IMG 222 THERMAL RESISTANCE

NORLIA MAHROR 15/12/2020

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Heat Is Lethal To Microorganisms But

Each Species Has Its Own Particular Heat Tolerance.

THERMAL DESTRUCTION OF MICROBES

- During a thermal destruction process, the rate of destruction is **logarithmic**, as is their rate of growth. Thus bacteria subjected to heat are killed at a rate that is **proportional** to the **number of organisms** present.

- The process is dependent both on the **temperature** of exposure and the **time** required at this temperature to accomplish the **desired rate of destruction**.

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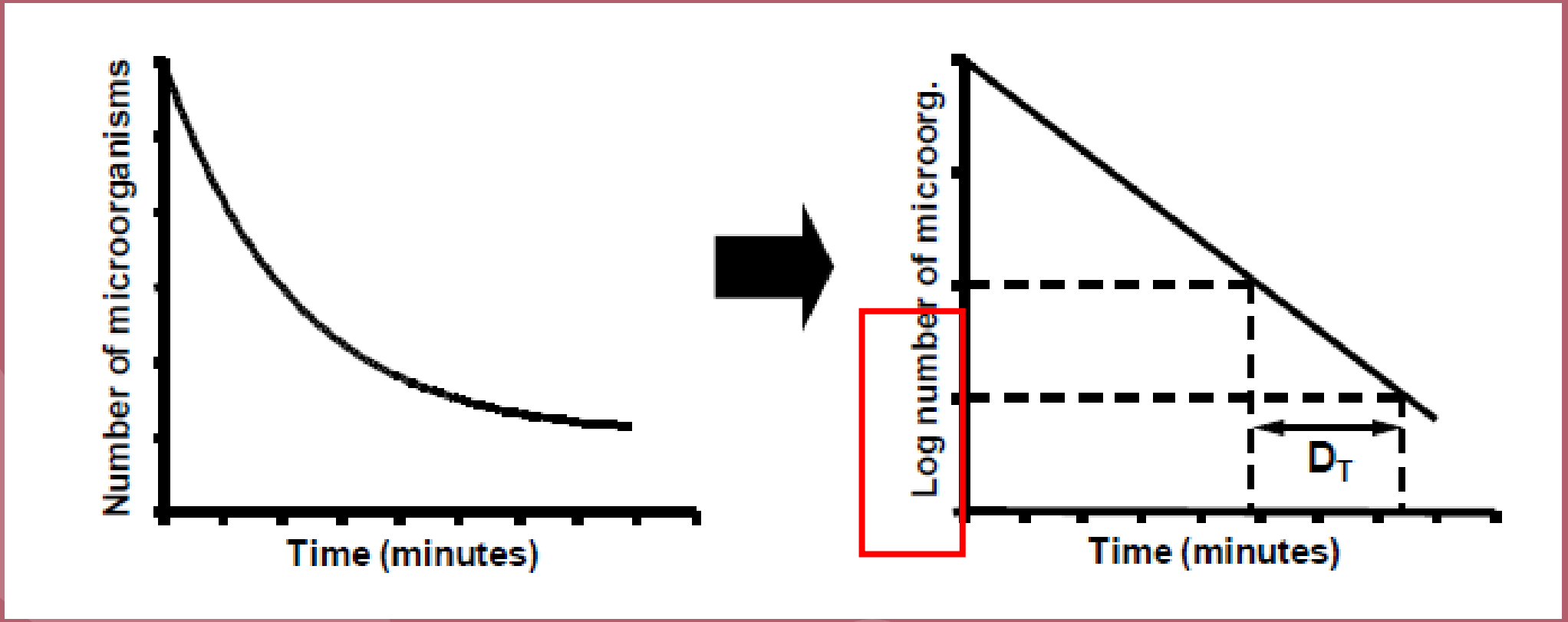
Information Required For Thermal Calculations

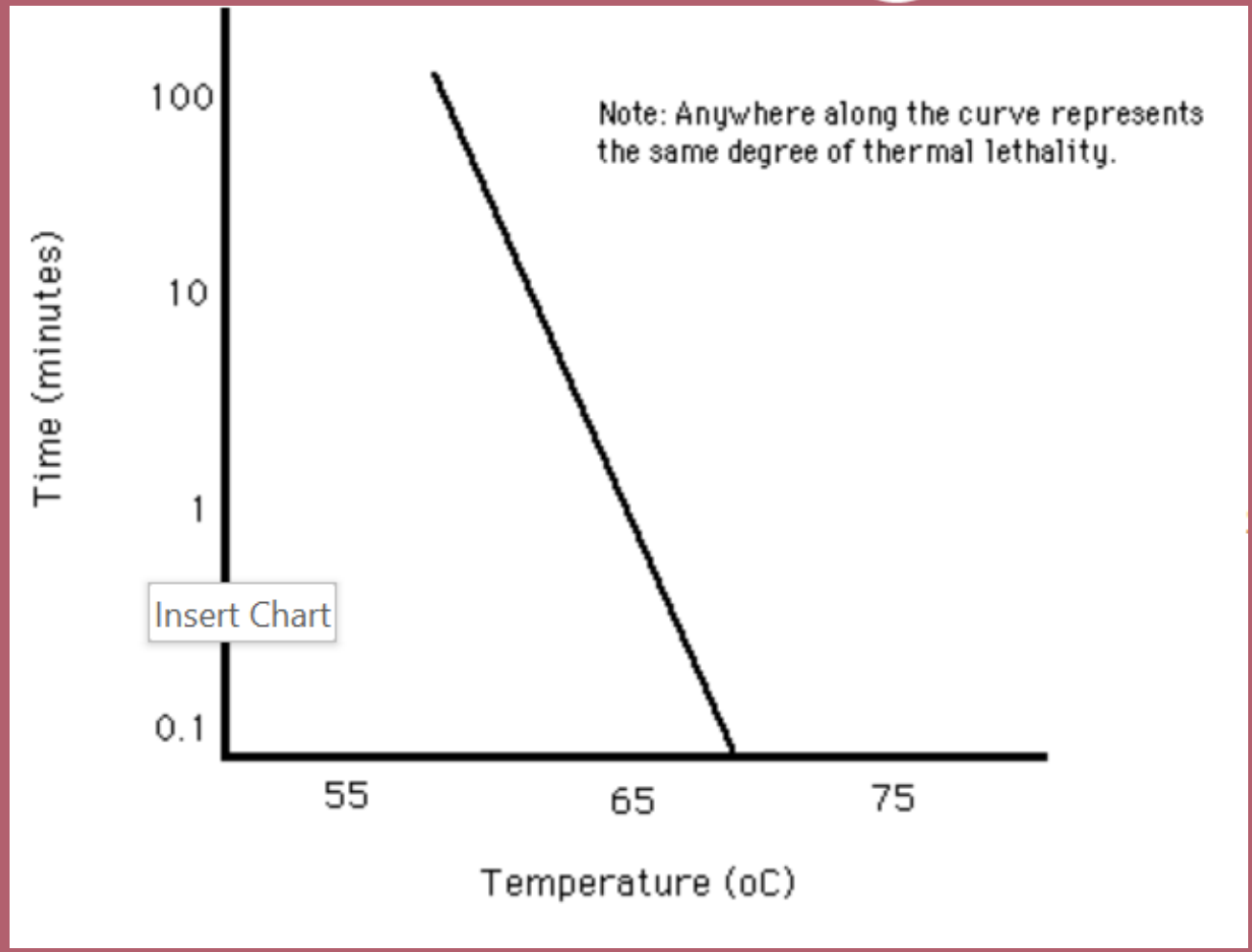
- The number of microorganisms to be destroyed
- The acceptable number of microorganisms that can remain behind (e.g. spoilage organisms, but not pathogens),
- The thermal resistance (D value) of the target microorganisms (the most heat tolerant ones)
- The temperature time relationship required for destruction of the target organisms.

THERMAL DEATH CURVE IS A LOGARITHMIC PROCESS



The destruction process is dependent on time and temperature

At a given time and temperature, the same percentage of the bacterial population will be destroyed regardless of the population present.



