

Heat Resistance of Bacteria

Cause of microbial death

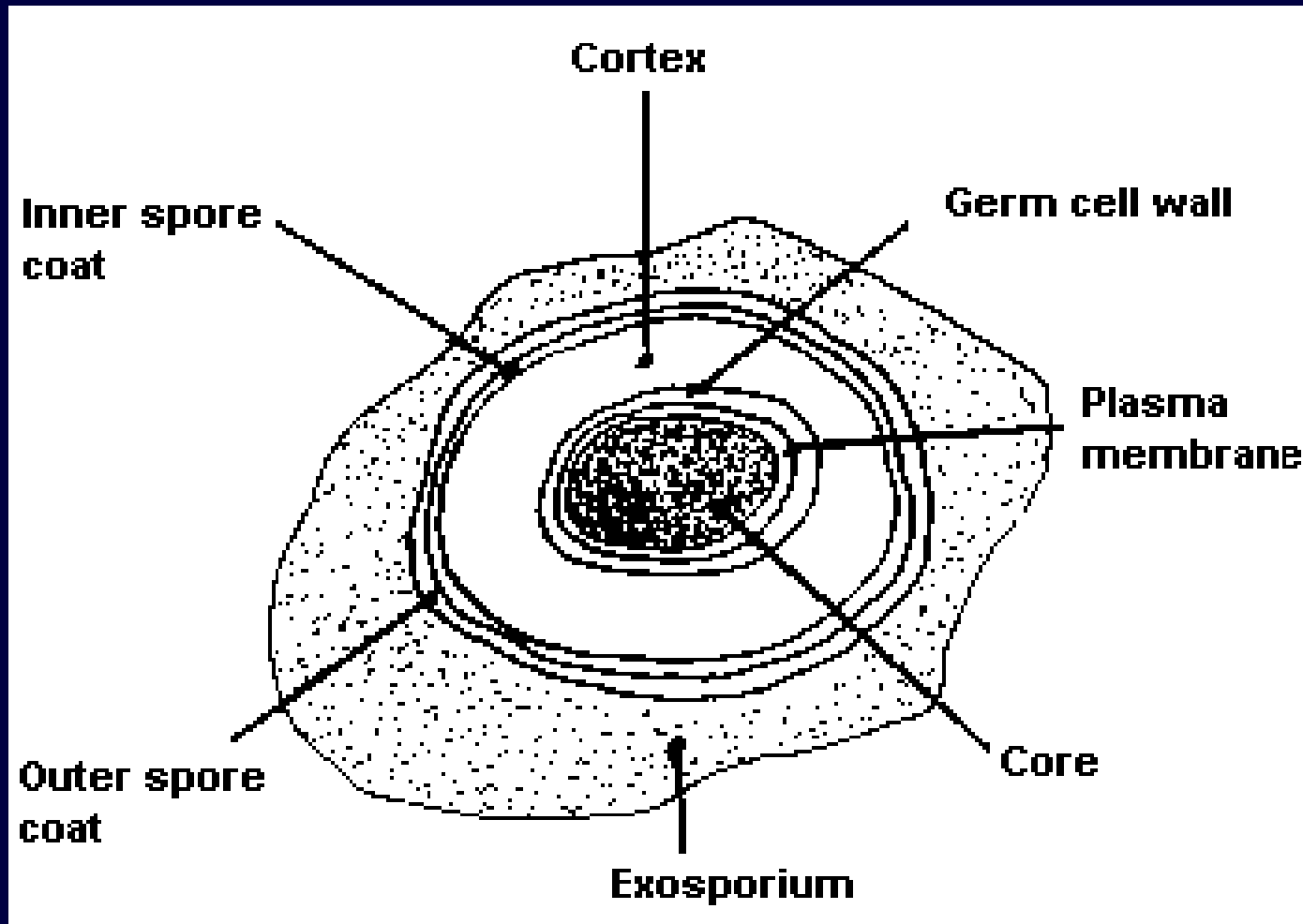
Death of vegetative bacteria due to

- Coagulation of proteins
- Inactivation of enzymes
- Release or activation of native nucleases that damage DNA
- Damage of cytoplasmic membrane causing leakage of cell constituents

Heat resistance of spores

- Endospore contains DNA, RNA, water, enzymes, Ca, Mg and dipicolonic acid (DPA)
- Heat resistance is due to
 - Role of **DPA** (5-15%)
 - High **calcium** (2-10 times more than vegetative forms)
 - Lack of water in spores for cell protein coagulation
- Heat resistance increases with high Ca:DPA ratios

Structure of bacterial spore



Structure of bacterial spore

Exosporium - A thin delicate covering made of protein.

Spore coats - Composed of layers of spore specific proteins.

Cortex - Composed of loosely linked peptidoglycan and contains dipicolinic acid (DPA)

- DPA cross links with calcium ions embedded in the spore coat
- This cross linkage contributes to the resistance
- It creates a highly impenetrable barrier
- The calcium DPA cross linkages compose 10% of the dry weight of the endospores

Core - The core contains cell wall, cytoplasmic membrane, nucleoid, and cytoplasm.

