigmatocystin

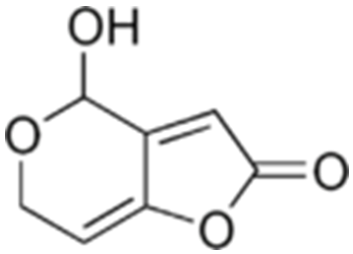
Aflatoxins
Aspergillus flavus
Aspergillus parasiticus



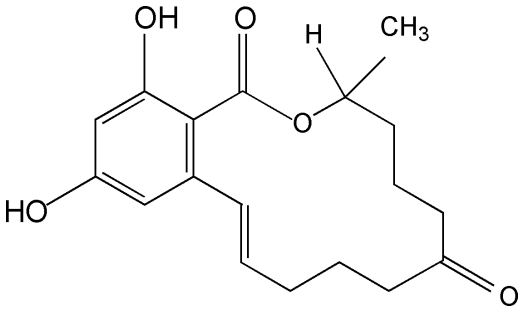
Vomitoxin (DON)
Fusarium graminearum



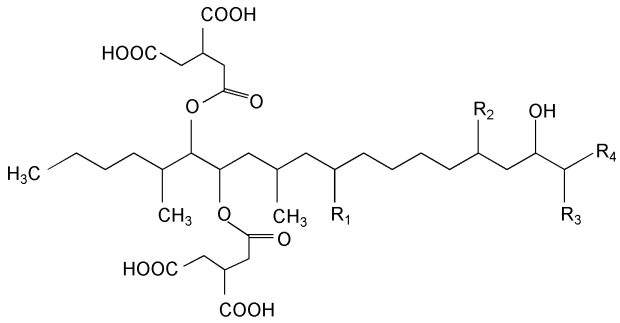
Ochratoxin A
Aspergillus ochraceous
Penicillium spp.



Patulin
Aspergillus, Penicillium



Zearalenone (F-2 Toxin)
Fusarium graminearum



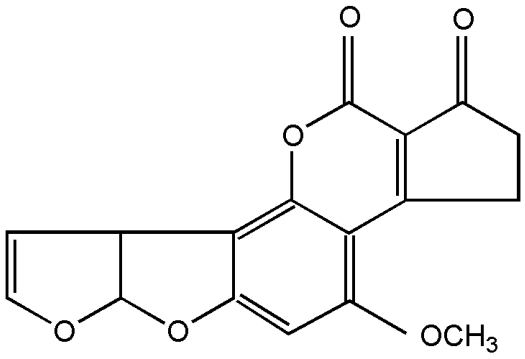
Fumonisin	R_1	R_2	R_3	R_4
FB_1	OH	OH	NH_2	CH_3
FB_2	H	OH	NH_2	CH_3
FB_3	OH	H	NH_2	CH_3
FC_1	OH	OH	NH_2	H
FA_1	OH	OH	$NHCOCH_3$	CH_3

Fumonisins
Fusarium verticillioides

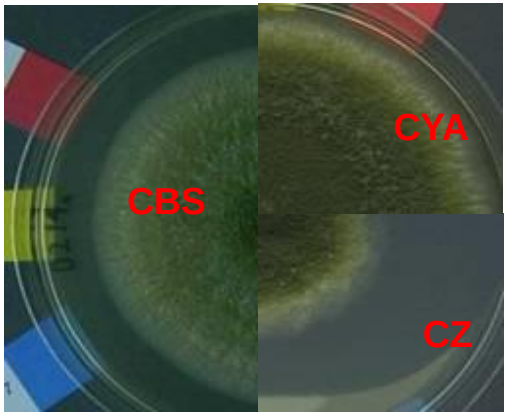
Mycotoxins associated with food and feed

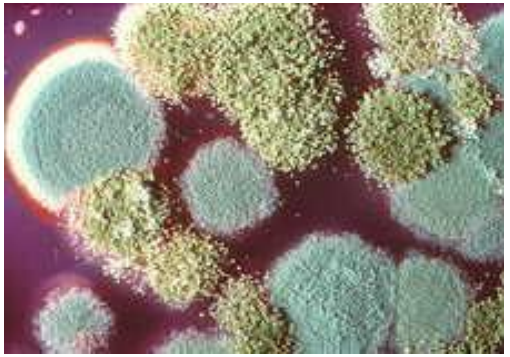
- **Aflatoxins (B1, M1)**
- **Ochratoxin A**
- **Zearalenone**
- **Fumonisins**
- **Trichothecenes**
- **Patulin**
- Moniliform
- Sterigmatocystin
- Citrinin
- Cyclopiazonic acid
- Kojic acid
- Maltoryzine
- ß-nitropropionic acid
- Aspergillic acid
- Penicillic acid
- Roquefortine C

Aflatoxins



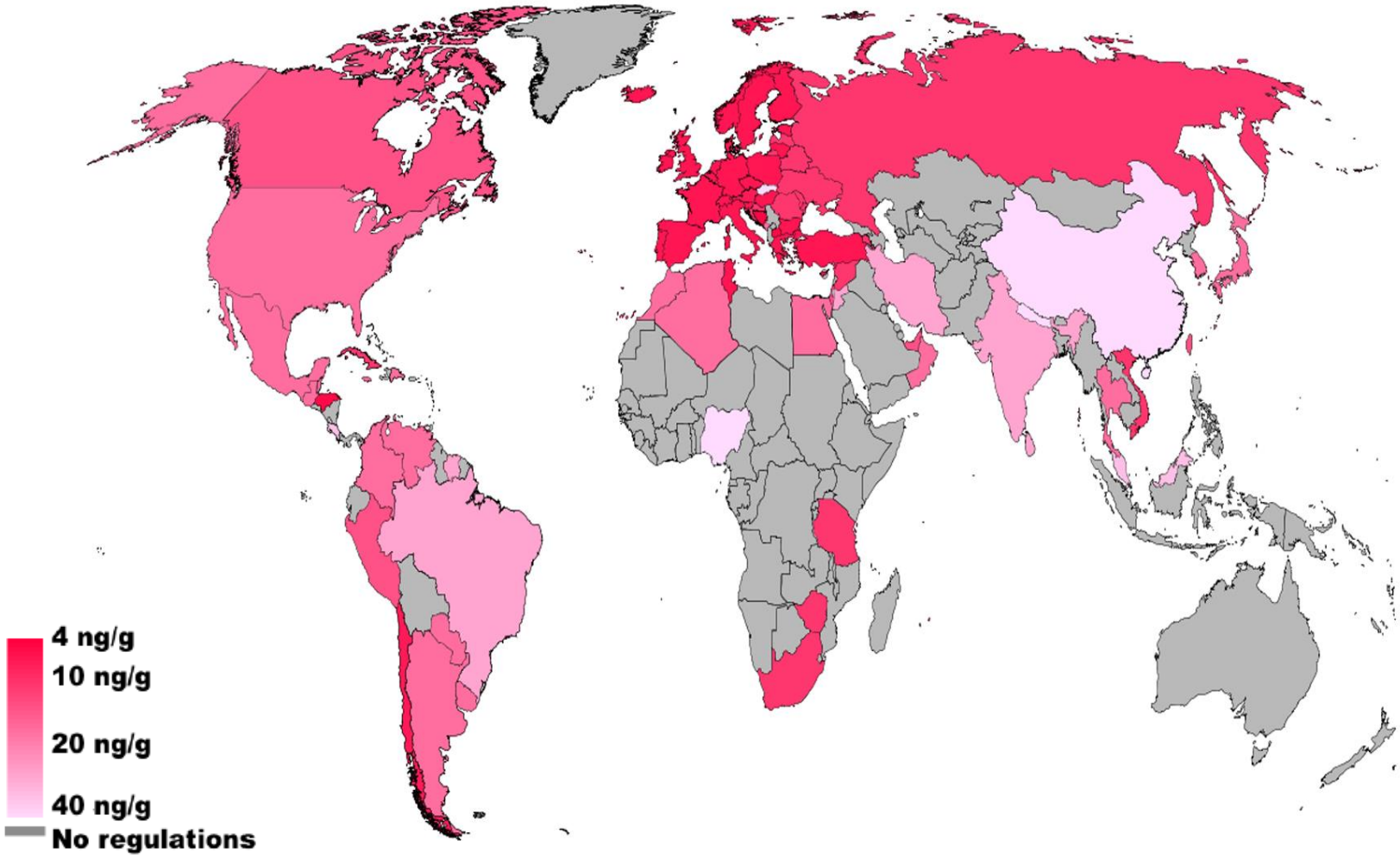
Aflatoxin B_1





- Commonly associated with maize, groundnuts, tree nuts, spices, dried fruit etc.
- Carry-over from animal feed to foods of animal origin for humans: e.g. Aflatoxin M1 in milk
- International guidelines exist for prevention and control