Thermal calculations

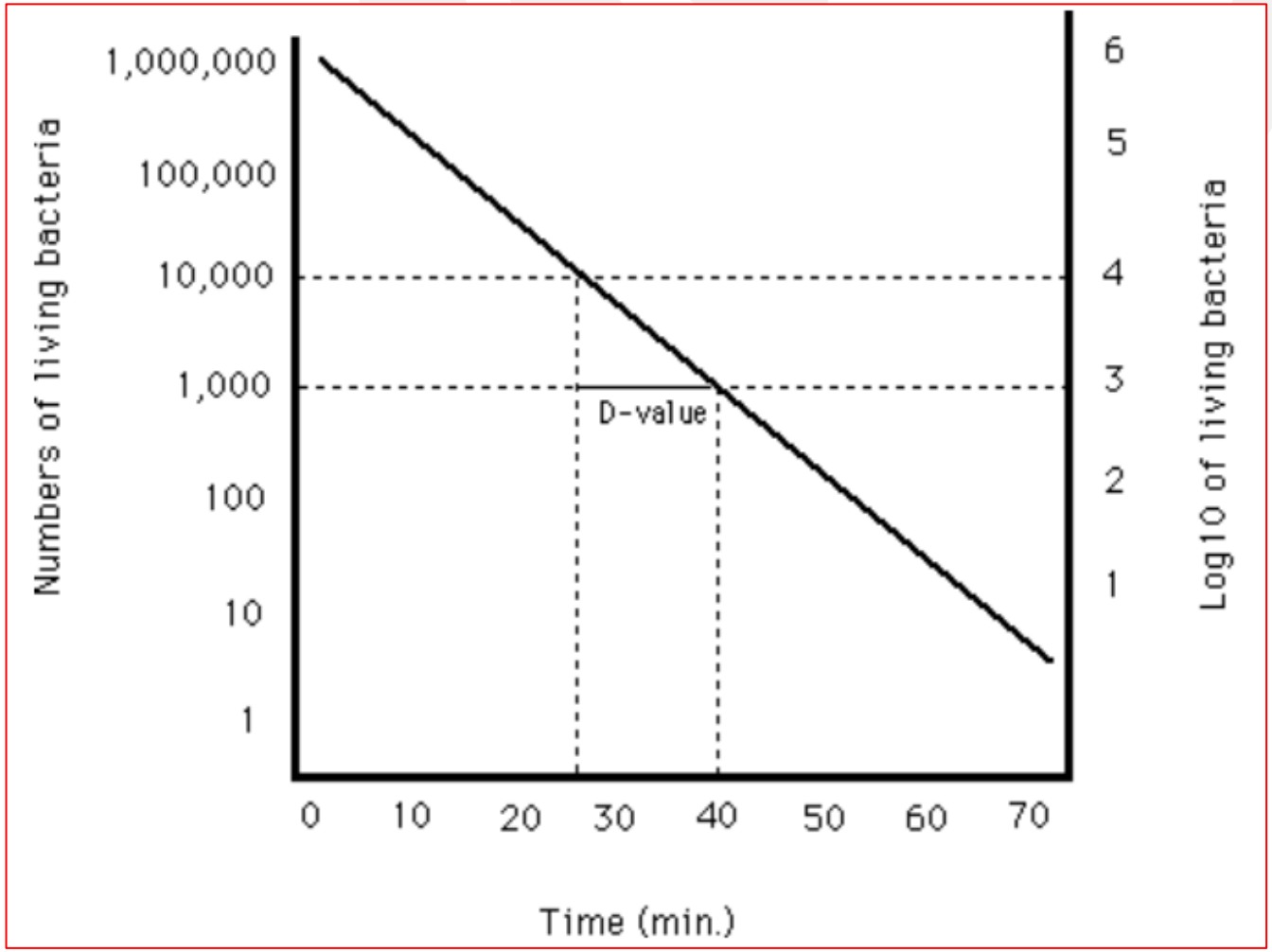
D value

(Heat resistant of microorganisms)

D value is the **TIME** (in minutes)

at a given TEMPERATURE

required to **DESTROY 1 LOG CYCLE (90%)** of the targeted microorganisms



Thermal calculations

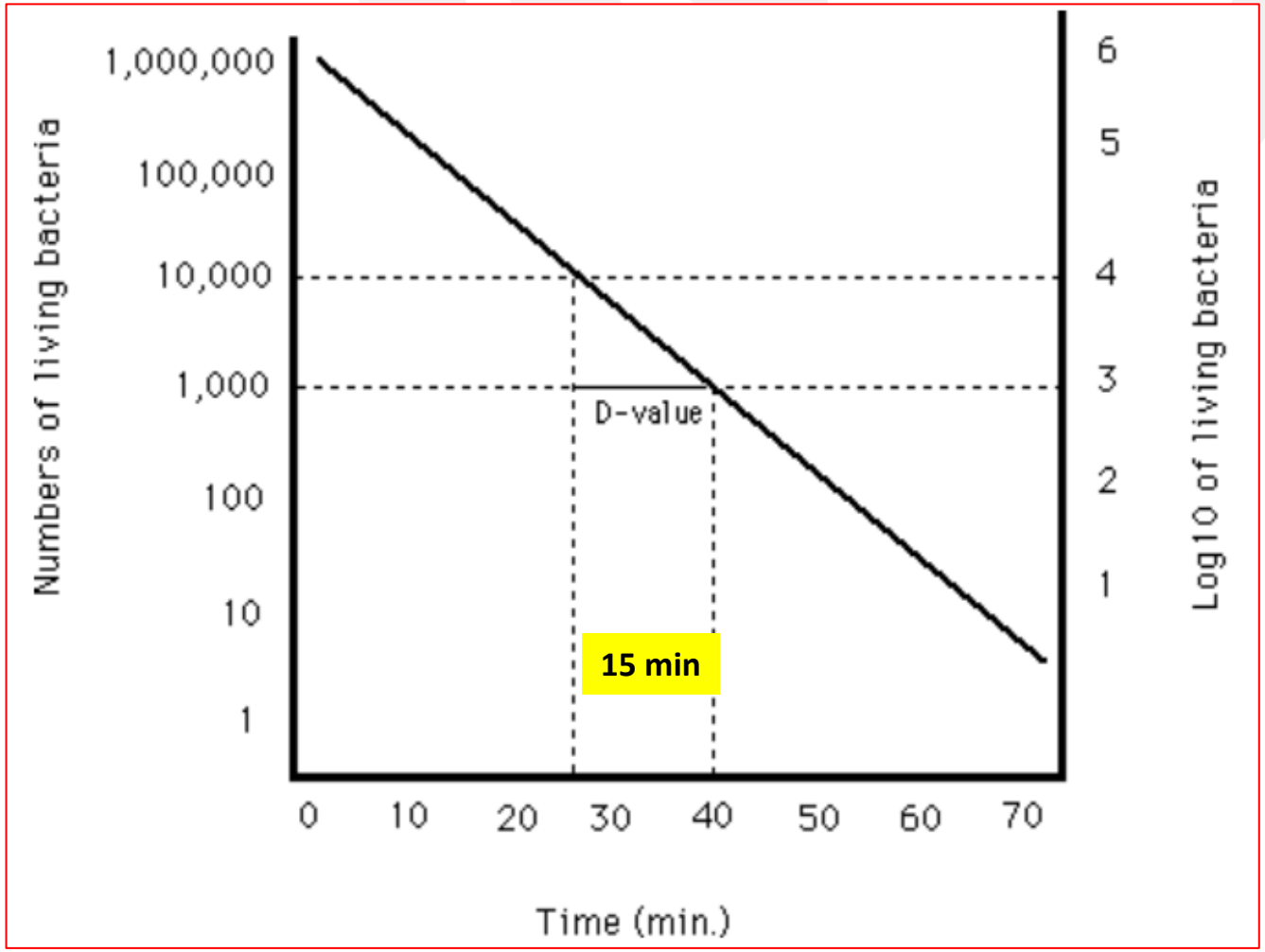
D value

(Heat resistant of microorganisms)

Example:

If a **D value** at **72°C** is **15 min**

It means that for each minute of processing at 72°C, the bacteria population of the target microorganism will be reduced by **90%**



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D VALUE OF DIFFERENT BACTERIAL SPECIES AND SPORES

Table 1. Range of D_T values of different bacterial species in buffers and foods (pH range 5.5–7.0; $a_w > 0.98$). Sources [5–15].

	Bacterial Species	Temperature (°C)	D_T (Minutes)	z (°C)
Vegetative cells	*Aeromonas hydrophila*	60	<0.02	5.2–7.7
	Campylobacter spp.	60	<0.01–0.11	4.1–4.7
	Yersinia enterocolitica	60	0.07–0.8	4.0–5.8
	Salmonella enterica	60	0.1–3.3	3.8–6.3
	Cronobacter sakazakii	60	0.05–2.0	4.1–6.2
	Escherichia coli	60	0.7–2.7	3.2–5.2
	Staphylococcus aureus	60	0.2–6.0	3.6–8.5
	Listeria monocytogenes	60	0.5–15	5.2–5.8
	Enterococcus faecium	60	5.0–30	4.3–8.0
Spores	*Bacillus subtilis*	100	3.31–>100	6.7–10.1
	Clostridium botulinum (proteolytic)	121	<0.01–0.22	7.6–12.1
	Geobacillus stearothermophilus	121	0.1–5.0	7.3–12.2

Heat resistance (1)

Vegetative organism	D values (min)		
	55°C	60°C	65°C
Escherichia coli	4		0.1
Salmonella spp.			0.02-0.25
Salmonella typhimurium			0.056
Salmonella senftenberg			0.8-1.0
Staphylococcus aureus			0.2-2.0
Listeria monocytogenes		5.0-8.3	
Campylobacter jejuni	1.1		

Heat resistance (2)

Bacterial endospores	D values (min)		
	100°C	110°C	121°C
C. botulinum type A and B	50		0.1-0.2
C. botulinum type E		< 1 sec	
C. perfringens	0.3-20		
C. sporogenes			0.1-1.5
Bacillus cereus	5		

Effects on proteins and vitamins

	D_{121} (min)
Protein degradation	5
Non - enzymatic browning	0.4 - 40
Lipase	1.2 - 1.7
Thiamin	38 - 380
Vitamin C	245
Betamin	48

