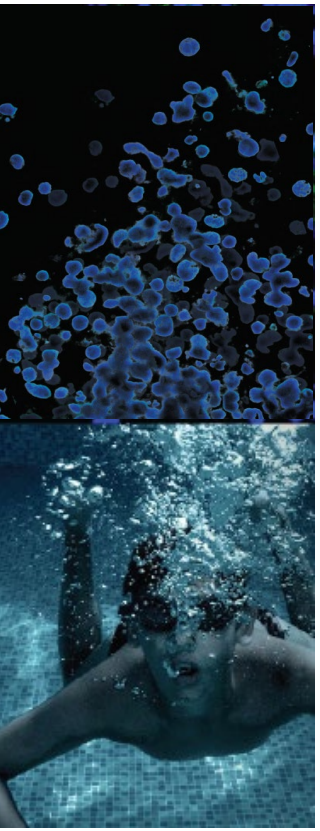
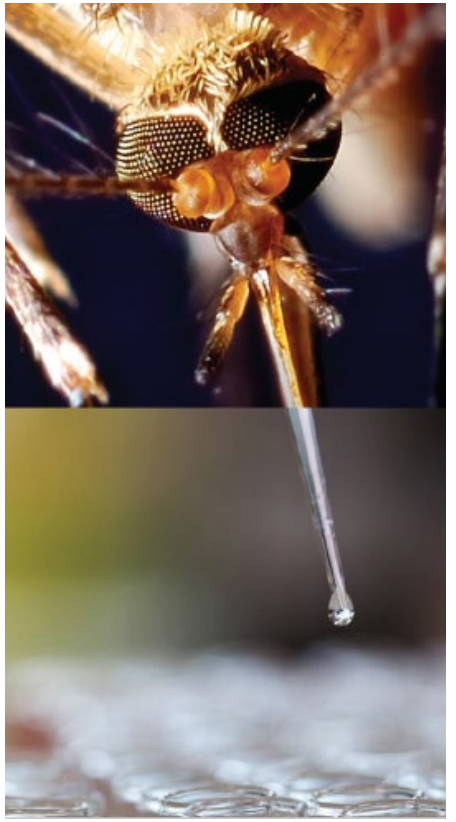
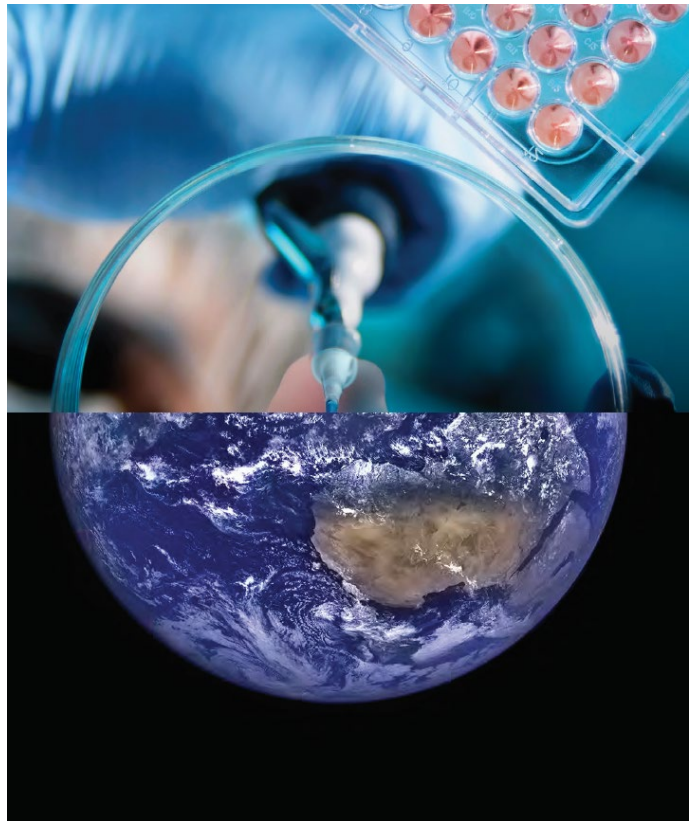
Formulation-based scientific approach for the long-term preservation of fungal microorganisms

