PHR 250
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MICROBIAL ECOLOGY OF FOODS

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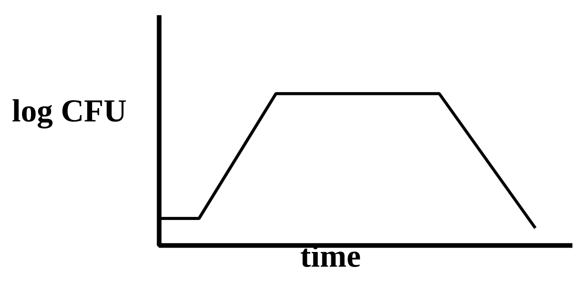
Every food is an ecosystem for the microorganisms it contains. **They don't "know" they are in food!**

- Bacteria and molds present may multiply, survive, or die.
- Viruses and parasites may only persist or be inactivated (die, lose infectivity).
- Most of our attention will be devoted to the fates of bacterial pathogens.

If pathogenic bacteria contaminate a food, there are several potential "outcomes":

- Persistence — the bacteria remain viable, numbers unchanged; this may be either a lag phase, a stationary phase, or (as appropriate) a spore stage
- Growth (multiplication) — this can usually be described by a rate parameter (not necessarily constant) based on the doubling time
- Death — this also can usually be described by a rate parameter; however, it should be noted that some bacteria enter a "viable-nonculturable" state (injured, but capable of resuscitation)
- Sporulation — another defense against unfavorable conditions, for certain bacteria
- Toxigenesis — bacteria that produce toxins must usually multiply in order to do so; however, conditions for toxigenesis are usually more restrictive than those for growth, so growth is necessary, but possibly not sufficient

The "classical" bacterial growth curve comprises four phases: lag, logarithmic growth, stationary, and logarithmic death.



Modeling (prediction of the fate of bacteria in a specific food under specific conditions) has focused on the logarithmic growth phase with some success; attempts to predict the lag phase have been less successful. You have been shown some examples and may copy the software.

Bacteria (and fungi) may be quiescent, metabolically active, multiplying, adapting, injured, dying, or dead.

- Spores and lag phase cells are essentially quiescent, but may be adapting to environmental conditions. Adaptation entails selection of needed enzymes (by activation of appropriate genes) from the broad bacterial repertoire.
- Multiplying cells are metabolically active, and often adapting; but not all metabolically active cells are multiplying; multiplication = doubling.
- Stress causes adaptation or injury.
- Stationary phase may represent quiescence or growth rate = death rate.
- Some injured cells are hard to distinguish from dead ("viable nonculturable").
- Some dead cells autolyze.

Bacteria may be studied while dispersed in broth ("planktonic cells"), but

- they tend to aggregate (or not separate after division) or
- attach to surfaces, growing as colonies or forming biofilms.
- Most foods provide a solid matrix that may include a variety of microenvironments.
- Sterile foods do not contain pathogens, but pathogens are likely to be outnumbered by other bacteria in non-sterile foods.

Research is often done with sterile substrate and "monoculture," but bacteria don't usually live like that.

- Most sources of contamination to food (water, air, soil, raw material, feces) have a mixed microflora.
- Occasionally, the food ecosystem is so selective that one best-adapted organism overgrows all others.
- At high enough levels, bacteria signal each other chemically.
- Usually, different species interact within the resources available, sometimes competitively, but sometimes beneficially.
- "Programmed" successions among species occur as bacterial growth modifies the environment.
- Genetic exchanges among strains or species are also known.
- Toxigenic agents (including molds) may grow under conditions that do not permit toxigenesis.

Major factors in the microbes' ecosystem (all of these interact intimately):

- Temperature
- E_h
- a_w
- pH (specific cations & anions)
- Nutrients available
- Physical structure (of food, etc.)
- Microflora
- Specific antimicrobial agents: preservatives, radiation, etc.

Temperature

Temperature ranges for procaryotic organisms (°C)

Group	Minimum	Optimum	Maximum
Thermophiles	40–45	55–75	60–90
Mesophiles	5–15	30–45	35–47
Psychrophiles	-5–+5	12–15	15–20
Psychrotrophs	-5–+5	25–30	30–35?

Cold—bacteria generally require a liquid environment for metabolism and growth, so these occur only above the freezing-thawing temperature of the "medium."

- Freezing kills some of the cells in a typical microbial population (gram-negatives more sensitive); further die-off in frozen storage is limited (persistence longer at colder temperatures); **frozen food**.
- Refrigerator temperatures generally preserve mesophilic bacteria; psychrophiles and psychrotrophs can multiply, with doubling times of several hours; rapid chilling may shock bacteria; **refrigerated food**.

