

ENTRAPMENT OF FLUORESCENT *E. COLI* CELLS IN ALGINATE GEL

ENTRAPAREA CELULELOR FLUORESCENTE DE *E. COLI* IN GEL DE ALGINAT

T. VINTILA¹, D. VINTILA¹, M. DRAGOMIRESCU¹, V. IGNA²

¹*Faculty of Animal Sciences and Biotechnologies, Timișoara, România,
tvintila@animalsci-tm.ro*

²*Faculty of Veterinary Medicine, Timișoara Romania*

By this experiment we will demonstrate the possibility to obtain genetically modified microbial strains that can be used as markers in different studies. The trait transferred in this study is the fluorescence in UV light expressed by a gene isolated from jellyfish. This gene was inserted into a plasmid carrying ampiciline resistance and in the operon for arabinose fermentation. The plasmid was called pGLO. E coli HB101 K-12, ampicillin resistant colonies has been obtained. The colonies on the LB/amp/ara plate fluoresce green under UV light and the transformed colonies can grow on ampicillin. Transformation efficiency = 362 transformed colonies/ μ g DNA. The cells where immobilized by entrapment in alginate gel to study the phenomenon involved in cells immobilization. After immobilization in alginate gel, 5×10^4 cells of E. coli pGLO / capsule and $1,4 \times 10^5$ cells of E. coli HB101/capsule has been found. Fluorescent microscopy revealed the presence of pGLO carrying cells into the capsules. After cultivation of alginate capsules containing E. coli in LB broth, and fluorescent microscopy of the capsule sections, several observations of the phenomenon involved in continuous fermentation using biocatalysts in has been made. These cells grow and migrate to the cortical part of the matrix where they are immobilized.

Keywords: fluorescent cells, alginate gell, entrapment

Introduction

The aim of this work is to obtain a marker-strain by genetic engineering. This strain can be subsequently used in the next studies, to find out the way the cells are attached or entrapped in the matrix of support in microbial products. This strain can be a model for further researches in genetics of microorganisms or industrial microbiology. More than that, by this experiment we will demonstrate and establish a way to transfer foreign genes in microbial cells coding traits of wide importance. By this experimental model we will demonstrate the possibility to

obtain microbial strains able to synthesize products of agro-industrial interest (enzymes, aminoacids, hormones, proteins etc).

Materials and Methods

In this work, first we tried to induce genetic transformation mediated by a plasmid in the following microorganisms: *Sacharomyces cerevisiae*, *Bacillus subtilis*, *Lactobacillus plantarum*, *Rhizobium meliloti*, *Escherichia coli HB101 K12*. However, only the last organism accepted the plasmid. The trait transferred in *E. coli HB101 K12* is the fluorescence in UV light expressed by a gene isolated from the jellyfish *Aequorea victoria*. This gene was inserted into a plasmid carrying ampiciline resistance and in the operon for arabinose fermentation. The plasmid was called pGLO. The process of bacterial transformation, when bacteria acquire the pGLO plasmid, can be explained in four steps: 1. Pre-incubation: Calcium chloride, a transformation solution, is added to the bacteria. The ionic character of CaCl_2 (calcium's positive ions) allows it to interact with the biochemistry of the cell membrane and the bacterial DNA to allow external DNA to more easily enter the cell. 2. Incubation: The pGLO plasmid is added to the cell culture. 3. Heat shock: Bacteria are subjected to extreme differences in temperature, a process which allows the cell wall to expand, contract, and more easily take in the new pGLO plasmid. 4. Recovery: The bacteria are grown in Luria broth and are briefly incubated to foster growth. These four steps enable gene expression of pGLO in the presence of arabinose sugar and ultraviolet light.

In the next step, the cells were immobilized by entrapment in alginate gel to study the phenomenon involved in cells immobilization.

