

A heat transfer analysis to assess the performance of cryopreservation protocols of *Saccharomyces eubayanus*, a wild Patagonian yeast relevant for the brewing industry

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INTRODUCTION & AIM

Saccharomyces eubayanus is a cryotolerant wild yeast known as the cold-adapted parent of *S. pastorianus*, the hybrid responsible for lager-beer production. Due to its industrial relevance, efficient cryopreservation methods are essential to improve culture viability during long-term storage. The standard protocol (CoolCell® devices) submits cryovials at a cooling-rate of 1 °C/min. An alternative technique is the direct freezing from ambient temperature to -80 °C, which avoids manipulation and is cost-effective.

Both methods involve different freezing stages governed by transient heat conduction that depend on overall heat transfer coefficients (U) which reflect conductive and convective heat exchange between the cryovial and its environment. The cryopreservation protocol can be represented by the characteristic freezing time (tc) which is inversely proportional to the freezing rate, and is defined as the time elapsed between the initial freezing temperature and a reference temperature (-40°C).

The objectives were to: i) determine tc and heat transfer coefficients for direct freezing of cryovials in Ultrafreezer at -80 °C (protocol A) and using CoolCell® (protocol B); ii) correlate these parameters with viability, vitality, phenotype, and genetic stability of *S. eubayanus* CRUB 1568^T.

RESULTS & DISCUSSION

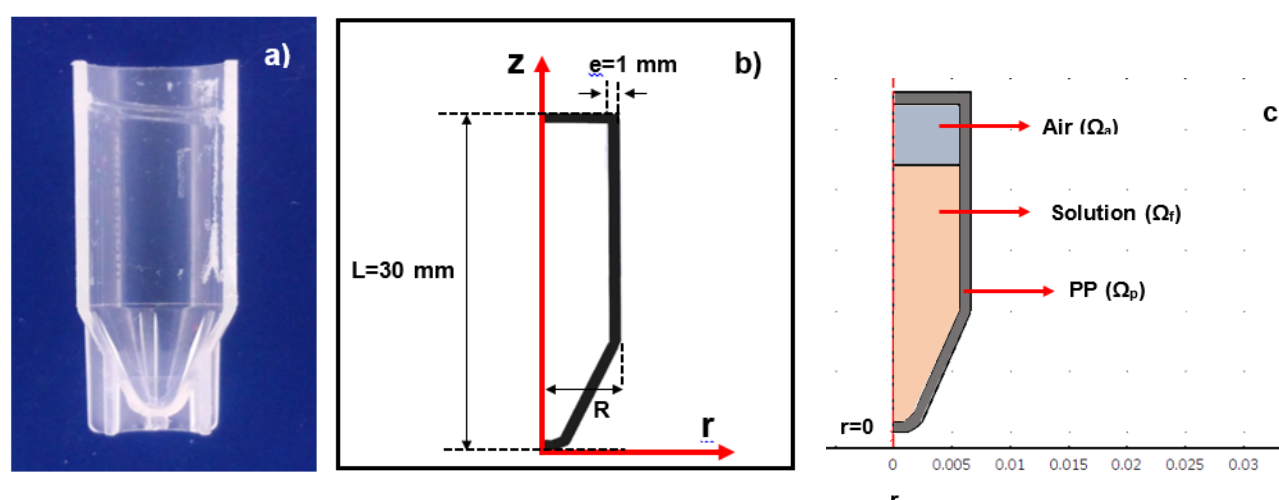


Figure 1a) photograph of the cross sectional area of the cryovial used for the numerical simulations, b) cylindrical coordinate system used and relevant dimensions of the cryovial, c) material domains considered in the model (Solution refers to distilled water or a 10% w/w glycerol suspension with the yeast culture, PP refers to the plastic material of the cryovial).