To protect populations from aflatoxin, >100 nations have regulatory standards in food

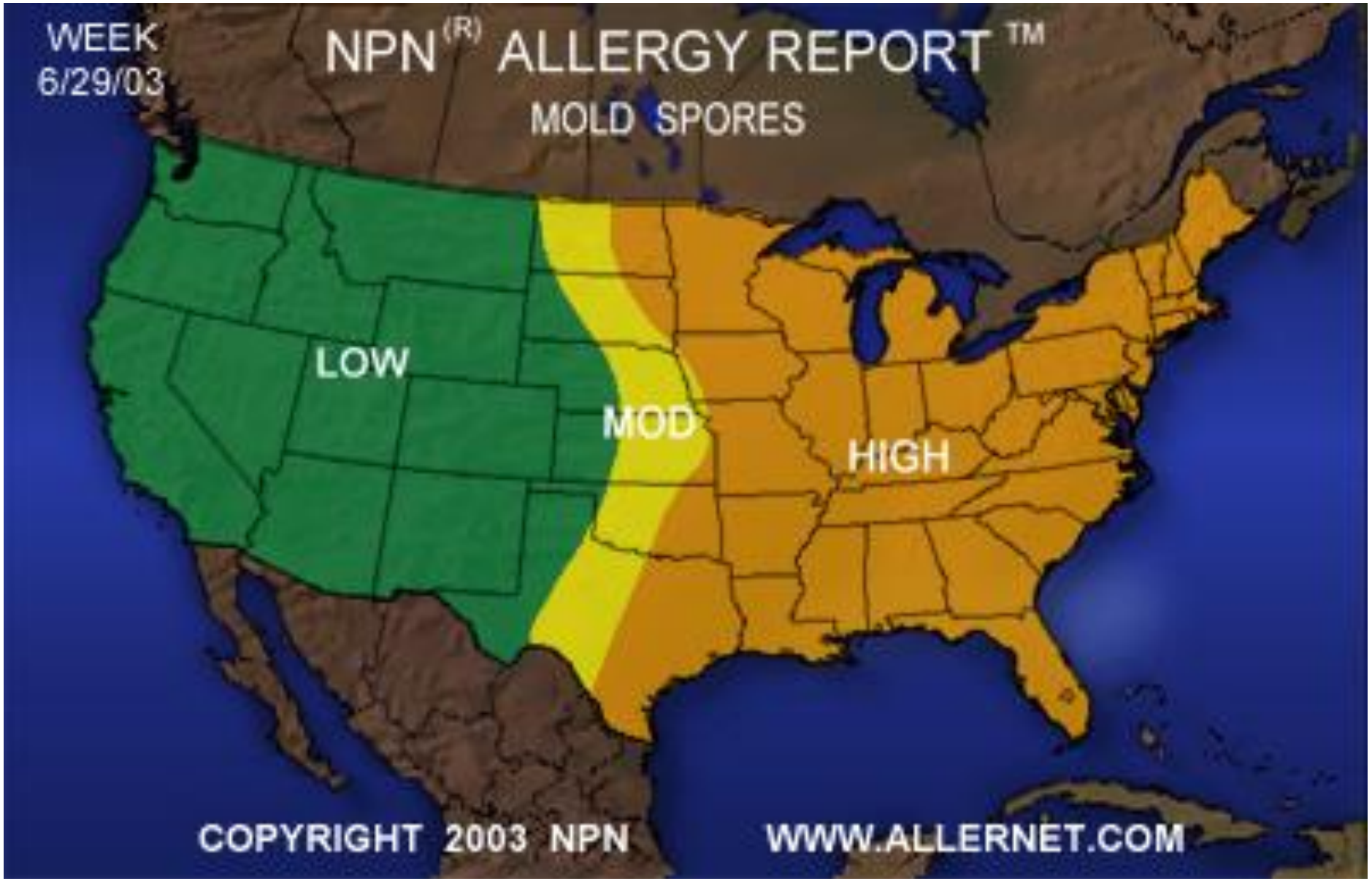


Other important mycotoxins

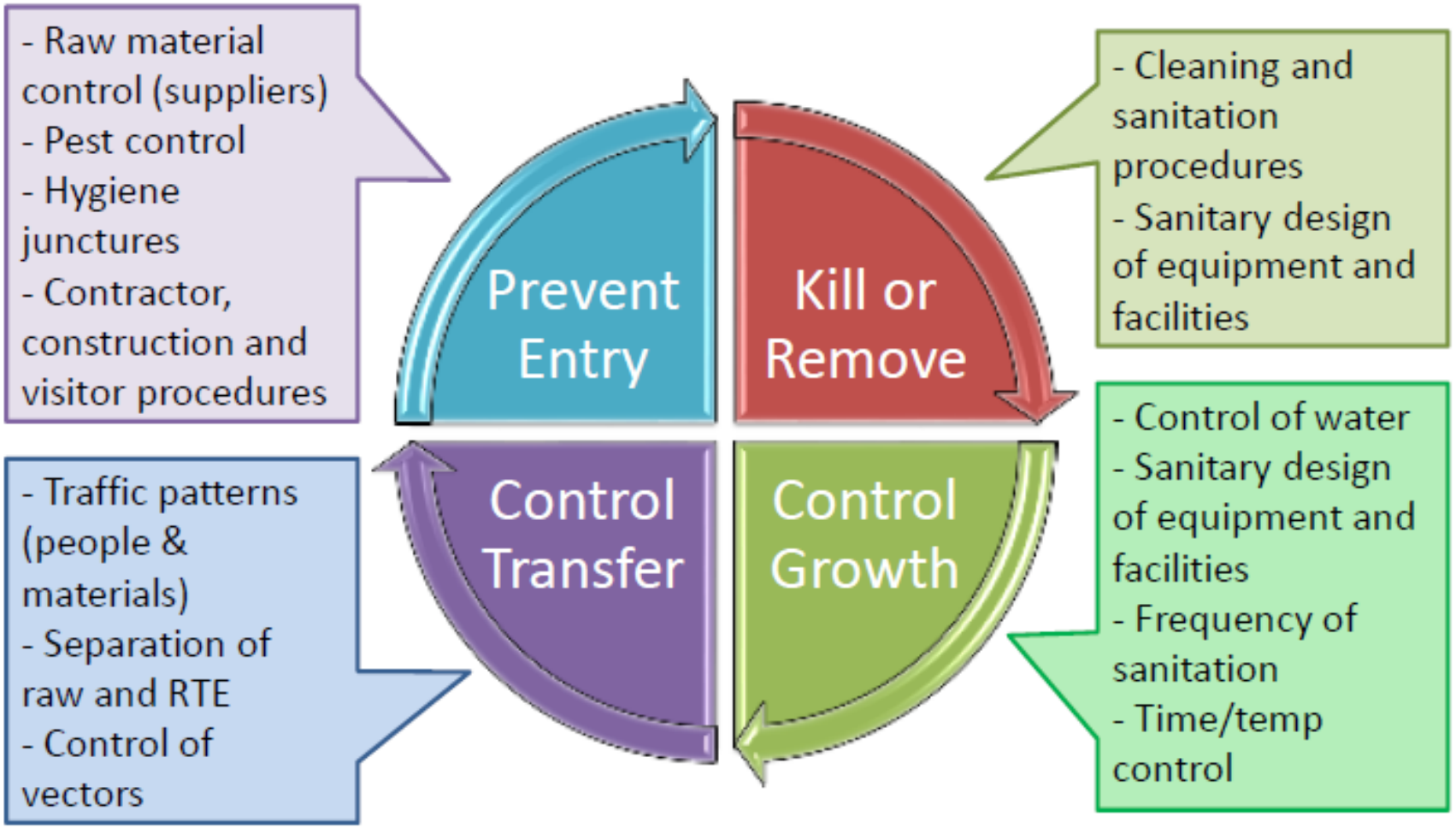
- Trichothecenes; DON – *Fusarium spp*
  - A variety of cereals and wet harvest conditions – FDA Advisory Levels 1 ppm ~
- Zearalenone – *Fusarium spp*
  - Maize grown in temperate climates
- Fumonisins – *Fusarium spp*
  - Primarily associated with maize – FDA Advisory Levels 2 ppm~
- Patulin - *Penicillium spp*, *Aspergillus spp*
  - Apple products – FDA **Action Level** 50 ppb
- Ochratoxin – *Aspergillus spp*, *Penicillium spp*
  - Cereals, wine, grape juice, dried fruit, coffee and cocoa

