

Cryopreservation of the *Pseudomonas aeruginosa* species

Comparison of several methods

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Abstract

Low-temperature cryopreservation is a common method for the preservation of microorganisms. However, this method has a major defect which is a strong rate of bacterial mortality. To limit this loss, many studies have shown the efficiency of additives in the cryopreservation medium. Called cryoprotectives, these additives allow to protect bacteria against freezing and thawing damages. However, this protection is only partial and it changes according to the used cryoprotective agent and preserved bacterial strain. This preliminary study compares four methods of cryopreservation, using as cryoprotective glycerol or skimmed milk. Our results demonstrate that the use of glycerol (18 %) in the cryopreservation of *Pseudomonas aeruginosa* strains allows to obtain higher bacterial viability than skimmed milk (10 %).

Introduction

The cryopreservation of bacteria at low-temperature is a method usually used within laboratories (1). However, the use of this method generates a strong loss of bacterial viability (2).



Culture of *Pseudomonas aeruginosa*

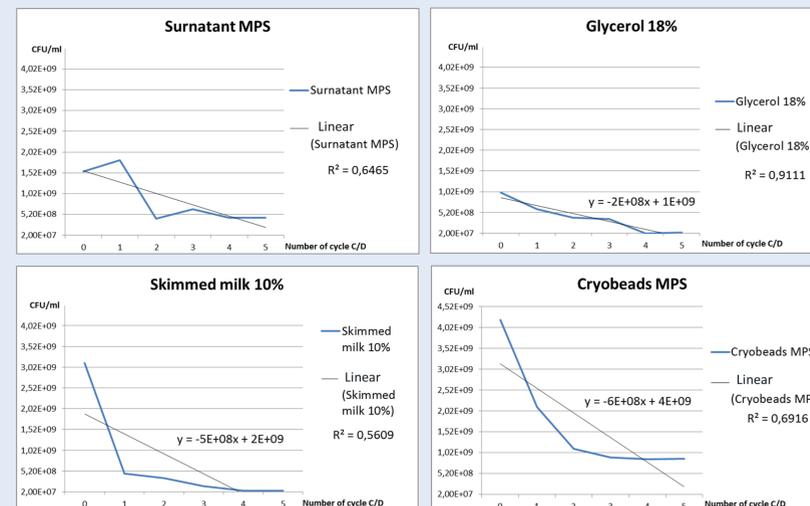
To limit this loss, it is usually recommended to use cryoprotectives agents such as glycerol or skimmed milk (3). Added in the medium of cryopreservation, their role is to protect bacteria from freeze and thaw damage.

The objective of this preliminary study was to determine the efficiency of four methods of cryopreservation in -80 °C for the preservation of the species *Pseudomonas aeruginosa*. These methods include the use of glycerol or skimmed milk as cryoprotective agents.

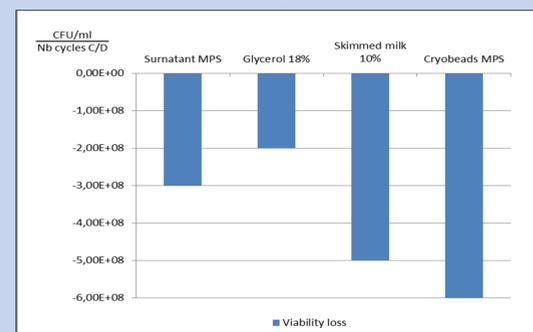
Results

Statistical Analysis

Statistical tests	Conclusions
Strain influence	P-value = 0,1344 > 0,05 : no significant influence Three tested strains show similar results
Inoculum standardisation at JO	P-value = 0,05056 > 0,05 : standardised inoculum Inoculum produced in similar manner in cryotubes



Estimation of bacterial viability (CFU/ml) for each method of cryopreservation according to the number of freezing/thawing cycle.
R² : coefficient of determination
y : equation of curve



Comparison of the loss of bacterial viability averages (slope of the linear trend curve) according to the method of cryopreservation.

These results show the fact that the method allowing to obtain the lowest loss of bacterial viability during the cycles of freezing/thawing is the one using as cryoprotective agents glycerol 18 %.

Conclusions

The method using as medium of conservation Brain Heart Infusion (BHI) added to glycerol 18 % as cryoprotective allows to preserve a bacterial viability superior to the other methods tested for the species *Pseudomonas aeruginosa*.

The loss of bacterial viability due to freezing/thawing is not completely compensated with the addition of cryoprotective agents tested in this study.

Perspectives

The perspectives will first of all be the comparison of these methods of cryopreservation for other bacterial species in order to confirm the existence of a depending species factor in the choice of the cryoprotective agent.

In a second phase, we shall test various concentrations of cryoprotective agents to improve the methods we use at the present time.

Materials & Methods

Strain

Genus	Species	Origin	Source	Health risk	Type of Strain
<i>Pseudomonas</i>	<i>aeruginosa</i>	USA	Blood	2	Clinic
<i>Pseudomonas</i>	<i>aeruginosa</i>	France	Blood	2	Clinic
<i>Pseudomonas</i>	<i>aeruginosa</i>	France	Water	2	Environmental

➤ Temperature of strain cryopreservation : -80 °C

➤ Time between each viability control : 7 days

Method of viability control

Method of cryopreservation	Step 1	Step 2	Step 3
Medium BHI Glycerol 18 %	Thawing	Dilution 1 µl/10ml	Growing in Petri dish + counting (estimation)
Medium BHI Skimmed milk 10 %			
Supernatant MPS*			
Cryobeads MPS*	Without thawing	Dilution 1 cryobille/10ml	

* Microorganism Preservation System (glycerol) Technical Service

References

- (1) : Cody W.L, Wilson J.W., Hendrixson D.R., McIver K.S., Hagman K.E., Ott C.M., Nickerson C.A., Schurr M.J. 2008. Skim Milk Enhances the Preservation of Thawed -80°C Bacterial Stocks. J Microbiol Method. 75(1) : 135-38
- (2) : Dong J., Malsam J., Bischof J.C., Hubel A., Aksan A. 2010. Spatial Distribution of the State of Water in Frozen Mammalian Cells. Biophysical journal. 99 (8) : 2453-59.
- (3) : Hubálek Z. 2003. Protectants used in the cryopreservation of microorganisms. Cryobiology. 46(3) : 205-29.