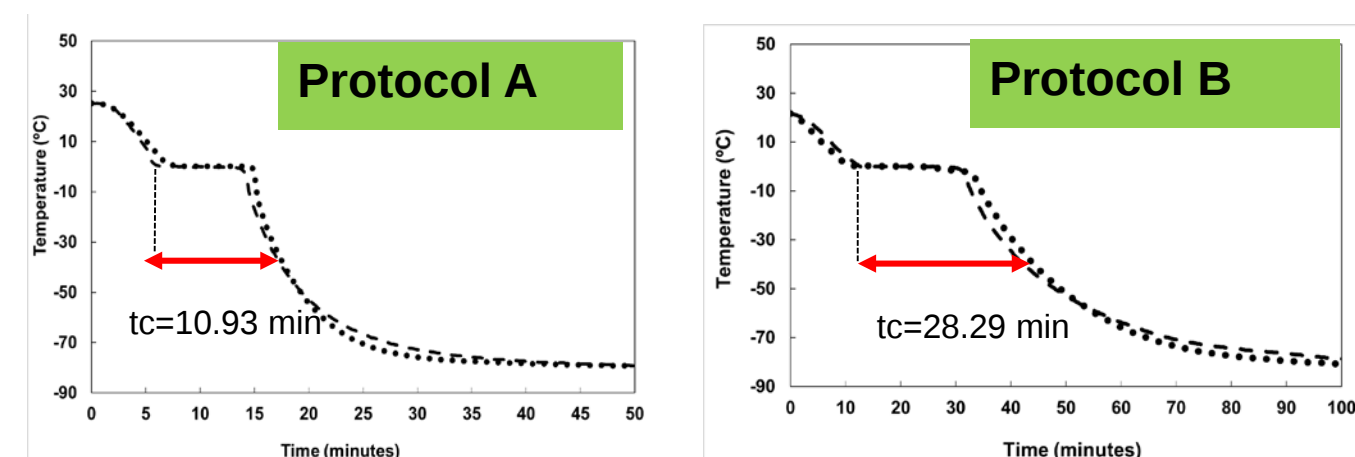


Figure 3) experimental results of temperature vs time (●) during freezing of cryovials containing yeast+glycerol, and predicted temperatures obtained by the numerical simulations(–).

CONCLUSION

For protocol B method, the obtained values were: tc=28.29 min, UB=4.76 W/m²K, and viability=51.21%. For protocol A a higher cooling rate was observed (smaller tc=10.93 min) associated to higher values of UA=18.04 W/m²K and viability (71.7%), with no observed genetic or fermentation alterations of the tested strain. In conclusion protocol A, with higher cooling rates improved cryopreservation of *S. eubayanus* being a simpler and cost-saving process.

