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Cell Division Gene from Bacteria in Minicell Production for Therapy

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1. Introduction

Drug resistance is one of our biggest problems in terms of cancer therapy. Chemotherapeutic drug therapy in cancer is seriously hampered by severe toxicity primarily due to indiscriminate drug distribution and consequent collateral damage to normal cells. Therefore, the cancer treatment requires the combination with pharmaceutical science, cell biology, chemistry, electronics, materials, science and technology to improve the cancer therapy development. The results of genome sequencing and studies of biological–genetic function (functional genomics) are combined with chemical, microelectronic and micro system technologies to produce medical devices, known as diagnostic 'Biochips'. The multitude of biologically active molecules is expanded by additional novel structures created with newly arranged 'gene clusters' and (bio-) catalytic chemical processes. With the nanotechnology involving the ability to arrange molecules and atoms into molecular structures, the drug development in cancer treatment is also limitted. The application of micro-machining techniques is growing rapidly and has applications in microfluidics (for labs-on-a-chip), in sensors as well as in fiber optics and displays. Nowsaday, direct-write technologies are of increasing importance in materials processing. Building the structures are made directly without the use of masks, allowing rapid prototyping. The techniques comprise plasma spray, laser particle guidance, matrix-assisted pulsed-laser evaporation, laser chemical vapor deposition, micro-pen, ink jet, e-beam, focused ion beam and several droplet micro-dispensing methods. Micrometer-scale patterns of viable cells are required for the next generation of tissue engineering, fabrication of cell-based microfluidicbiosensor arrays, and selective separation and culturing of microorganisms. The patterns of viable *Escherichia coli* bacteria have been transferred onto various substrates with laser-based forward transfer technique. These tools can be used to create three-dimensional mesoscopically engineered structures of living cells, proteins, DNA strands and antibodies and two co- fabricate electronic devices on the same substrate to generate cell-based biosensors and bioelectronic interfaces and implants. Discrete nanoparticles with controlled chemical composition and size distribution are readily synthesized using reverse micelles and microemulsions as confined reaction media, but their assembly into well-defined superstructures amenable to practical use remains a difficult and demanding task. This usually requires the initial synthesis of spherical nanoparticles, followed by further processing such as solvent evaporation, molecular crosslinking or template patterning. The

interfacial activity of reverse micelles and microemulsions can be exploited to couple nanoparticle synthesis and self-assembly over a range of length scales to produce materials with complex organization arising from the interdigitation of surfactant molecules attached to specific nanoparticle crystal faces. The construction and the evolutionary improvement of micro- and nanodevices may be carried out using evolving populations of artificial creatures. The biological life is in the control of its own means of reproduction, which generally involves in the complex, auto-catalyzing chemical reactions. However, this autonomy of the design and manufacture has not yet been realized artificially. Therefore, the requirements for cancer treatment are essential and developed rapidly with the involvement in numerous physical and chemical methodologies.

Gene therapy and tissue engineering are new concepts in the treatment of cancer disease. The oligonucleotides used in gene therapy with the potential to alter cell function for an extended period of time in relation to more established therapeutic agents. Tissue engineering aims to regrow tissue structure lost as a result of trauma or cancer through the application of engineered materials. Both fields can demonstrate initial success, such as the replacement of adenosine deaminase genes in children with severe immunodeficiency and the use of synthetic materials to accelerate healing of burns and skin ulcers. Major limitations are the low level of expression in gene therapy and the tissue engineers have not yet learned to reproduce complex architecture, such as vascular networks, which are essential for normal tissue function. A combination of both methods has been used as a new strategy to overcome problems remaining. DNA delivery has been described in situ from polymer coatings, microspheres and synthetic matrices. DNA material hybrid systems promise to enable forms of gene therapy not possible with other gene delivery systems, e.g., polymeric microspheres facilitate the expression of orally administered genes.

Besides, an F1-ATPase biomolecular motor and fabricated nanopropellers was constructed. The molecular dynamics of the F1-ATPase at the molecular level were described and the suitability of biomolecular devices as nanomolecular machines comprehensively reviewed. This chapter aims to present an integrated view of the developments on the discovery of new therapy for cancer disease.