The following procedure was followed: 2 ml of fresh culture in LB broth is centrifuged 10 minutes at 5000 r.p.m. The pellet is resuspended in 2 ml phosphate buffer pH 7. The result was mixed with 100 ml solution of sodium alginate 2,5%. The obtained mixture was dropped into sterile 0,2 M CaCl_2 solution. Alginate drops were solidified upon contact with CaCl_2 , forming capsules and thus entrapping bacterial cells. The capsules were allowed to harden 24 hours at $+4^\circ\text{C}$ and washed with sterile saline solution to remove excess of Ca^{2+} and cells. The obtained biocatalyst was preserved at 4°C in sterile saline solution.

The drying of biocatalyst was carried out under sterile condition, in a laminar flow hood. The total drying time (from the filtration to the end of vacuum drying) was 24 hours.

Samples of 20 wet capsules and 20 dry capsules were put in 10 ml 0.05 M sodium hexametaphosphate sterile solution for 2 hours on an orbital shaker at 200 rpm. If needed, the undissolved fragments of biocatalyst were crushed with a sterile blunt end glass stick. The suspension is then well mixed on a vortex until a homogenous suspension without any residual fragments that may contain

entrapped cells is obtained. The suspension is then diluted in sterile saline solution, plated on nutrient agar and the CFU are counted.

Results and Discussion

Procedure of transformation mediated by pGLO plasmid applied in this study has been successful only in *Escherichia coli* HB101 K-12 and ampicillin resistant colonies has been obtained. Whitish color of bacterial colonies is one of the traits originally observed for *E. coli* that did not seem to become altered. Also, the colony size is similar both before and after transformation. But, the colonies have gained a new trait, and that is the colonies on the LB/amp/ara plate fluoresce green under UV light and the transformed colonies can grow on ampicillin. The pGLO plasmid, which has been inserted into *E. coli* cells by transformation express a gene for ampicillin resistance (the protein product of the *bla* gene, which codes for betalactamase, the protein that breaks down ampicillin). How can we prove that these changes that occurred were due to the procedure that we performed? The best way is to compare the control to the experimental plates. Cells that were not treated with the plasmid, ((-) DNA and LB/amp plate) could not grow on ampicillin, whereas cells that were treated with the plasmid ((+) DNA) can grow on the LB/amp plate (figure 1). Thus, the plasmid must confer resistance to ampicillin.

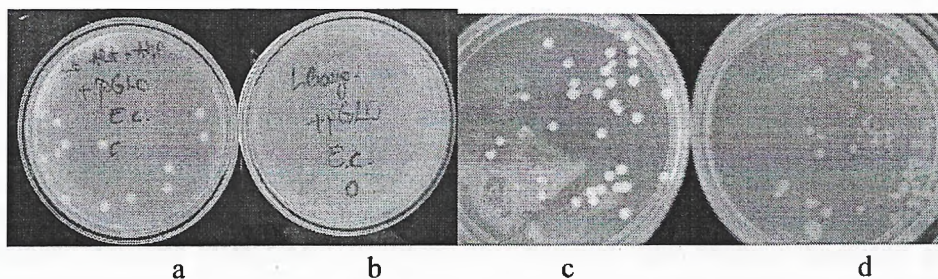


Figure 1. Colonies of genetically transformed E. coli
a) and c) – colonies on LB/amp/ara; b) and d) – colonies on LB/amp

The *ara* genes that code for the digestive enzymes involved in the breakdown of arabinose are clustered together in what is known as the arabinose operon. In the same operon was inserted the *GFP* gene as well. In the presence of arabinose, *araC* protein promotes the binding of RNA polymerase and GFP is produced. Cells fluoresce brilliant green as they produce more and more GFP. In the absence of arabinose, *araC* no longer facilitates the binding of RNA polymerase and the GFP gene is not transcribed. When GFP is not made, bacteria colonies will appear to have a wild-type (natural) phenotype—of white colonies

with no fluorescence. This is an excellent example of the central molecular framework of biology in action: DNA→RNA→PROTEIN→TRAIT.