Warm—somewhere near optimum for the group of organisms in question

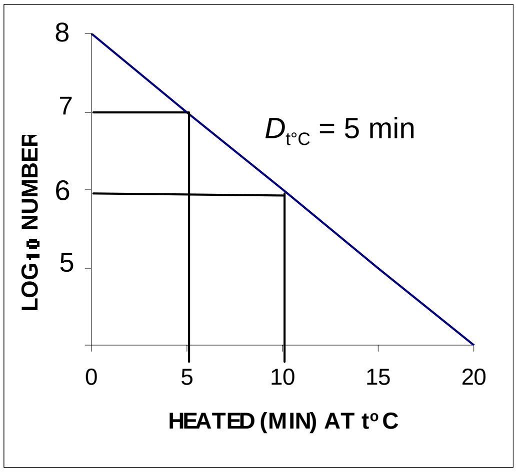
- Food spoilage organisms are generally mesophiles, psychrophiles (which build up due to poor sanitation), or psychrotrophs—sometimes thermophiles (also poor sanitation).
- **"Danger Zone": 4° to 60°C** (40° to 140°F; or 5 to 57°C = 41 to 135°F) see page 5; rapid transition from hot to cold or cold to hot

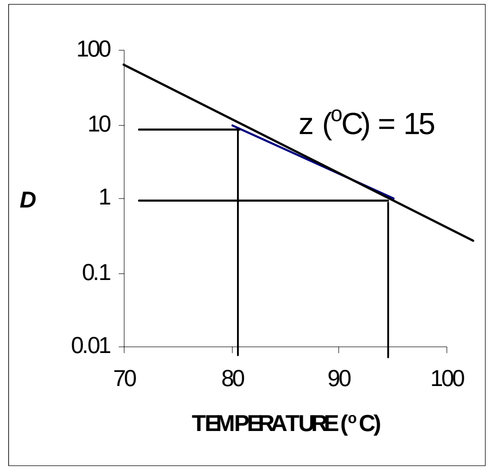
Hot—temperatures above maximum growth temperature cause death

- *D* value: time for decimal reduction at t°C; organisms are in log death phase
- z value: temperature change (°C) to reduce the *D* value 10-fold
- Heat treatments called cooking, blanching, or pasteurization are not intended to produce even "commercial sterility."
- Cells in logarithmic growth phase are more heat-sensitive than otherwise.
- Cells adapt to heating by producing "heat-shock" proteins that help them adapt and survive; some of these adaptive proteins are also produced in response to other stresses.
- Pathogens are generally mesophiles or psychrotrophs—infectious agents must be able to multiply at body temperature.

Purposeful temperature fluctuation—e.g., tyndallization

- Boil a food (internal temperature ~100°C) on 3 successive days
- Day 1: vegetative cells killed, spores heat-shocked
- Day 2: vegetative cells from germinated spores killed, remaining spores heat-shocked
- Day 3: vegetative cells from final, germinated spores killed
- Endpoint: sterility





E_h: oxidation-reduction potential

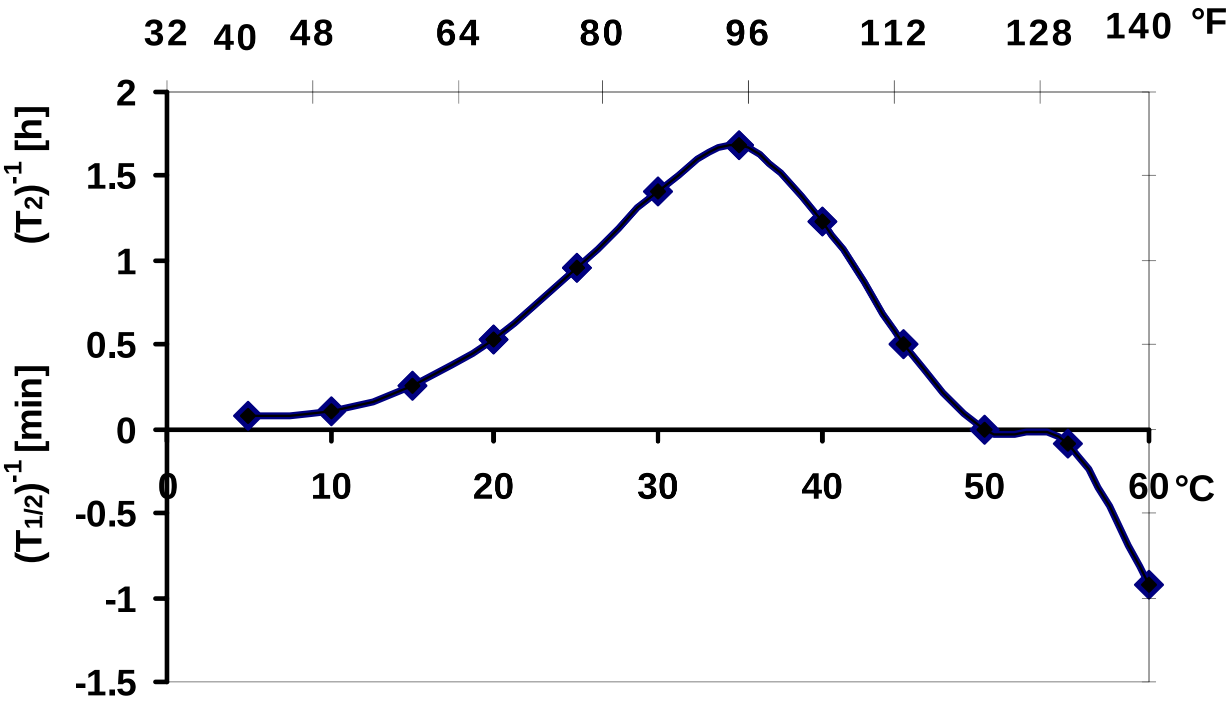
- Bacteria are classified as aerobes, microaerophiles, facultative, or anaerobes, on the basis of their preferences for an oxidizing or reducing atmosphere.
- "Strict" aerobes may not grow at E_h < 0 mV, and "obligate" anaerobes may grow only at E_h < -300 mV; facultative organisms often use available 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 of foods is difficult to measure accurately and may differ in different parts of a food: muscle of animals, including fish, will afford anaerobic conditions in its interior. Vegetables that are alive when packaged may metabolize oxygen and drastically reduce their E_h during storage.
- Packaging materials and the atmosphere within a food package can be selected with a view to controlling the E_h of the stored product.
- Molds are generally strict aerobes