Thiruganesh Ramasamy, PhD
Senior Scientist, ATCC







Credible Leads to Incredible™

About ATCC®

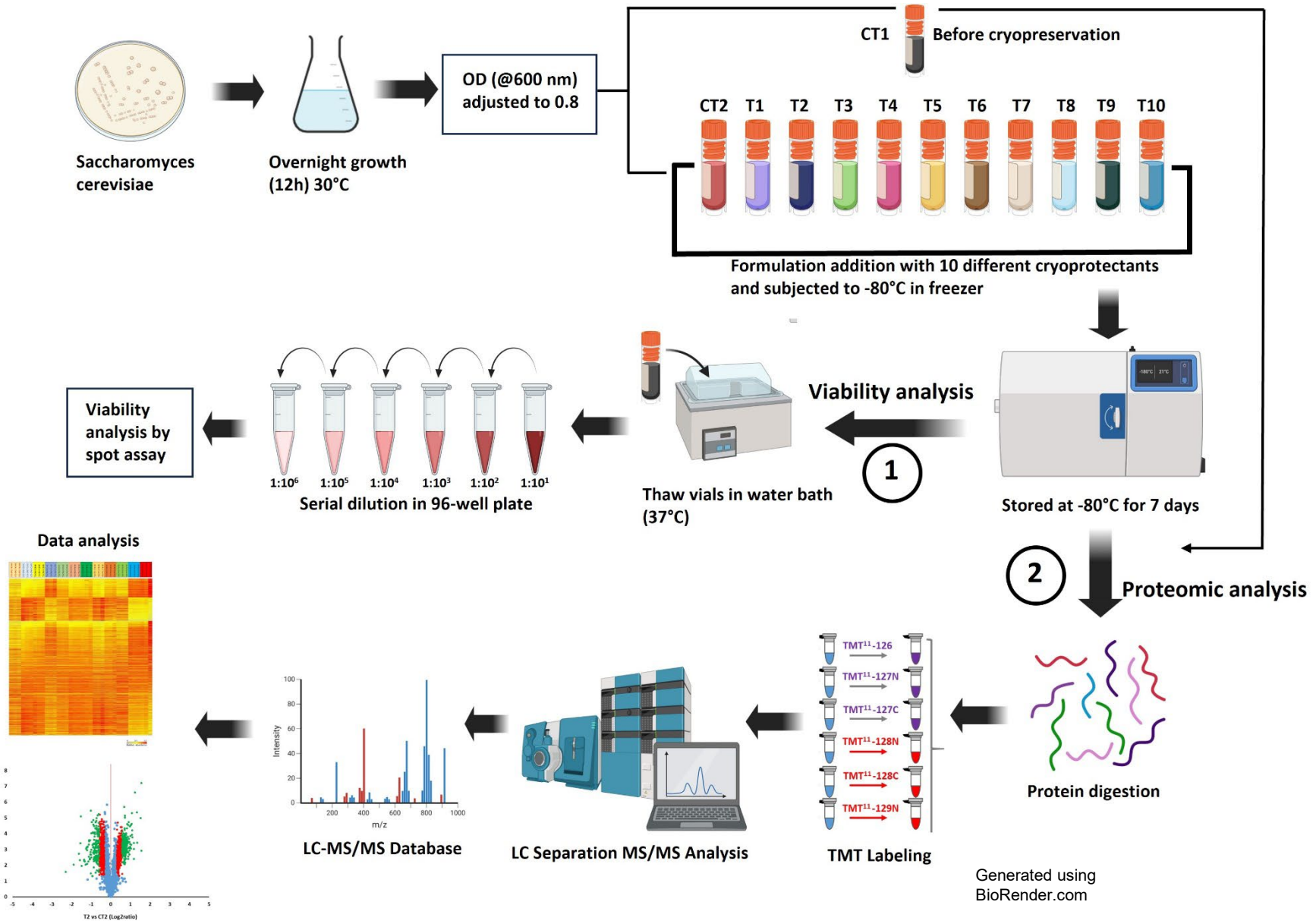
- Founded in 1925, ATCC® is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's premier biological materials resource and standards development organization
  - 5,000 cell lines
  - 80,000 microorganisms
  - Genomic & synthetic nucleic acids
  - 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
- Talented team of 500+ employees, over one-third with advanced degrees