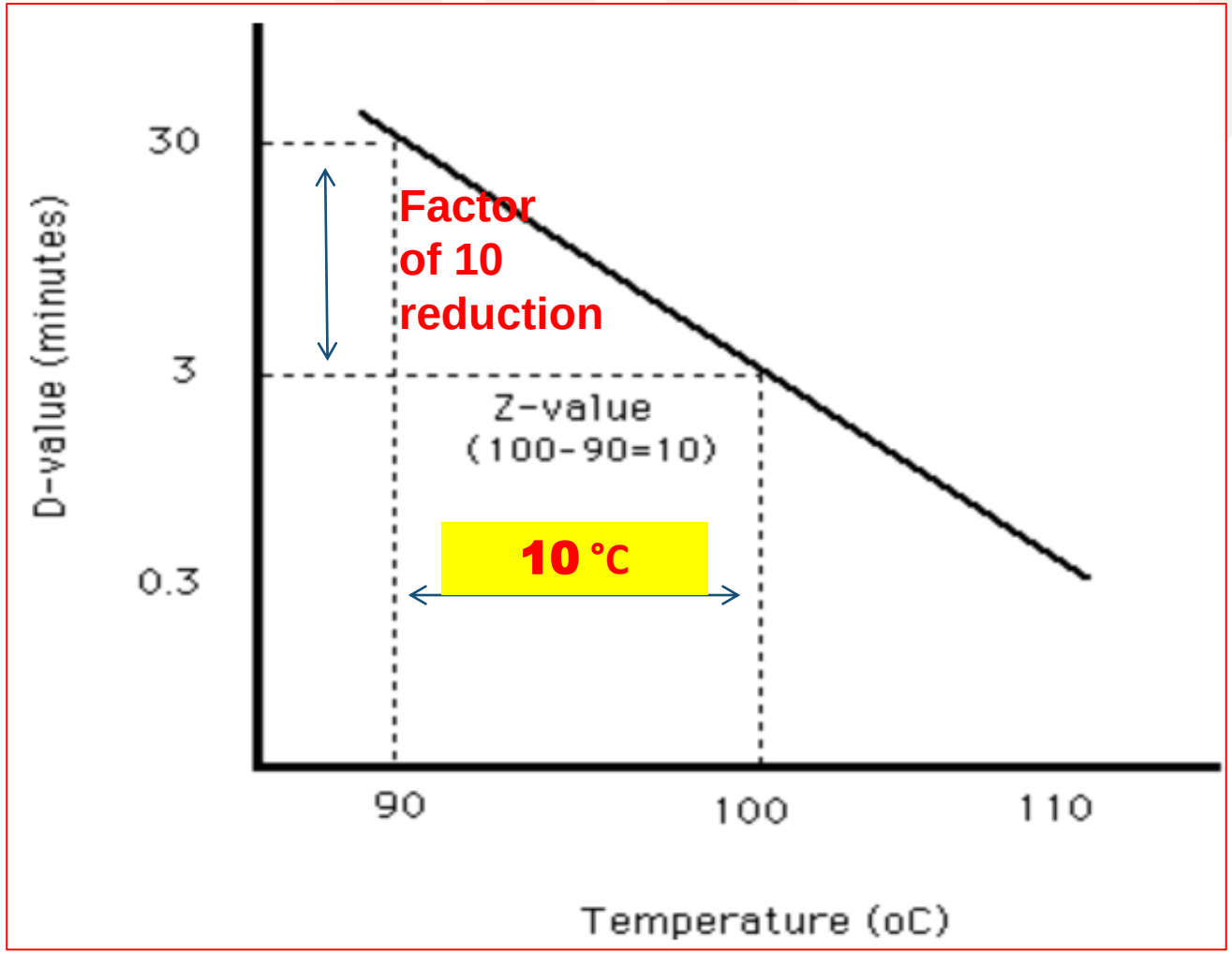
Thermal calculations

Z value

Z value is the **temperature change required to change the D value** by a **factor of 10 / 1 log cycle / 90%**

Reactions with large Z values require larger changes in temperature to reduce the time.

Spore forming bacteria has a Z value of 10°C



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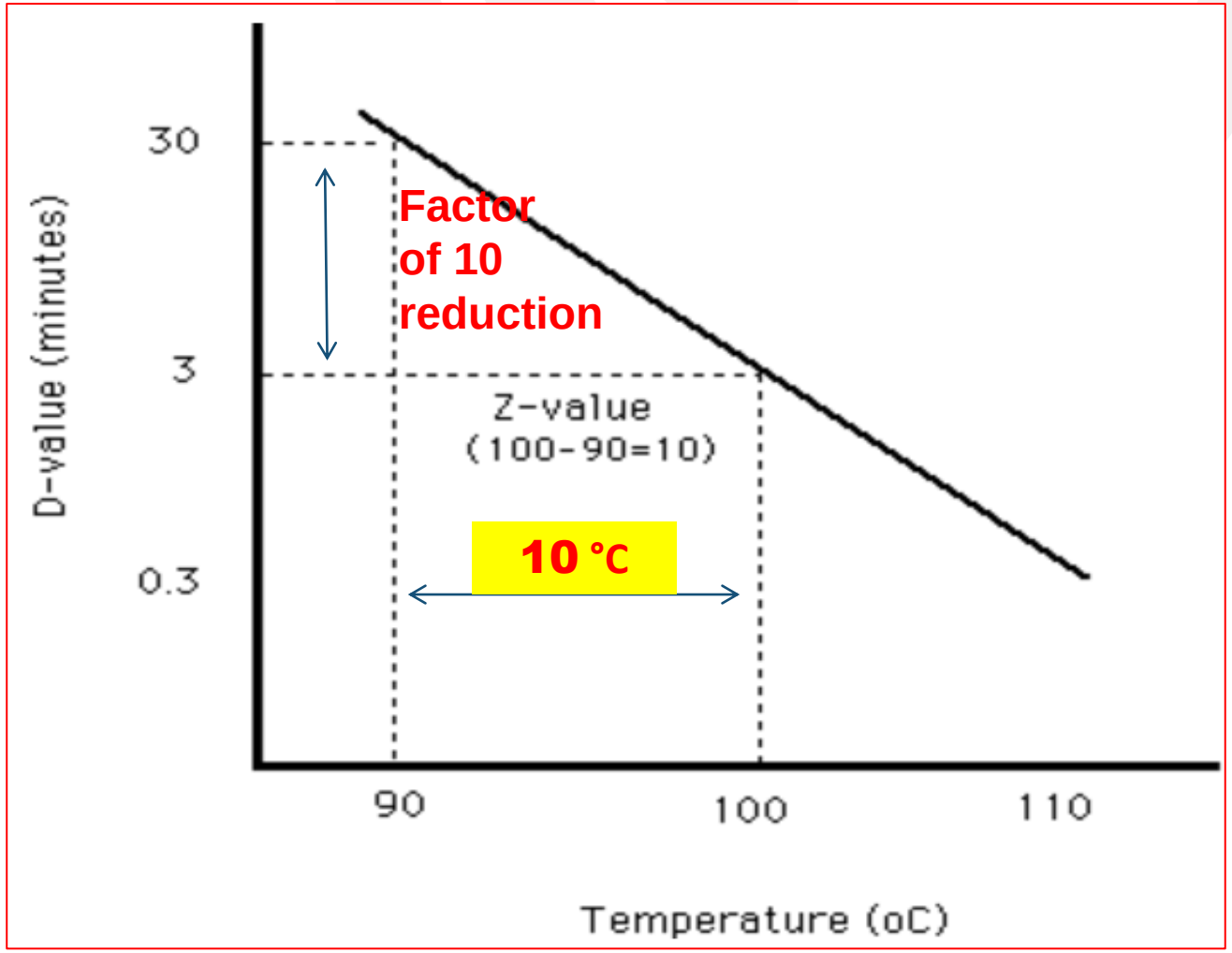
Thermal calculations

Z value

Heat induced chemical changes have much larger Z values than microorganisms

Example:

- Bacteria Z (°C) 5-10; D_{121} (min) 1-5
- Enzymes Z (°C) 30-40 D_{121} (min) 1-5
- Vitamins Z (°C) 20-25 D_{121} (min) 150-200
- Pigments Z (°C) 40-70 D_{121} (min) 15-50



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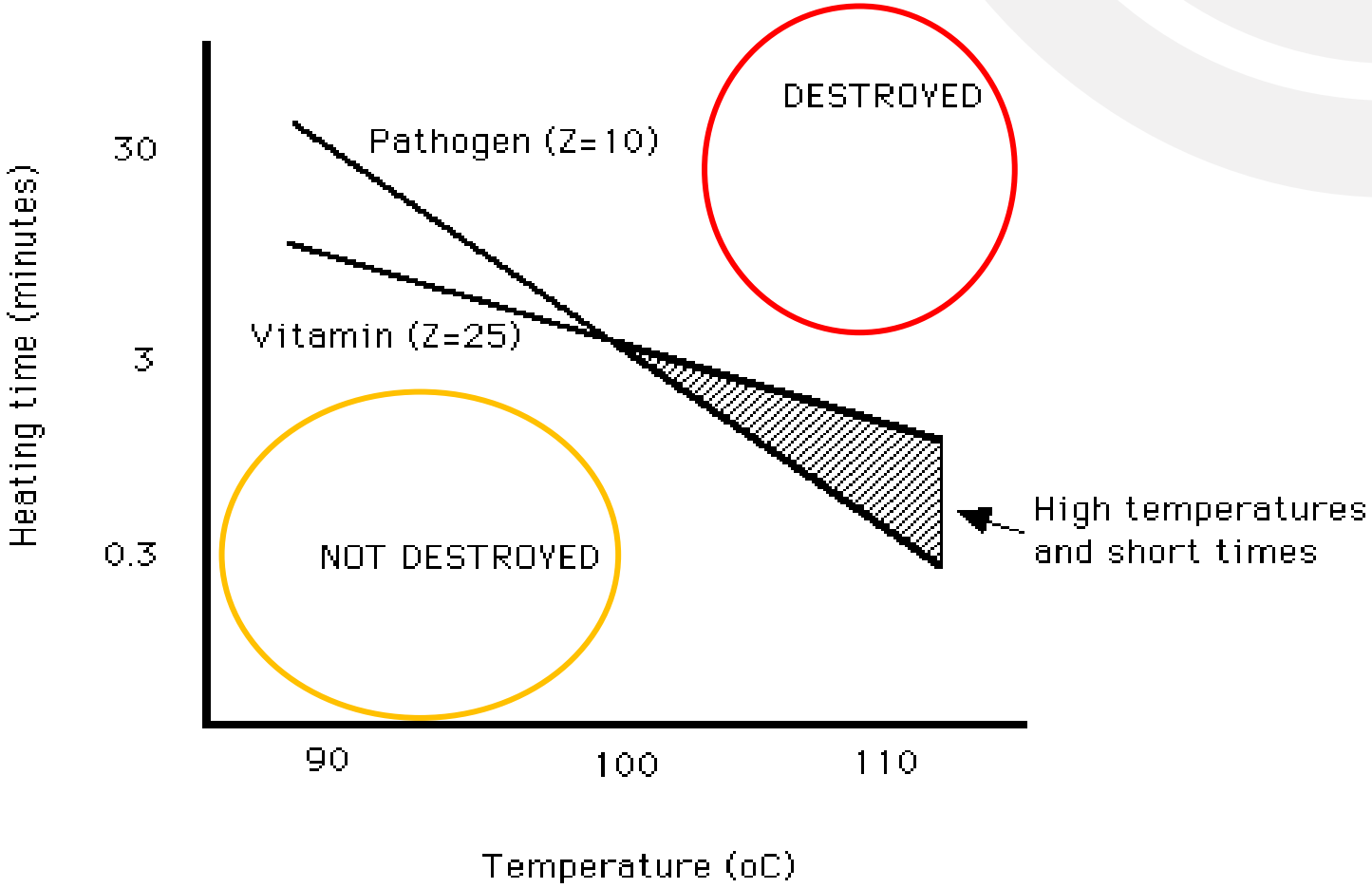
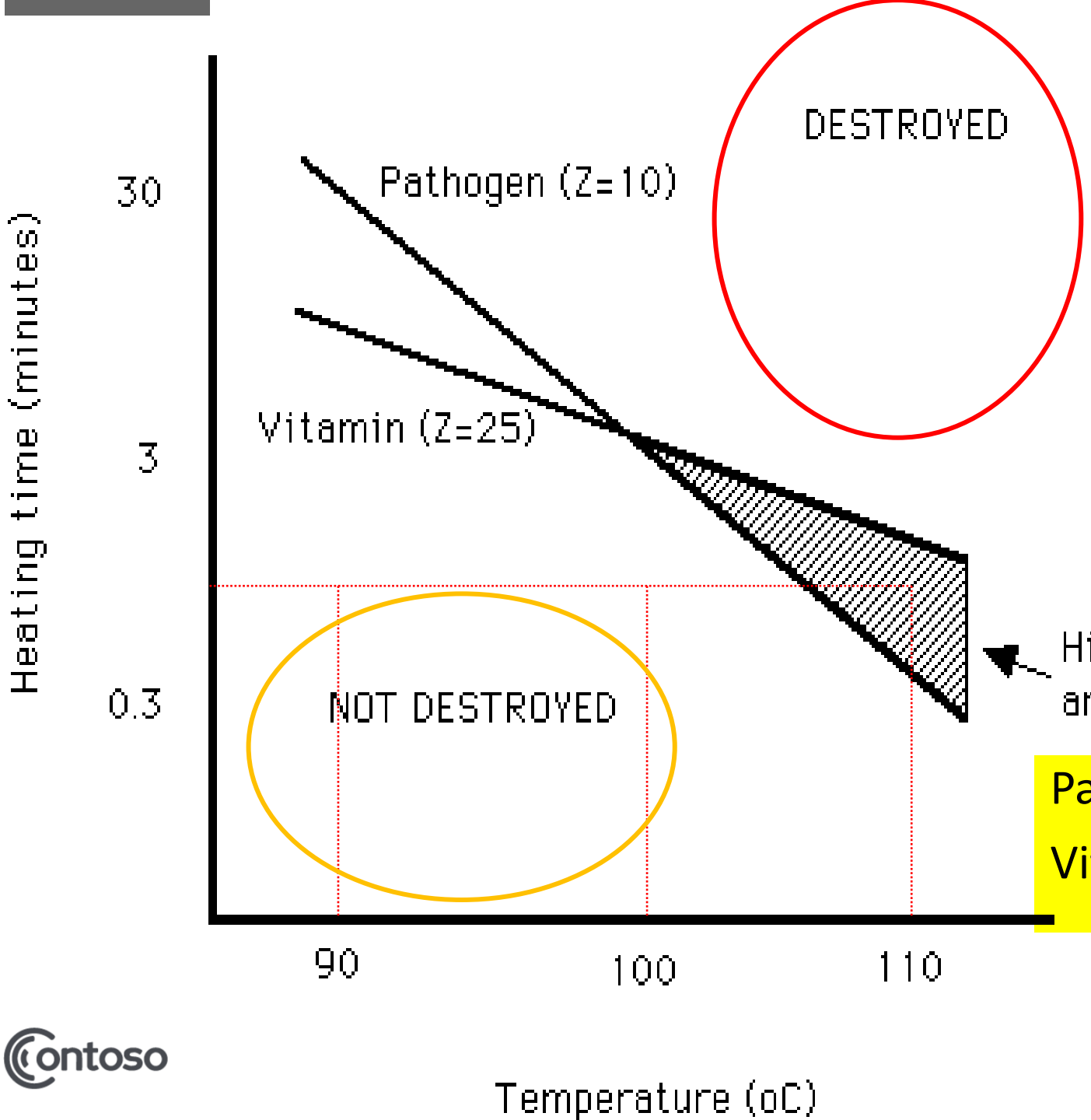
Relative changes in time temperature profiles for the destruction of microorganisms.