a_w: water activity, defined as the ratio of the water vapor pressure of a food to the vapor pressure of water at the same temperature (range is 0 to 1).

- a_w of fresh meats, fish, fruits, and vegetables is generally at or above 0.98 (see p.10).
- Reduction of a_w by removal of water or by adding solute such as salt or sugar is one of the oldest means of food preservation.
- Gram-negative bacteria generally have the highest a_w requirements for growth, followed by gram-positive bacteria and then molds and yeasts.

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*



pH: hydrogen-ion potential

- Foods range naturally from near neutrality (pH 7) downward, with few exceptions (see p. 10). Acidification of food, which inhibits spoilage and the growth of many pathogens, has long been used in food preservation; it is achieved by fermentation or by adding fermented products such as vinegar.
- The single pH value that is most important in food safety is **4.6**, in that *Clostridium botulinum* will not grow at this pH, even if other conditions are optimal; therefore, foods above this pH are “low acid” and subject to special regulation if they are preserved.
- In addition to pH as it may be measured with a meter, there is great significance to the types of acids or anions (and sometimes cations) in the solute: “organic” acids (e.g., lactic, acetic, etc.) at a given pH tend to be much more effective antimicrobial substances than are mineral acids; organic acids are usually most effective when undissociated, so it is important to note that at a given pH the molar quantity of organic acid is likely to be much higher than that of a mineral acid.

Nutrients available

- Bacterial growth basically requires a source of carbon and a source of nitrogen; some bacteria require specific growth factors as well.
- In general, foods are good sources of both C and N, and some provide needed growth factors for organisms that need them.
- At least initially, bacterial growth is more likely to be determined by factors discussed earlier than by nutrient availability; however, competition among organisms for nutrients can have a very significant effect on which predominates.

Physical structure (of food, etc.)

- As was mentioned above, bacteria grow on surfaces when they can.
- Some food surfaces (melon rind, eggshell) limit the access of contaminating bacteria to the nutrients inside—until the surface is disrupted.
- Foods that have a highly viscous or cellular structure limit the ability of bacteria to diffuse through the food matrix; molds tend to have enzymes that facilitate mycelial growth in foods.
- Because water and solutes cannot diffuse freely in some foods, local variations in E_h, a_w, and pH are highly possible in some foods.
- High viscosity or strongly cellular structure can greatly limit heat transfer (both heating and cooling) in foods because conduction is slower than convection.

Microflora

- As was mentioned above, bacteria in foods seldom occur in monoculture: there is almost always variety and competition.
- In addition to the competition for nutrients, microbial growth in food may lower E_h and pH; molds that use organic acids as carbon sources may raise the pH.
- 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.

- Accumulation of metabolites, rather than depletion of nutrients, may be responsible for the transition from logarithmic growth to stationary phase or death. In milk, the rapid producers of lactic acid (lactococci) are succeeded by slower acid producers (lactobacilli) that tolerate lower pH's, followed by acid-stable putrefactive (proteolytic) bacteria and finally molds, if the process runs its course. This is determined at least as much by how each organism tolerates the metabolites of its predecessors as by what nutrients are left.