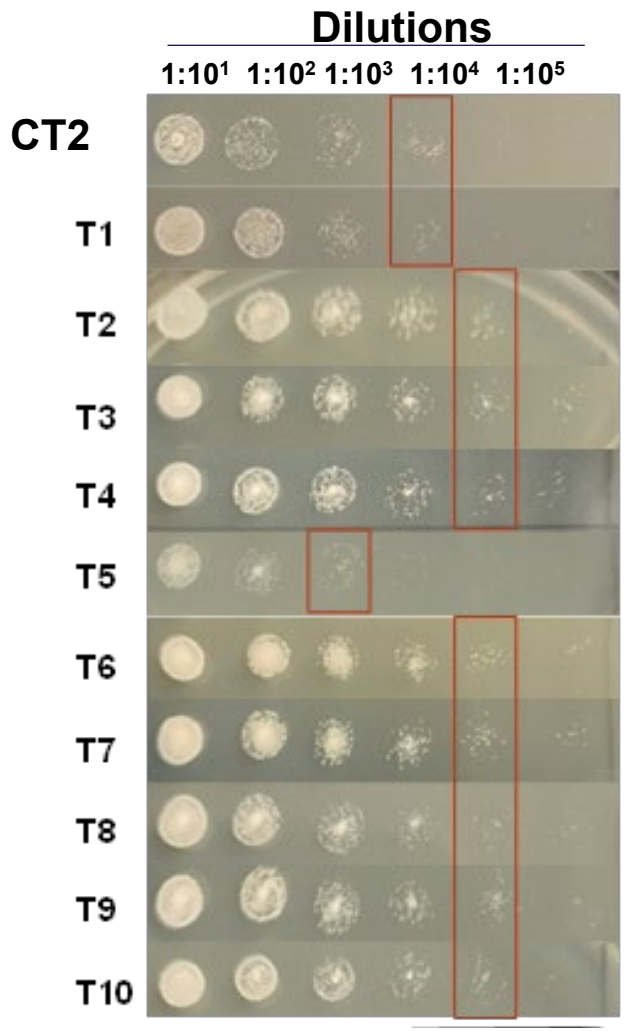
Background and significance

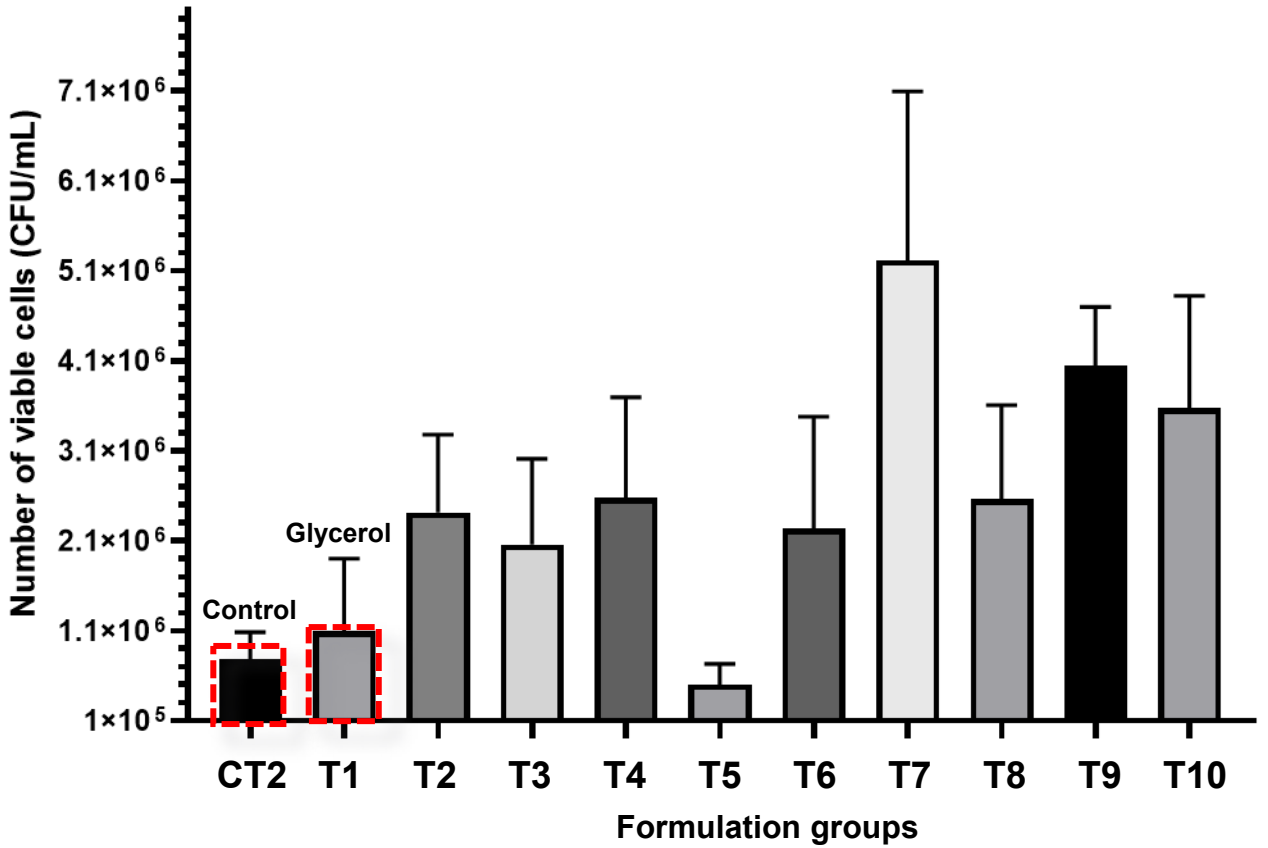
- Cryopreservation is a vital technique used to achieve the long-term storage of cells, bacteria, fungi, and other biological materials, and it underpins all forms of biomedical research.
- To date, the most commonly used cryoprotective agents (CPAs) include dimethyl sulfoxide (DMSO) and glycerol (chance discoveries), which were originally discovered 60 years ago.
- Despite their widespread use, these traditional CPAs present numerous challenges for the biological materials.
- Newer and advanced cell models require advanced tools to preserve the biological samples, maximize recovery/function, enable quicker recovery, and are adept for assay-ready format.

