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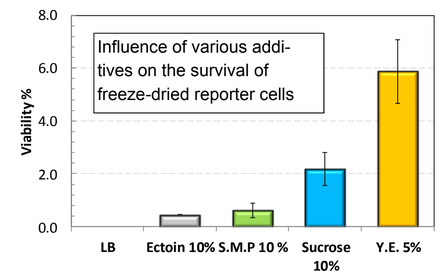
Survival rate and conditioning of dried *E. coli* DH5α 1598 ArsR reporter cells

Rémy Dufresne, Gordana Pistoletti, Jacqueline Prim, Simon Crelier

HES-SO Valais, Institute of Life Technologies, Route du Rawyl 64, CH-1950 Sion

Hes·so VALAIS WALLIS
Haute Ecole d'Ingénierie
Hochschule für Ingenieurwissenschaften



1. Introduction and goals

The proof of concept has been successfully established for reporter bacteria such as *E. Coli* DH5α 1598 ArsR [1], but some aspects need to be addressed before it can be routinely used in a portable detection and quantification device:

1. The bacteria has to be produced in significant amounts, collected and dried for storage
2. Cell viability and response to As (III) stimulation must be reproducibly preserved over the whole shelf-life
3. Insertion and "activation" of the bacteria in the portable device must be easy to do directly on the measurement site

This part of the project aims at optimizing drying conditions in order to preserve both cell viability and activity.

It also addresses the formulation issue, looking for an easy insertion and reactivation of cells in the portable device.

The experiments were performed according to the protocol described in **Figure 1**.

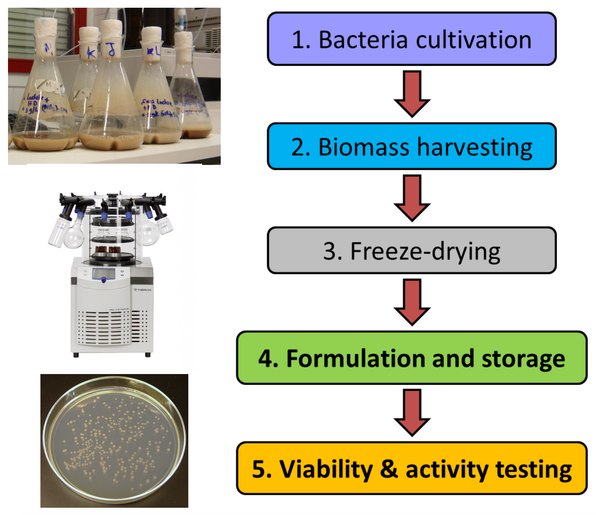


Figure 1: Experimental protocol in this investigation

2. Cell cultivation and harvesting

Precultures: 50 mL LB medium in a 300 mL shake-flask. Incubation with 10 µL of a cryopreserve and cultivation overnight at 37 °C with shaking at 180 rpm.

Cultures: 1L LB medium in a 2L baffled shake-flask. Inoculation with preculture and growth at 37°C and 180 rpm.

Biomass collection: Broth was centrifuged 20 min at 8'000 g and biomass resuspended in the same volume of fresh medium before freeze-drying. **Figures 2a and 2b** shows typical results

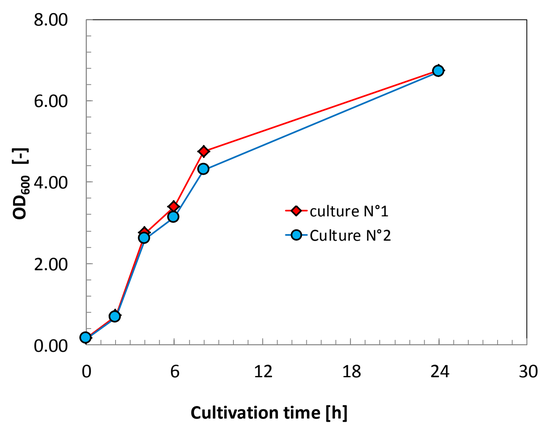


Figure 2a: Growth curves for two cultures of reporter cells. 1L medium in 2L flask, 37°C, 180 rpm

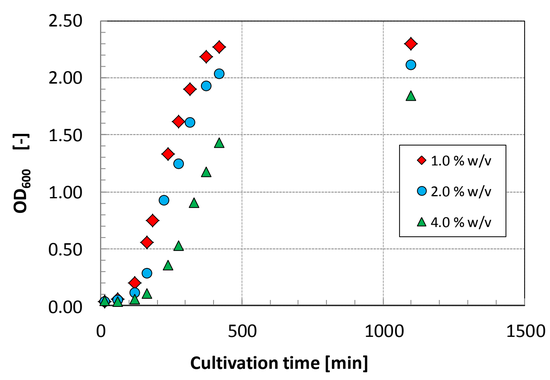


Figure 2b: Growth curves for reporter cells under osmotic stress at 1.0, 2.0 and 4.0 % w/v NaCl

3. Survival and activity after freeze-drying & storage

A variety of additives were tested in order to check their influence on dried *E. coli* survival rate and activity. **Figure 3** shows that the combination of glucose, glycerol and yeast extract (sample E) gave the best results with 40% survival.

