Molecular Studies as a Guide for Designing an Optimal Lyophilization Process for Microbial Preservation

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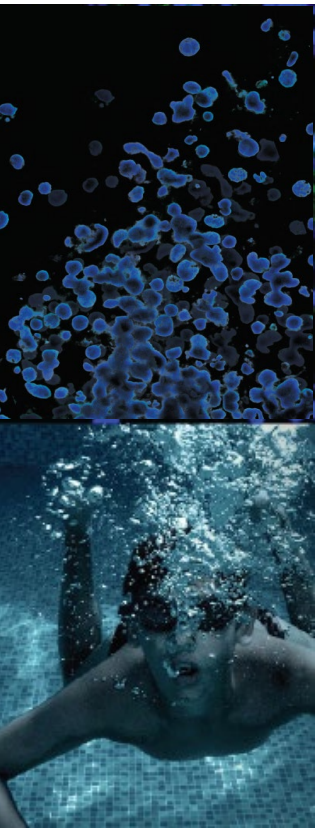
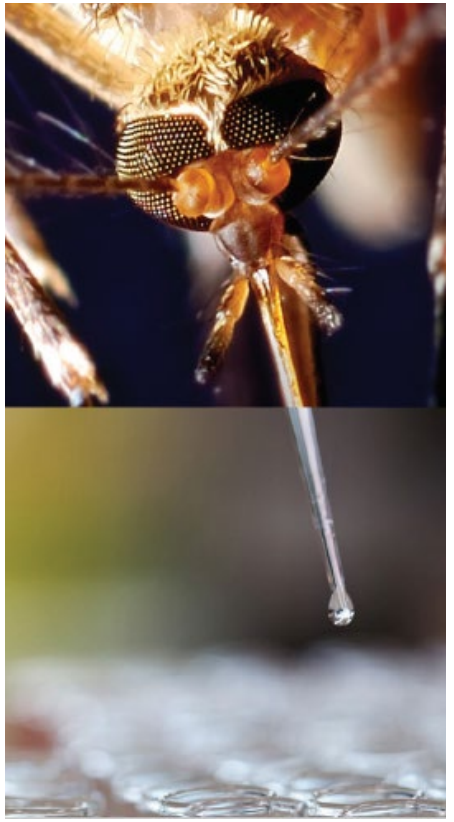
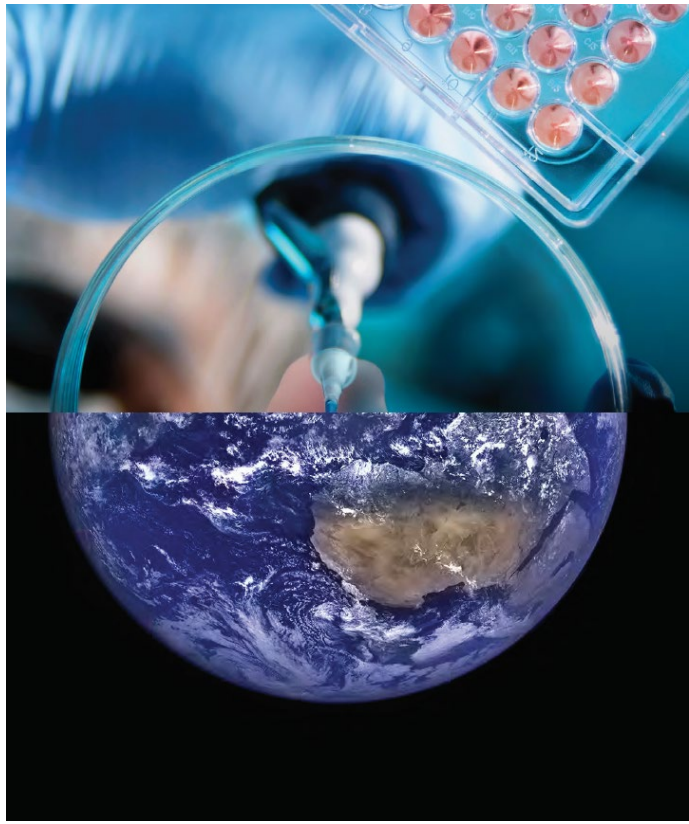


Credible Leads to Incredible®





About ATCC®

- Founded in 1925, ATCC® is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's premier biological materials resource and standards development organization
  - 5,000 cell lines
  - 80,000 microorganisms
  - Genomic & synthetic nucleic acids
  - Media/reagents

- ATCC® collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 19 international distributors
- Largest portfolio of microbial strains for the academia and industry