Schematic illustration of design



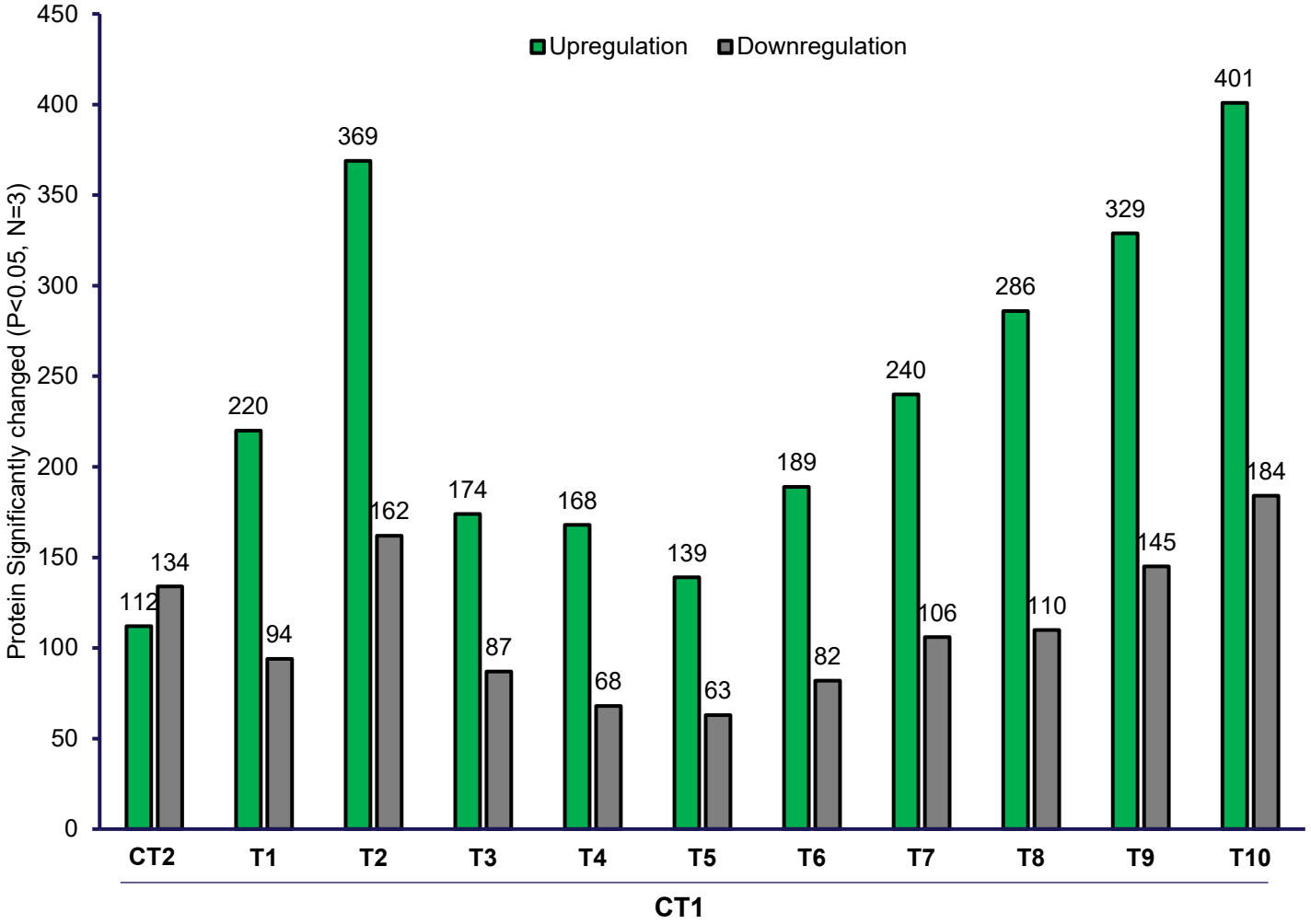
Effect of formulations on fungal cell viability





