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The Antibiotic Induction of Apoptotic – Like Changes in Bacteria *E. Coli* – GFP Encapsulated in Hollow Fibers

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Encapsulation of bacteria in a semipermeable polymer membrane gives a lot of technological possibilities. Our aim was to evaluate the performance of bacteria encapsulated in hollow fibers when treated with chosen antibiotic. The antibiotic application may cause release of biologically active substances for which production the bacteria may be genetically modified. The encapsulated in HF bacteria *Escherichia coli* transfected with pQE-GFP (green fluorescent protein) plasmid were incubated with addition of gentamycin or tetracycline. The encapsulated in hollow fibers *E. coli* culture with addition of tetracycline proves the tetracycline impact on the bacteria viability increasing the necrotic bacteria share. Polypropylene modified membranes allow to avoid permeation of the bacteria through the membrane wall. *E. coli* encapsulated in HF may be used in future, in systems releasing the therapeutic factor.

K e y w o r d s: hollow fiber, encapsulation, bacteria *Escherichia coli*, antibiotic

1. Introduction

Encapsulation of bacteria in a semipermeable polymer membrane gives a lot of technological possibilities: improves the bacteria stability during passage under adverse conditions of the gastrointestinal tract in food industry applications [1–4], increases the rate of substrat biodegradation by the bacteria in waste treatment applications. For example degradation 90% of petrol hydroxycarbons runs 3 times more quickly with application of the encapsulated bacteria then non-encapsulated. Also, such a system allows avoiding an attack of the implanted microorganisms to the host and the host

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immunological response after implantation into the animal. Some encapsulated microorganisms may carry a transfected human gene, and thus become a source of valuable regulatory factors. Such factors released in strategic locations may direct or modify the biological processes in the eukaryotic organism [5–7].

The most commonly performed procedure is based on calcium-alginate gel capsule formation and the bacteria applied in therapeutic purposes are encapsulated in microcapsules. For example genetically modified bacteria *E. coli* DH5 encapsulated in microcapsules may act in diminishing of creatinine, urea, ammonia and another metabolites level during renal or liver dysfunction [6, 7].

The polypropylene surface modified hollow fiber [8] was applied for bacteria *E. coli* isolation. The membrane surface was double layer modified with different siloxanes. Such modification improves biocompatibitity of the membrane and allows to avoid bacteria escaping from the lumen of the membrane (what is not equal to avoiding of bacteria adhesion which problem was the subject of many authors' interest [9–13]).

Our aim was to evaluate the performance of the bacteria encapsulated in the hollow fibers when treated with a chosen antibiotic. The antibiotic application may cause release of biologically active substances for which production the bacteria may be genetically modified.

2. Materials and methods

2.1. Materials

Hollow fibers (HF) – polypropylene K600 PP, Accurel (Akzo-Nobel, Germany; inner diameter 0.6 mm, wall thickness 0.2 mm) with surface modified by siloxanes, sterilized in 70% ethanol, and washed with sterile physiological saline.

R*eagents* – Isopropyl β- D- 1- thiogalactopyranozyd (IPTG) (ICN, USA), tetracycline (ICN, USA), gentamycin (ICN, USA), propidium iodide (PI) (Sigma, USA).

Media – RPMI 1640 (GIBCO, USA); Luria-Bertan, Broth, Miller (Difco, USA); media mixture (LB): Luria-Bertan and RPMI1640 1:10 supplemented with100 μg/ml kanamycin and 100 μg/ml streptomycin.

Bacteria – Escherichia coli (*E. coli*) strain SG3103 (Qiagen) was transfected with pQE-GFP (green fluorescent protein) plasmid by dr S. Świeżewski, (IBB, Poland). The pQE-GFP plasmid was created by cloning into the pQE60 (Qiagen) vector of the GFP gene. The GFP sequence amplified was: 5'CAT G*CC ATG G*CA ATG AGT AAA GGA GAA GAA CTT-3' and GFP_1 5'CG*G GAT CC*A TGT TTG TAT AGT TCA TCC ATG CC 3' primers from pBINmGFP5er kindly provided by J. Haseloff (Cambridge, UK).

The expression of the GFP in the bacteria was induced by 2 mM IPTG for 2 hours, before the encapsulation (bacteria *E. coli* GFPI).

