

Microbial Ecology of Foods

Dean O. Cliver

Epidemiology teams

Team 1

Tyann Blessington
Rebecca Garabed
Kullanart Tongkhao

Team 2

Kristin Ahrens
Sharon Hunt Gerardo
Bradley Olson

Team 3

Thuyvan Lam
Anika Singla
Christopher Theofel

Team 4

Shirin Jamshidi
Thomas Kohler

A food is an ecosystem for microbes

- ◆ They don't "know" they are in food!
- ◆ Bacteria & molds may multiply, survive, or die.

A food is an ecosystem for microbes

- ◆ Viruses & parasites can only persist or be inactivated (die, lose infectivity).
- ◆ Most attention devoted to fates of bacterial pathogens.

Pathogenic bacteria in food: potential "outcomes"

- ◆ Persistence: viable, numbers unchanged (lag or stationary phase or sporulation)
- ◆ Growth (multiplication): rate parameter (variable) based on doubling time

Pathogenic bacteria in food: potential "outcomes"

- ◆ Death: another rate parameter (cf. viable-nonculturable)
- ◆ Sporulation: another defense (species)
- ◆ Toxigenesis: growth is necessary, but possibly not sufficient

Growth curve biology

- ◆ Spores & lag phase cells quiescent; adaptation to environmental conditions = selecting needed enzymes (activating appropriate genes) from broad bacterial repertoire.

Growth curve biology

- ◆ Multiplying (doubling) cells are metabolically active, often adapting; not all metabolically active cells are multiplying.
- ◆ Stress causes adaptation or injury.

Growth curve biology

- ◆ Stationary phase may represent quiescence or (more often) growth rate = death rate.
- ◆ Some injured cells appear dead (“viable nonculturable”).
- ◆ Some dead cells autolyze.

Bacteria in broth vs food

- ◆ Broth: “planktonic cells”
- ◆ Bacteria tend to aggregate, attach to surfaces, form colonies or biofilms
- ◆ Foods = solid matrix, microenvironments
- ◆ Pathogens outnumbered

Research vs real food

- ◆ Food contaminants (water, air, soil, raw material, feces) have mixed microflora.
- ◆ Food ecosystem may select one organism

Research vs real food

- ◆ At high levels, bacteria signal each other chemically (“consensus”)
- ◆ Different species interact competitively, but sometimes beneficially

Research vs real food

- ◆ “Programmed” successions
- ◆ Genetic exchanges among strains or species
- ◆ Toxigenic agents (including molds) *grow* under conditions that do not permit toxigenesis.

Major factors (interact)

- ◆ Temperature
- ◆ E_h
- ◆ a_w
- ◆ pH (specific cations & anions)
- ◆ Nutrients available
- ◆ Physical structure
- ◆ Microflora
- ◆ Antimicrobial agents

Temperatures for Thermophiles

- ◆ Minimum: 40–45°C
- ◆ Optimum: 55–75°C
- ◆ Maximum: 60–90°C

Temperatures for Mesophiles

- ◆ Minimum: 5–15°C
- ◆ Optimum: 30–45°C
- ◆ Maximum: 35–47°C

Temperatures for Psychrophiles

- ◆ Minimum: -5–+5°C
- ◆ Optimum: 12–15°C
- ◆ Maximum: 15–20°C

Temperatures for Psychrotrophs

- ◆ Minimum: -5–+5°C
- ◆ Optimum: 25–30°C
- ◆ Maximum: 30–35°C
(cf. handout)

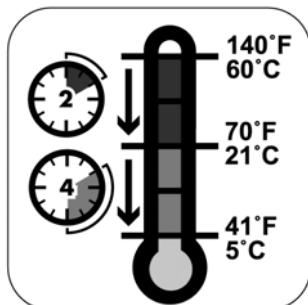
Cold: liquid or solid water?

- ◆ Freezing kills some cells, frozen storage preserves
- ◆ Psychrotrophs grow slowly in refrigerated food

Warm = near optimum?

- ◆ Food spoilage promoted; test of sanitation
- ◆ “Danger Zone”: 4–60°C (40–140°F) or 5–57°C (41–135°F)
- ◆ Rapid transition from hot to cold or cold to hot

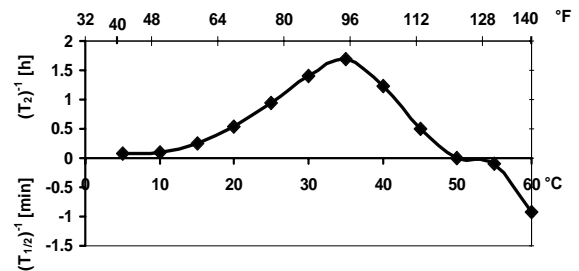
“Danger zone” depicted



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DANGER ZONE FOR NEUTRAL FOODS