Microbial preservation and industrial requirements

Methods of quantitative preservation and industrial requirements

- **Microbes used in industrial applications**
  - Quality control and compendial assays (high- and low-titer microbes)
  - Quantitative viable microorganisms
- **Methods of preservation**
  - Frozen microbes (Storage: -80°C and vapor phase of liquid nitrogen)
  - Lyophilized microbes (Storage: 4°C and -20°C)
- **Potential challenges**
  - Universal formulation for microbial preservation
  - Storage temperature for quantitative microorganisms

Overview of the microbial lyophilization process

Freezing, primary drying, and secondary drying

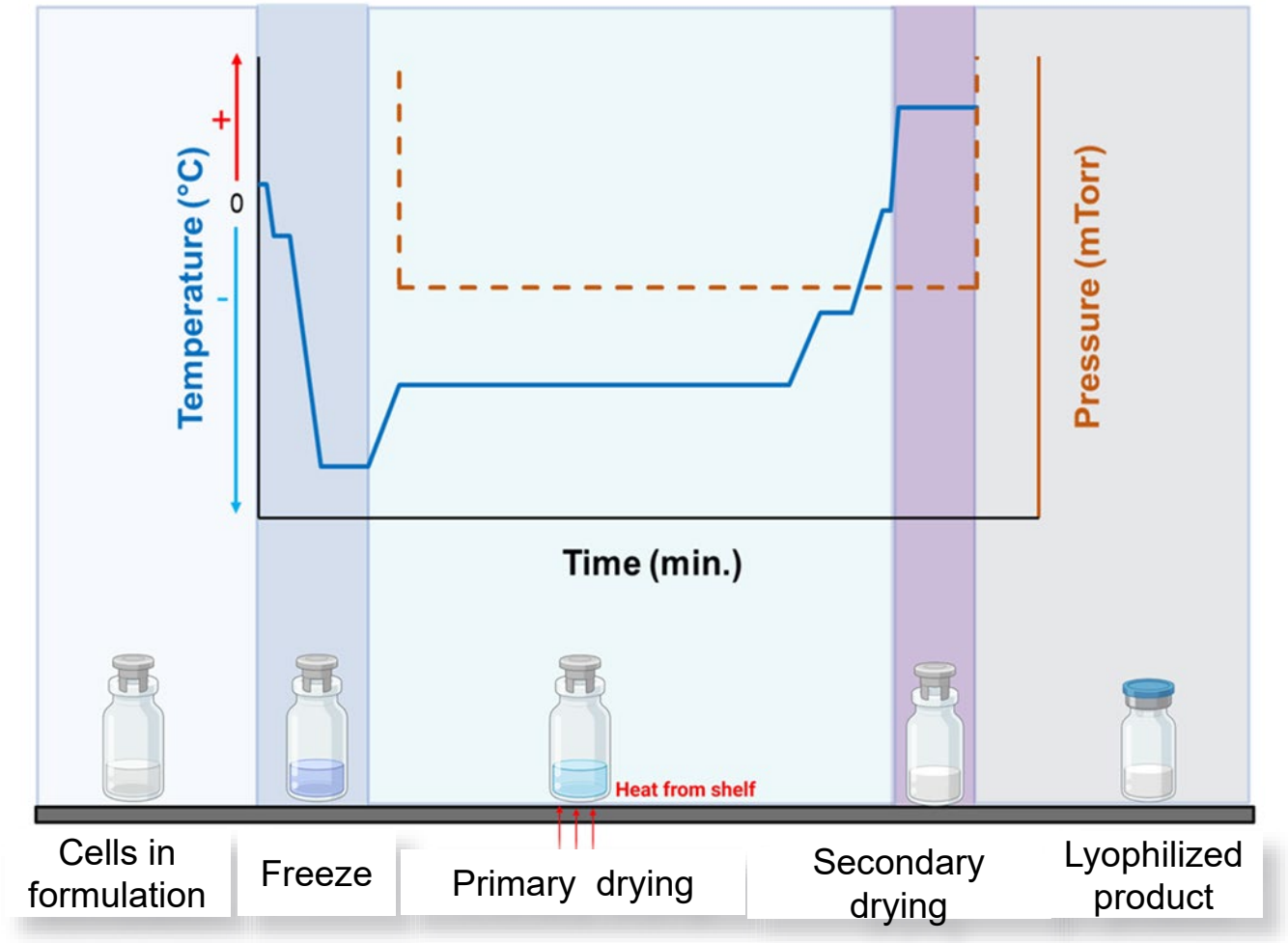
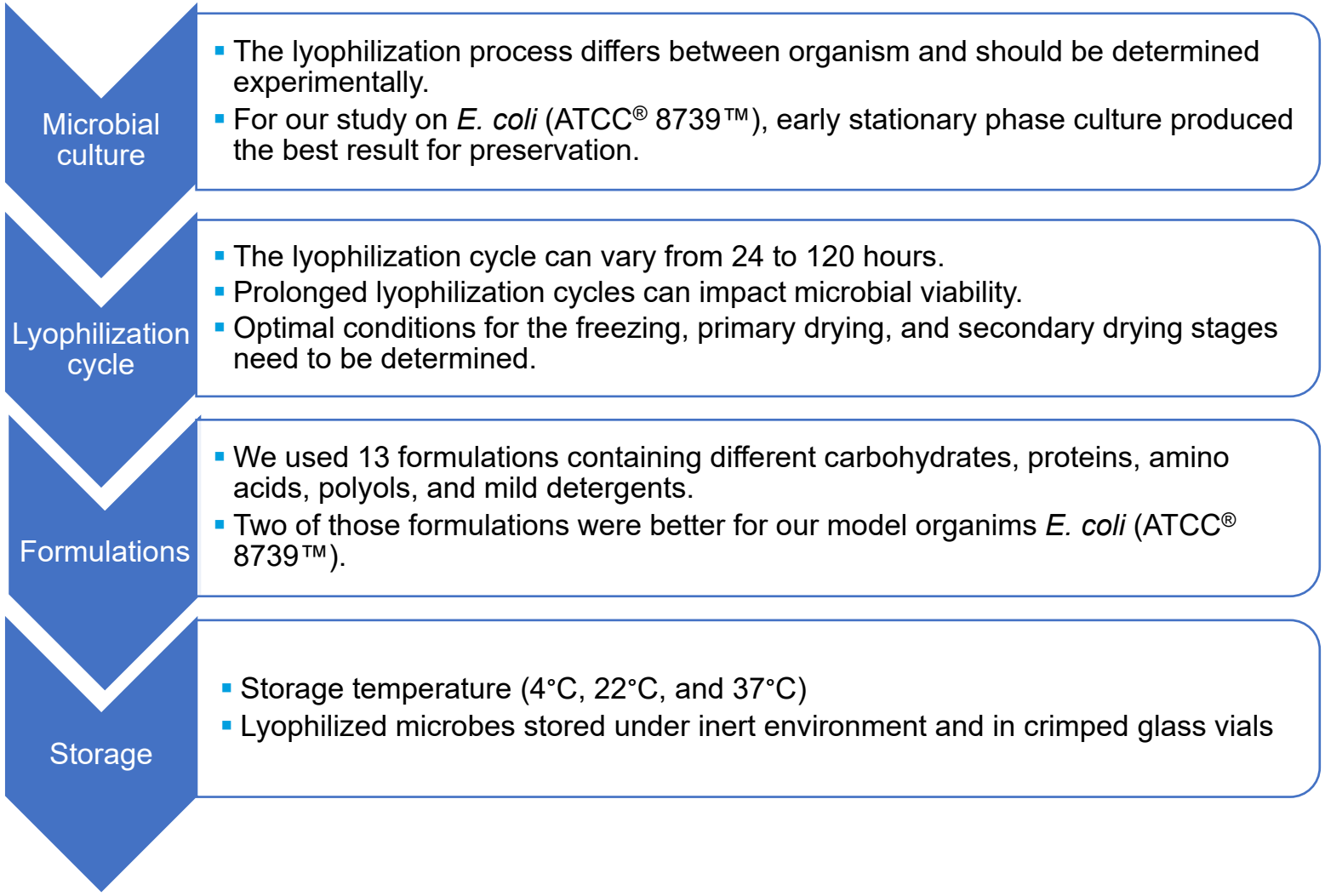