2.2. Procedures

Evaluation of the antibiotic impact on bacteria E. coli

The bacteria *E. coli* (GFPIs) at the concentration of about 1.5×10^8 bacteria/ml (the initial concentration of the bacteria was set by spectrophotometer at the wave length of 550 nm to absorbance 0,125) were incubated for 2 hours in LB culture medium with addition of gentamycin or tetracycline in concentration 1mg/ml (35ºC). As a negative control the encapsulated in HF bacteria *E. coli* was incubated in the LB medium. After 2-hour incubation the samples of bacteria suspension were evaluated in cytochemical reaction with PI in a flow cytometer to assess the presence of GFP fluorescence of organisms as well as the PI fluorescence of organisms with eventually damaged cell membrane.

Evaluation of the optimal antibiotic concentration

The bacteria *E. coli* (GFPI) at concentration about 6.8×10^8 bacteria/ml or 20-times diluted in concentration about 3.4×10^7 bacteria/ml or 40-times diluted in concentration about 1.7×10^7 bacteria/ml were incubated (35ºC) in the culture medium LB with addition of tetracycline in concentration 0.01 mg/ml, 0.1 mg/ml, 1mg/ml for 2 hours. After incubation the samples of bacteria suspension were taken and analyzed in cytochemical reaction with PI in the flow cytometer to assess the presence of GFP fluorescence of organisms as well as the PI fluorescence of organisms with eventually broken cell membrane.

Evaluation of the tetracycline impact on encapsulated bacteria E. coli

The encapsulated in HF bacteria *E. coli* (GFPI) at concentration about 1.5×10^8 bacteria/ml were incubated (35ºC) for 1-, 2-, 24-, 48-hours in the LB culture medium with addition of tetracycline in concentration 1mg/ml (35ºC). As a negative control the encapsulated in HF bacteria *E. coli* was incubated in the LB medium. After 1-, 2-, 24-, 48- hour incubation the HF content was evaluated in the cytochemical reaction with PI in the flow cytometer to assess the presence of GFP fluorescence of organisms as well as the PI fluorescence. The samples of the culture medium in which the HF encapsulated *E. coli* were cultured were analyzed for the presence of bacteria as well.

Flow cytometry

The presence of organisms was assessed using the FACS Calibur flow cytometer (Becton Dickinson Immunocytochemistry Systems, USA) equipped with an argon ion (488 nm) laser. The results were processed by the CellQuest software system (Becton Dickinson, USA). The organisms were separated from other events on light scatter characteristics (the gate of FSC and SSC).

3. Results and Discussion

Evaluation of the antibiotic induced changes in GFP expression on bacteria E. coli

The *E. coli* GFPI were incubated for 2 hours in the culture medium LB or LB supplemented with tetracycline or gentamycin. As a negative control the *E. coli* GFPI were incubated in the LB medium.

The percent number of the $GFP^{+-}PI^{+}$ *E. coli* population (with damaged cell membrane) was for gentamycin 0.54±0.0, for tetracycline 20.74±4.51 (Fig. 1).

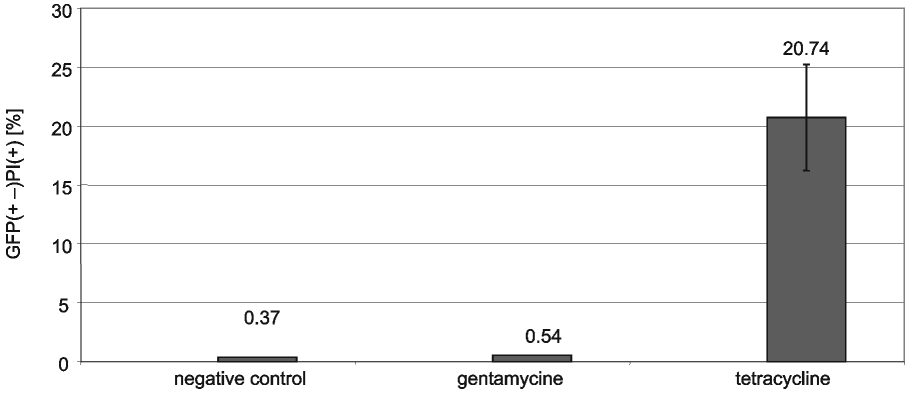

Fig. 1. The percentage of the $GFP^{+-}PI^{+}$ *E. coli* population with damaged cell membrane during culture with different antibiotics

Evaluation of the optimal antibiotic concentration

It was observed no tetracycline influence in concentrations 0.01 and 0.1 mg/ml on the bacteria *E. coli* viability in the initial bacteria concentration as well as diluted. The bacteria viability decreased about 80% (from about 90% to 10%) with antibiotic concentration increase for 20-times diluted bacteria as well as for 40-times diluted bacteria. The viability decrease for the initial bacteria concentration (6.8×10^8 bacteria/ml) was 10% (Fig. 2).