Above --> right: microorganisms or quality factors would be destroyed

Below --> left of: microorganisms or quality factors would not be destroyed





HTST (72°C, 16 s) and UHT (140 ° C, 1-2 s) is favoured compared to LTLT in milk pasteurization

HTST results in a lower loss of vitamins and better sensory quality

UHT eliminate bacterial spores but maintained the quality

Pathogens destroyed

Vitamins remained

A Working Example Of How To Use D And Z Values In Pasteurization Calculations:

- Pooled raw milk at the processing plant has bacterial population of **$4x10^5$/mL.**

  It is to be processed at **79°C for 21s**.

  The average **D value at 65°C** for the mixed population is **7 min.** The **Z value is 7°C**.

  How many organisms will be left after pasteurization?

  What time would be required at 65°C to accomplish the same degree of lethality?

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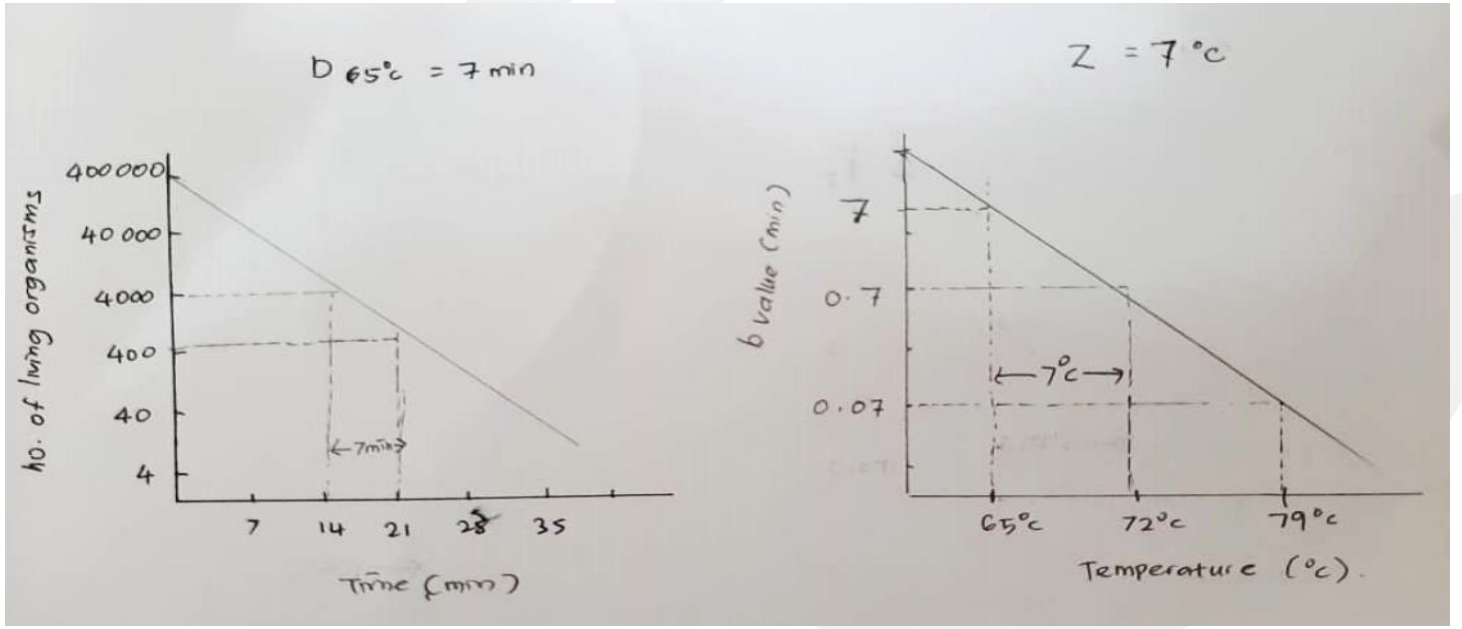
EXAMPLE

Initial microbial population: **$4x10^5$ /mL**

D_{65} = 7 min
Z value = 7°C

Pasteurisation process at **79°C, 21 sec**

- How many organisms will be left after pasteurization?
- What time would be required at 65°C to accomplish the same degree of lethality?



Z value = 7°C means every 7 °C, D value will be reduced by 1 log cycle

So, we need to find D value at 79°C:

- At 79°C, D value has been reduced by 2 log cycle: (79-65=14=2 log cycle)

Hence, D_{79} = 0.07 min.

The milk is processed for 21s = 0.35 min.

- 0.07 min --> 0.35 min = 5 cycle
  (400,000 reduced to 4 = 5 log cycle reduction)
- 4 organism left after pasteurization

At 65 °C, D value =7 min.
So, 35 min is needed to accomplish a 5 log cycle reduction.

MILK

- Milk pasteurization was designed based on the pathogens *Mycobacterium tuberculosis* and *Coxiella burnetti*
- Direct estimation of pathogens in milk is time consuming
- Therefore, a simple test for phosphatase activity is routinely used to estimate the presence of pathogens – inadequate heat treatment, contaminated pasteurized milk
- Alkaline phosphatase is a natural enzyme in raw milk which has similar Z value to heat resistant pathogens.

Table: Milk Pasteurizing Temperatures

Temperature	Time
63°C	For 30 min (low temperature long time LTLT)
72°C	For 15 sec (primary high temperature short time, HTST method)
89°C	For 1.0 sec
90°C	For 0.5 sec
94°C	For 0.1 sec
100°C	For 0.01 sec

- These conditions are sufficient to destroy most of heat sensitive of the non-spore-forming pathogenic bacteria
- It can also destroy yeast, moulds
- 2 groups of bacteria might survive
  - Thermoduric – survive but does not necessarily grow (Streptococcus and Lactobacillus)
  - Thermophilic – survive and require high temperature to grow