The chapter will drive why cell division gene from bacteria may play a role in drug delivery to treat the cancer diseases. The topics will deal with the affects of hyaluronan in cell morphology of *Lactobacillus acidophilus* in minicell producing study and the role of the cell division gene from bacteria in minicell production for therapy. In order to study the minicell production from bacteria, the function of cell division genes in bacteria as well as minicells in cancer treatment. The role of *min* gene from *Streptomyces lavendulae* in minicell formation for therapy will be studied as the first contribution to cancer therapy.

2. Minicell production for therapy

Up to now, the major factor limiting cancer chemotherapeutics is toxicity to the host cells. 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 anucleate nanoparticles produced as a result of inactivating the genes controlling the normal bacterial cell division (de Boer et al., 1989) 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. Therefore, to find out many methods in the minicell formation without the above mentioned discussion is essential. This chapter will point the difficulties in minicell production in the hyaluronic acid combination and then suggest the role of minicell production in drug resistance that may be the better way for cancer treatment.

2.1 Affects of hyaluronic acid in cell morphology in *Lactobacillus acidophilus*

In order to study the role of minicell formation, this chapter had also used probiotic named *Lactobacillus acidphilus* having a positive impact to the host by helping balance the intestinal flora. Relying on the benefit properties of *Lactobacillus acidphilus*, the new application of this probiotic in minicell formation is discussed in this chapter.
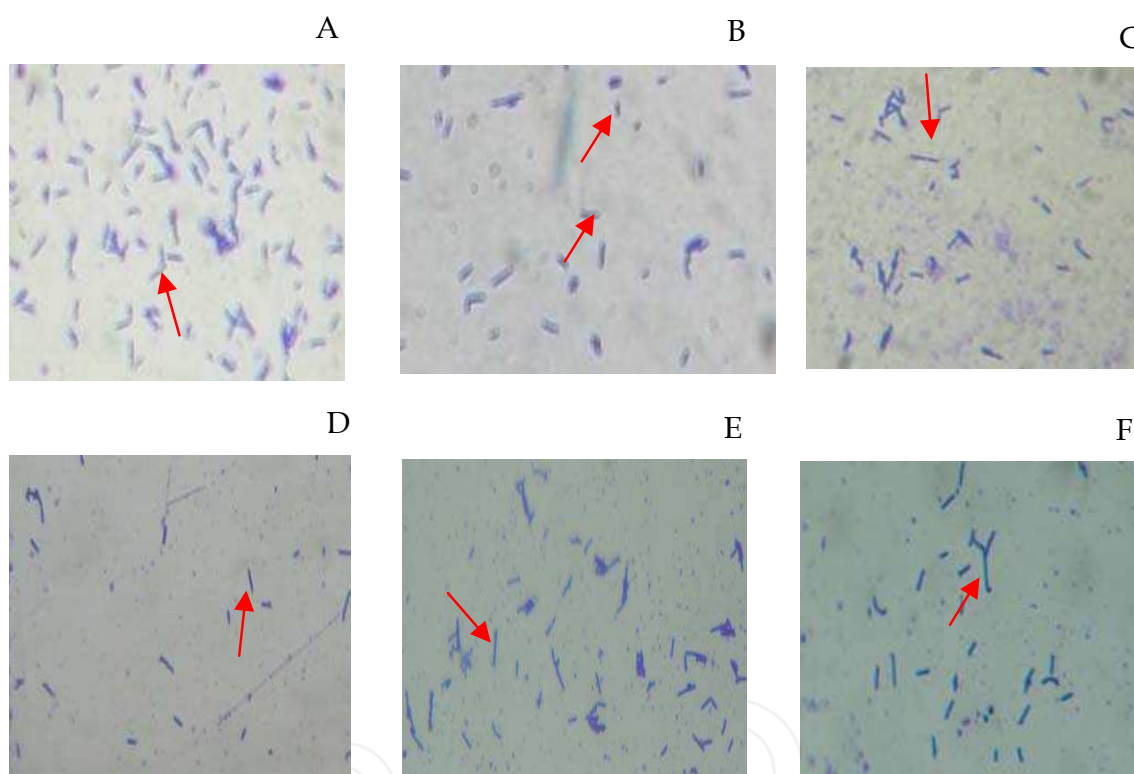

Fig. 1. The gram stainning of *L. acidophilus* with different HA concentration A: 0% ; B: 0.02%; C: 0.03% ; D: 0.05%; E: 0.08% ; F: 0.1%. The arrows showed the shape differentiation in medium with different concentration of hyaluronic acid.