Why, where, and how are we getting molds in food and beverage?

Mold and mold spores are part of our daily life.



> Control of Microorganisms



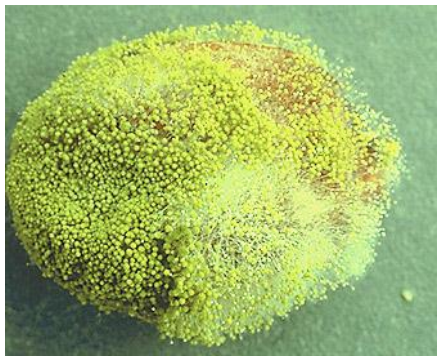
LAND O'LAKES, INC.

Courtesy of Dr. Benjamin Warren

Table 2.2 Comparative heat resistance of ascospores and conidia[a]

Fungus	Spore type	Initial viable count/ml	Survivors (%)		
			50°C	60°C	70°C
Eurotium amstelodami	Ascospores	5.0×10^2	93	85	3
	Conidia	7.3×10^2	107	0.3	0
Eurotium chevalieri	Ascospores	1.0×10^3	103	62	21
	Conidia	8.9×10^2	128	0.1	0
Xeromyces bisporus	Ascospores	1.0×10^3	93	30	0.3
Aspergillus candidus	Conidia	3.8×10^2	102	0	0
Wallemia sebi	Conidia	7.1×10^2	42	0	0

[a] Heated at temperatures shown for 10 min. Data from Pitt and Christian (1970).



SUMMARY

(1) The holder process of pasteurization, in which milk was heated to 145° F. (62.8° C.) and maintained at that temperature for 30 minutes, killed the conidia of every species investigated, except those of *Aspergillus repens*, *A. flavus*, and *A. fumigatus*. The molds which survive are found only occasionally in milk.

(2) The flash process of pasteurization, where milk was heated to 165° F. (73.9° C.) for a period of 30 seconds, destroyed the spores of all the molds tested with the exception of many spores of one form and occasional spores of three more forms. At 175° F. (79.5° C.) only occasional spores of two forms developed.

(3) When the heating process was performed in dry air for a period of 30 seconds at 200° F. (93.3° C.), 31 out of 42 forms of Penicillium and 7 out of 24 forms of Aspergillus were destroyed, but none of the cultures of the mucors. A temperature of 250° F. (121.1° C.) over a period of 30 minutes killed all the forms of *Penicillium* spp. tried, but left an occasional living spore in one species of Aspergillus and three out of six mucors.

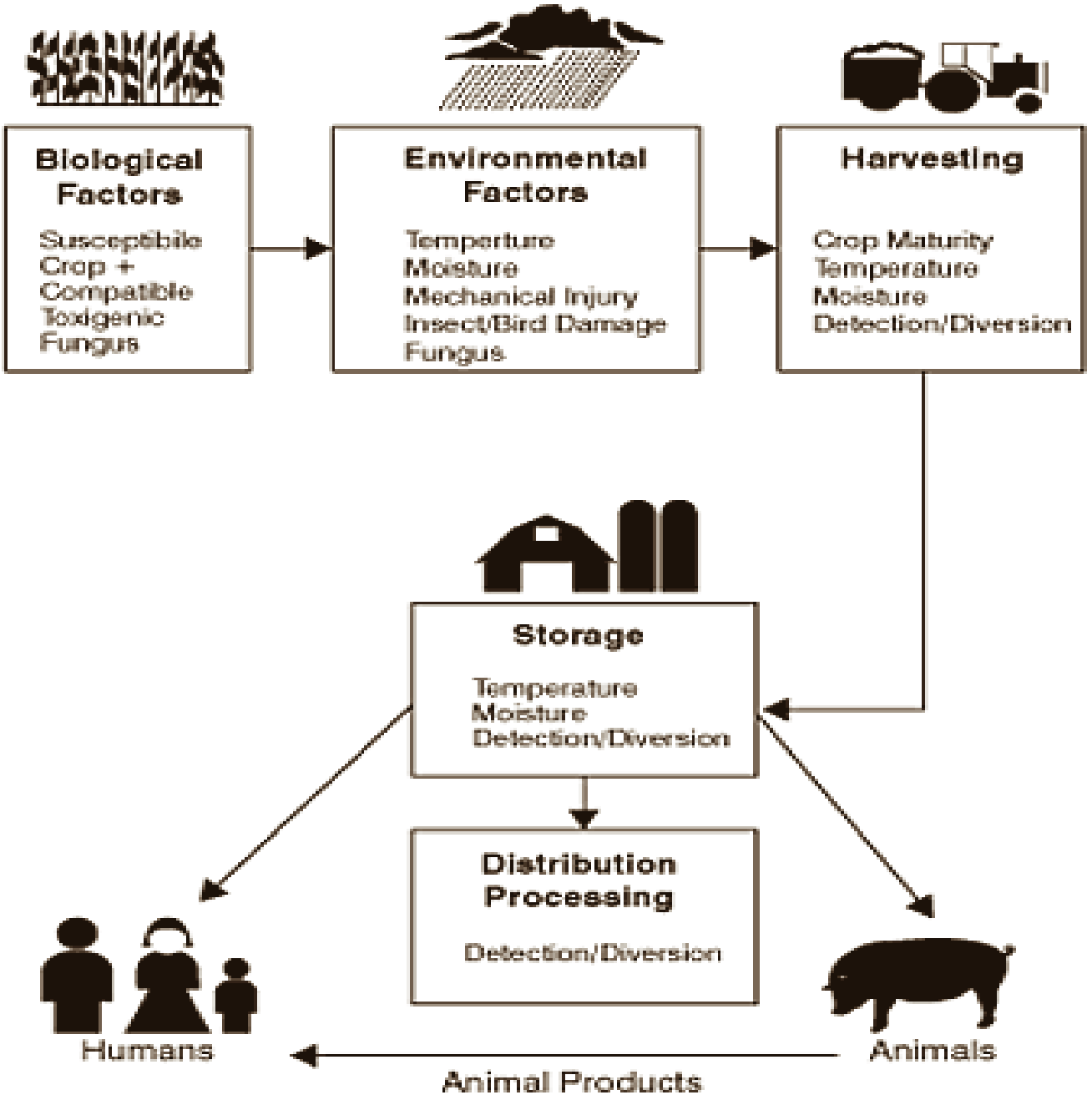
Heat Restraint Fungi

Those can survive thermal treatment by **minimum 30 minutes using 75°C or higher**: more restrictive than those used in industrial pasteurization.