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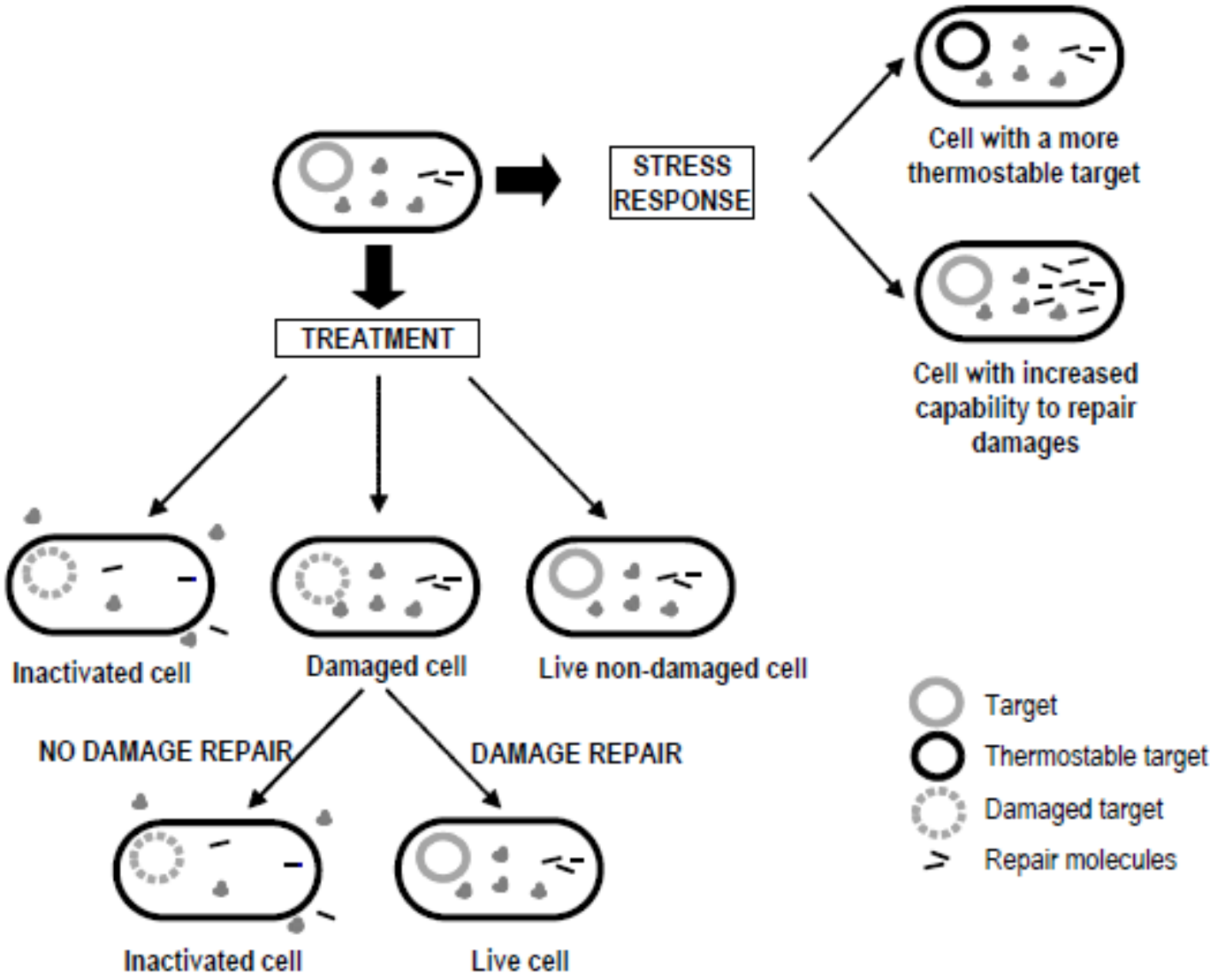
DIFFERENT SCENARIOS THAT CAN DETERMINE CELLULAR SURVIVAL OR INACTIVATION UPON HEAT EXPOSURE



Cebrian et al., 2017

LIVING CELLS

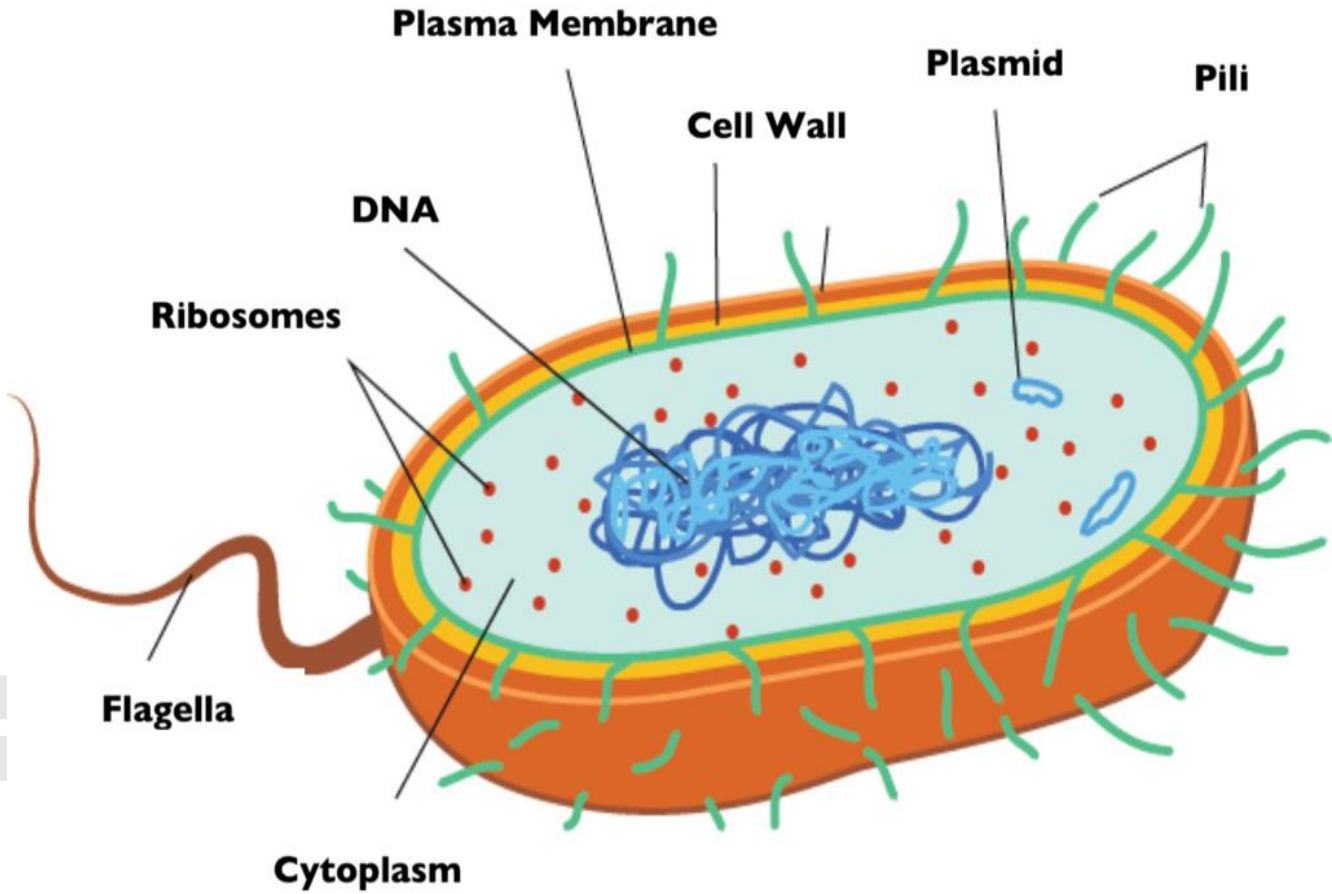
- Cells that has the potential to multiply and grow under suitable conditions

INACTIVATED CELLS

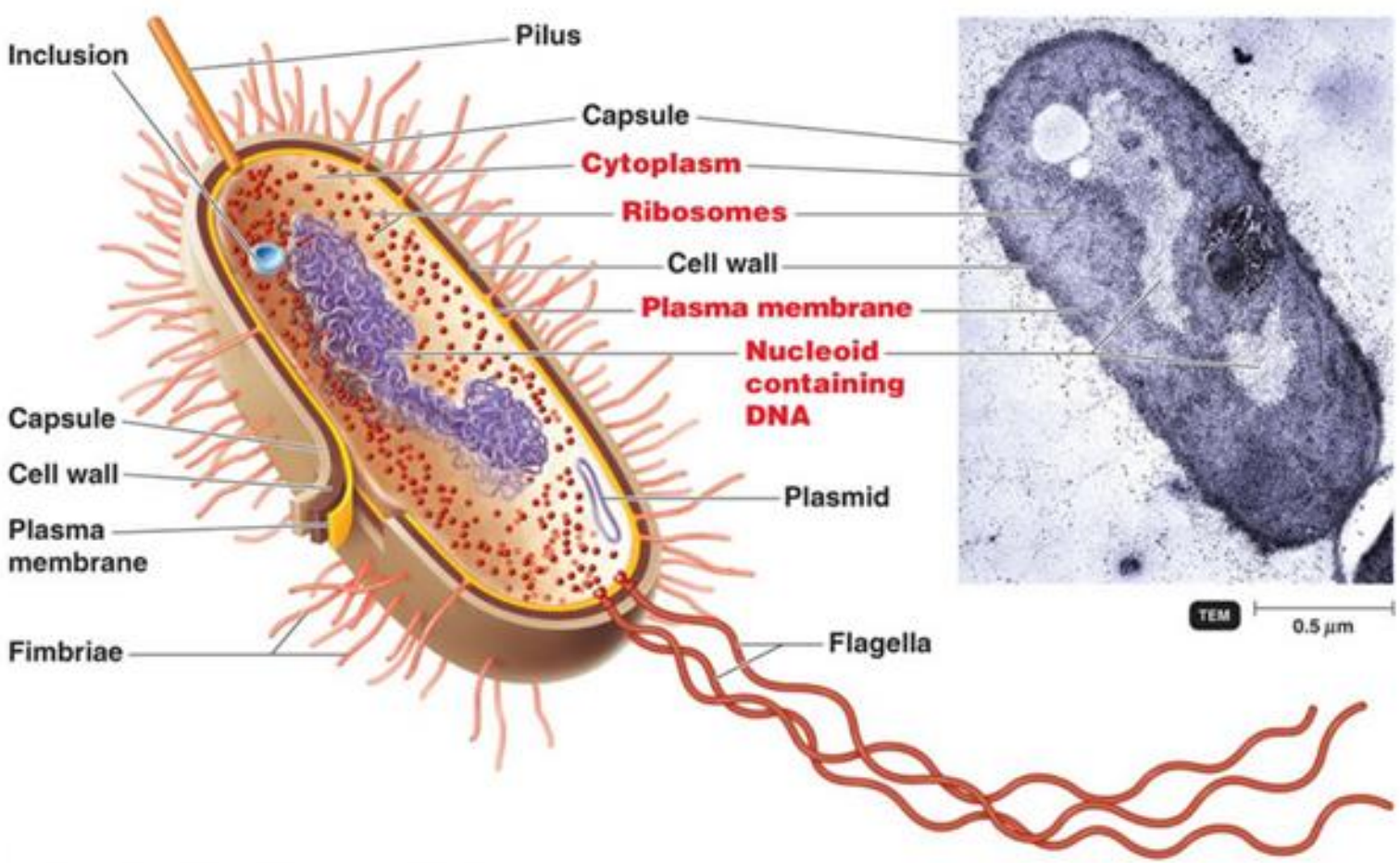
- Cells lose the potential to multiply
- This is due to the alteration of one or more cellular structures or functions known as cellular target
- Alteration of critical component may lead to cell death (e.g. RNA polymerase, ribosomes, cytoplasmic membrane)
- This is the objective of food preservation