Application of tetracycline concentration 1mg/ml for bacteria in concentration below about 10^8 bacteria/ml allows to observe the antibiotic impact on viability of the microorganisms.

Evaluation of the tetracycline induced changes in GFP expression on encapsulated bacteria E. coli

There was a difference between the percent number of the $GFP^{+-}PI^{+}$ *E. coli* population as compared to a negative control. It was observed that the percent number of the population

$GFP^{+-}PI^{+}$ of the encapsulated *E. coli* cultured with tetracycline increased during 2 hours about 20 times (18.24±2.19%) as compared to a negative control (0.95±0.78%).

After 24-hour culture it was 61.70±21.18% (7.12± in negative control), after 48-hour culture it was 85.78±12.70% (11.57± in negative control) (Fig. 3). There was no microorganisms GFP observed in the culture medium where the encapsulated *E. coli* were cultured.

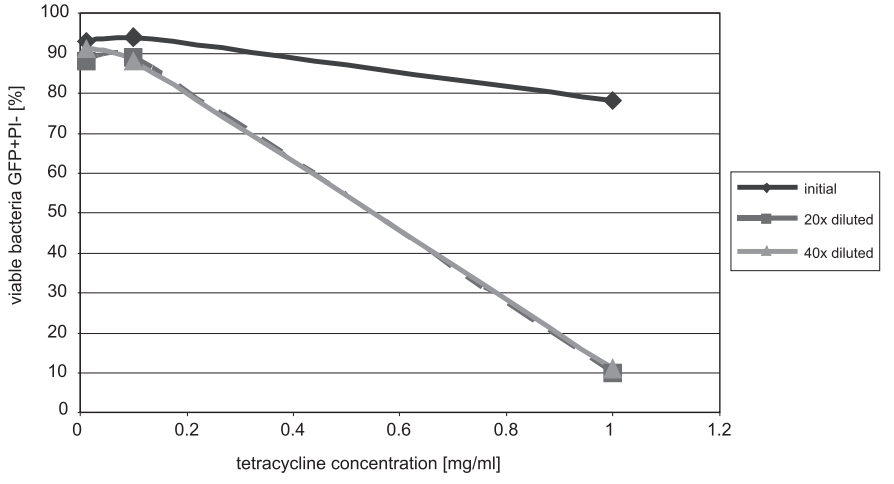

Fig. 2. The bacteria viability dependence on the antibiotic concentration

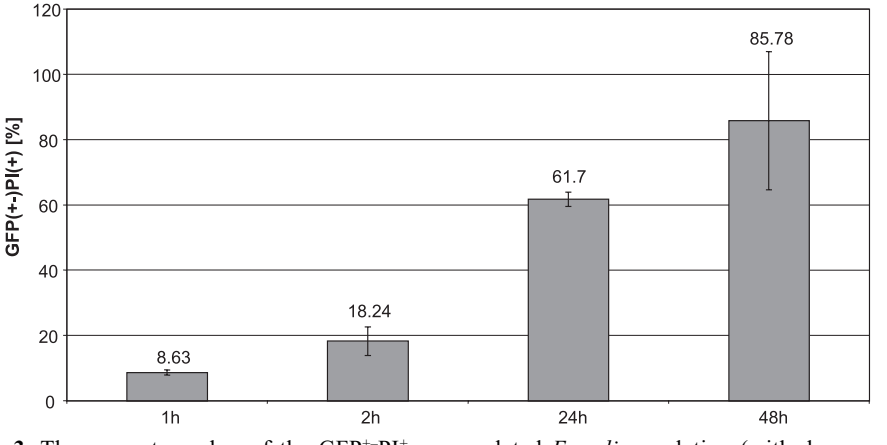

Fig. 3. The percent number of the $GFP^{+-}PI^{+}$ encapsulated *E. coli* population (with damage cell membrane) during the culture in tetracycline presence

4. Conclusions

(1) *E. coli* are not susceptive on gentamycin

(2) Polypropylene modified membranes allow to avoid permeation of the bacteria through the membrane wall.

(3) The encapsulated in hollow fibers *E. coli* culture with addition of tetracycline proves the tetracycline impact on bacteria viability increasing the necrotic bacteria share.

(4) *E. coli* encapsulated in HF may be used in future, in systems releasing the therapeutic factor.

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