Species most commonly implicated in fruit and fruit product disintegration are *Byssochlamys fulva*, *Byssochlamys nivea*, *Neosartorya fischeri*, *Talaromyces flavus*, and *Eupenicillium brefeldianum*.

Sexual spore: Ascospore

How can we prevent molds growth and/or mycotoxin contamination in food?



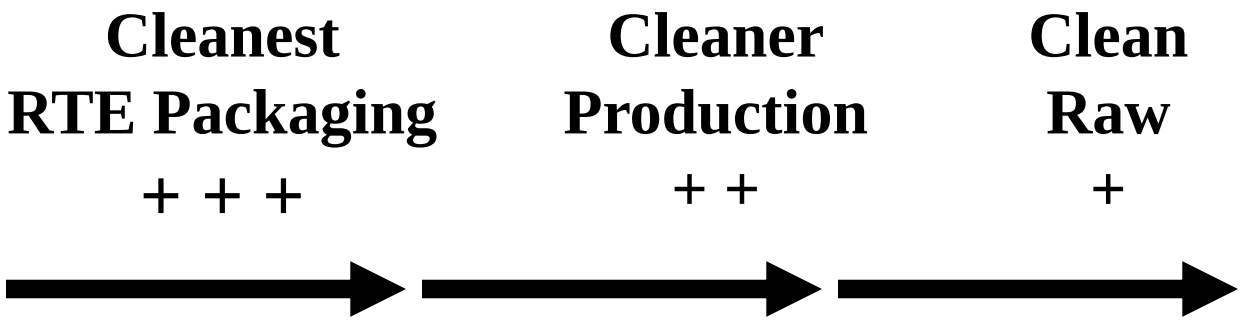
http://www.foodtech-international.com/papers/images/mycotoxins/figure1.gif

Sources of Spoilage Organisms

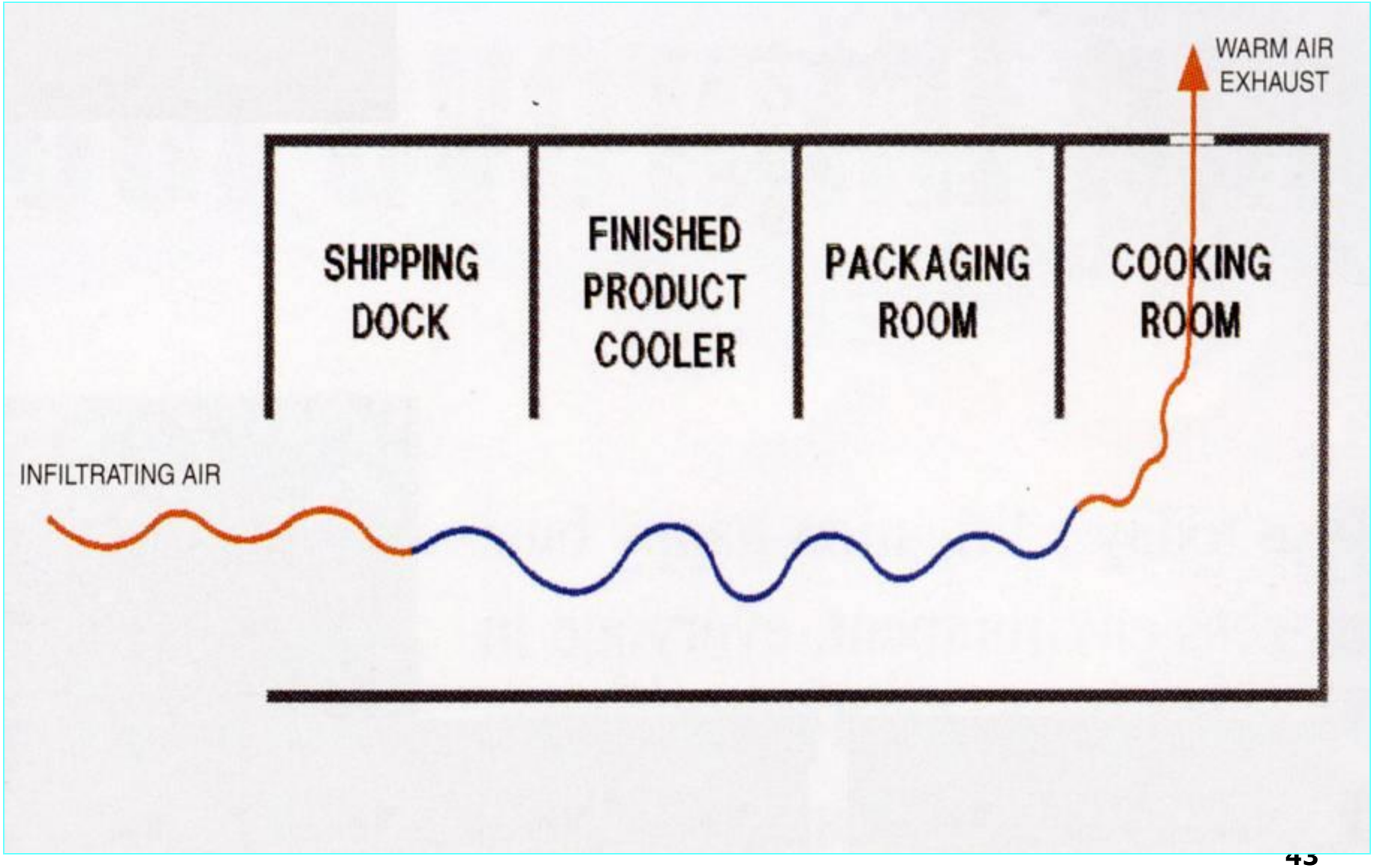
- Ingredients, Raw Products
- Food processing surfaces/equipment
- Airborne contaminants
- Other materials entering the facility
- Humans

Air flow control is important!