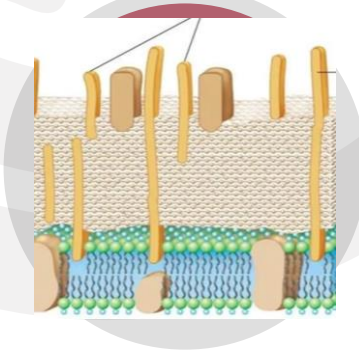
SUBLETHAL INJURY & RECOVERY

- Cell that present damages in non-critical components / low damage in critical component
- The damages affect the cellular structures and functions
- But can be repaired by the cellular machinery
- Under favourable conditions



EFFECT OF HEAT ON CELLULAR TARGET



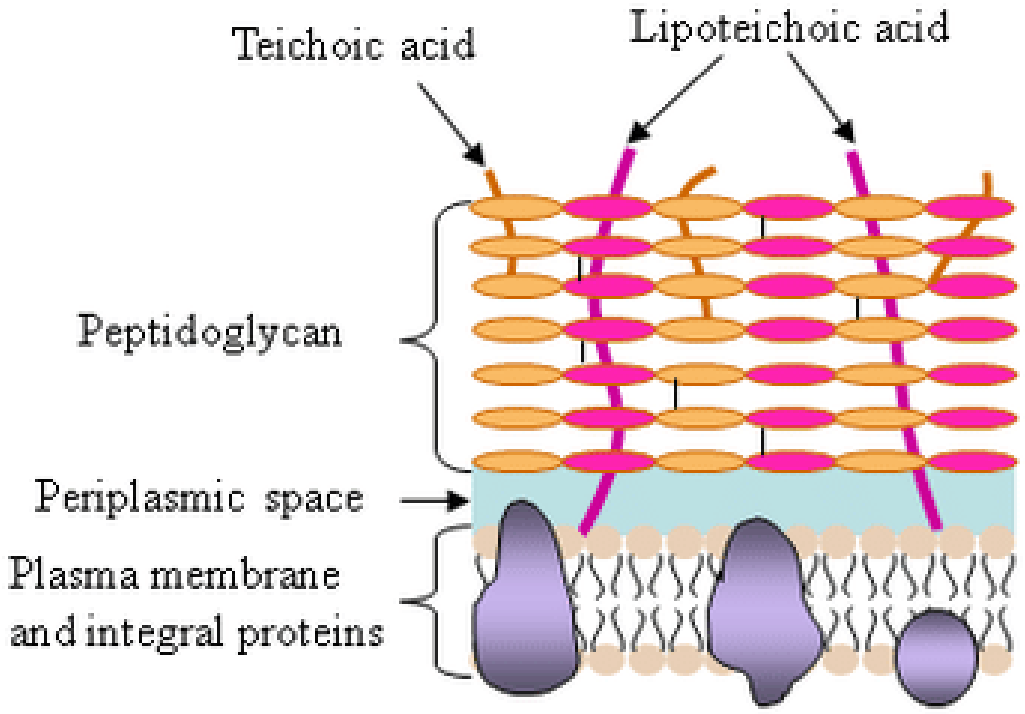


PEPTIDOGLYCAN CELL WALL

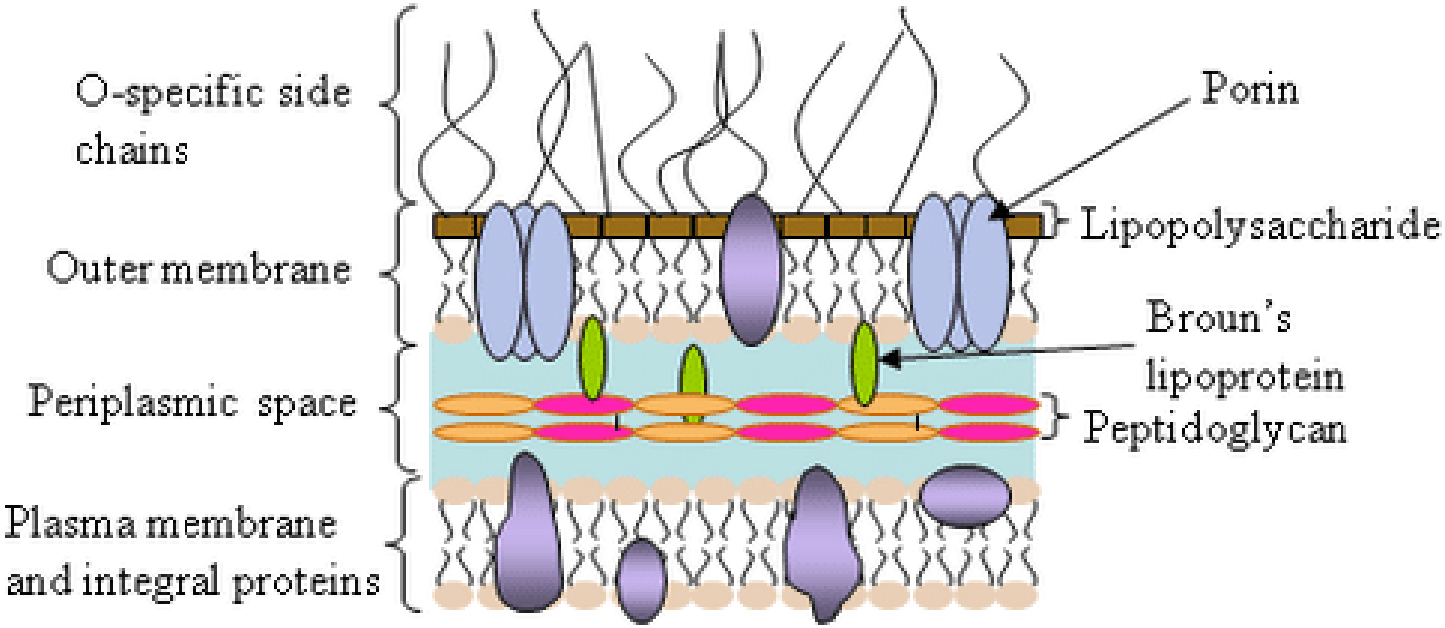
S. aureus cells lose D-alanine from the teichoic acid- preventing their role in certain essential metabolic processes