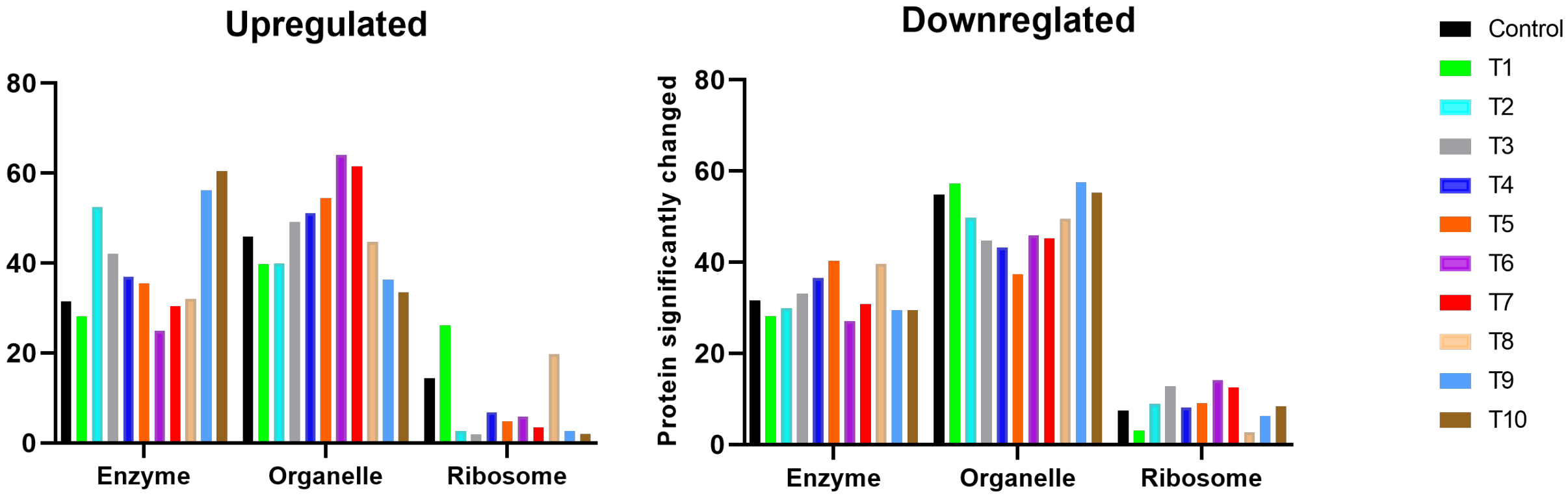
- Individual CPA or CPA-based combinations showed greater recovery as compared to either glycerol or the control
- Glycerol alone didn't show any improvement over the control and showed insignificant recovery