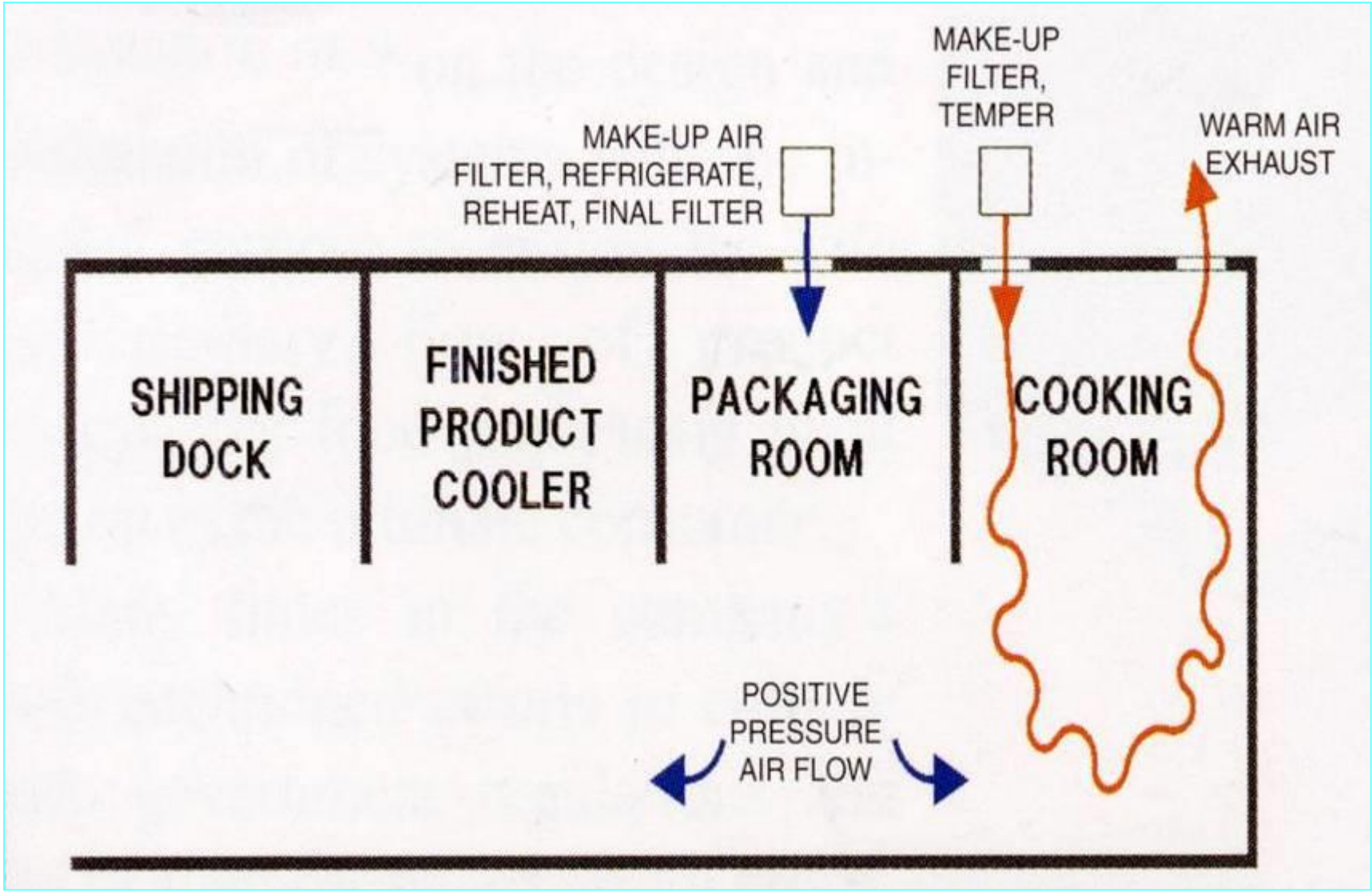
All rooms should have their pressure controlled to ensure airflow will be **from more clean to less clean areas**



Very Bad Ventilation Design



Good Ventilation Design



Prevention of Spoilage

- Do not allow biologically active waste to accumulate: CLEAN CLEAN CLEAN!!!!
- Clean equipment immediately after use, not just before next use.
- Identify source of contamination promptly.
- Minimize outside sources of contamination: know your raw products (ingredients)!!
- Use effective anti-microbials with a hurdle concept
- Monitor signs of spoilage constantly

6 Factors for fungal growth…

- **F** Food/Fungi
- **A** Acidity
- **T** Temperature

- **T** Time
- **O** Oxygen
- **M** Moisture

Requirements for Fungal Growth

- **Substrates**: Nitrogen and Energy source
- **Temperature**: Broad ranges, 0°C ~ 58°C
- **Oxygen**: Fungi are aerobic
- **Unbound Water**: measured by water activity
  - **Moisture is the most important factor!**

Three groups of foods: based upon rate of spoilage

- highly perishable
  - meat
  - fruit
  - milk
  - vegetables
  - eggs
- semi perishable
  - potatoes
  - nuts
- stable
  - rice
  - flour
  - dry beans

WET

Dry

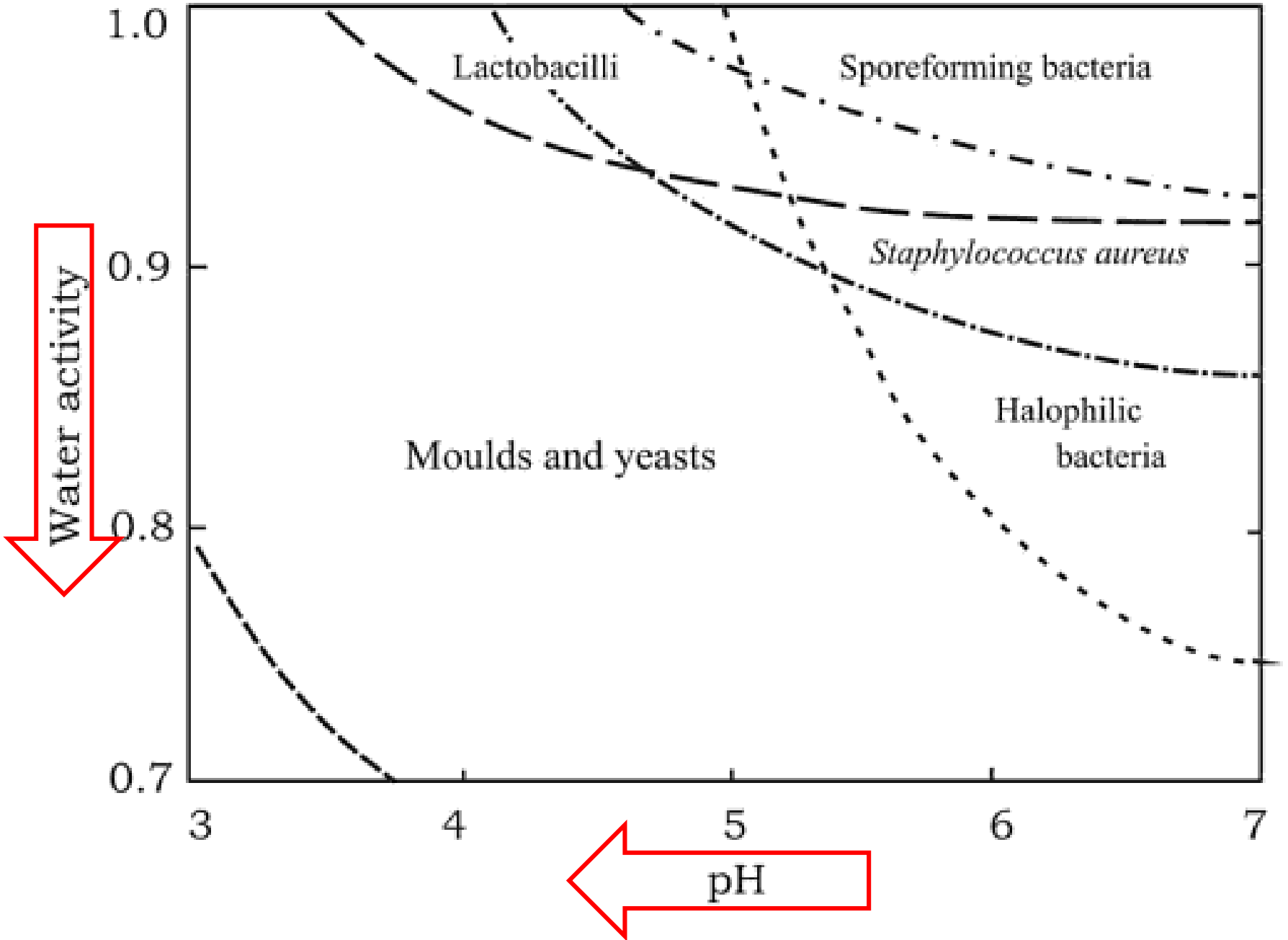
What defines each group?