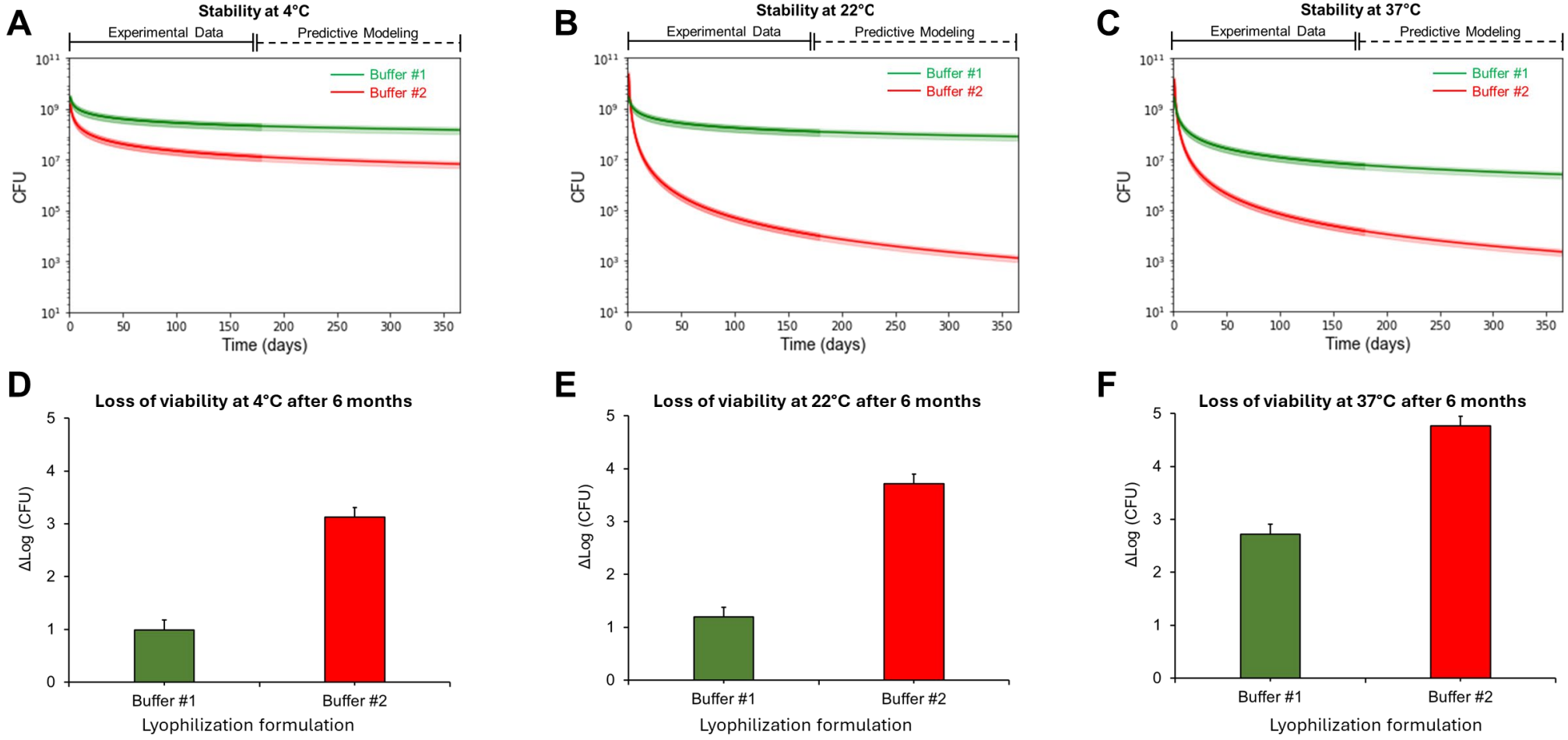
Figure was adapted from https://app.biorender.com/biorender-templates