Specific antimicrobial agents

- Preservatives are materials added to food specifically to inhibit microbial growth.
    - ♦ Nitrite, a component of "curing" salts for meats, has an important effect in inhibiting growth of *C. botulinum*.
    - ♦ Sorbates, benzoates, and many other salts of organic acids can delay food spoilage and often prevent multiplication of pathogens; these are not necessarily bactericidal (e.g., *E. coli* O157:H7 in chemically preserved apple cider).
    - ♦ Gases such as carbon dioxide and sulfur dioxide have a long history of use in foods; the latter is highly toxic to a small segment of the population.
    - ♦ Spices — especially those with strong flavors — are often viewed as preservatives or disinfectants. Although some are able to inhibit bacterial growth under some circumstances (bacteriostasis), few or none are capable of killing bacteria in food.
- Radiation, both nonionizing and ionizing, have important antimicrobial applications
    - ♦ Ultraviolet light is widely applicable to decontamination of food surfaces, food contact surfaces, and water used in food processing; its penetrating ability is limited, but efficiency of surface action can be enhanced by pulsed laser. application (some antimicrobial pulsed laser applications are based on visible light).
    - ♦ Ionizing radiation (gamma rays, x-rays) can penetrate food and kill pathogens with very limited applications of energy; "pasteurization" treatments are more commercially attractive than "sterilization" treatments, but special diets for immune impaired people (as well as astronauts) and laboratory animals may be radiation sterilized.

EVERYTHING AFFECTS EVERYTHING ELSE!

- The pH that permits growth of a bacterium near its optimal temperature may be limiting at a less favorable temperature.
- Safe foods can be "designed" by combining slightly unfavorable conditions for several parameters to ensure that target pathogens and spoilage organisms do not grow.
- Because this kind of food design has heavy safety implications, modeling (discussed last time) is used to make choices which are usually validated by inoculated-pack, product-abuse trials before a new food product is marketed; we have seen that this fits into the HACCP approach as well.

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Pathogen Modeling Program:
http://www.arserrc.gov/MFS/PATHOGEN.HTM

ECOLOGIC VALUES FOR SOME FOODBORNE BACTERIAL PATHOGENS (ICMSF, 1996)

Species	°C			pH			a_w		
	≥	Opt.	≤	≥	Opt.	≤	≥	Opt.	≤
Bacillus cereus	4	30–40	55	5	6–7	8.8	0.93		
Campylobacter jejuni	32	42–43	45	4.9	6.5–7.5	~9	>0.997		
Clostridium botulinum[a]									
Gp. I (proteolytic)	10–12			5			~0.94		
Gp. II (nonproteolytic)	3.3			5.2			~0.97		
Clostridium perfringens	12	43–47	50	5.5–5.8	7.2	8.0–9.0	0.93	0.95–0.96	0.97
Escherichia coli (pathogenic)	~7–8	35–40	~44–46	4.4	6–7	9.0	0.95	0.995	
Listeria monocytogenes	–0.4	37	45	4.39	7.0	9.4	0.92		
Salmonella spp.[b]	5.2(7)	35–43	46.2	3.8	7–7.5	9.5	0.94	0.99	>0.99
Shigella sonnei	6.1		47.1	4.9		9.34	5.18[c]		
Staphylococcus aureus[a,b]	10	40–45	48	4.5	7–8	9.6	0.87	0.98	>0.99
Streptococcus pyogenes	10–15	37	40–45	4.8–5.3	7	<9.3	4–6.5[c]		
Vibrio cholerae	10	37	43	5	7.6	9.6	0.97	0.984	0.998
Vibrio parahaemolyticus	5	37	43	4.8	7.8–8.6	11	0.94	0.981	0.996
Vibrio vulnificus	8	37	43	5	7.8	10	0.96	0.98	0.997
Yersinia enterocolitica	–1.3	25–27	42	4.2	7.2	<10	<7[c]		

[a] Conditions for toxin production, rather than growth
[b] Other conditions optimal
[c] Lowest % NaCl that inhibits, not expressed as a_w

Addendum, from: Gavin, A., and L. M. Weddig (eds.). 1995. Canned foods: Principles of thermal process control, acidification, and container closure evaluation, 6th ed. Food Processors Institute, Washington, DC.

Water activities of some common foods

Food	a_w
Liverwurst	0.96
Cheese spread	0.95
Fudge sauce	0.83
Semi-moist pet food	0.83
Salami	0.82
Soy sauce	0.80
Peanut butter—15% total moisture	0.70
Dry milk—8% total moisture	0.70

Additional foods (other references)

Food	a_w
Fresh fruit or vegetables	$\geq$0.97
Fresh poultry or fish	$\geq$0.98
Fresh meats	$\geq$0.95
Juices, fruit & vegetable	0.97
Cheese, most types	$\geq$0.91
Honey	0.54-0.75
Cereals	0.10-0.20
Crackers	0.10

Influence of product pH on required degree of thermal processing