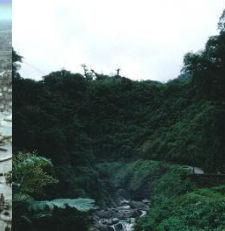


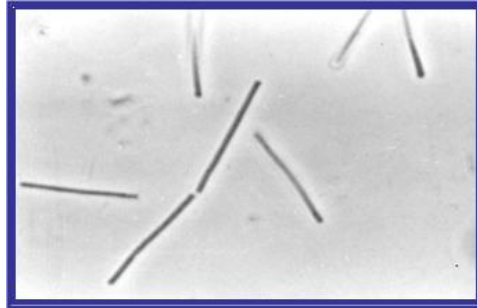
Monosodium Glutamate as Lyoprotector: Effectiveness during Freeze-drying and Storage of *Lactobacillus delbrueckii* subsp. *bulgaricus*

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L. bulgaricus



Mozzarella



Tarhana

***Lactobacillus delbrueckii* ssp.
*bulgaricus***

Kishk

Tacharas

Chondros

Kushik



Provolone



Kefir



yogurt



Emmental



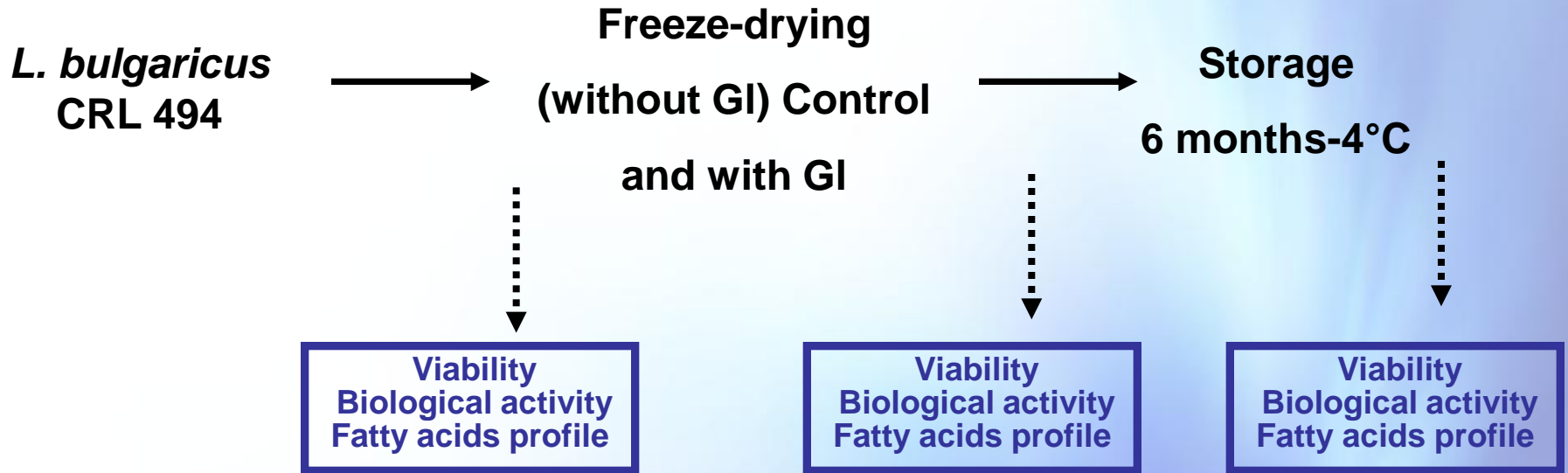
Gruyere



Objectives

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

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



Cell viability: plate dilution

Biological cell activity: direct conductimetry