Effect of formulations on proteomic analysis

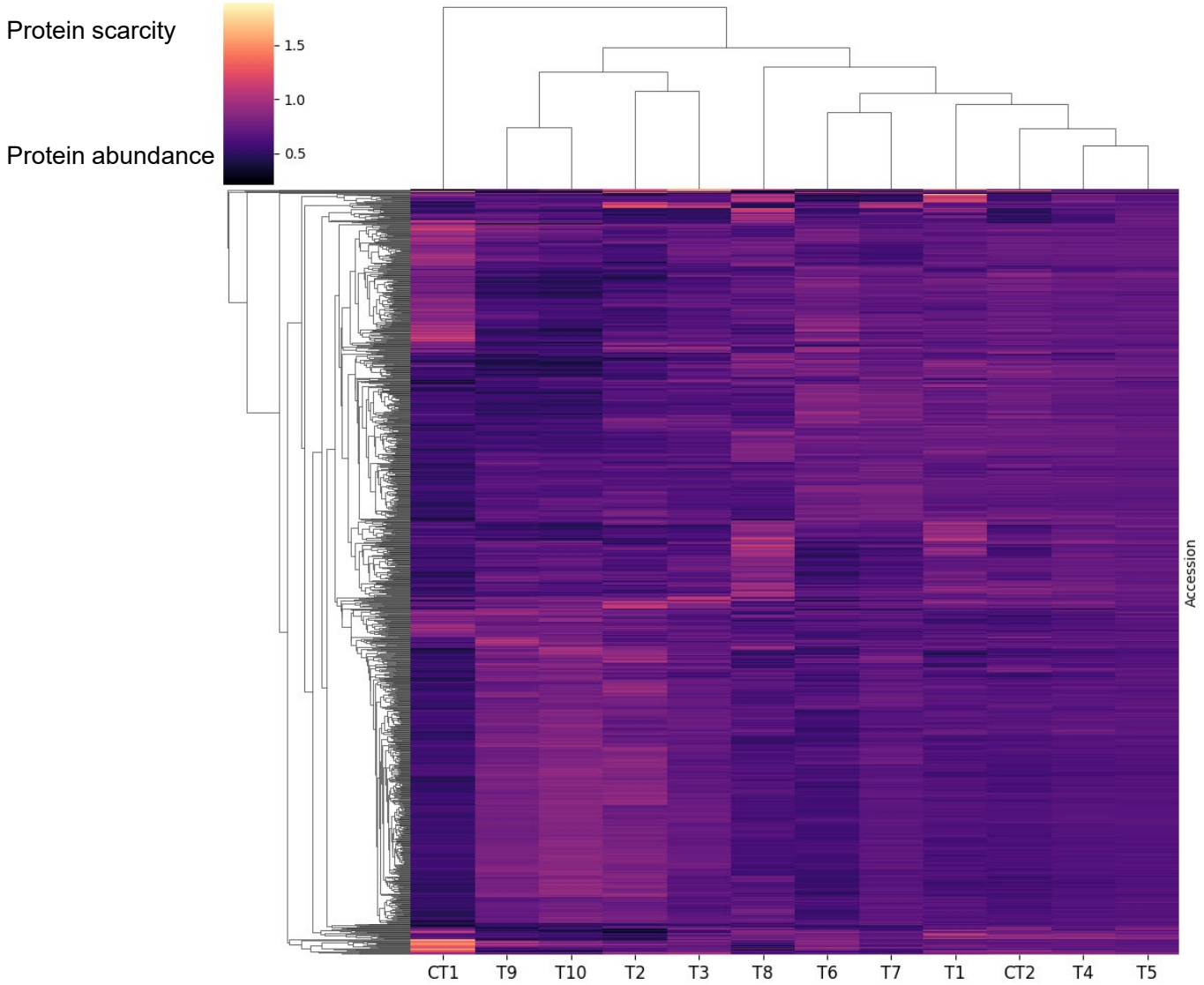


Cells using formulation T7 almost showed almost similar levels of protein changes as compared to those using T1 while exhibited 4-fold higher recovery. This suggests the superior advantage of a better cryopreservation formulation.