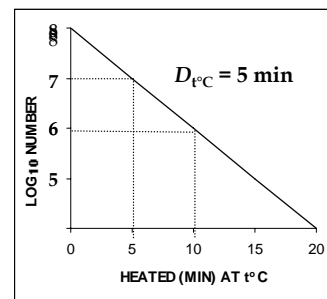
Averages of *Aeromonas hydrophila*, *Bacillus cereus*, *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, & *Yersinia enterocolitica*



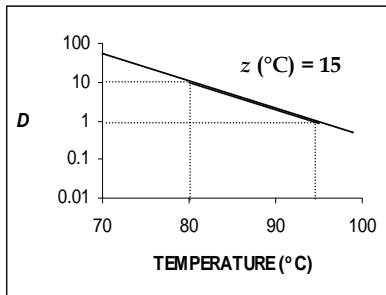
Hot—temps > max for growth cause death

- ◆ *D* value: time for decimal reduction at $t^{\circ}\text{C}$; organisms are in log death phase
- ◆ *z* value: temperature change ($^{\circ}\text{C}$) to reduce the *D* value 10-fold

D value example



z value example



Heat

- ◆Cooking, blanching, pasteurization not for “commercial sterility”
- ◆Cells in log phase are more heat-sensitive

Heat

- ◆“Heat-shock” proteins aid adaptation; some produced in response to other stresses.
- ◆Mesophiles or psychrotrophs – infectious agents must be able to multiply at body temperature.

Tyndallization: boiling on 3 days

- ◆Day 1: vegetative cells killed, spores heat-shocked
- ◆Day 2: veg cells from spores killed, last spores heat-shocked
- ◆Day 3: vegetative cells from final spores killed; endpoint: sterility

Eh

- ◆Aerobic (>0 mV), microaerophiles, facultative, anaerobic (<0 mV)
- ◆“Strict” aerobes $E_h > 0$ mV, “obligate” anaerobes $E_h < -300$ mV

Eh

- ◆Facultative organisms often use available energy more efficiently under aerobic conditions
- ◆*C. perfringens* may not start growing under aerobic conditions, but is not inhibited by oxygen once growth begins.

E_h

- ◆ E_h hard to measure in foods
- ◆ Live foods metabolize or bind oxygen
- ◆ Packaging, modified atmosphere
- ◆ Molds generally strict aerobes

Water activity—"a_w"

Water available for microbial growth, based on water present and on binding by solutes such as salt or sugar; equilibrium relative humidity ÷ 100; range is 0 to 1.00

Approximate a_w of some foods

- ◆ Fresh fruit or vegetables ≥ 0.97
- ◆ Fresh poultry or fish ≥ 0.98
- ◆ Fresh meats ≥ 0.95
- ◆ Juices, fruit & vegetable 0.97
- ◆ Cheese, most types ≥ 0.91
- ◆ Honey 0.54–0.75
- ◆ Cereals 0.10–0.20

Minimum a_w for some foodborne pathogens

- ◆ *Salmonella* 0.93
- ◆ *C. botulinum* 0.93
- ◆ *Staphylococcus aureus* 0.85
- ◆ (Most yeasts) 0.88
- ◆ Most molds 0.75

pH: hydrogen-ion potential

- ◆ Foods range from pH 7 downward.
- ◆ Acidification inhibits spoilage & growth of many pathogens.
- ◆ "Low acid" (bot) pH ≥ 4.6

pH values of some foods

- ◆ Egg white 7.6–9.5
- ◆ Milk 6.3–6.8
- ◆ Chicken 5.5–6.4
- ◆ Beef 5.3–6.2
- ◆ Cheeses, most 5.0–6.1
- ◆ Tomatoes 3.7–4.9
- ◆ Apples 2.9–3.5

**Important minimum pH values
for growth of microbes in foods**

- ◆ *Clostridium botulinum* 4.8-5.0
- ◆ *Salmonella* (most types) 4.5-5.0
- ◆ *Staphylococcus aureus* 4.0-4.7
- ◆ Yeasts & molds 1.5-3.5

- pH**
- ◆ “Organic” acids (e.g., lactic, acetic, etc.) more effective antimicrobials than mineral acids
 - ◆ Most effective undissociated; at a given pH, molar quantity of organic acid >> than that of a mineral acid.

Nutrients available

- ◆ C & N sources required, sometimes “growth factors”
- ◆ Foods generally good C & N sources
- ◆ Other factors, then nutrients decide which organism predominates

Physical structure

- ◆ Bacteria grow on surfaces when they can.
- ◆ Some surfaces (melon rind, eggshell) limit access to nutrients.
- ◆ Food matrix: molds often penetrate better than bacteria.

