Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(7):328-331

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The minicell generation in *Escherichia coli* harboring *minD* of *Lactobacillus*

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ABSTRACT

Minicells have been studied as drug delivery with highly significant anti-tumor effects in vivo. Minicells were produced by mutant bacteria without min locus. In this study, the minicell generation was done in the glucose condition in combination with different antibiotic concentrations when culturing the Escherichia coli BL21(DE3)plysS harboring pET 21(a+) and pET 28(a+) inserted with minD gene of Lactobacillus acidophillus VTCC-B-871. The minD gene gives a filamentous phenotype in 1% glucose and ampicillin at concentration at 100μg/ml for the host cells harboring pET 21(a+)/minD and kanamycin at 50μg/ml for the host cells harboring pET 28(a+)/minD. However, the filaments changed into minicells when chloramphenicol presented in the culture with the long incubation time to 24 hours. Especially, glucose could induce the expression of MinD. The morphology differentiation was observed by microscope. The study supported the minicell generation with high rate and enough amount for drug delivery.

Keywords: glucose, *Lactobacillus acidophilus* VTCC-B-871, *minD* gene, minicell

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INTRODUCTION

Resistance of drug is one of our biggest problems in therapy, especially cancer therapy. Therefore, the requirements for disease treatment are essential and developed rapidly with the involvement in numerous physical and chemical methodologies. The efforts to the develop the targeted drug delivery systems as nanoparticles, polymer therapeutics to cancer cell via cell-surface receptors are also not perfect in leakage in vivo or production scale up. Remarkably, bacterial minicells are anucleated nanoparticles produced as a result of inactivating the genes controlling the normal bacterial cell division [1], [2] due to depressing the polar sites of cell division, may provide the better way for cytotoxic drug delivery. The minicells are also prepared from genetically defined *minCED(-)* chromosomal deletion bacteria and then the subsequent minicells were purified. The deletion of *minCED(-)* out of the bacteria cell may affect on their growth under their control so far [3]. MinD might show other function [4], [5]. MinD oscillation depend on temperature [6]. Therefore, to find out many methods in the minicell formation without the above mentioned discussion was essential. The study suggested the way of minicell production that may be the better way for cancer treatment.

The study selected the cell division of *Lactobacillus acidophilus* as object because of the use of *Lactobacillus acidophilus* in medicinal products.

EXPERIMENTAL SECTION

Bacterial strains, plasmids, and growth conditions

Escherichia coli BL21(DE3)pLysS were purchased by Promega. The pET21 (a+) used for overexpression was purchased by Novagen. *Lactobacillus acidophilus* VTCC-B-871 purchased by Vietnam type culture collection (VTCC). *Escherichia coli* BL21(DE3)pLysS was used as an expression strain. *Lactobacillus* strains were grown on MRS for 72-96 hours at 30^{o}C. *Escherichia coli* strains were grown in Luria-Bertani for 18-24 hours at 37^{o}C with shaking at 200 rpm. When required, antibiotics as ampicillin, chloramphenicol, kanamycin were added to media. Glucose was used to test at 1%(w/w). IPTG was used at 0.5 mM.

Introduction of *MinD* into *E. coli* and light microscopy

The *Lactobacillus acidophilus minD* was amplified by PCR with a sense primer (5'-CATATGGGGACAGCGTTAGTAGTGACTTC-3') (the *NdeI* site is underlined) and an antisense BHE2 (5'-CTCGAGGATGGCGATGGAACAATTTTGAC-3') (the *XhoI* site is underlined). The amplified *minD* was cut by *NdeI* and *XhoI* double-digestion and inserted into the same sites of pET-21(a+) to produce pET-21(a+)*/minD*. The transformation was performed according to Sambrook [7]. *E. coli* BL21(DE3)pLysS transformed with pET-21(a+)/*minD* was grown in LB medium supplemented with appropiate antibiotics at 37^{o}C to OD600 = 0.3, after which 0.5 mM IPTG or glucose was added to culture to induce at 28^{o}C from 5 to 24 hours. Light microscopy was used to observed the morphological changes in *E. coli*.