Lactobacillus bulgaricus injured after heating at 64C

Gram (+) cell-wall



Gram (-) cell-wall



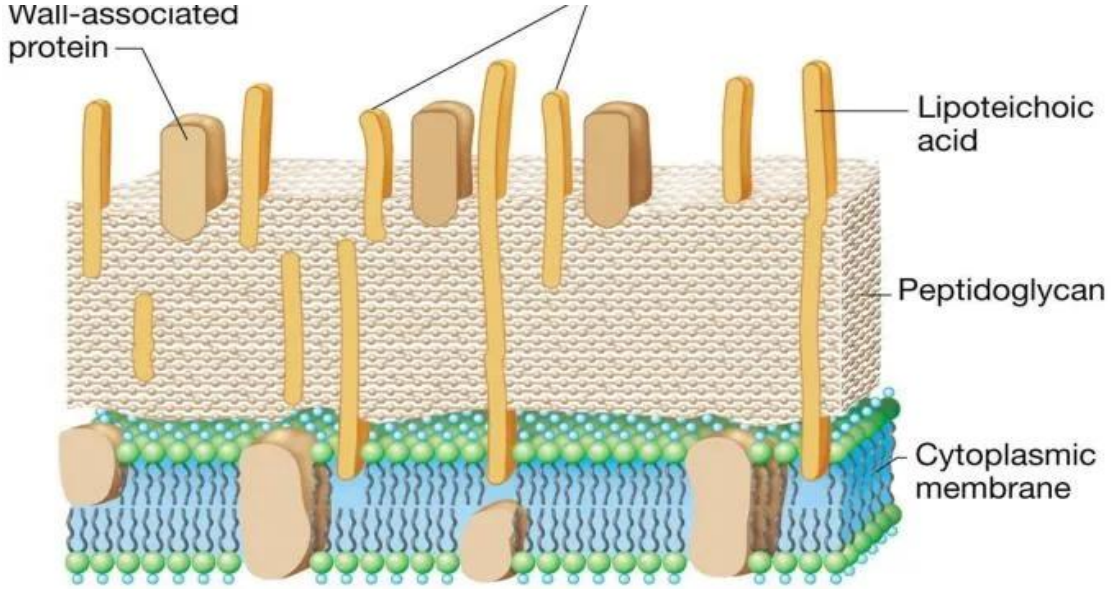
GRAM POSITIVE CELL WALL WITH TEICHOIC ACID

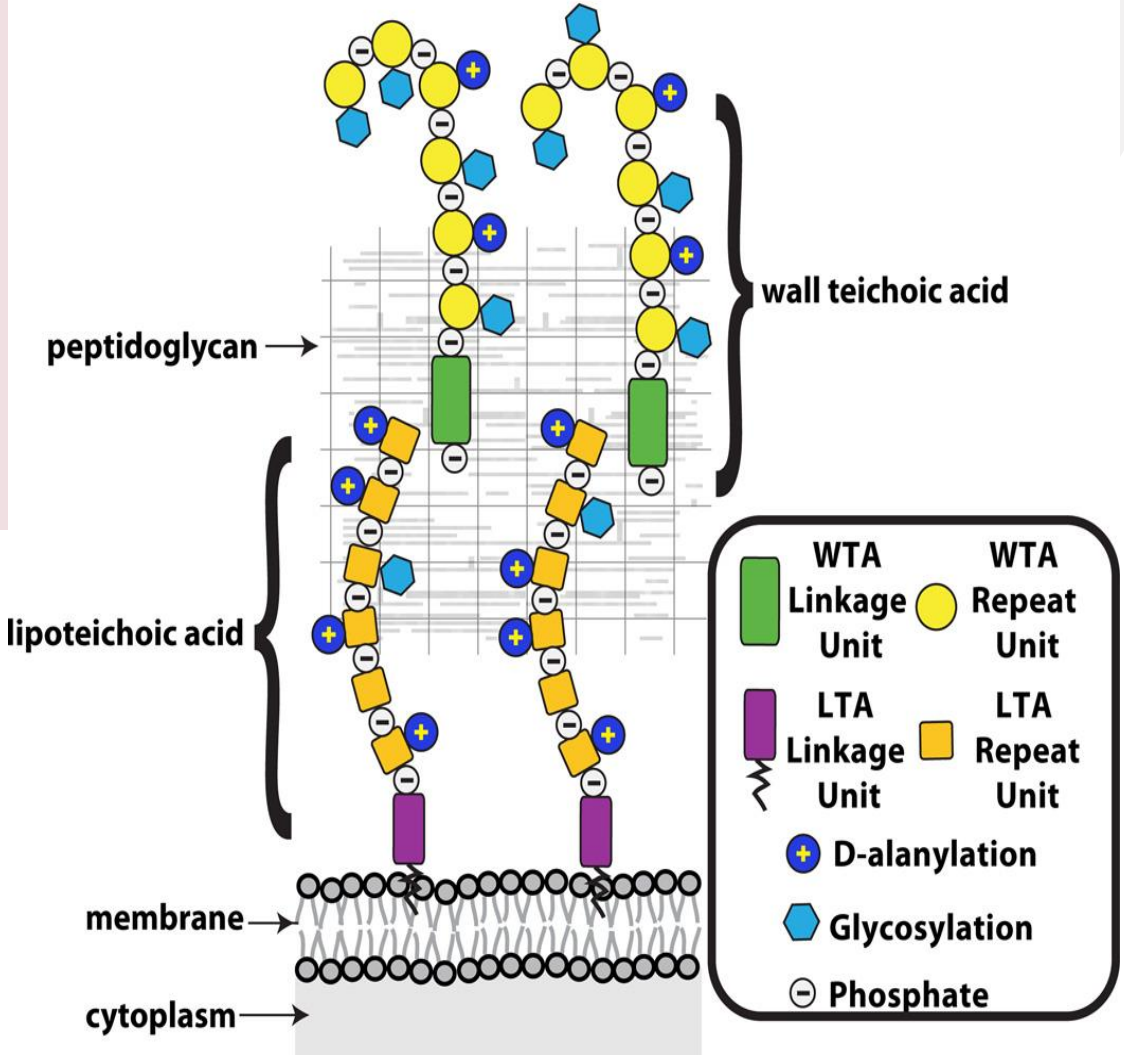
Teichoic acid polymers are located within the gram-positive cell wall

Maintaining the cell shape

Teichoic acid play role in pathogenesis – promoting adherence to host tissue . E.g. mediate the attachment of *Staphylococci* to mucosal cells

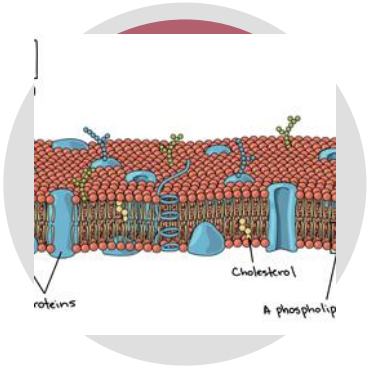
D-alanine ester attach to WTA and LTA





Cebrian et al., 2017

EFFECT OF HEAT ON CELLULAR TARGET

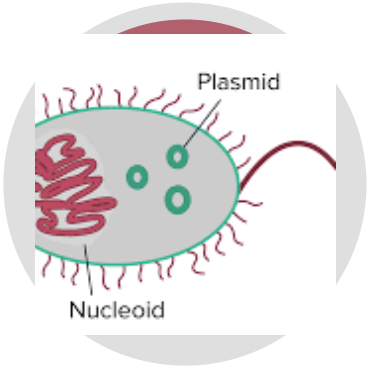


CYTOPLASMIC MEMBRANE

Loss of membrane materials & integrity

Loss of respiration activity (ETC is located in plasma membrane)

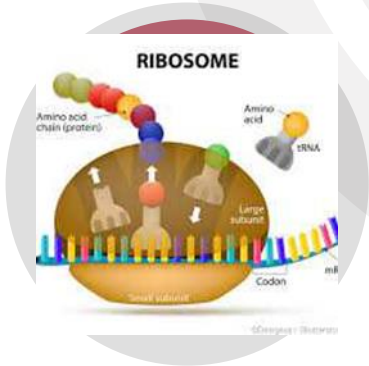
Loss of osmotic and pH homeostasis



NUCLEOID

Sterilization temp: DNA denatured, esp. under heat dry condition

Les intense treatment: DNA will damage & increase mutations

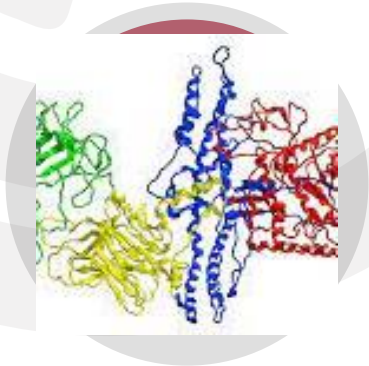


RNA & RIBOSOMES

RNA denatured

Ribosomes damaged

(Irreversible denaturation)



PROTEIN

Structural proteins or enzymes (e.g. RNA polymerase)

Transport pump & channels also susceptible to heat denaturation

Protein denaturation lead to loss of functionality

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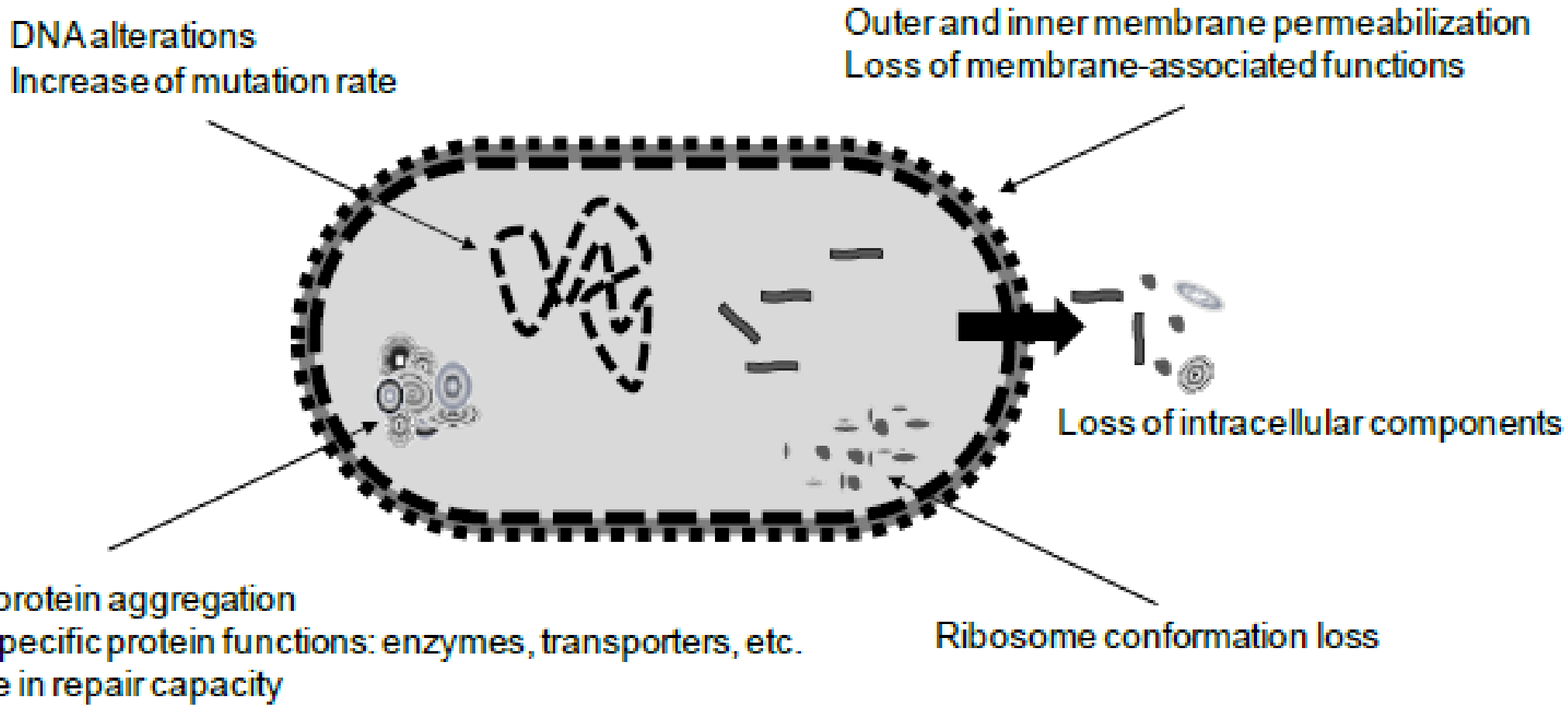
MAIN EVENTS THAT OCCUR IN A VEGETATIVE BACTERIAL CELL EXPOSED TO HEAT



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FACTORS AFFECTING HEAT RESISTANCE

WATER

Heat resistance of microorganism increases with decrease in moisture or humidity.

- Less water, Heat Resistance Increase
- More water, Heat Resistance Decrease

Protein denaturation occurs at a faster rate when heated in water than air.

Possibility:

1. Heating of wet proteins causes formation of free-SH groups with a consequence increase in water binding capacity of proteins.
2. Presence of H_2O allows for thermal breaking of peptide bonds which require more energy in the absence of H_2O

FACTORS AFFECTING HEAT RESISTANCE

FAT

General increase in the heat resolution

Fat protection : probably due to availability of moisture. Long chain fatty acids are more protective than short chain.

