Monosodium Glutamate as Lyoprotector: Effectiveness during Freeze-drying and Storage of *Lactobacillus delbrueckii* subsp. *bulgaricus*

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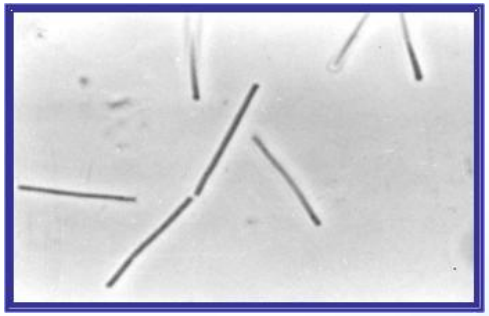


Mozzarella

Lactobacillus delbrueckii ssp. _bulgaricus_

(L. bulgaricus)

Tarhana

Kishk

Tacharas

Chondros

Kushik

Provolone

Kefir

yogurt

Emmental

Gruyere

Objectives

To evaluate the damage occured during lyophilization and the role of monosodium glutamate as lyoprotector in preserving cell viability and metabolic activity of *L. bulgaricus* CRL 494

Material and Methods

1. FREEZE-DRYING (FD): Effect of monosodium glutamate (Gl)

L. bulgaricus **CRL 494** → **Freeze-drying (without Gl) Control and with Gl** → **Storage 6 months-4°C**

Viability Biological activity Fatty acids profile	Viability Biological activity Fatty acids profile	Viability Biological activity Fatty acids profile

Cell viability: plate dilution

N/Ni index

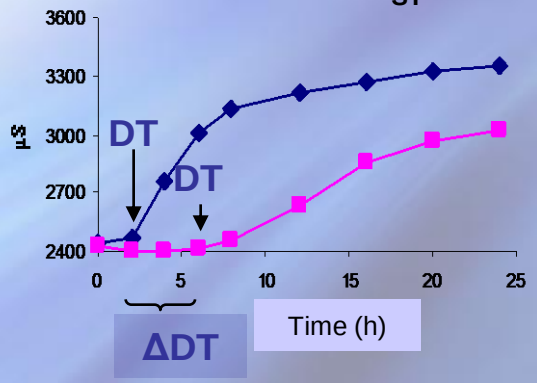
N: log CFU/ ml after a given treatment (freeze-drying or storage)

Ni: log CFU/ ml before the referred treatment

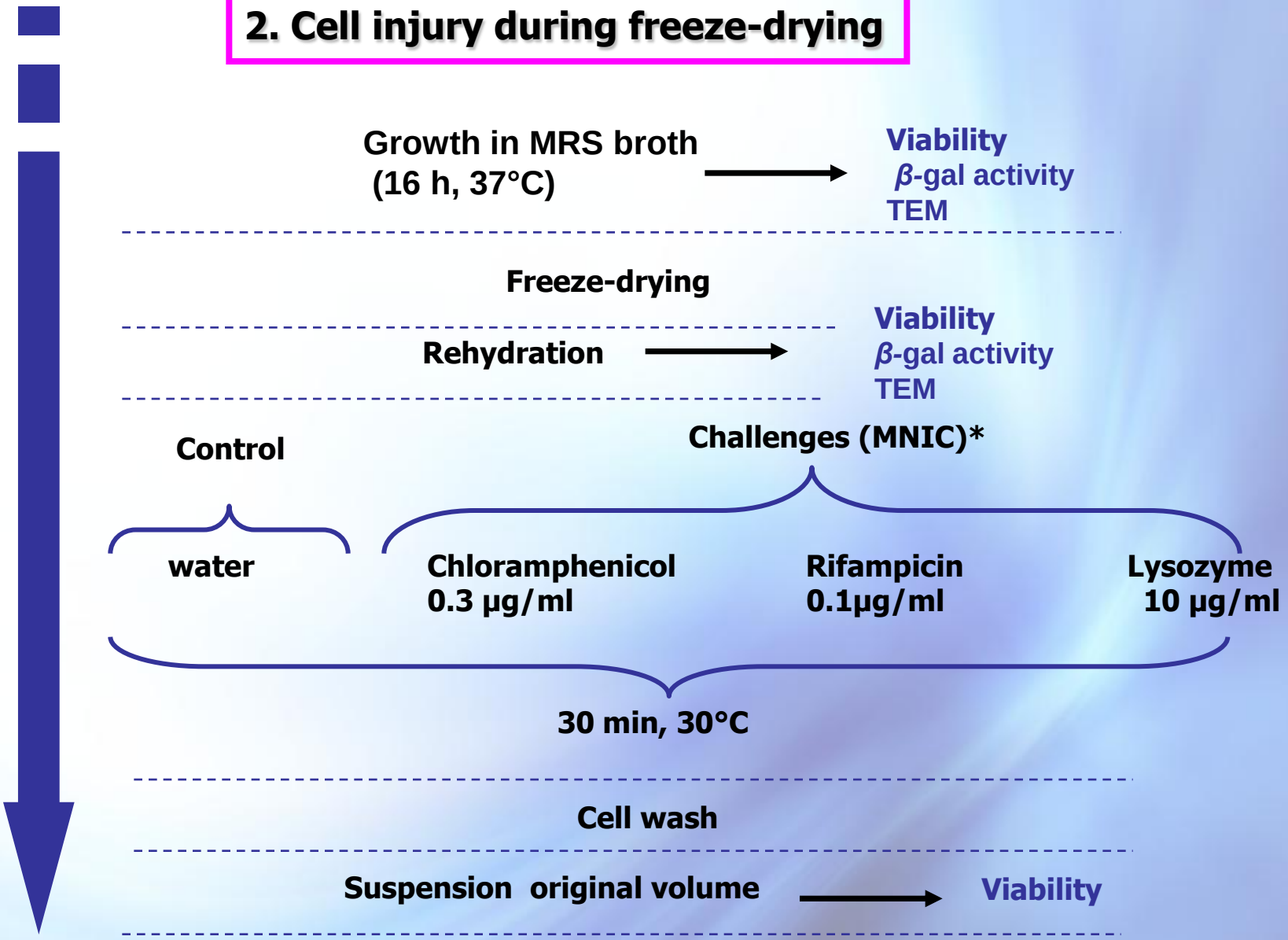
Biological cell activity: direct conductimetry