Amount of water

Table 2.1 Water activity and microbial water relations in perspective[a]

a_w	Perspective	Foods	Moulds	Yeasts
1.00	Blood, plant wilt point, seawater	Vegetables meat, milk fruit		
0.95	Most bacteria	Bread	Basidiomycetes Most soil fungi	Basidiomycetes
0.90		Ham	Mucorales *Fusarium*	Most ascomycetes
0.85	*Staphylococcus aureus*	Dry salami	*Rhizopus, Cladosporium*	*Zygosaccharomyces rouxii* (salt)
0.80			*Aspergillus flavus* Xerophilic Penicillia	*Zygosaccharomyces bailii*
0.75	Salt lake Halophiles	Jams Salt fish Fruit cake	Xerophilic Aspergilli *Wallemia* *Eurotium*	*Debaryomyces hansenii*
0.70		Confectionery Dried fruit Dry grains	*Chrysosporium* *Eurotium halophilicum*	
0.65			*Xeromyces bisporus*	*Zygosaccharomyces rouxii* (sugar)
0.60	DNA disordered			

[a] Modified from data of J.I. Pitt as reported by Brown (1974). Water activities shown for microorganisms approximate minima for growth reported in the literature.


1.0
0.9
0.8
0.7
Water activity
Lactobacilli
Sporeforming bacteria
Staphylococcus aureus
Halophilic
bacteria
Moulds and yeasts
3
4
5
6
7
pH

Fungal spoilage of starch-based foods in relation to its water activity

Days before visible appearance of fungi on six starch-based food at 25°C. Average of three determinations

a_w	Rice	Glutinous rice	Rice flour	Glutinous rice flour	Wheat flour	Corn flour
0.98	7 ± 2	7 ± 2	11 ± 3	14 ± 2	8 ± 1	22 ± 3
0.95	9 ± 1	9 ± 2	15 ± 4	17 ± 4	10 ± 1	51 ± 2
0.90	10 ± 2	10 ± 3	20 ± 1	24 ± 3	10 ± 1	69 ± 2
0.85	10 ± 2	10 ± 0	27 ± 1	39 ± 2	14 ± 3	124 ± 4
0.80	13 ± 1	17 ± 1	28 ± 3	64 ± 4	17 ± 1	[a]
0.75	20 ± 2	19 ± 1	32 ± 1	91 ± 3	27 ± 2	[a]
0.65	57 ± 2	73 ± 1	[a]	[a]	[a]	[a]

[a] No fungal development at 6 months incubation.

(Abdullah *et al*., 2000)

How to reduce available water?

- drying
  - sun
  - heat
  - freeze - dried (expensive!)

How to reduce available water?

- addition of salt or sugar
  - water needed to keep salt and sugar in solution

Antimycotics controlling the growth of yeasts and molds

- **Organic acids**
- **Chelating agents (EDTA, HMETA)**
- **Gaseous compounds (ethanol, sulfur dioxide, chlorine dioxide, propylene oxide, ozone, hydrogen peroxide, acetic acid)**
- **Biocontrol (mycoviruses, lactic acid bacteria, propionibacteria, and yeasts)**
- **Modified atmosphere packaging**
- **Fungicides**
- **Naturally occurring compounds: vanillin, phenolic compounds, phytoalexins, chitosan**

Table 41.5 Major Groups of Chemicals Used in Food Preservation

Preservatives	Approximate Maximum Use Range	Organisms Affected	Foods
Propionic acid/propionates	0.32%	Molds	Bread, cakes, some cheeses, inhibitor of ropy bread dough
Sorbic acid/sorbates	0.2%	Molds	Hard cheeses, figs, syrups, salad dressings, jellies, cakes
Benzoic acid/benzoates	0.1%	Yeasts and molds	Margarine, pickle relishes, apple cider, soft drinks, tomato ketchup, salad dressings
Parabens[a]	0.1%	Yeasts and molds	Bakery products, soft drinks, pickles, salad dressings
SO_2/sulfites	200–300 ppm	Insects and microorganisms	Molasses, dried fruits, wine, lemon juice (not to be used in meats or other foods recognized as sources of thiamine)
Ethylene/propylene oxides	700 ppm	Yeasts, molds, vermin	Fumigant for spices, nuts
Sodium diacetate	0.32%	Molds	Bread
Dehydroacetic acid	65 ppm	Insects	Pesticide on strawberries, squash
Sodium nitrite	120 ppm	Clostridia	Meat-curing preparations
Caprylic acid	—	Molds	Cheese wraps
Ethyl formate	15–200 ppm	Yeasts and molds	Dried fruits, nuts

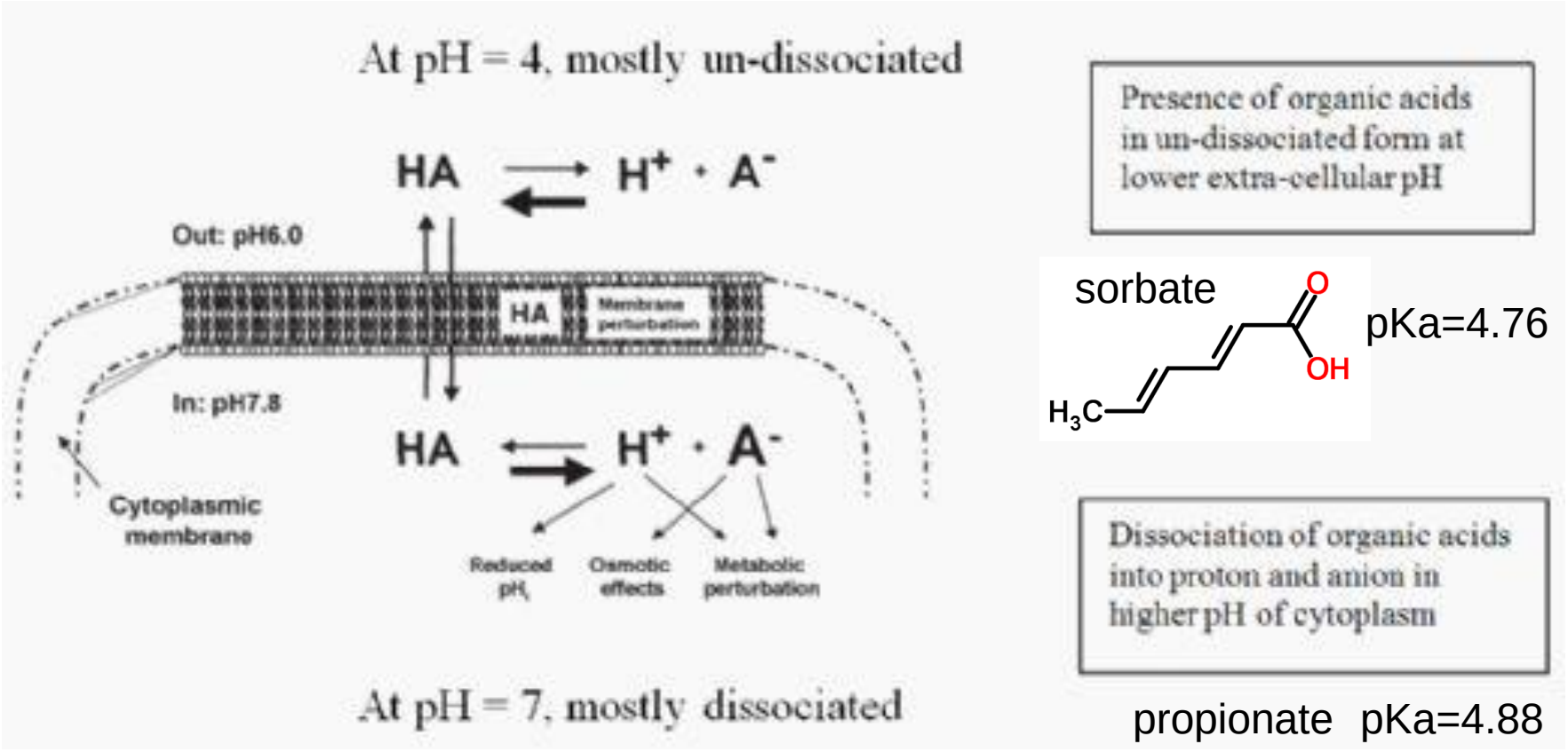
From James M. Jay. 2000. *Modern Food Microbiology,* 6th edition. Reprinted by permission of Aspen Publishing, Frederick, Md.