METHODS

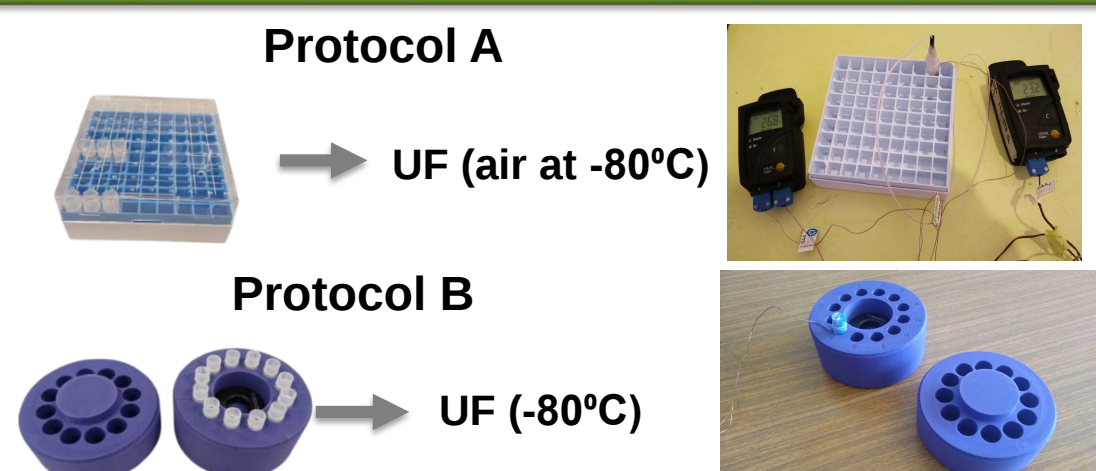


Figure 2) Schematic representation of the cryopreservation protocols

Cells were harvested at early stationary phase, suspended in 10% glycerol, and stored for one year at -80°C. Viability and vitality were evaluated, along with experimental fermentations and PCR fingerprinting to assess phenotypic and genetic stability. Thermal histories of cryovials were recorded using thermocouples to measure tc. Heat transfer coefficients were determined by computational modeling using the finite element method, incorporating temperature-dependent properties.

The heat conduction equation in cylindrical coordinates with phase change transition can be written as follows for the fluid inside the cryovial

$$\rho_f(T)C_{pf}(T)\frac{\partial T}{\partial t}r = \frac{\partial}{\partial r}\left(k_f(T)r\frac{\partial T}{\partial r}\right) + \frac{\partial}{\partial z}\left(k_f(T)r\frac{\partial T}{\partial z}\right)$$

T the temperature, t the time, r and z the coordinates, $k_f(T)$ is the thermal conductivity, $C_{pf}(T)$ the apparent specific heat, $\rho_f(T)$ the density

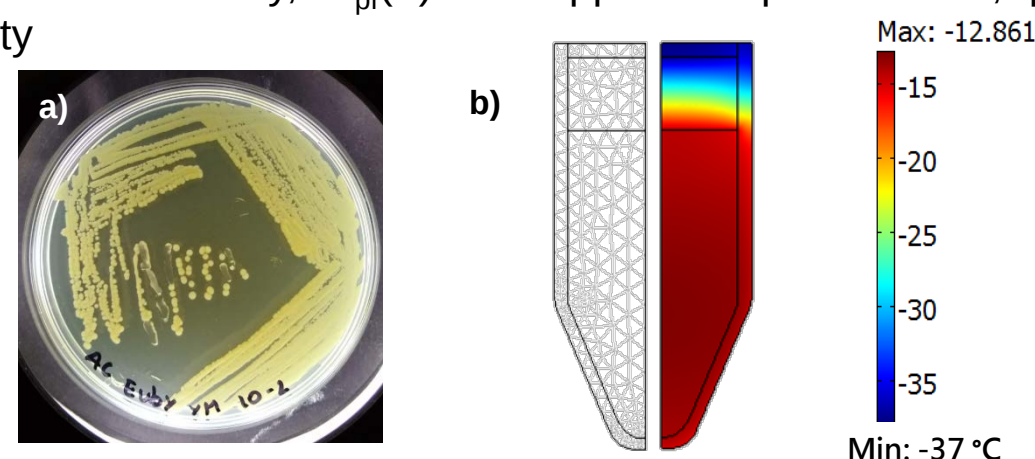


Figure 4) a) Plate with *S. eubayanus*, b) triangular mesh used in the numerical simulations (left) and temperature spatial distribution predicted from the numerical simulation for the cryovial after 2000 s of being submitted in the ultrafreezer inside the CoolCell®, $T_i = 19.5$ °C, $T_{ext} = -78$ °C, $U_B = 4$ W/m² K.

A complex mathematical model was developed which numerically solved the transient heat transfer equation with simultaneous phase change transition using the finite element method. Experimental time temperature data validated the computer simulations, enabling the estimation of overall surface heat transfer coefficients

	PROTOCOL A		PROTOCOL B	
	Distilled water	Yeast + Glycerol	Distilled water	Yeast + Glycerol
tc (min)	10.86 (0.35)	10.93 (0.59)	26.85 (5.79)	28.29 (2.44)
U (W/m ² K)	UA= 18.04 (1.16)		UB=4.76 (0.84)	
%Viability		71.67* (3.46)		51.21 (3.61)
%Vitality		72.90 (2.19)		74.76 (1.00)

Higher U and lower tc values achieved higher viability after cryopreservation