Minicell generation

The cells was expressed in the medium contaning 1% glucose and different chloramphenicol at 5, 20, 34 µg/ml. After inducing at 37^{o}C from 5 to 24 hours, the cell was taken out and observed under light microscope to make sure the minicell was generated and analysis.

RESULTS AND DISCUSSION

Differentiation of filamentous morphology

The *minD* gene introduced into *Escherichia coli* by transformation of pET21(a+)/*minD* and pET28(a+)*/minD*. The clone selection was based on the ampicillin resistant gene in pET21(a+) and kanamycin for pET28(a+). The ampicillin was used at 100µg/ml and kanamycin was used at 50µg/ml. At this step, chloramphenicol was not used. After induction, the *E. coli* cells became filaments (Fig. 1 and Fig. 2). The *E. coli* harbored pET21a+ and pET28a+ without *minD* shown the normal rod (Fig. 1A, 2A). Without chloramphenicol, the result of filamentous phenotype have occurred because *Lactobacillus* MinD enhanced MinC-mediated inhibition of cell division at all potential division sites in *E. coli* cells (Fig. 1B, 2B) and MinD was determined in non-dividing division in condition of beta-lactam. As a result, *minD* of *Lactobacillus acidophilus* is functional across species.

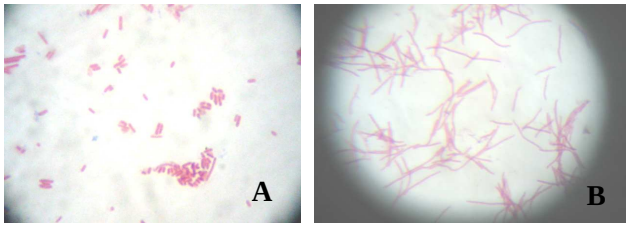

Figure 1: Morphology of *Escherichia coli* in medium containing ampicillin (A) *E. coli* harboring pET21(a+); (B) *E. coli* harboring pET21(a+)/*minD*

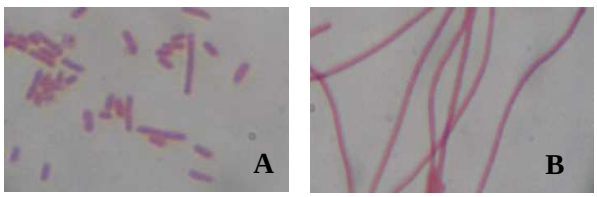

Figure 2: Morphology of *Escherichia coli* in medium containing ampicillin (A) *E. coli* harboring pET28(a+); (B) *E. coli* harboring pET28(a+)/*minD*

Minicell generation

The previous studies only pointed the introduction of *minD* gene into pET vector system, leading to filamentous with IPTG as inducer. However, this study found that different concentration of chloramphenicol would cause filaments in the 5 hours of induction and minicell formation in the 5 hours of induction. Normally, MinCDE prevents minicell formation. But, in this study, depending on chloramphenicol concentration and the length of induction time, the *Lactobacillus* MinD in *E. coli* produced minicell with high rate (Fig. 3B, 3D, 3F). In case of induction by glucose and chloramphenicol concentration at 5µg/µl, the cell showed the filaments after 5 hour and 24 hour induction, respectively (Fig. 3A, 3B). The cell could form both mincell and filaments at 24 hours of induction at with the concentration of chloramphenicol at 20µg/µl (Fig. 3D). The cell could form mincell at 24 hours of induction at with the concentration of chloramphenicol at 34µg/µl (Fig. 3F). To confirm this phenomenon and the hypothesis, the study also perform the overexpresion using the pET28 (a+) carrying kanamycin resistant gene. Clearly, kanamycin can not cause the minicell morphology (Fig. 2A, 2B). The study also compared with *E. coli* BL21(DE3)plysS harboring plasmid without *Lactobacillus minD* and revealed that *E. coli* phenotype could not be affected. The complicated study will be useful to study other MinD function like human MinD whether that was one of the root of cancer disease or cancer resistance in therapy. The study also supported the way to produce minicell for drug delivery or vector for nucleic delivery.