Lyophilization process optimization for viable strains

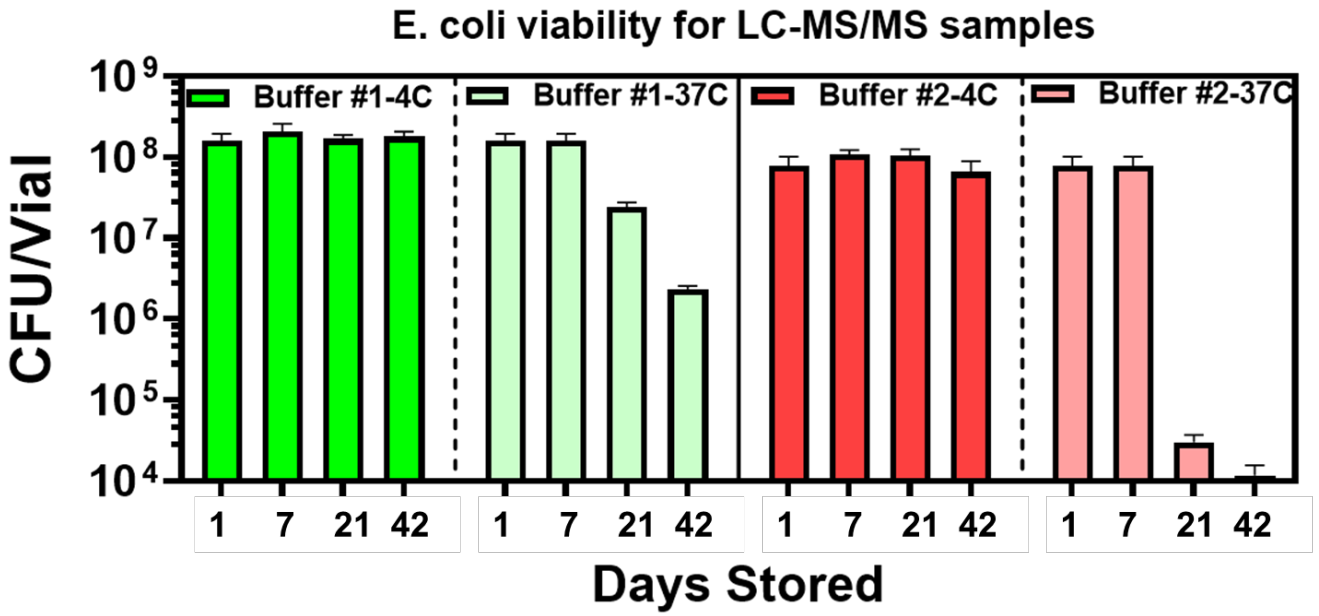


Stability of *E. coli* (ATCC® 8739™) in two different formulations

Overnight growth of *E. coli* culture → Centrifugation and buffer exchange → Lyophilization → Storage (4°C, 22°C, 37°C) → Viability (CFU) determination (up to 6 months) (Dilution/plate based)



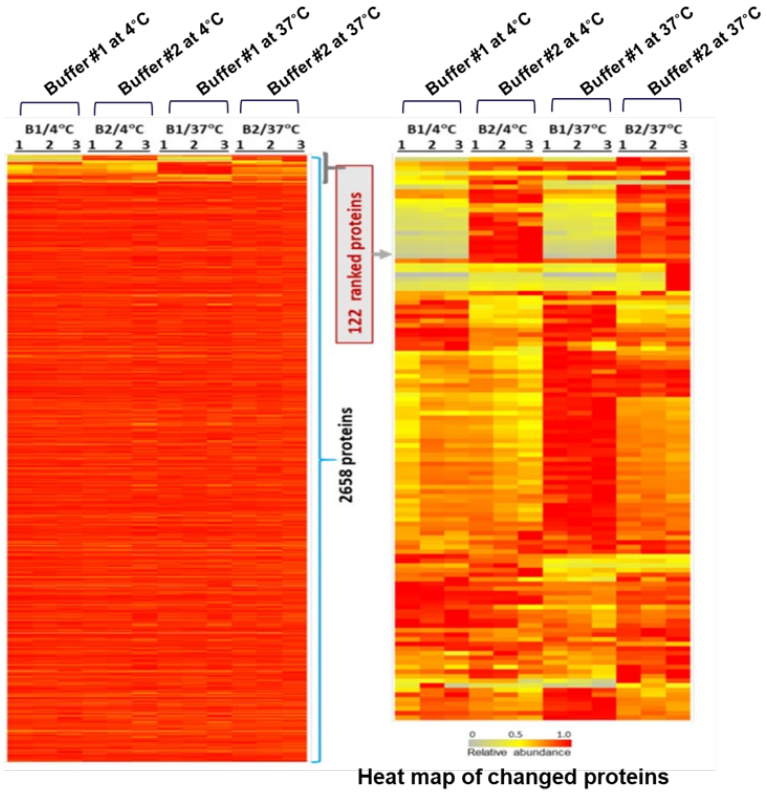
Lyophilization and viability determination before LC-MS/MS



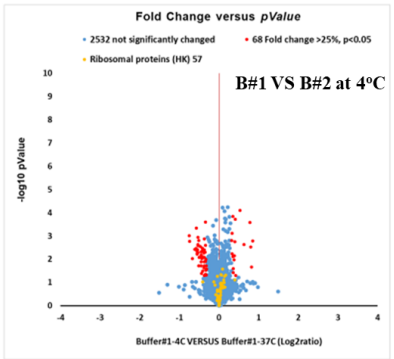
Proteomic characterization of lyophilized *E. coli*

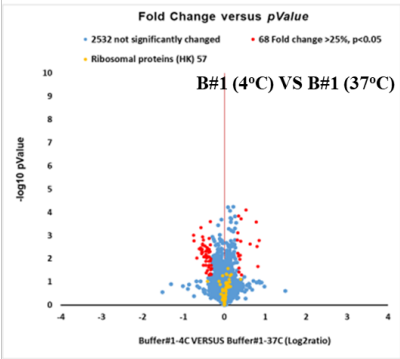
Proteomic characterization of *E. coli* in different buffers and storage conditions

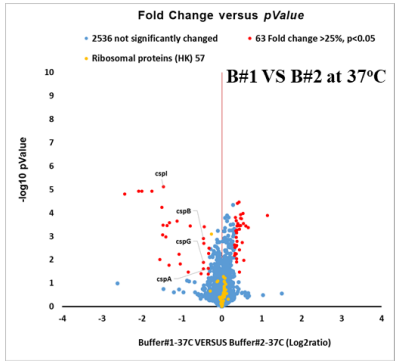
Overnight growth of *E. coli* culture → Centrifugation and buffer exchange → Lyophilization → Storage (4°C) / Storage (37°C) → **Protein extraction & LC-MS/MS after 42 days**; Viability (CFU) determination 1, 7, 21 & 42 days