ΔDT_{FD} = DT after FD - DT before FD

ΔDT_{ST} = DT after sto - DT after FD

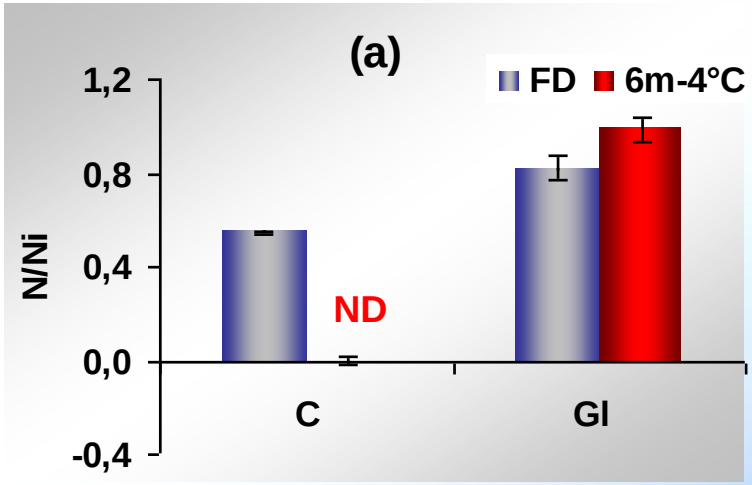


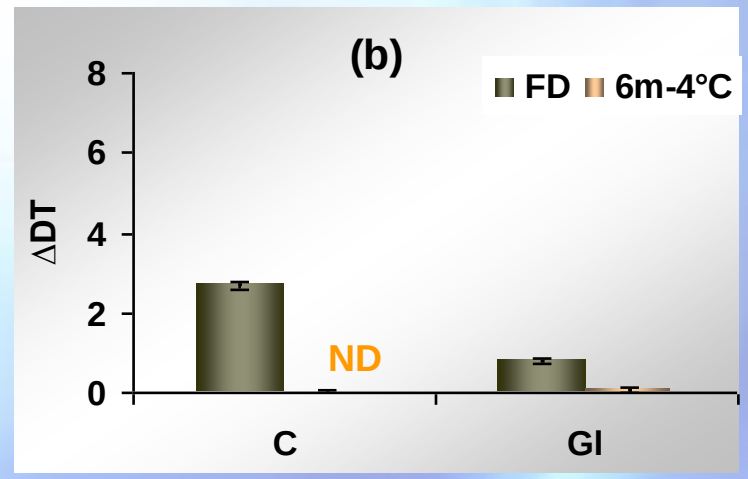
2. Cell injury during freeze-drying



*MNIC: Minimal Non Inhibitory Concentration

1. Survival of *L. bulgaricus* CRL 494 subjected to freeze-drying and storage (6 months-4°C). Effect of sodium glutamate 5%





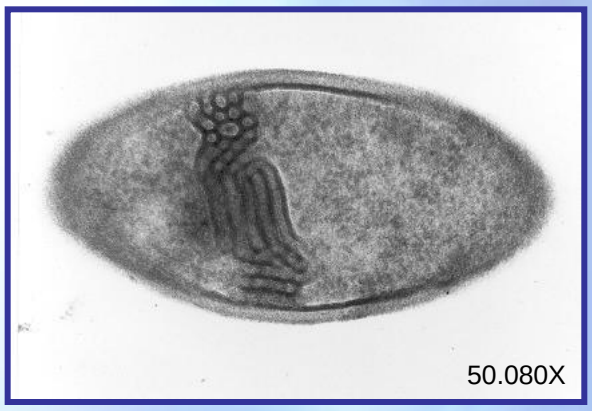
- **Lyophilization causes decrease in (a) viability and (b) metabolic activity of *L. bulgaricus* CRL 494.**