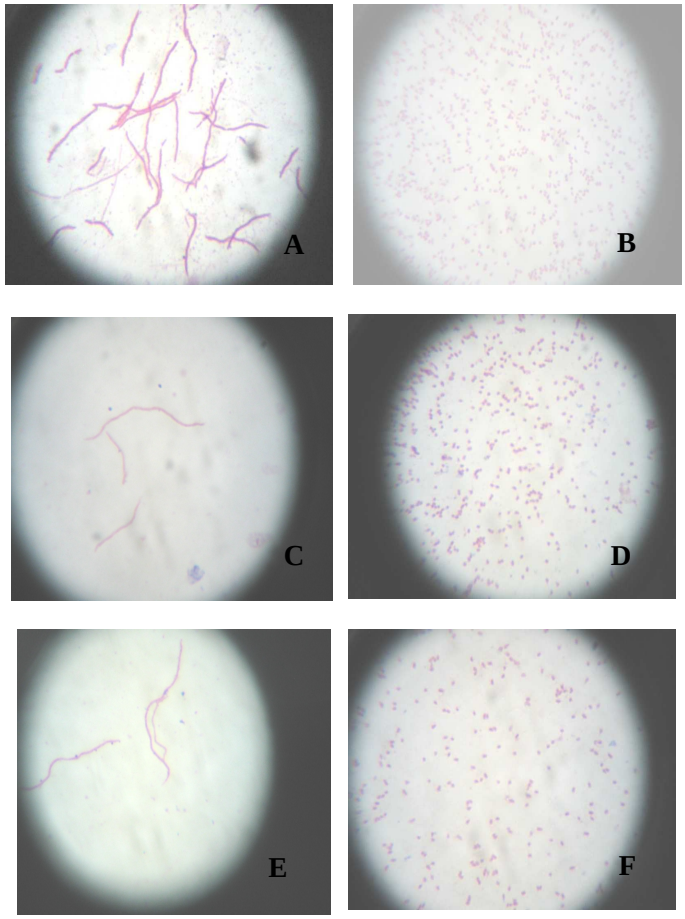

Figure 3: Morphology of *Escherichia coli* in medium containing ampicillin and chloramphenicol under induction of IPTG (A) chloramphenicol concentration at 5µg/µl and induction time at 5 hours; (B) chloramphenicol concentration at 5µg/µl and induction time at 24hours; (C) chloramphenicol concentration at 20µg/µl and induction time at 5 hours; (D) chloramphenicol concentration at 20µg/µl and induction time at 24hours; (E) chloramphenicol concentration at 34µg/µl and induction time at 5 hours; (F) chloramphenicol concentration at 34µg/µl and induction time at 24hours

Possible mechanisms of action by glucose on cell division

The minicell produced under higher chloramphenicol or exposure to chloramphenicol in the presence of glucose. The mechanism should be studied. However, the study hypothesized (1) the hydroxyl group of chloramphencol interacted

with switch I or II domain of MinD and then prevented the activation of MinC of *E. coli* and MinD-MinD because MinD homolog probably interacted with *E. coli* MinD like *Neisseria* [8] and (2) ATP and glucose were similar because they were both chemical sources of energy used by cells. They are very different in terms of composition and structure. Glucose is made up of carbon, hydrogen and oxygen only whereas ATP has phosphorus and nitrogen in addition to the aforementioned three elements. Glucose is gradually broken apart during cellular respiration, and the energized electrons from glucose are carried by the carrier molecules NAD and FAD to the electron transport chain. When the H+ concentration was built up enough, they rushed back into the inner mitochondrial space through special "ATP-maker enzymes" that span and built ATP. In the study, switch I or II domain of MinD play a role in cell division.

The study tried to overexpress *minD* gene by inducing with IPTG and glucose. The results showed that glucose could give the phenomenon of cell differentiation. Glucose is the cheap source to use in minicell generation. That will be benefit in drug delivery in pharmaceutical field. However, the mechanism of glucose used in induction of cell division *minD* gene was not understood. The study will bring out the explanation of the glucose consuming in human because MinD homolog also exist in human. The morphology alteration occured when using glucose for MinD. It meant that glucose might be play a role in cell division in human hopefully. MinD oscillation might be an other reporter which is different from the previous study [9]. More studies should be performed to understand well so far.

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