[a]Methyl-, propyl-, and heptyl-esters of *p*-hydroxybenzoic acid.

Undissociation of acids affected by pH

		Percentage undissociated acid at pH:						
Acid	pK_a	2.5	3.5	4.5	5.0	5.5	6.0	7.0
Acetic	4.74	99	95	63	35	14	5.2	0.55
Citric	3.13	81	30	4.1	1.3	0.4	0.13	0.01
Formic	3.75	95	64	15	5.3	1.7	0.56	0.06
Lactic	2.74	64	15	1.7	0.5	0.2	0.06	0.01
Malic	3.40	89	44	7.4	2.5	0.8	0.25	0.03
Tartaric	2.98	75	23	2.9	0.9	0.3	0.10	0.01
Benzoic	**4.19**	**98**	**83**	**33**	**13**	**4.7**	**1.5**	**0.15**
Propionic	**4.87**	**100**	**96**	**70**	**43**	**19**	**6.9**	**0.74**
Sorbic	**4.76**	**99**	**95**	**65**	**37**	**15**	**5.4**	**0.57**

From Beuchat, In Minimally Processed Fruits and Vegetables (Alzamora, Tapia, and Lopez-Malo, ed.). Aspen Publ., Chapter 4, pp. 63-77, 2000.



Presence of organic acids in un-dissociated form at lower extra-cellular pH

sorbate

pKa=4.76

Dissociation of organic acids into proton and anion in higher pH of cytoplasm

propionate pKa=4.88

benzoate pKa=4.2

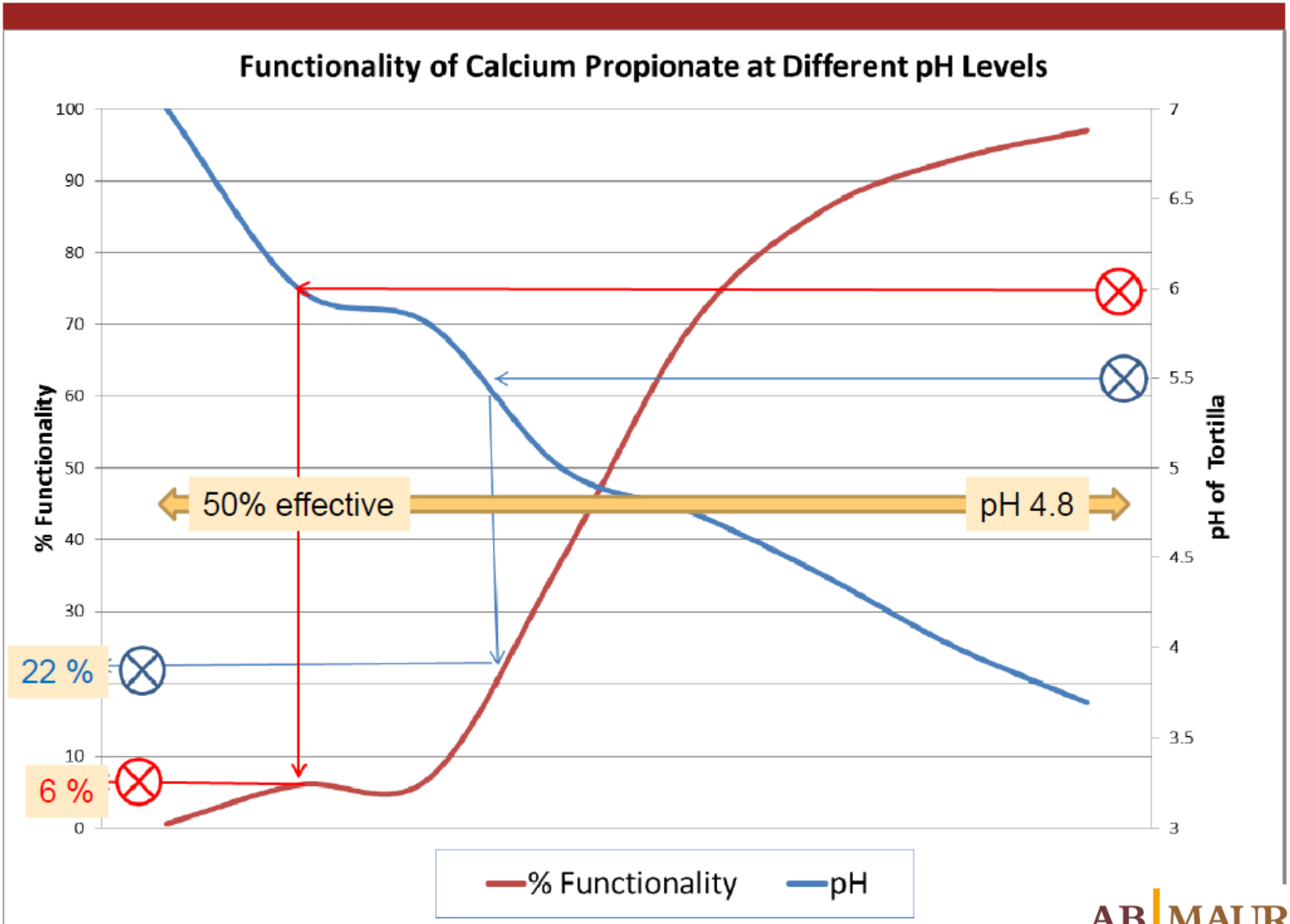
MOA of organic acids inhibiting mold growth:

The un-dissociated organic acids (HA) penetrates cell membrane then, HA → H+ + A-.

1. Cell needs to pump out H+, depleting ATP.

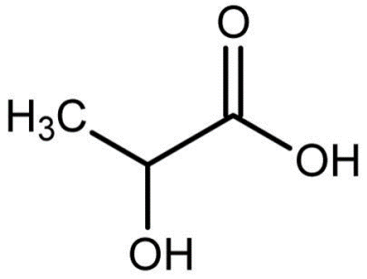
2. The disrupted membrane permeability causes reduction in cellular uptake of amino acids and nutrients.

