Title: PRESERVATION OF MICROORGANISMS STRAINS IN COMMON FREEZING

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Abstract:

Standard strains are microorganisms from a collection of cultures recognized nationally and/or internationally. The preservation of its original features and viability are essential requirements for reproduction in laboratory routines and research centers. The goal of any maintenance method is to preserve the viability and provide genetic stability as long as possible. The lack of knowledge of maintenance methods increases the difficulty of compliance with good practices in relation to the use of standard strains. Therefore, many laboratories do not follow this procedure or adopt inappropriately and need to acquire the standard strains more frequently. The cryoprotectants agents are substances added to the cell suspension in order to protect the cells from damage during the freezing medium to long term. They are characterized by low molecular weight, high aqueous solubility and low toxicity. The common freezing method is based on preservatives at relatively low temperatures of -4 to -20 °C. It is presented as one of the most simple and inexpensive methods. This study aimed to compare this method with the use of cryoprotectants such as glycerol to 20% and 15% skim milk for the preservation of bacteria and yeast. Cryopreservation was performed on domestic freezer at temperature -10 to -20°C in December 2013. The feasibility of 10 microorganisms were analyzed*: Staphylococcus aureus* subsp. *aureus* ATCC 25923, *Enterococcus faecalis* ATCC 19433, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC14028, *Escherichia coli* ATCC 25922*, Salmonella enterica* subsp. *enterica* serovar Choleraesuis ATCC 7001, *Klebsiella pneumoniae* subsp. *pneumoniae* ATCC 13883, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853, *Saccharomyces cerevisiae* ATCC 9763, *Enterobacter aerogenes* ATCC 13048. In March 2015, after 1 year and 3 months of the preservation, the recovery in both cryoprotectant was 100%. This storage period there was also no contamination. All methods were efficient for the preservation of the tested microorganisms and is a practical technique to be used for routine monitoring.

Keywords: cryopreservation, glycerol, preservation methods, skim milk.