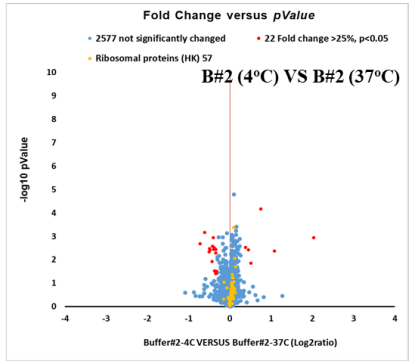


Heat map of changed proteins



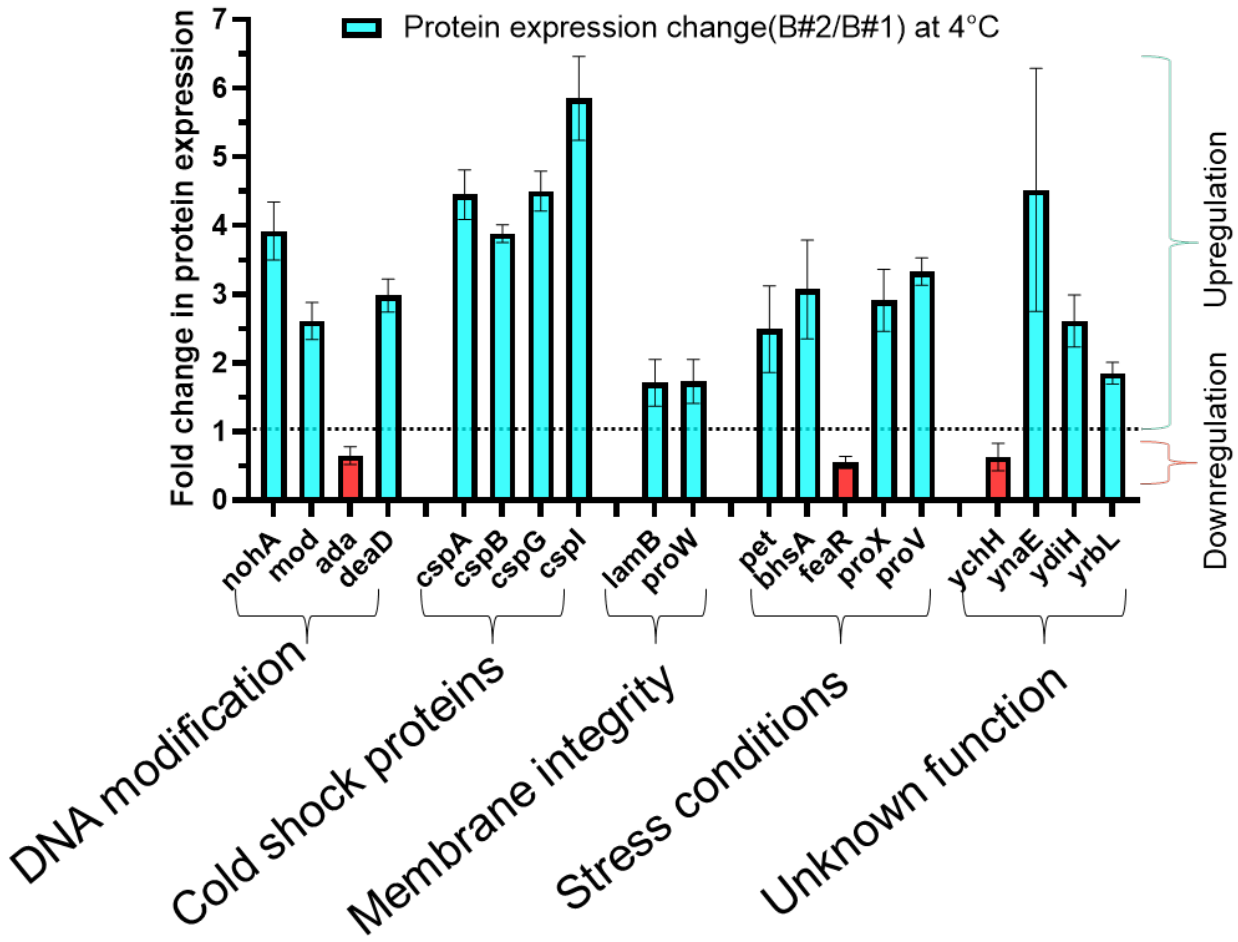






Proteomic characterization of lyophilized *E. coli*