Effect of formulations on proteomic analysis



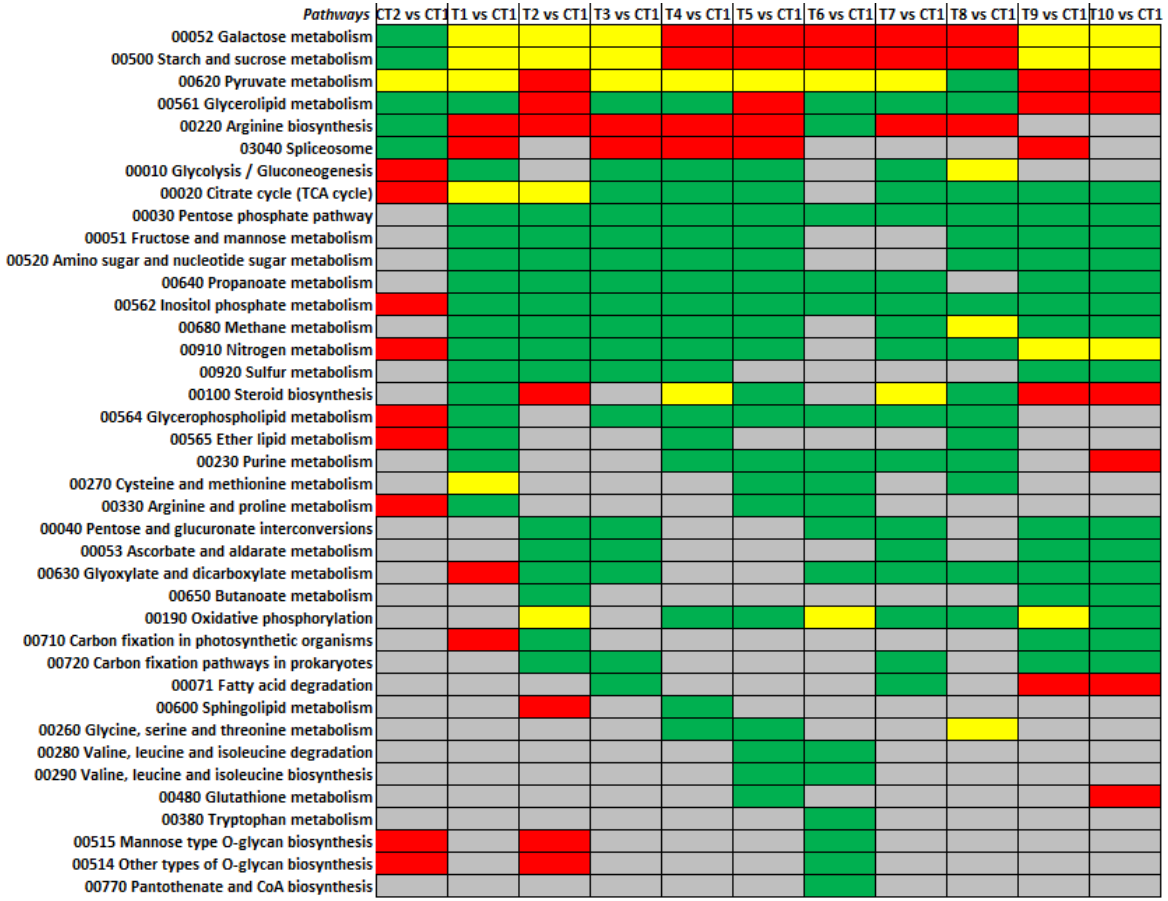
Quantitative Heat map analysis

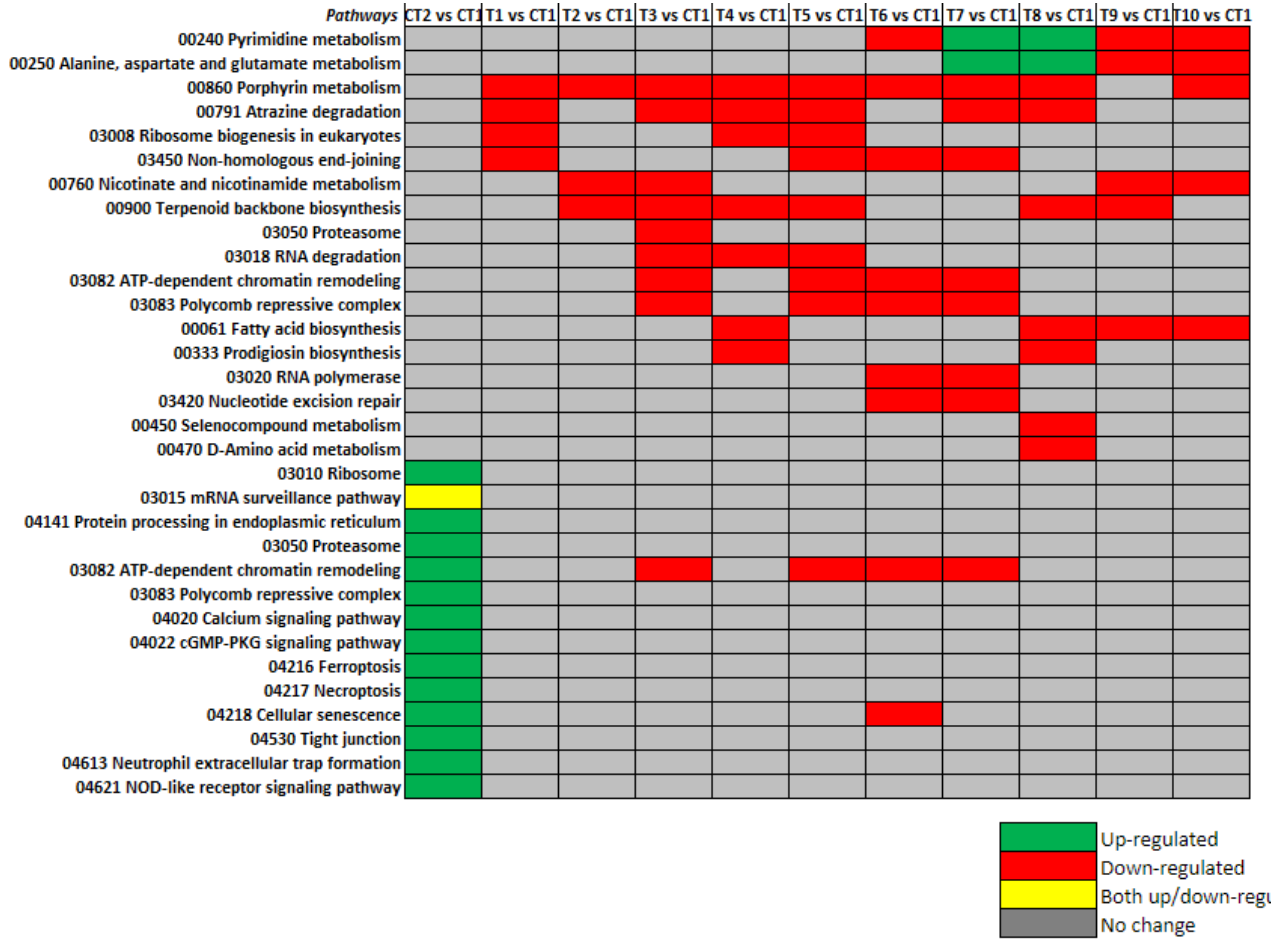


- CT1 protein profiling is completely different than the rest of the group, clearly highlighting the difference before and after cryopreservation.
- Key identical CPAs play major role in proteomic change while a key additional ingredient further pushes the cryopreservation efficiency.