Effects is variable and dependent on type of salt & its concentration

Salt decrease water activity, and increase heat resistance, or

Salt can have protective effect & stabilize molecule. E.g. Ca2++ and Mg2 ++

- Supplementation of growth medium of *B. megaterium* with CaCl2 yielded heat resistant spores

Salts such as phosphates, polyphosphates, and nitrites tend to reduce heat resistance

SALT

FACTORS AFFECTING HEAT RESISTANCE

CARBOHYDRATE

Presence of sugars cause increase in Heat Resistance. Probably due to decrease in a_w.

Effect of sugars and alcohol – variation on Heat Resistance

Decreasing Order of D value:

Sucrose> glucose> sorbitol> fructose> glycerol

Maximum heat resistance at optimum growth pH (close to neutral)

Move away from optimum growth pH, heat resistance decreases.

Acidic condition cause cytoplasmic acidification due to the altered permeability of cell membrane – lead to rapid protein denaturation inside the cell

Therefore, high acid foods requires low temperature for processing compared to low acid foods.

pH

FACTORS AFFECTING HEAT RESISTANCE

TIME & TEMPERATURE

Time of heating do not greatly affect heat resistance but temperature do.

The higher the temperature, the greater the killing effect

NUMBER &TYPE OF ORGANISMS

Larger the number of microorganism, greater the Heat Resistance.

Gram positive is more resistant than Gram negative

Coccoid cells are more resistant than bacilliary cell

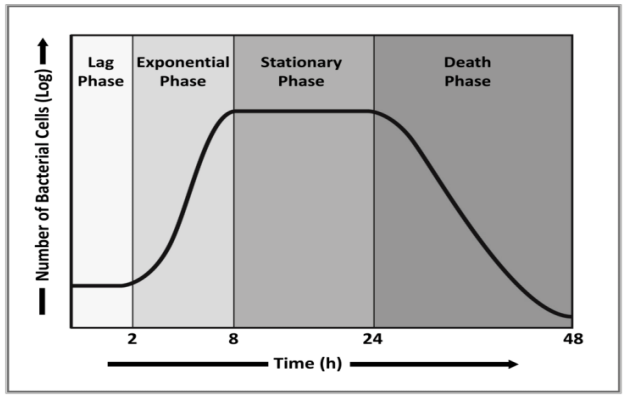
Intraspecies variation. E.g. *Salmonella* Seftenberg had D value 10x higher than other strains of the same species

FACTORS AFFECTING HEAT RESISTANCE

GROWTH PHASE

Stationary phase cells are more heat resistant than log phase (exponential) cells

AT this stage, cell increase the expression of sigma factor which control the transcription of genes involved in stress resistance, e.g. genes involved in DNA stabilization and repair.



Heat resistance increases with increasing incubation temp.

Bacteria can modify the fatty acid, and protein composition of membrane – cell fluidity is adjusted to maintain the same cell permeability properties

eg. *Salmonella* Seftenberg grown at 44°C was 3 times more resistant compared to cells grown at 35°C

GROWTH TEMPERATURE

FACTORS AFFECTING HEAT RESISTANCE

PRESENCE OF INHIBITORY SUBSTANCE

Heat resistance decreases in the presence of antibiotics, SO_2 and other microbial inhibitors

Heat in combination with antibiotics or nitrite is more effective in controlling microbes

The presence of ethanol, niasin, essential oils, sorbic acids, may interfere the integrity of the outer and cytoplasmic membrane & decrease heat resistance

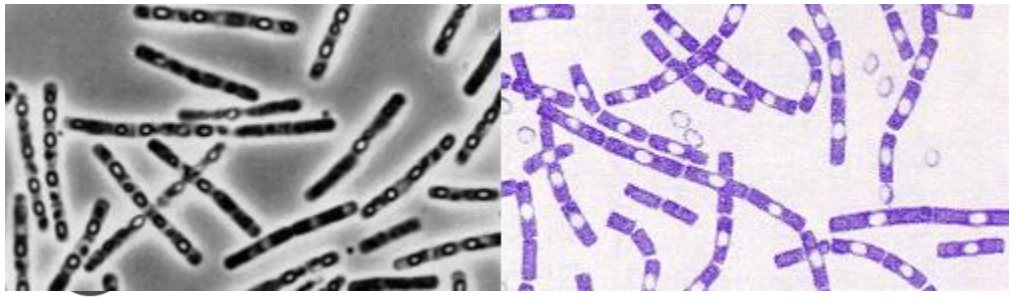
– inhibit recovery process of injured cells

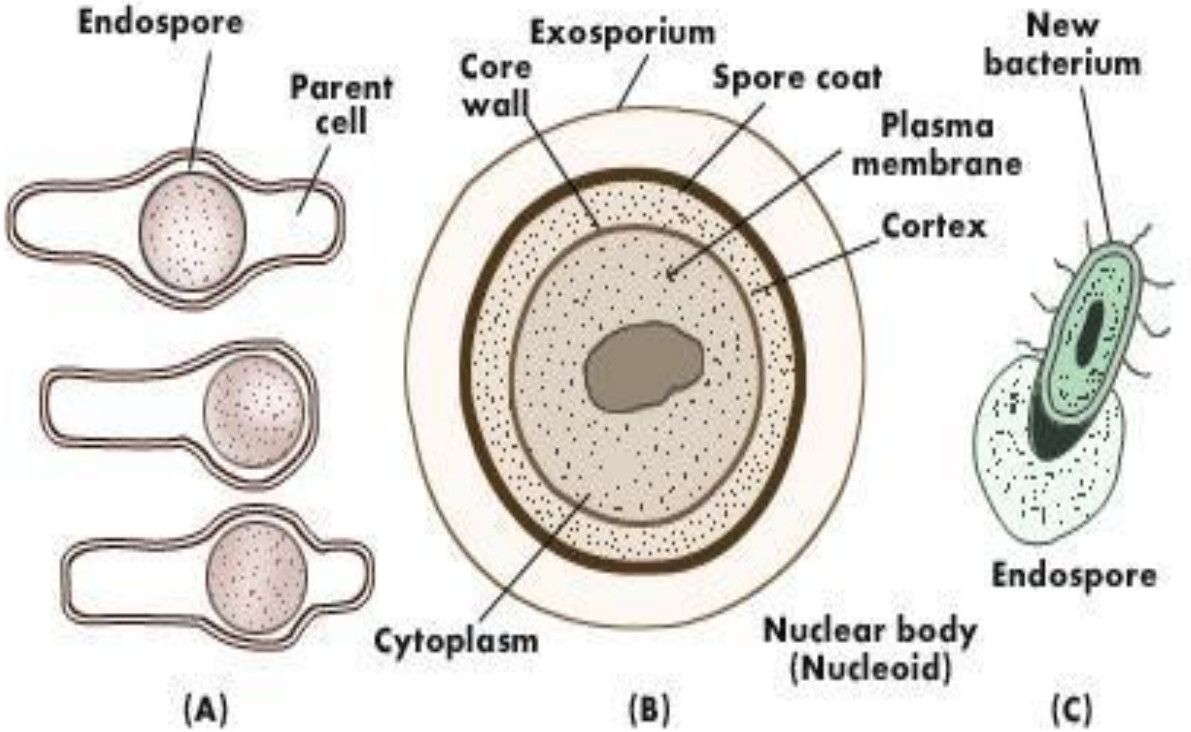
– extra safety to certain thermally-processed foods

HEAT RESISTANCE OF ENDOSPORES

Endospores are formed as a result of nutrient depletion or environmental stre

Endospores are not only resistant to heat, but also to drying, cold, chemicals and other adverse environmental factors

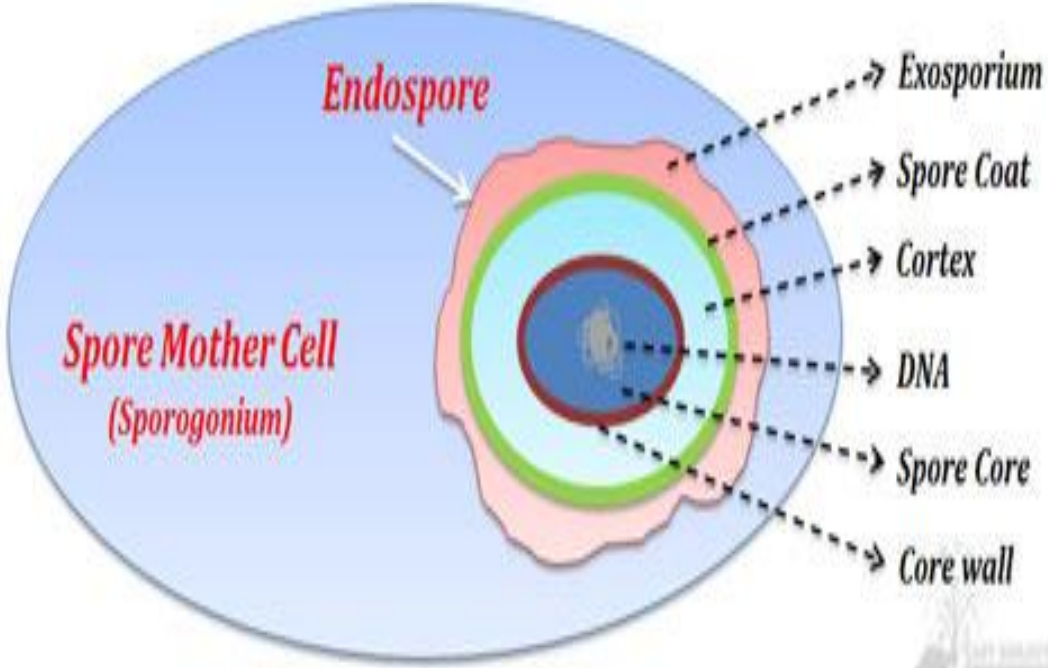




Endospore formation. A, Endospores according to their position in parent cells. B, An edospore in cross-section. C, Germination of endospore

ENDOSPORE STRUCTURES

1. **Exosporium,** a thin delicate covering made of protein.
2. **Spore-coat** is thick and consisting of several protein layers. The spore-coat is impermeable and responsible for the spores resistance to chemicals
3. **Cortex** is a thick layer composed by peptidoglycan, and occupy half of the spore volume
4. **Core wall** covers the central protoplast (core of endospore)
5. **Spore core** (Protoplast) contain cytoplasm, nucleoid, ribosomes etc. but is metabolically inactive
6. The spore core contains about 10-25% of water of the normal vegetative cell



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THANK YOU !