The hypothesis was to apply a polymer named hyaluronic acid (HA) commonly used in foods, cosmetics, pharmaceutical field to form minicell. Besides, HA was also used to study in drug delivery. The delivery system prepared from *Lactobacillus acidophilus* and probiotic was thought to prevent many side effects in cancer treatment. *Lactobacillus acidophilus* was used and maintained in MRS agar and MRS broth. The incubation was performed at 25 – 30oC in anaerobic condition for 3-4 days. The HA was added into the MRS broth in 5 tubes to obtain the final concentrations as 0, 0.03%, 0.05%, 0.08% and 0.1%, respectively. *Lactobacillus acidophilus* in medium with 0.03%, 0.05% of HA was shorter than in the medium without HA (Fig.1C).

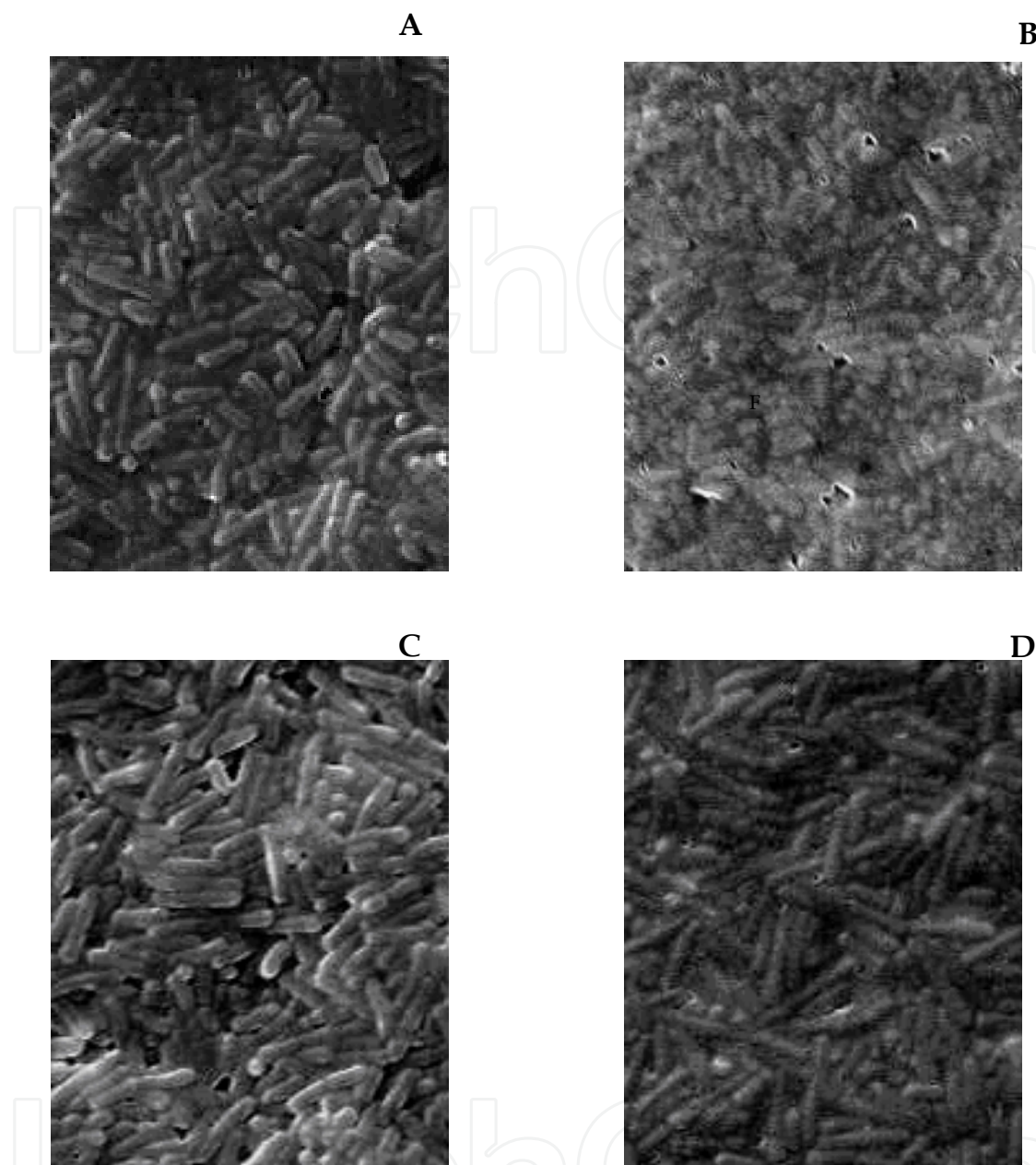**A**

B

C

D

Fig. 2. *L. acidophilus* with HA concentration at A: 0%; B: 0.03%; C: 0.08%; D: 0.1% with the zoom out of 1.300 times of scanning electron microscope. Photographs of scanning electron microscope with the scale bar is 10μm.

The cell was shortest in the medium with 0.03% HA and become longer in the medium with 0.08% and 0.1% than in the medium without HA (Fig.1E and F). To confirm the morpholgical differentiation of *L. acidophilus* with HA, the scanning electron microscope was used to observe the cells and the results were showed (Fig.2 A, B, C, D).

The differentiation of cell caused by HA depends on the different HA concentration . This results showed that the minicell phenotype produced by HA were the simple way. Maybe HA participate in the gene expression in *Lactobacillus acidophilus* that will be studied so far. Therefore, looking for the new tools to form minicell is neccesary. The chapter suggested the way to produce minicell obviously for healthcare.

2.2 Cell division gene from bacteria in minicell production for therapy
2.2.1 Min gene in cell division function

In *E. coli*, a number of proteins involved in cell division were identified by Hirota and co-workers who isolated thermo-sensitive mutants that failed to divide at elevated temperatures (Hirota et al., 1968). Since these mutants form long filaments, fts (<u>f</u>ilamentous <u>t</u>emperature <u>s</u>ensitive) was designated. Additional cell division proteins have been identified. For example, 10 proteins such as FtsZ, A, K, Q, L, B, W, I, N and ZipA, which are required for cell division, are