3. The anions (A-) formed inhibit the synthesis of cell wall components, DNA, RNA, lipids and proteins.

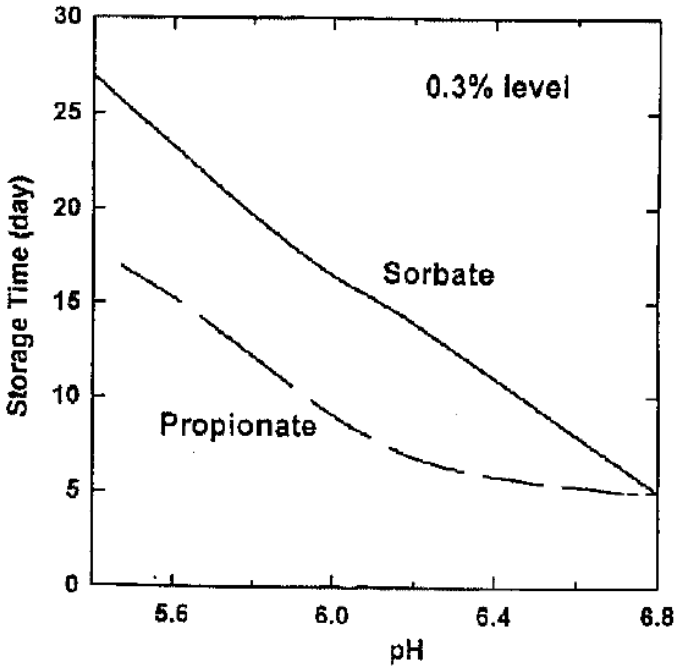


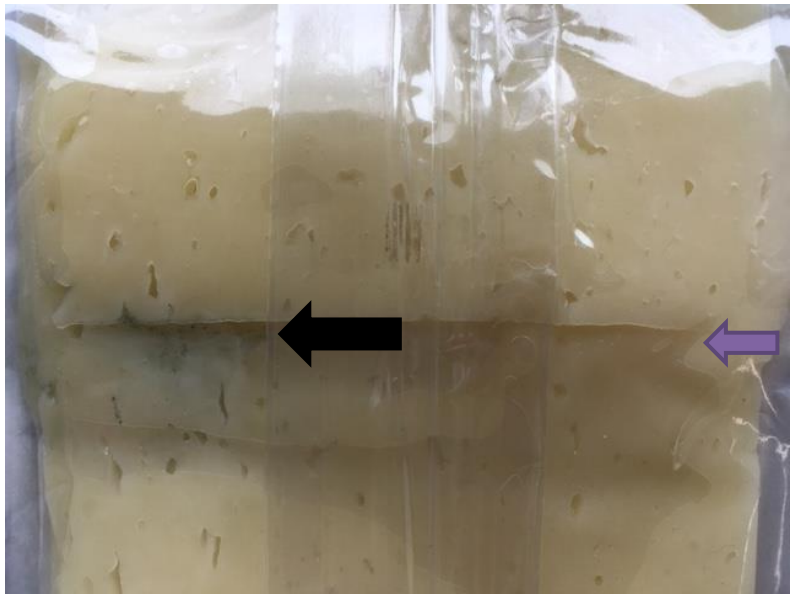
AB MAURI
BAKERY INGREDIENTS

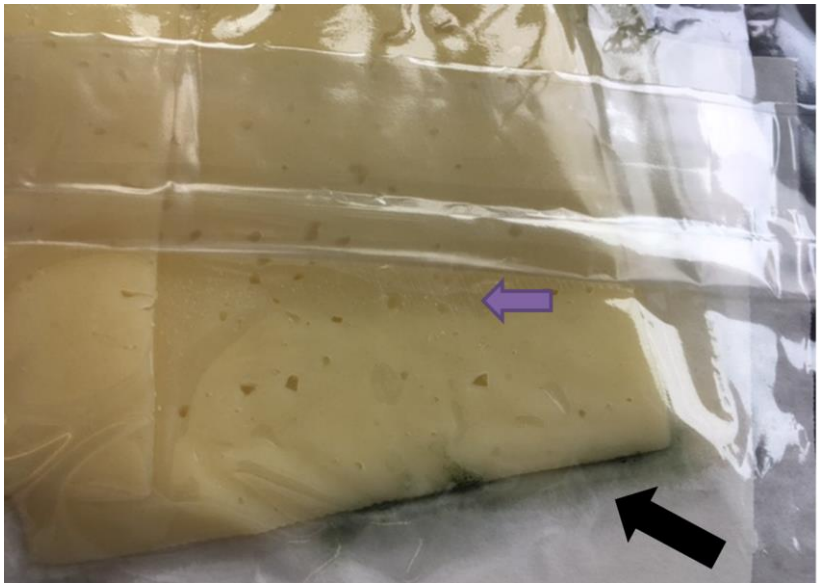
Organic Acids and Salts

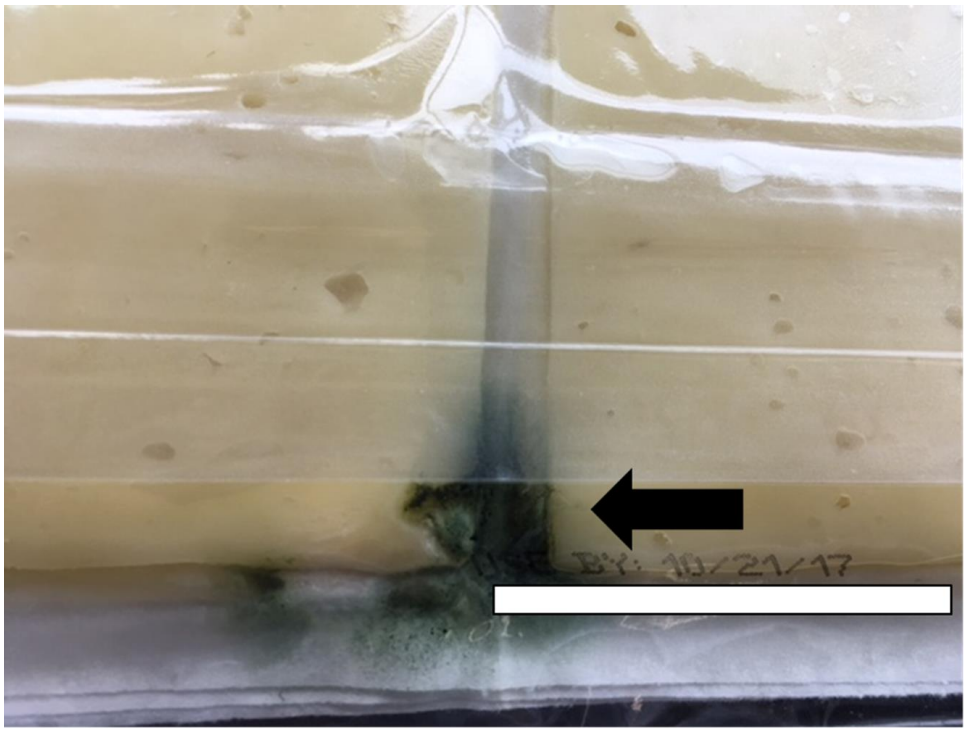


- Optimizing efficacy
  - Lower pH values (e.g. <5.5; near or below pK_a)
  - Lower temperatures (4 vs. 7 or 10°C)
    - Exception: pH ≤4.6; then combined stress with higher temperatures increases inactivation rate
  - Combined with other antimicrobials








BY: 10/21/17

Hurdle concept

- **Simultaneous application of two or more stress or inhibitory factors (e.g., temperature, pH, acidulant, low a_w, salt, phenolic compounds, etc.)**

→ **synergistic interactions lead to a combined effect of greater magnitude than the sum of constraints applied individually**

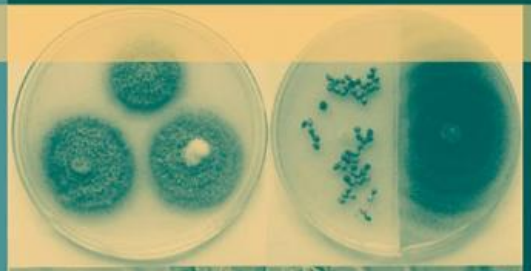
Key to minimize mold problems

- Basics of mold problems: Use your common sense:
  - **1. Inoculum (Molds)**
  - **2. Substrates (Foods)**
  - 3. Temperature (Broad range)
  - 4. Oxygen
  - **5. Water**
- ***MOISTURE CONTROL*** is the key!

THIRD EDITION

John I. Pitt
Ailsa D. Hocking

Fungi and Food Spoilage



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