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ENGINEERING OF ADENOVIRAL VECTOR CARRYING THE GENE OF HUMAN LACTOFERRICIN

V. S. Ugriumov[1,2], R. Z. Prusakov[1,2], V. M. Vakhtinskii[2], K. A. Pervoykina[2]

[1]*Mendeleev University of Chemical Technology of Russia, Moscow*
[2]*Gamaleya National Research Center for Epidemiology and Microbiology, Moscow*

✉ slavapok2@mail.ru

Abstract

Antimicrobial peptides are one of the alternatives to antibiotics, but their widespread use as therapeutic agents is limited by a short half-life and by the toxicity that occurs when large doses are injected. Adenoviral vectors carrying antimicrobial peptide genes help circumvent these limitations. In this work we obtain a recombinant adenovirus carrying the gene of human lactoferricin.

Introduction

According to the statistical data, provided by WHO, in 2019, more than 1.27 million deaths were attributable to antibiotic-resistant bacteria, with an additional estimated 5 million associated deaths [1]. In 2024 WHO released an updated version of Bacterial Priority Pathogens List (BPPL). Among them, a significant role belongs to gram-positive antibiotic-resistant bacteria such as *S. aureus*, *E. faecium*, and different species of *Streptococci* [1].

Antimicrobial peptides are a promising tool for combating antibiotic-resistant strains of pathogens. Most of the well-studied AMP have an amphipathic alpha-helical structure with a cationic and hydrophobic surface that helps them interact with the bacterial membrane disrupting it. The development of resistance to AMP in bacteria is hampered by the fact that modification of membranes to counteract AMP can cause functional changes unfavorable to the vital activity of the bacteria themselves.

Among numerous known AMP, we chose human Lactoferricin, as one of the most studied and safe. Lactoferricin interacts with the outer bacterial membrane, and causes its destruction, as well as penetrates into bacterial cells and affects bacterial intracellular mechanisms that leads to consequent cell death [2]. Widespread use of AMP is limited due to a number of problems. The first problem is the short half-life because peptides are rapidly degraded *in vivo* by the action of proteases. The second problem is related to their toxicity to normal cells when a large dose of the drug is administered.

One of the possible solutions is the use of viral vectors. Prolonged *in vivo* expression of desired AMP at a certain level provides almost its constant concentration, which is enough for effective therapy. The aim of our work was to construct an adenoviral vector, carrying the gene of human Lactoferricin.

Results

During the first step of our work we obtained a pShuttle-CMV plasmid (Agilent #240007) carrying the gene of human lactoferricin using the circular PCR. We used the primers, having the gene of lactoferricin in their 5` extensions and the region of their complementarity to each other (Fig. 1) Sequencing of the expression cassette showed the correct insertion of gene.

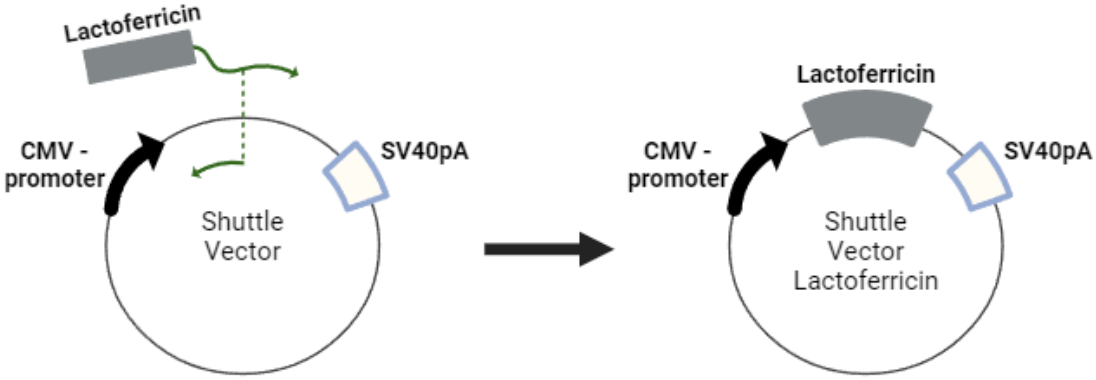

Fig. 1. Scheme of circular PCR using the primers, carrying the gene of Lactoferricin in the extensions

On the next step we linearized pShuttle-CMV-Lactoferricin using PmeI and co-transformed BJ5183 strain of *E. coli* (Agilent #200154) with pAdEasy-1 vector (Agilent 240005), containing the genome of human Ad5 virus. Consequential selection positive recombinants (pAd-lactoferricin) were held by addition of Kanamycin to the medium.