A z-score of 0 represents protein abundance and purple color represents higher z-scores and thus higher protein abundances

KEGG Pathway analysis





The identified proteins were related to 73 different signaling pathways with a mix of upregulating and downregulating pathways

Summary

- The present study provided insight into the different cryopreservation formulations and their influence on the long-term preservation of *S. cerevisiae*.
- The T7 formulation group enabled multifold higher fungal recovery as compared to the control group and other formulation groups.
- The T4 formulation group enabled intact proteomics and higher recovery of the fungal strain as compared to that of the non-treated control or glycerol (T1).
- The molecular mechanism of cold-stress response of *S. cerevisiae* was investigated by functional proteomic analysis and KEGG pathway analysis.
- A total of 2299 proteins were identified. Depending on the formulation group, 116 proteins (for T4) to 1241 proteins (for T9) have been found to be significantly changed indicating the influence of individual formulations.

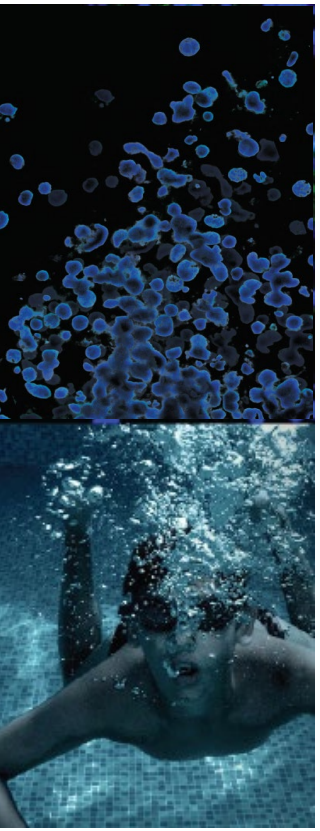
Acknowledgements

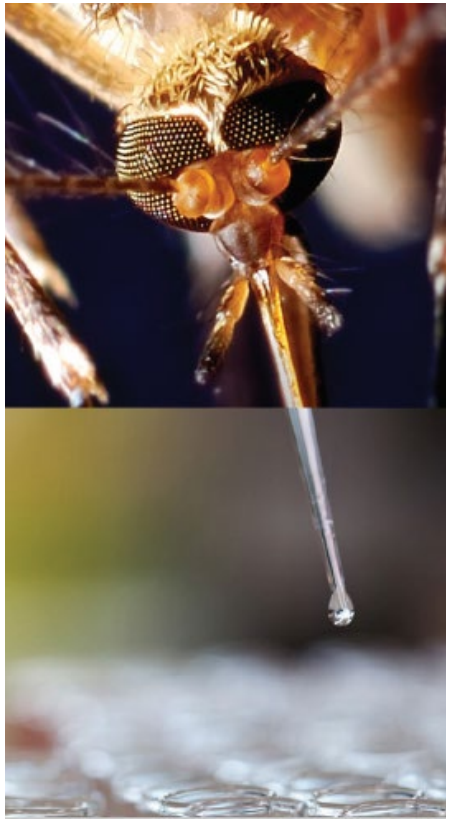
- Dr. Nilay Chakraborty
- Dr. Victoria Knight-Connoni
- Dr. Rahul Tevatia
- Dr. Shaheen Ali
- Anthony Muhle

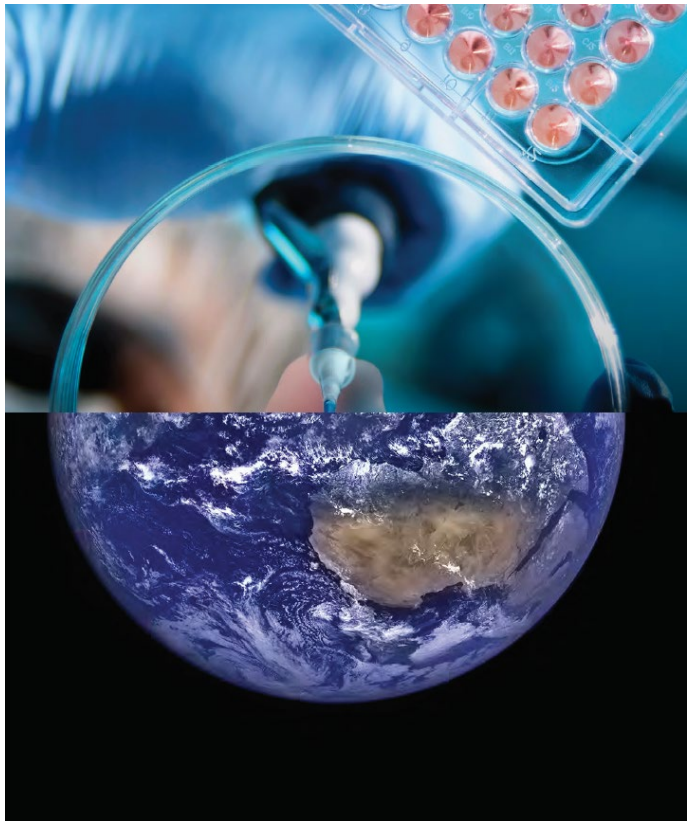
ATCC

ATCC®

THANK YOU







Credible Leads to Incredible™