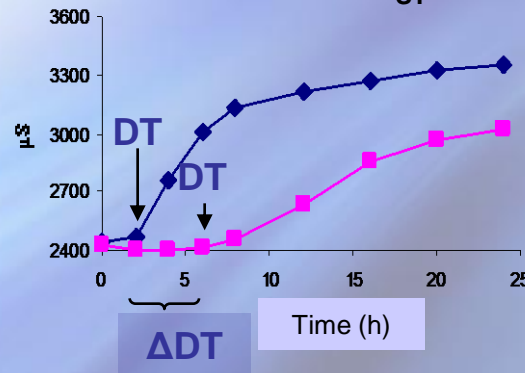
N/Ni index

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

Ni: log CFU/ ml before the referred treatment

$$\Delta DT_{FD} = DT \text{ after FD} - DT \text{ before FD}$$

$$\Delta DT_{ST} = DT \text{ after sto} - DT \text{ after FD}$$



2. Cell injury during freeze-drying



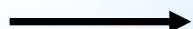
Growth in MRS broth
(16 h, 37°C)



Viability
 β -gal activity
TEM

Freeze-drying

Rehydration



Viability
 β -gal activity
TEM

Control

Challenges (MNIC)*

water

Chloramphenicol
0.3 μ g/ml

Rifampicin
0.1 μ g/ml

Lysozyme
10 μ g/ml

30 min, 30°C

Cell wash

Suspension original volume

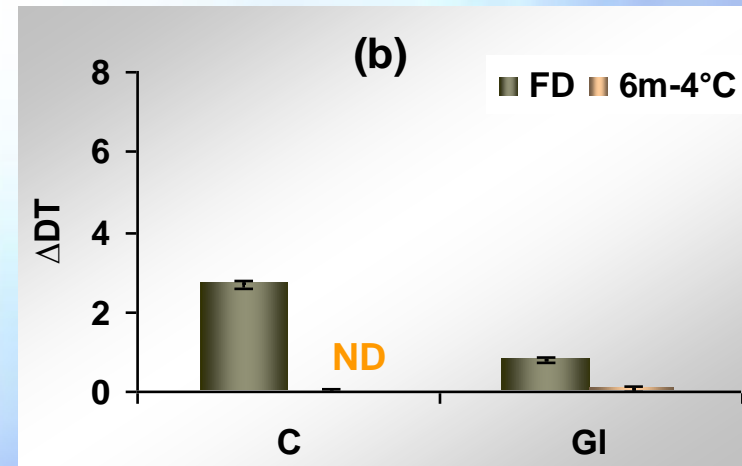
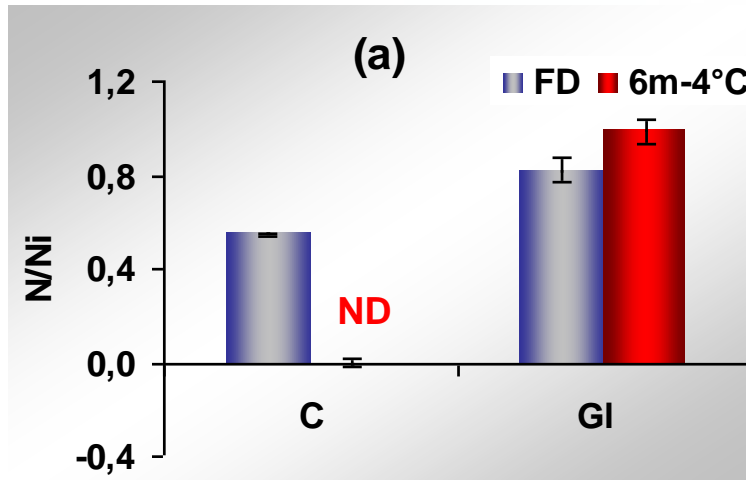