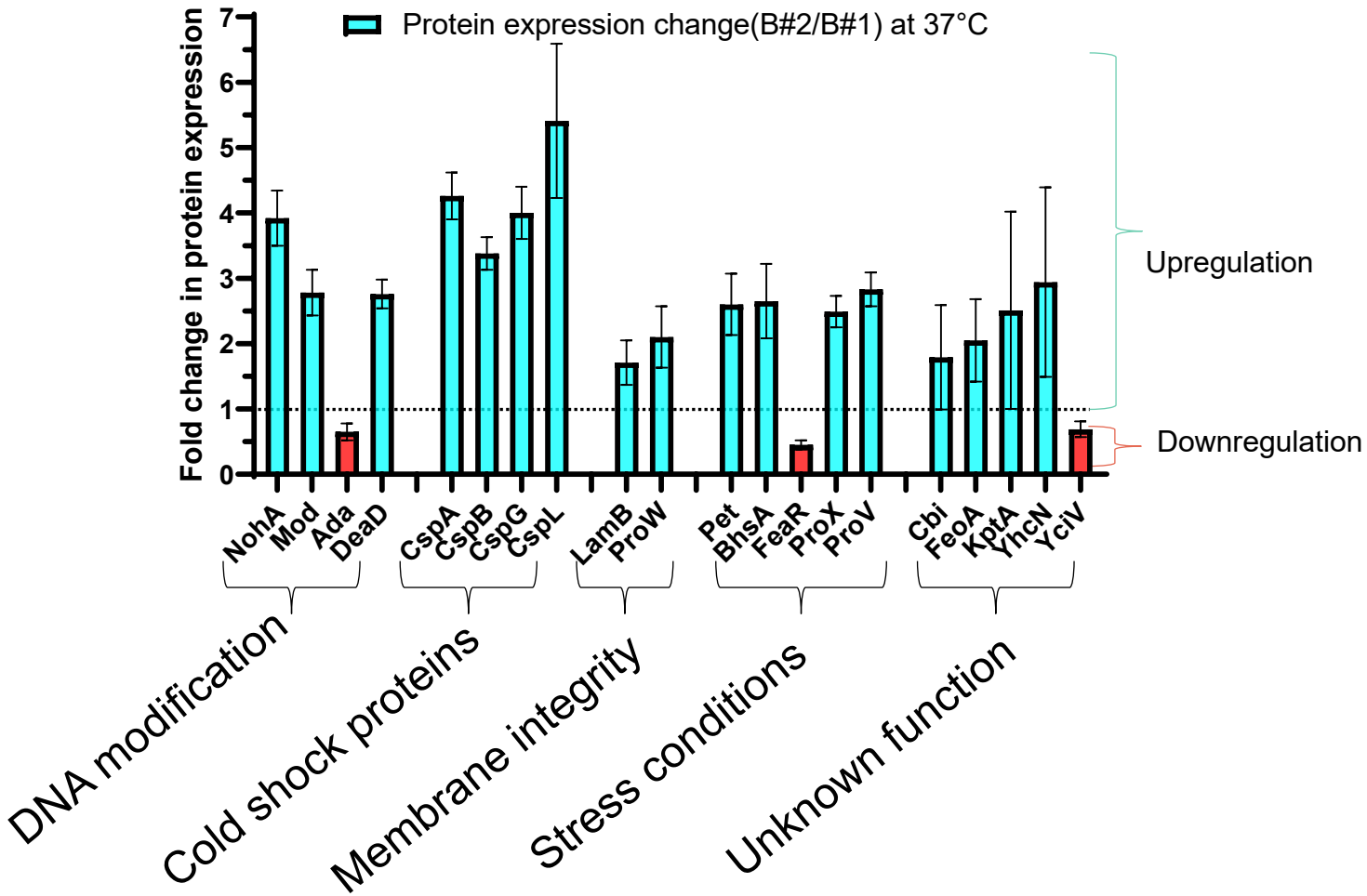
Fold change in protein expression of *E. coli* in buffers 1 and 2 while stored at 4°C