known until now (Addinall & Holland, 2002; Buddelmeijer & Beckwith, 2002; Errington et al., 2003; Margolin, 2000; Rothfield et al., 1999). These 10 proteins assemble at the site of division into a multi-protein complex called the septal ring. Formation of this complex is thought to initiate with the assembly of FtsZ into a ring-like structure, the Z-ring, which requires the activity of at least one of the two essential FtsZ binding proteins, ZipA or FtsA (Pichoff & Lutkenhaus, 2002). Following addition of both ZipA and FtsA to the Z-ring, the remaining division proteins are recruited to mature the septal ring (Addinall & Holland, 2002; Buddelmeijer and Beckwith, 2002; Errington et al., 2003; Hale & de Boer, 2002; Mangolin, 2000; Pichoff & Lutkenhaus, 2002; Rothfield et al., 1999). After the assembly is completed, the proteins for the formation of the septal ring function in concert to mediate the coordinated invagination of three cell envelope layers such as the inner membrane, the peptidoglycan layer, and the outer membrane. Unfortunately, very little is known about the roles of many of the division proteins in this process. Spatial regulation of Z-ring assembly by the Min system is independent of nucleoid occlusion, which is evident in anucleate cells produced by chromosome segregation mutants. In the cells, the Z-rings assemble at or near mid-cell, suggesting that the Min system alone is sufficient to regulate the proper placement of the Z-ring (Sun et al., 1998). In contrast, in cells lacking a functional Min system, nucleoid occlusion is not sufficient to direct Z-ring assembly, and the division occurs consequently at any one of the three potential division sites dictated by the nucleoid occlusion. If the cell division occurs at a polar potential division site, a chromosomeless-minicell and a multinucleate filament are produced (Adler et al., 1967; de Boer et al., 1989). The first minicell mutant in *E. coli* has been isolated in 1967 (Adler et al., 1967). The mutations responsible for the minicell phenotype were mapped to the *minB* locus (Adler et al., 1967), which was later cloned and characterized (de Boer et al., 1989). The *minB* locus encodes three proteins, designated MinC, MinD, and MinE, all of which are required for proper the Min system function. In *B. subtilis*, the Min system includes MinC, MinD and DivIVA.