Viability

*MNIC: Minimal Non Inhibitory Concentration

Results and Discussion

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



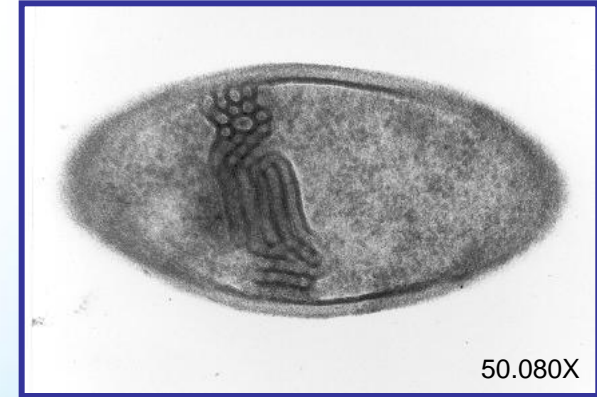
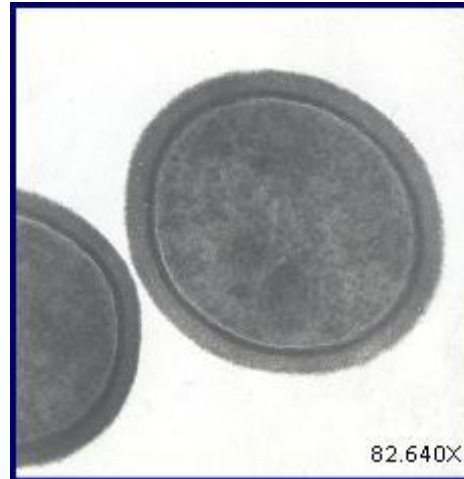
✚ Lyophilization causes decrease in (a) viability and (b) metabolic activity of *L. bulgaricus* CRL 494.

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

2. Cell injury during freeze-drying

Changes in cell envelope (TEM)

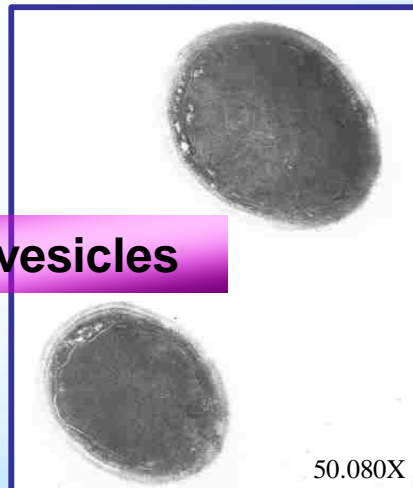
Before freeze-drying



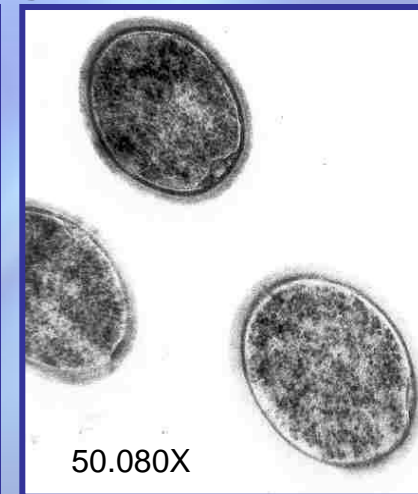
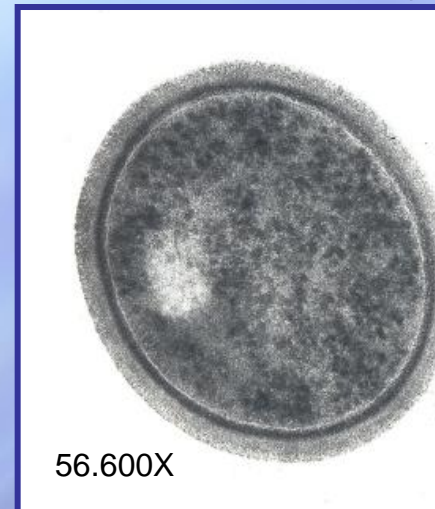
After freeze-drying without GI



Sacs and vesicles



After freeze-drying with GI



Cell damage indicators

After freeze-drying	
Absorbance	Δ OD
$\lambda=260$ nm	0,726
$\lambda=280$ 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 !!