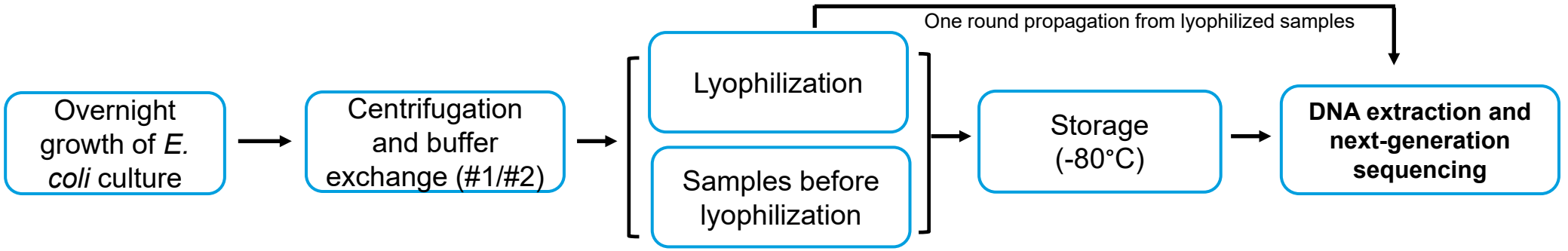
	Protein	Family	Function
DNA Modification	Mod	Type III restriction-modification system	DNA cleavage
	NohA	Terminase small subunit	Impairing DNA sysnthesis
	Ada	DNA repair enzyme	Methylated DNA repair
	DeaD	ATP-dependent RNA helicase	RNA degradation
Cold shock proteins	CspA	Cold shock protein	Reduces global protein synthesis
	CspB		
	CspG		
	CspL		
Membrane integrity	LamB	Maltporin	Loss in membrane integrity
	ProW	Transport system	Modulation of transport
Stress Condition	Pet	Serine protease autotransporter	Enterotoxic effect
	BhsA	Stress protein	Increase cellular stress
	FeaR	Transcriptional activator	Abnormal cell production
	ProX	Transport system	Modulation of transport/stress
	proV	Transport system	Modulation of transport/stress

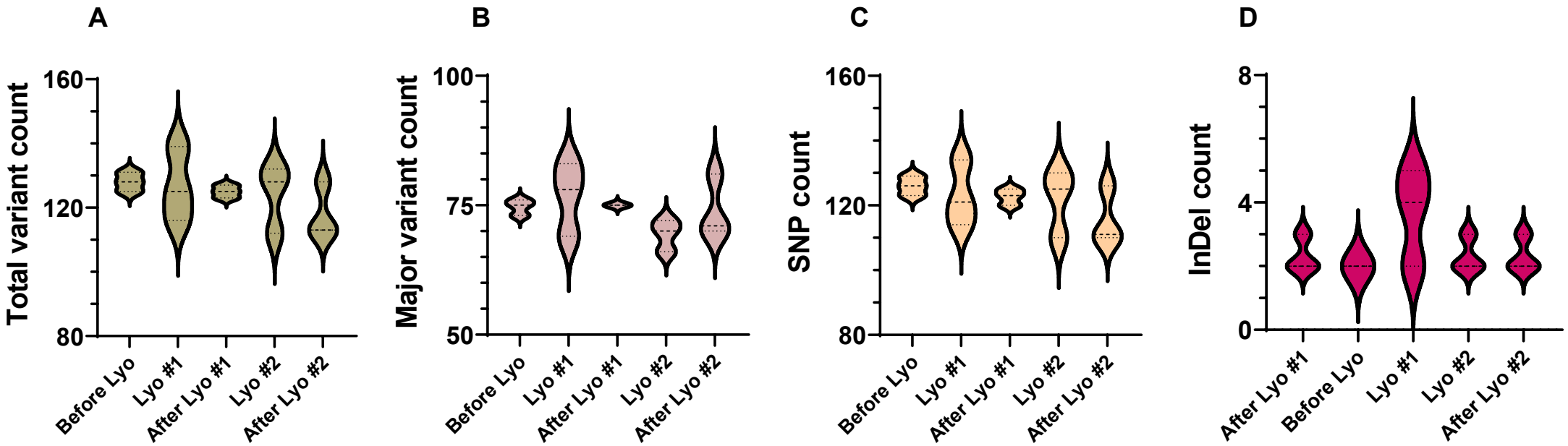
Proteomic characterization of lyophilized *E. coli*

Fold change in protein expression of *E. coli* in buffers 1 and 2 while stored at 37°C



Impact of lyophilization on the *E. coli* genome





Changes in *E. coli* genome

Closing remarks

Conclusions

- We found that an optimized proprietary formulation (buffer #1) stabilizes *E. coli* better than a conventional formulation (buffer #2).
- Using global proteomic analysis, we demonstrated that the overexpression of cold shock proteins, DNA methylation repair genes (CspA, B, G and L), and a restriction modification enzymes (Mod, NohA) and the underexpression of abnormal cell production genes (FaeR) contributed to the improved stability of *E. coli* in buffer #1 as compared to buffer #2.
- Our whole-genome sequencing analysis of *E. coli* before and after lyophilization with the optimized proprietary formulation indicated that no significant genomic changes occur during lyophilization or one round of propagation after.

Future work

- Evaluate how the proprietary formulation (Buffer #1) affects the stability of other microorganisms.
- RNA-seq analysis of the sample to understand the global transcriptome.

Special thanks to:

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Questions?

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