Lyophilization

Introduction

Lyophilization (also known as freeze-drying) is a technique used in cellular biosensors to dehydrate a sample without applying extreme heat that might destroy it. In this case, the sample will be composed of E. coli cells that will be kept in this lyophilized state for a long time, facilitating storage and transport for a long time. This protocol is mostly derived from Prévéral et al, 2017, changing the machine used for the freeze-drying and the time spent lyophilizing

Materials

- LB media
- Bacterial E. coli strain
- Sucrose
- Machinery
    - Incubator with shaker.
    - -80ºC freezer
    - SpeedVac™ SPD1030/2030 (or any other freeze-drying device available)
    - Spectrophotometer

Procedure

Preparation of the materials

Prepare sterile LB media and sterile 12% sucrose LB media. Be careful if you decide to autoclave the sucrose solution, given that it tends to degrade and caramelized at high temperatures. Filtration is advised.

Growth of the cells

Grow desired cells overnight at 37ºC in LB media under shaking.

The next day, make a dilution 1/100 in fresh LB media until OD600 = 0.3, at the start of the exponential phase.
Pellet the cells by centrifuging 5 minutes at 5000 rpms, and resuspended to an OD600 = 0.5 in LB + sucrose 12%.
Aliquot the cells by adding 500 µL in 2 mL eppendorf tubes then freeze them at -80ºC.

Lyophilization

The next day, lyophilize for 6 hours in a SpeedVac Vacuum Concentrator. The lyophilized cells were stored for a week at 4 ºC before their use, but according to the literature, they should stay alive and maintain protein induction after a year of storage at that temperature.

References

Prévéral, S., Brutesco, C., Descamps, E. C., Escoffier, C., Pignol, D., Ginet, N., & Garcia, D. (2017). A bioluminescent arsenite biosensor designed for inline water analyzer. Environmental Science and Pollution Research, 24(1), 25-32.