- The core only has 10-30% of the water content

Factors affecting HR of spores

Important factors affecting heat resistance are

- ☀ Initial number of spores
- ☀ Age of the spores
- ☀ Time-temperature relationship
- ☀ Environmental factors
 - ☀ During spore formation
 - ☀ During heating
 - ☀ Effect of recovery medium
 - ☀ Effect of sub-lethal heat treatment

Initial number of spores

- Destruction follows a logarithmic pattern
- High initial number, longer time for inactivation

Age of spores

- Young moist spores less resistant than old spores
- Maximum heat resistance attained after 4-8 days
- Spores in soil more resistant than broth or agar
- Aging (1 year) increases resistance of aerobic spore formers

Time-temperature relationship

- Time for destruction decreases with temperature increases
- Analogous results obtained if spore or vegetative cells are heated at different temperatures

Time-temperature relationship

Temp (°C)	Time taken to destroy spores (min)
100	1200
105	600
110	190
115	70
120	19
130	3
135	1

Environmental factors

1. Spore formation

- Changes in composition of nutritive medium
Spores produced in pasteurized or sterilized meat, 3 times more HR than spores produced in raw meat
- Reduction of Fe^{++} and Ca^{++} conc. decreases HR
- Long chain fatty acids increases HR

2. Heating

✿ pH of suspending medium

- Max HR at neutral
- Little loss in range 6-8
- Decreases more if < 6
- Acidic more effective

✿ a_w of the medium

- Spores less HR to moist heat than dry heat
- Spores exhibit high HR if a_w is low

✿ Protective substances

- Sugars in 10% conc increases HR
- NaCl above 3-4% reduces HR
- Phosphates increases spore germination and HR

✿ Fatty or oily media

- More HR than aqueous media
- Fat protect due to poor conductivity
- Vary with the degree of saturation

✿ **Proteins, starch and spice oils** offers protection to spores

✿ **Antibiotics**, subtilin, nisin & tylosin at low conc reduce HR

3. Recovery medium

- ✿ Depends on composition of medium and temperature
- ✿ Medium should initiate spore germination and stimulate or support veg. growth
- ✿ Incubation at low temp 24-31°C

4. Sub-lethal heat treatment

- ✿ Delay germination and dormancy of heated spores
- ✿ Correlation between dormancy and Ca:DPA
- ✿ Ca⁺⁺ is the probable factor for dormancy
- ✿ Recovery medium eliminate or reduce dormancy

Methods for estimating heat resistance

Determination of thermal death time at different temperatures

- ☀ Suspend a known number of spores of the reference bacteria in a specified medium in a sealed container is heat in an oil bath maintained at a constant temperature
- ☀ Draw samples at specified intervals and sub-culture in a suitable medium
- ☀ **Death** is defined as the inability of the organisms to form a visible colony on subculture
- ☀ **Thermal death time** (TDT) is defined as the time in minutes required to inactivate an arbitrarily chosen number of spores of a given bacteria at a specified temperature

Different methods

1. Single tube method
2. Multiple tube method
3. Can method
4. Thermoresistometer
5. Culture tube method
6. Bulbed capillary method

1. Single tube method (Bigelow and Esty)

- ☀ Suspend a known no. of bacteria or spores in suspension in sealed tubes and heat by immersing in oil bath maintained at a constant temp.
- ☀ Remove a single tube at known short intervals, rapidly cool and culture in a suitable medium
- ☀ Thermal death time lies between the time of heating the last tube showing growth and the time of heating of the first tube showing no growth
- ☀ Serious shortcoming – occurrence of 'skips'
- ☀ **Skip** – A tube which shows no growth on subculture although heated for a shorter time than the tubes containing viable organisms

2. Multiple tube method (Esty and Williams)

- ✱ Instead of single tube, a large number of tubes 25-30 removed at each of the four conveniently spaced timings
- ✱ Percentage of tubes showing growth at each heating period is plotted on a semi log paper
- ✱ From the straight line graph, the TDT at the temperature used can be found by extrapolation of the curve
- ✱ Problems of skips – non-significant

3. Can method (Townsend et al)

- ✦ TDT in raw food products
- ✦ Special cans, 2" dia x 3/8" height are used to contain the raw material and test organisms
- ✦ Cans are sealed under vacuum and heated in specially designed small retorts
- ✦ After heating, cans are cooled and incubated
- ✦ Bulging of cans caused by gas formation is taken as indication of growth

4. Thermoresistometer

- ☀ Stumbo Thermoresistometer – designed for heat destruction studies in the range 115.6 to 132.2°C
- ☀ Apparatus consists of three chambers connected to a steam reservoir and each chamber can be pressured or exhausted independently
- ☀ Inoculated samples are spread in small metal cups (7mm dia x 1.5 mm deep), which rest in aluminum boat are moved through the three previously sterilized chambers
- ☀ Sample is loaded in the first chamber
- ☀ Second contact chamber is pressured by steam at working temp
- ☀ Boats with cups and samples, cooled by evaporation fall into the tubes of culture medium in the third chamber
- ☀ Process time in central chamber is accurately measured

Advantages of thermoresistometer

- ✦ Greater accuracy because of rapid heating and cooling
- ✦ Simple in operation
- ✦ Entire sample is cultured
- ✦ Chances of contamination are greatly reduced

5. Culture tube method (Schmidt)

- ✦ Test suspension heated in small glass tubes fitted with cotton wool plugs
- ✦ This facilitates addition of culture medium directly into the tubes
- ✦ Heating done in a miniature retort fitted with a pressure cooling system
- ✦ Accurate measurement of temperature is made using a thermocouple inserted into one of the tubes

6. Bulbed capillary method

- ✦ Spore suspension introduced into a small bulb blown from capillary tubings, sealed and is placed at the slowest heating point in a can of food
- ✦ Actual time of exposure of bulb to the processing temperatures is calculated taking into consideration the come-up-time and cooling time