Class: BSc. Hons. Botany (VI Semester)
Paper: BOB:604 (PLANT AND MICROBIAL TECHNIQUES)
Topic: Cryopreservation
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Cryopreservation

Cryopreservation is a process to preserve cells, tissue, organelles or any biological materials at very low temperature

Cryoprotectants agents (CPAs)

Two classes of CPAs

First: Chemicals agents that passively move through the plasma membrane to equilibrate between the extracellular solution and the cell interior (penetrating or permeating CPAs)

Examples:1,2-propanediol; Glycerol and Dimethylsulphoxide (DMSO)

Second: They do not passes through the plasma membrane and remain in the extracellular solution (non-penetrating or non-permeating CPAs).

Examples: Polyvinylpyrrolidone (PVP), Hydroxyethyl starch, Polyethylene glycol (PEG) and sugar

Process of cryopreservation

- Slow freezing process
- Vitrification process

Slow freezing process

- Cooling rate should be 0.5 ◦C to -100 ◦C /min. keep it for 20-45 min before storage in liquid nitrogen.
- Slow cooling reduces the amount of intracellular water due to ice formed outside the cells.
- This approach particularly useful for cell suspensions.

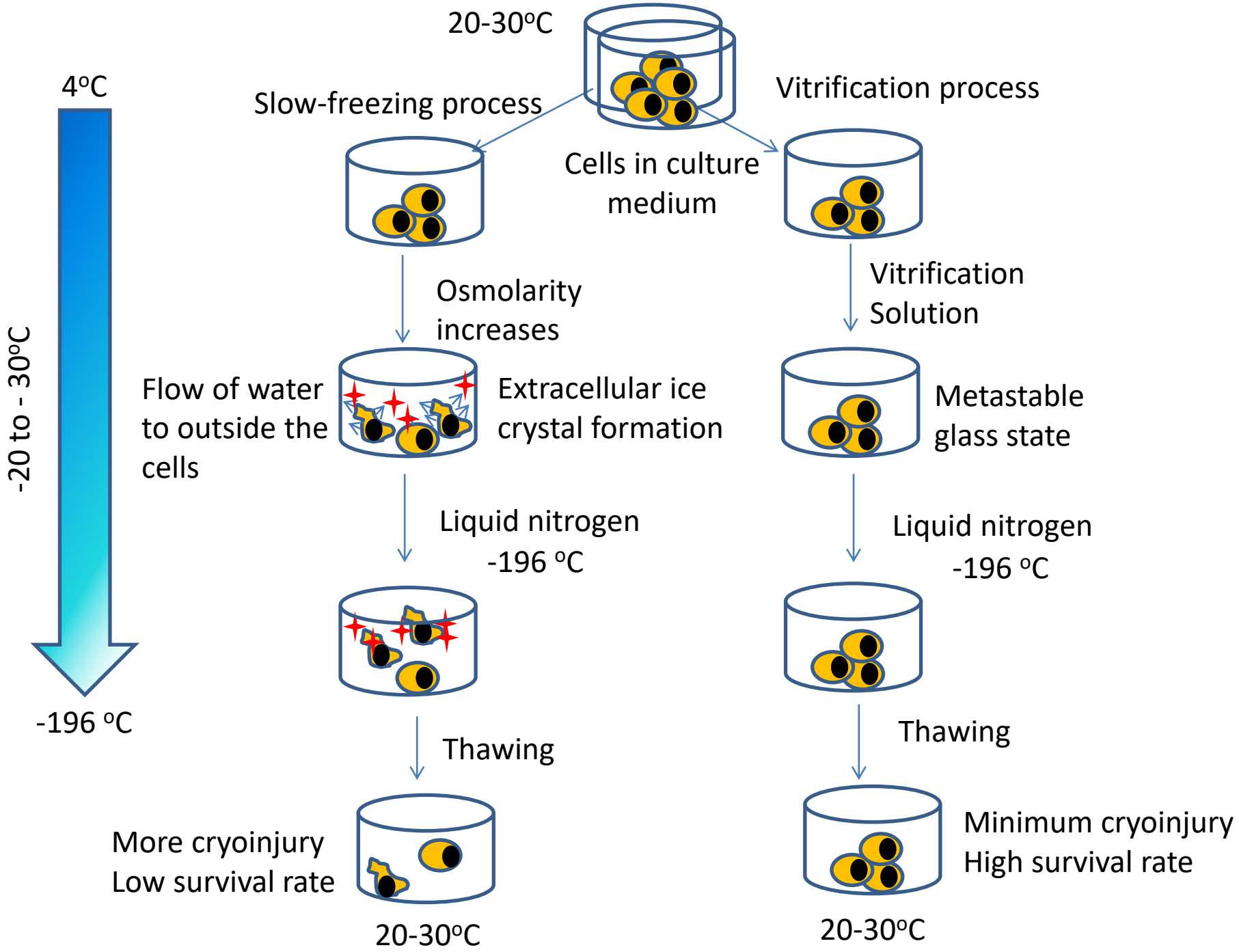
Vitrification

- At very low temperature, cell suspensions containing cryoprotective agents are directly transformed from aqueous phase to metastable glass state without formation of ice crystals.

- The process requires cryogenic temperatures with high concentrations of CPAs (40–60%, w/v).

- Vitrification is dependent on three factors: (1) viscosity; (2) cooling rates; and (3) sample volume

- This approach particularly useful for cryopreservation of cell culture, protoplasm tissue and shoot tips.


20-30ºC
4ºC
Slow-freezing process
Vitrification process
Cells in culture medium
-20 to - 30ºC
Osmolarity increases
Vitrification Solution
Flow of water to outside the cells
Extracellular ice crystal formation
Metastable glass state
Liquid nitrogen -196 ºC
Liquid nitrogen -196 ºC
-196 ºC
Thawing
Thawing
More cryoinjury
Low survival rate
Minimum cryoinjury
High survival rate
20-30ºC
20-30ºC

Applications of cryopreservation

(1) Cryopreservation of cells or organs
(2) Biochemistry and molecular biology
(3) Food sciences
(4) Ecology and plant tissue culture
(5) Cryosurgery

References

- Jang TH et al. (2017) Cryopreservation and its clinical applications. Integrative Medicine Research 6:12-18.

- Singh BD (2013) Biotechnology Expanding Horizons. pp. 321-325.