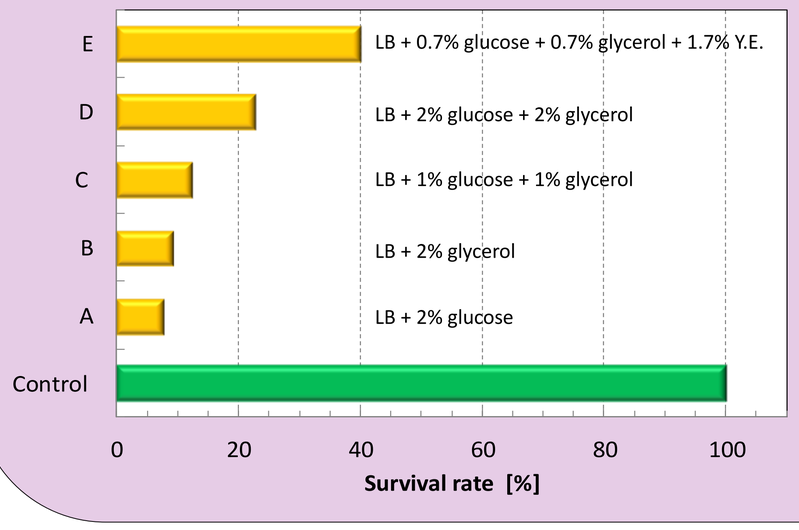


Figure 3: Survival rate of reporter cells freeze-dried in the presence of various protective agents

4. Alternative formulations and conditionings

4.1 Freeze-drying inside HPLC vials

1 mL HPLC vials offer a low-cost, geometrically standardized kind of container which permits freeze-drying, storage as well as a direct reconstitution through the septum. Figure 4 shows such vials after freeze-drying of the cells (left side) and after application of an arsenic-containing sample (right side).

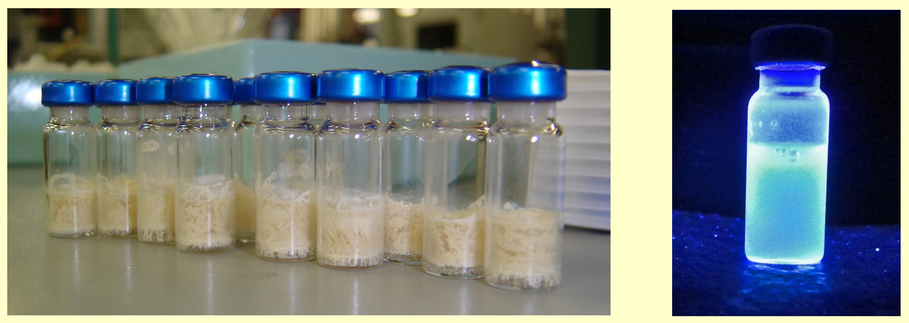


Figure 4: HPLC vials with freeze-dried cells before (left) and after addition of an arsenic-containing sample followed by incubation. Right sample was placed under a UV light

4.2 Freeze-drying inside cell-counting microtubes

Freeze-drying could also be performed successfully in microcentrifuge tubes used to measure Packed Cell Volume (PCV). These tubes also allow reconstituion and sample addition. A short centrifugation steps then drives the cells into the capillary where fluorescence can be measured, as shown in **Figure 5**.

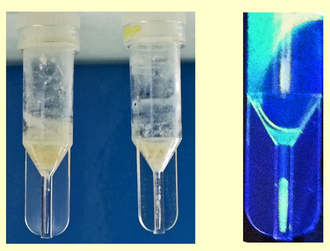


Figure 5: Microcentrifuge tube with Pack Cell Volume capillary on bottom. Left picture shows tubes with freeze-dried reporter cells. Right picture shows fluorescence in the capillary after rehydration, arsenic addition, incubation and illumination

4.3 Freeze-drying inside glass capillaries (Figure 6)

This approach would allow an easy connection to a microfluidic system provided one end of the channel can be fitted with a cell retention device (porous polymer plug or membrane). First results are encouraging but not conclusive.

Figure 6: Tip of a capillary plugged by a porous monolith of polymer (poly-BuMA and EDMA) for cell retention.

5. Conclusion and perspectives

Escherichia coli is notoriously difficult to dry and store in a viable state [2], [3] and the observed survival rates are generally low.

Best results regarding viability and activity freeze-dried *E. coli reporter* cells were obtained with combined glucose, glycerol and yeast extract.

Storage at room temperature led to fast inactivation of reporter cells. Storage stability is significantly increased at 4 °C.

Although fluorescence measurement is difficult in HPLC vials or microcentrifuge tubes, these offer a very convenient «one pot» system for storage and assaying.

Freeze-drying of the cells in capillaries appears to be feasible, provided thawing and leakage can be prevented. Further efforts will concentrate on the porous cell retention monolith plug.

6. Bibliography

[1] K. Siegfried et al. (2012): Environ. Sci. Technol. 46, 3281-3287.

[2] M.B. Gu et al. (2001): J. Biotechnology 88 (2), 95-105

[3] T. J. Sinskey & G. J. Silverman (1970): J. Bacteriol. 101 (2), 429-437