Physical structure

- ◆ If water & solutes cannot diffuse freely, local variations in E_h , a_w , and pH are highly possible.
- ◆ High viscosity or strongly cellular structure can greatly limit heat transfer (both heating and cooling) in foods.

Microflora

- ◆ Bacteria in foods: variety & competition
- ◆ Microbial growth may lower E_h & pH; molds use organic acids as carbon sources & raise pH.

Microflora

- ◆ Bacteria may produce acetic, lactic, and other acids as fermentation products.
- ◆ Some produce bacteriocins – proteins that have a highly-specific lethal effect on closely related organisms.

Competing organisms

- ◆ *Staphylococcus aureus*
- ◆ *Clostridium botulinum*

“Programmed succession”

- ◆ Milk: rapid lactic acid producers (lactococci), then
- ◆ Slower acid producers (lactobacilli) that tolerate lower pH's, then
- ◆ Acid-stable putrefactive (proteolytic) bacteria and finally,
- ◆ Molds (metabolite tolerance).

Antimicrobials: preservatives

- ◆ Materials added specifically to inhibit microbial growth
- ◆ Nitrite for “curing” meats, vs *C. botulinum*.
- ◆ Sorbates, benzoates, & other salts of organic acids
bacteriostatic, not bactericidal

Antimicrobials: preservatives

- ◆ CO₂ & SO₂ long used in foods; SO₂ is highly toxic to a small segment of the population.
- ◆ Spices – especially those with strong flavors – often viewed as preservatives or disinfectants. Probably bacteriostatic, at best.

Antimicrobials: radiation

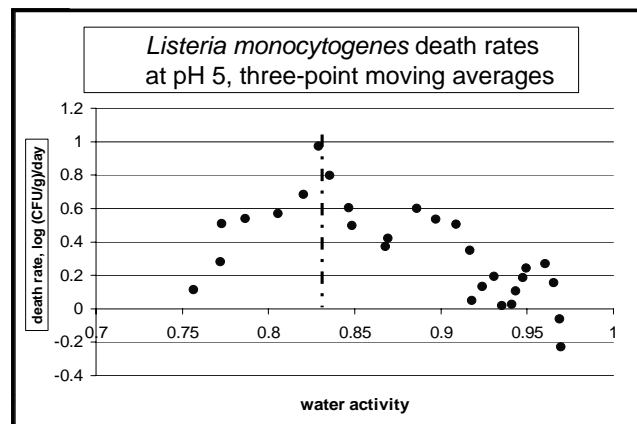
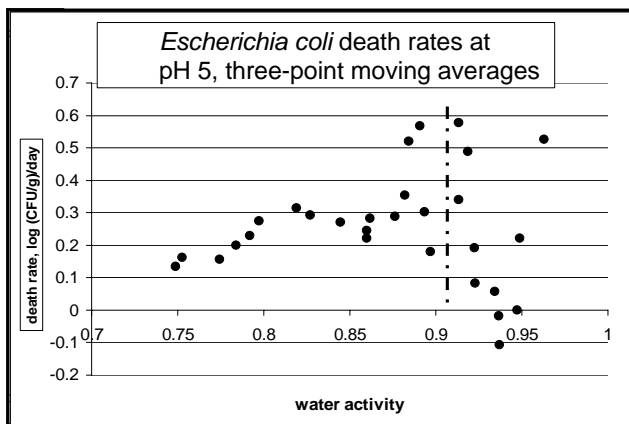
- ◆ UV widely applicable to decontamination of food surfaces, food contact surfaces, & water used in food processing; limited penetration.

Antimicrobials: radiation

- ◆ Surface efficiency enhanced by pulsed laser application (some pulsed laser applications use visible light).
- ◆ Ionizing radiation discussed earlier in course.

Interactions

- ◆ The pH that permits growth of a bacterium near its optimal temperature may be limiting at a less favorable temperature.



Interactions

- ◆ Safe foods “designed” combining slightly unfavorable conditions for several parameters to stop target pathogens and spoilage organisms.

Interactions

- ◆ This kind of food design has heavy safety implications; modeling (discussed last time) is used to make choices, then validated by inoculated-pack, product-abuse trials before a new food product is marketed.
- ◆ Applied in HACCP.

Pathogen Modeling Program (PMP)

<http://www.arserrc.gov/MFS/PATHOGEN.HTM>

Summary

- ◆ Food ecosystems govern which microorganisms may grow in them.
- ◆ Factors, such as temperature, a_w , pH, etc., interact to determine the microbiologic safety of a food.
- ◆ Food processing takes account of these factors to ensure food safety.