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Technical note

# Inhibition of lysosomal vacuolar proton pump down-regulates cellular acidification and enhances *E. coli* bactofection efficiency

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## Highlights

- Bactofection efficiency is constrained by cellular barriers such as plasma membrane, endosomal escape and nuclear entry.
- Bafilomycin $A_1$, a specific inhibitor of the lysosomal $H^+$ vacuolar proton pump, decreases the cellular acidification.
- Low acidification likely assists *E. coli*'s endosomal escape.
- Cells pre-treated with bafilomycin $A_1$ achieved significantly higher gene delivery by the *E. coli* vector.

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ABSTRACT

Endosomal escape is considered a crucial barrier that needs to be overcome by integrin-mediated *E. coli* for gene delivery into mammalian cells. Bafilomycin, a potent inhibitor of the H+ proton pump commonly employed to lower endosomal pH, was evaluated as part of the *E. coli* protocol during delivery. We found an increase in green fluorescent protein expression up 6.9, 3.2, 5.0, 2.8, and 4.5 fold in HeLa, HEK-293, A549, HT1080, and MCF-7 respectively, compared to untreated cells. Our result showed for the first time that Inhibition of lysosomal V-ATPase enhances *E. coli* efficiency.

## 1. Introduction

*E. coli*-based vectors commonly utilize exogenous genes such as invasin from *Yersinia pseudotuberculosis* and listeriolysin O from *Listeria monocytogene,* to enable the transfer of expression plasmids into eukaryotic cells [1–3]. However, the transfection efficiency of *E. coli* vectors has generally been low compared to viral vectors due to several cellular barriers [4], including cellular uptake of the vector, endosomal escape, and nuclear entry.

Of these, endosomal escape has been one of the major pitfalls that limit the endocytosed *E. coli* vectors from achieving higher gene delivery efficiencies [5]. Like other non-viral vector, the inability of the endocytosed *E. coli* vector to escape the endosome will result in lysosomal degradation. Lysosomal degradation is suggested to be as a result of decreasing intracellular pH as the vector navigates through the endo-lysosomal compartment [6]. Therefore, overcoming this lysosomal barrier by inhibiting its acidification could potentially enable our vector to deliver gene at a much higher rate. To the best of our knowledge, inhibition of the lysosomal acidification has not been tested as a method to improve the efficiency of gene transfer by the *E. coli* vector.

In a previous study, neutralization of the endosome and lysosome was achieved by lysosomotropic drug chloroquine that increased the efficiency of *E. coli* delivery by 6-fold in B16F10 cells [7]. This endosomal enhancer induced swelling of the endosome and prevented the fusion of the endosome to the lysosome without any effect on the vacuolar proton-translocating ATPases (V-ATPase).

The lysosomal V-ATPase is responsible for the continuous production of hydrolase that plays a crucial role in the acidification of the endosome and degradation of the internalized vector [8–10].

Based on these observations, we hypothesized that inhibiting the V-ATPase would decrease lysosomal acidification, resulting in a significant increase in *E. coli* gene delivery efficiency. To test this, we pre-incubated cells with bafilomycin $A_1$, a macrolide antibiotic that is known to inhibit the vacuolar-type $H^+$-ATPases, before invasion with the *E. coli* vector [11–13]. Specifically, HeLa, HT1080, HEK-293, A549 and MCF-7 cells were incubated with DMEM containing 1/100 volume of bafilomycin $A_1$ dissolved in DMSO for 1 h at 37 °C in a 5% of $CO_2$ atmosphere. Bafilomycin $A_1$ was prepared by reconstitution in DMSO before it was utilized. DMSO was used as a positive control in this experiment. DMSO is considered a small amphiphile that enhances cell permeability and works as a tool for drug delivery into mammalian cells [14].

Next, the cells were invaded with our *E. coli* vector carrying a pEGFP-N2 plasmid that contains a GFP reporter driven by a mammalian CMV promoter, using our established invasion protocol [1]. Cells were presented with *E. coli* at 1000 multiplicity of infection (MOI) in 6 well plates and centrifuged for 10 min at 250×*g* before incubation for 1 h. This was followed by treatment with 80 μg/ml of gentamicin to selectively kill the extracellular *E. coli* for 1 h, after which the plates were incubated for 48 h with DMEM containing 20 μg/ml of gentamicin.

To determine the gene delivery efficiency of *E. coli* vector,

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