After that we transfected HEK-293 cell line (ATCC) with previously PacI-linearized pAd-lactoferricin, using the Lipofectamine® 2000 Reagent (TM 11668-027). Every 3 days we replaced conditioned medium with fresh DMEM 10% FBS until we saw a CPE. Infected cells were harvested and frozen thawed three times. Virus stock was subcloned through a single plaque. Recombinant viruses were tested for presence of Ad5-Hexon and lactoferricin genes as well as the absence of E1 region of wild type adenovirus (Fig. 2).

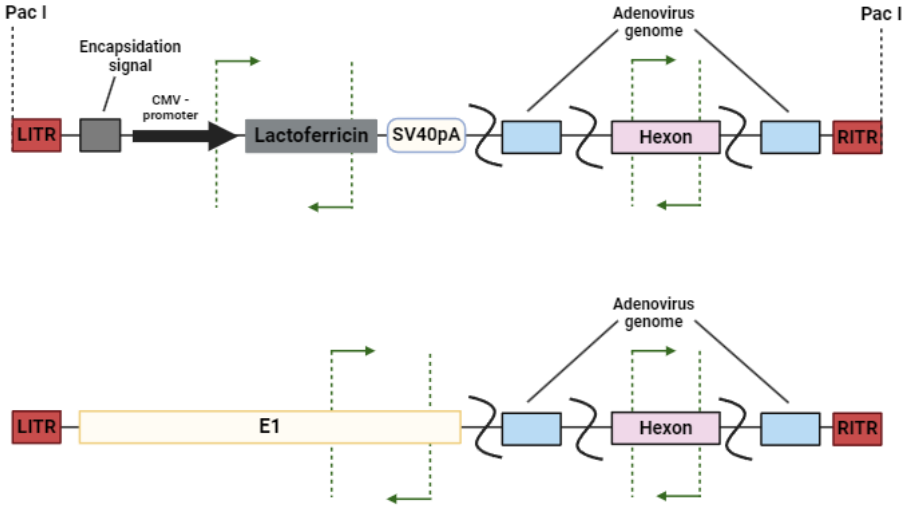

Fig. 2. Comparison of recombinant and wild types of Ad5

The results of PCR analysis provided at Fig. 3.

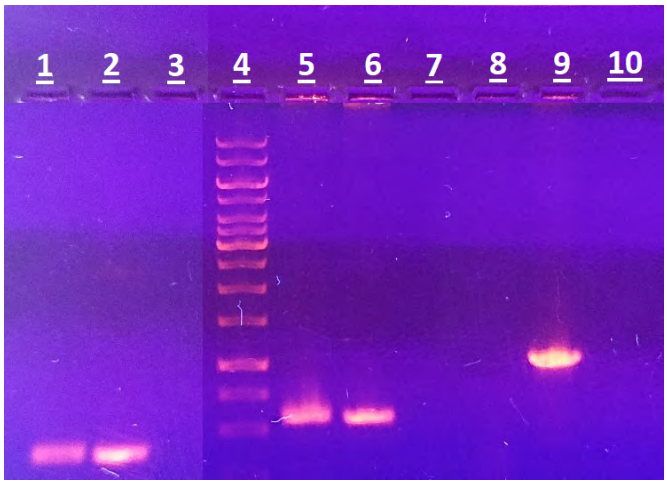

Fig. 3. Visualization of the results of PCR analysis using electrophoresis in 0.8% agarose gel.
Lanes *1–3*: PCR analysis for the presence of the lactoferricin gene. *1* — Infected cells, *2* — Positive control, *3* — Negative control; *4* — GeneRuler 1kb DNA Ladder (SM0311). Lanes *5–7*: PCR analysis for the presence of the adenovirus hexone gene, *5* — Infected cells, *6* — Positive control, *7* — Negative control; Lanes *8–10*: PCR analysis for the presence of the E1 region. *8* — Infected cells, *9* — Positive control, *10* — Negative control

The results of PCR show that the obtained virus is recombinant replicative deficient human adenovirus 5 serotype, carrying the gene of lactoferricin under the control of CMV promoter.

Our next step is to confirm the expression of lactoferricin by our vector and to study its antibacterial effect on Gram-positive strains of *in vitro* and *in vivo*.

References

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