2.2.2 MinC

MinC comprised of two domains of roughly equal size (de Boer et al., 1989; Hu & Lutkemhaus, 2001). The *N*-terminal domain interacts with FtsZ and is required and sufficient to inhibit FtsZ polymerization in vitro and Z-ring formation in vivo (Hu & Lutkenhaus, 2003). The mechanistic basis of this inhibition is unknown. The C-terminal domain is required for homodimerization of the protein and for interaction with activators of Min function, MinD and DicB (Hu & Lutkenhaus, 2003; Johnson et al., 2002; Szeto et al., 2001b). The DicB protein is encoded on the cryptic prophage Kim, and under normal conditions, expression of DicB is actively repressed (Cam et al., 1988). In the absence of these activators, MinC blocks cell division only when present at a level at least 25-fold greater than normal. In the presence of either activator, physiological levels of MinC are sufficient to inhibit division (de Boer et al., 1990; 1992b).

2.2.3 MinD

MinD is a part of the ParA superfamily of ATPase and is the most highly conserved of the Min proteins (Szeto et al., 2003). It generally associates with the inner membrane and is required for the binding and activation of MinC (Zhou et al., 2004). It contains five distinct domains. Three of which (P-loop, Switch I and Switch II) are thought to be important for ATP-binding (Gérard et al., 1998). The P-loop and switch II sites are deviant Walker A and B-type ATP binding motifs. Mutations in the P-loop, Switch I and Switch II domains are effective to ATP-binding causing the mislocalization of MinD and a concurrent mislocalization of MinC (Karoui et al., 2001). This suggests that ATP-binding is essential for membrane binding and activation of MinC. The ATPase domain is also required for an appropriate MinE-dependent localization to the cell poles (Maston et al., 1999b). Mutations in the ATPase domain of *B. subtilis* MinD are no longer restricted to the cell poles (Karoui et al., 2001). Instead, they diffuse throughout the inner membrane, suggesting a loss of interaction with DivIVA. However, MinD can localize to the division sites, in the presence or absence of DivIVA (Lee & Price, 1993). Mutations of the Switch I and Switch II in *E. coli* disrupt MinC activation, but not MinD localization (Zhou et al., 2004). Initial attempts for *B. subtilis* MinD with GFP (green fluorescence protein) at the *C*-terminal end have suggested that the protein does not function (Marston et al., 1998). Sequence analysis revealed a conserved *C*-terminal amphipathic helix (Hu et al., 2003; Szeto et al., 2002), that was likely disrupted by the addition of GFP. Even though structure prediction algorithms and the crystal structure failed to predict any membrane or membrane binding domains (Hayashi et al., 2001), deletion analysis has shown that the last 10 amino acids are required for membrane binding in *E. coli* (Zhou et al., 2003). *B. subtilis* also contains a *C*-terminal amphipathic helix required for membrane binding (Szeto et al., 2002). Truncations or mutations in this region disrupt MinD's ability to bind the membrane. Mutations that allow MinC activation but disrupt DivIVA interaction have also been identified (Karoui et al., 2001). *E. coli* MinD binds the membrane in an ATP-dependent manner in vitro (Lackner et al., 2003). MinD will associates with the phospholipids vesicles in the presence of ATP and Mg^{2+}, while the ADP-bound form will not. MinE stimulates the ATPase activity of MinD to release it from the membrane. Substitution with non hydrolysable forms of ATP cause MinD to remain associated with the membrane even in the presence of MinE. The ability of MinE to release MinD, and thus MinC, from the membrane is thought to be the driving force behind the oscillations in *E. coli* (Howard et al., 2001).

2.2.4 MinE

MinE is an 88 amino acid protein with two known functional domains. The *N*-terminal domain ([D]MinE, amino acids 1-33) is required and sufficient to interact with MinD and to counteract MinCD-mediated division inhibition (Huang et al., 1996). The *C*-terminal topological specificity domain ([TS]MinE, amino acids 34-88) is required to suppress the inhibitory activities of MinCD specifically at mid-cell. Additionally, this domain mediates homodimerization of the protein (King et al., 1999; 2000; Pichoff et al., 2002). The structure of the C-terminal domain has been solved (King et al., 2000), revealing that the protein dimerizes in an anti-parallel fashion. The *N*-terminal domain is not shown in the structure, but is predicted to be a nascent helix (King et al., 1999).