The results regarding the colonies in different plates are presented in the next table:

Plate	Observation
pGLO+, LB+amp	Whyte colonies of <i>E. coli</i> , (mean = 58 colonies)
pGLO+, LB+amp+ara	Colonies of <i>E. coli</i> , whyte in natural light and fluorescent in UV (mean = 60 colonies)
pGLO-, LB+amp	No colonies
pGLO-, LB	High concentration of whyte colonies of <i>E. coli</i> , (mean = 227 colonies)

Transformation efficiency was 362 transformed colonies/ μ g DNA.

The new strain of fluorescent *E. coli* will be named *E. coli pGLO*

Figure 2 represent microscopic images of *E. coli* cells, in visible light (a – wild type and transformed cells have the same morphology and characteres) and in UV (b – only transformed cells can be seen).



Figure 2. Microscopy of *E. coli* (x800)

a) *E. coli* cells – wild type in visible light; b) transformed cells of *E. coli* in fluorescence microscopy ($\lambda=450-490$ nm) – cells are fluorescent.

After immobilization in alginate gel, 5×10^4 cells of *E. coli pGLO* / capsule and $1,4 \times 10^5$ cells of *E. coli HB101* / capsule has been found. Fluorescent microscopy revealed the presence of fluorescent cells into the capsules, although the conventional *E. coli HB101* cells couldn't be visualized nor in visible or UV light. The *E. coli* cells didn't survive during drying the alginate capsules. They can be stored in 0,85% sterile saline solution at +4°C for less than two weeks, when the viability drops to very low levels. After cultivation of alginate capsules containing *E. coli* in LB broth, and fluorescent microscopy of the capsule sections, several observations of the phenomenon involved in continuous fermentation using biocatalysts in has been made: The substrate fed into the system to be transformed by the biocatalyst, is used by the immobilized cells as nutrient. These cells grow

and migrate to the cortical part of the matrix where they are immobilized (figure 3). This way, the catalysis of the substrate is carried out with higher intensity at the interface between biocatalyst and substrate. More than that, the cells can pass from the matrix into the liquid substrate, or can excrete enzymes which can be found into the liquid medium.

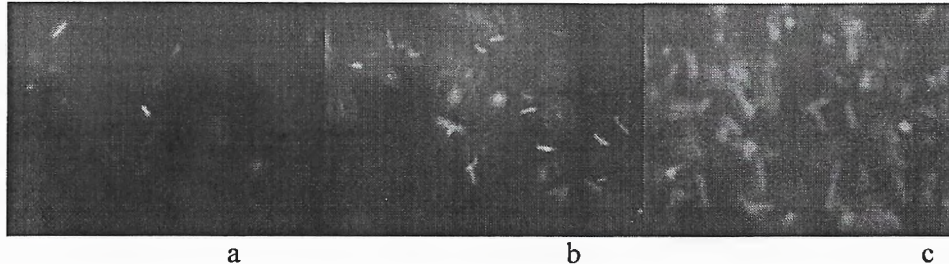


Figure 3.1. E. coli pGLO immobilized in calcium alginate capsules: low density of cells in the central part of the capsule (fig. 3.a), higher density close to the cortical part of the capsule (fig. 3.b) the highest density of the cells are found on the surface of the capsule (fig. 3.c).

It was impossible for us to visualize the immobilized cells of *E. coli* HB101 (the wild type) in entrapping matrix, although they are viable concluding to CFU method.

Conclusions

This study offered answers regarding the phenomenon involved in immobilization of biocatalysts by cells entrapping and in the continuous systems of fermentation using immobilized biocatalysts. It can be concluded that the biocatalysts consisting of immobilized cells into the matrix, represent in fact an inoculum into the bioreactor. This system made possible withdrawal of the products resulted in the biocatalysis and addition of new substrates in bioreactor without being necessary reinoculation with fresh culture, technique used in batch system.

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