- **Monosodium glutamate 5% was effective in preserving cell injury from freeze-drying.**

2. Cell injury during freeze-drying

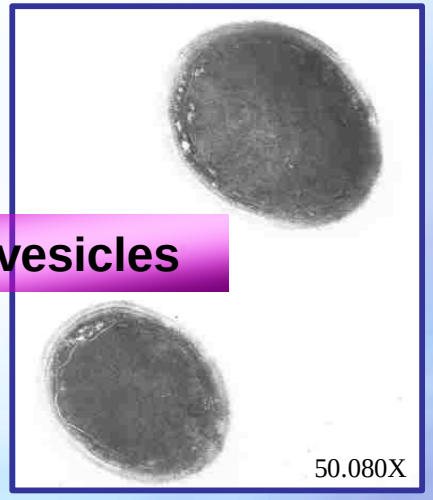
Changes in cell envelope (TEM)

Before freeze-drying

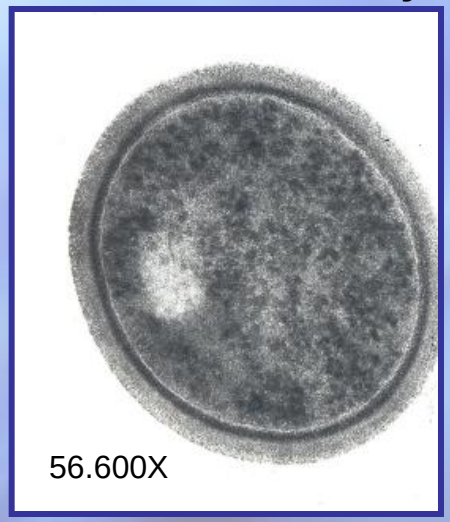


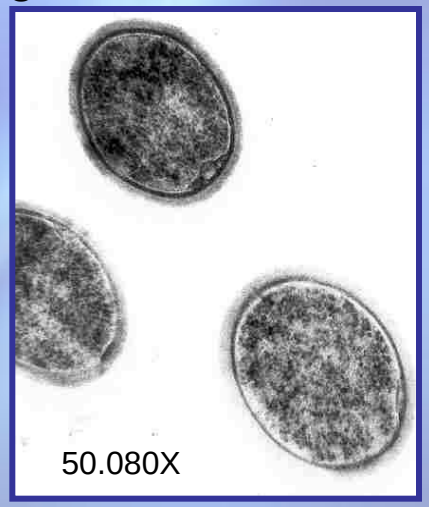
After freeze-drying without GI

Sacs and vesicles



After freeze-drying with GI





Cell damage indicators

After freeze-drying

Absorbance	**Δ OD**
λ=260 nm	0,726
λ=280 nm	0,291
***β*-galactosidase activity**	**(%)**
Cell suspension (non permeabilized cells)	38,2
Supernatant (non permeabilized cells)	10,7
Sensitivity to selective compounds	**(%)**
Chloramphenicol	3,1
Rifampicin	0,5
Lysozyme	25

Plasma membrane

Cell wall

Changes in fatty acid composition

Peroxidation

FA	Control	AFD	Storage
			6m-4°C
C13:0	ND	ND	7.9 ± 1.1
C14:0	8.5 ± 1.3	10.2 ± 0.9	0.4 ± 0.1
C16:1	19.4 ± 0.5	17.0 ± 0.6	15.1 ± 0.5
C16:0	44.6 ± 1.1	41.4 ± 0.5	40.1 ± 0.9
C17:0	ND	ND	2.9 ± 0.5
C18:1	11.9 ± 0.6	11.7 ± 1.2	9.7 ± 0.3
C18:0	1.7 ± 0. 1	3.8 ± 0.3	3.6 ± 0.1
C19:0 cyc	13.7 ± 3.9	13.5 ± 1.5	15.9 ± 0.9
C18:0,10 OH	ND	2.2 ± 0.2	4.3 ± 0.1
Unsaturated	45.0[1]	↓ 42.2[2]	↓ 40.7[2]
Saturated	54.8[1]	55.4[1]	54.9[1]
U/S	0.82[1]	0.76[2]	0.74[3]

2. Cell injury during freeze-drying

L. bulgaricus **CRL 494 displayed sensitivity to lysozyme and increased permeability of the cell envelopes possibly related to the membranous forms and retraction of the cytoplasm and changes in the lipid profile**

CONCLUSIONS

- **The envelope of *L. bulgaricus* CRL 494 was the major cell structure damaged by freeze-drying.**

- **Sodium glutamate reduced the deleterious effects of freeze-drying enhancing cell viability and recovery of metabolic activity.**

THANKS !!