2.2.5 DivIVA

A major difference between *E. coli* and *B. subtilis* is that *B. subtilis* uses DivIVA to sequester MinCD to the cell poles. Unlike the MinCD oscillations caused by MinE in *E. coli*, the

localization of *B. subtilis* MinCD is static (Edwards et al., 1997). Furthermore, *E. coli* does not contain a DivIVA homologue, nor does *B. subtilis* have a MinE homologue (Lee & Price, 1993). DivIVA in *B. subtilis* is a 19.5 kDa protein, but soluble DivIVA exists as a 145 kDa oligomer *in vivo* (Muchova et al., 2002). The structure of membrane associated DivVIA (~40%) remains unknown. Transmissible electron microscope images of DivIVA (Glu162Lys), a functional point mutant, reveals a 'doggy-bone' like structure capable of further assembly into two dimensional structures (Stahler et al., 2004). DivIVA localizes to the poles and midcell division sites, sometime, during the assembly of the cell division machinery. The timing and mechanism of localization is still unclear. After division, DivIVA remaining at the division sites is not essential, however. DivIVA must then have some function beyond that of localizing MinCD. DivIVA$_{SC}$ from the *S. coelicolor*, which is homologues to DivIVA from *Bacillus subtilis*, is essential and directly involved in hyphal tip growth and morphogenesis. A DivIVA$_{SC}$-EGFP hybrid was distinctively localized to hyphal tips and lateral branches. DivIVA$_{SC}$ is a novel bacterial morphogenesis, and it is closely localized at the apical sites of peptidoglycan assembly in *Streptomyces* hyphae.

2.2.6 Min system dynamics regulate the activity of MinC

The cell division is determined by the cellular distribution of MinC, which is regulated by both MinD and MinE. In addition, MinD and MinE influence the other cellular distribution. MinD, MinC and MinE are cytoplasmic, and MinC is unable to efficiently inhabit Z-ring assembly (Hu & Lutkenhaus, 2001). As a result, cells show a minicelling division phenotype (Min cells), where the cells frequently divide close to either cell pole (de Boer et al., 1989). In MinE cells, MinC and MinD are assembled along the entire membrane. This static, uniform distribution of MinC blocks the Z-ring assembly at all sites, resulting in the formation of long, non-septate filamentous cell (Sep-cells) (de Boer et al., 1989; Hu & Lutkenhaus, 2001; Rowland et al., 2000). In cells expressing three Min proteins (MinC, D and E), a wild-type scenario, MinC undergoes a rapid and dynamic localization cycle in which the protein oscillates from one cell pole to other every 20-30 seconds (Hu and Lutkenhaus, 2001). As the result of this pole-to-pole oscillation, the time-averaged concentration of the division inhibitor is greatest as the cell poles and lowest at mid-cell, and this concentration differential is proposed to direct Z-ring assembly to mid-cell (Hale et al., 2001; Howard et al., 2001; Huang et al., 2003; Kruse, 2002; Meinhardt and de Boer, 2001). This dynamic distribution of MinC is driven by MinD and MinE, which themselves undergo a coupled oscillatory localization cycle (Hale et al., 2001, Shih et al., 2002). At the start of this cycle, MinD and a portion of MinE assemble on the membrane along one half of the cell in a pattern resembling a test tube (MinD/E tube), while another portion of MinE assembles at the rim of this tube forming a ring (E-ring). The MinD/E tube undergoes a continuous cycle of disassembly from the membrane at one cell end and concurrent reassembly on the membrane at the opposite cell end. The E-ring closely follows the location of the MinD/E tube, always appearing to be associated with its rim. MinC plays no role in the mechanism of oscillation, and the cellular location of MinC follows that of MinD (Hale et al., 2001; Rowland et al., 2000). While the MinD/E tube and E-ring do not appear to have an underlying structure in live cells analyzed by conventional 2-dimensional fluorescence microscopy, spiral-like accumulations of the proteins in what would represent the MinD/E tube and E-ring have been observed in fixed cells analyzed by 3-dimensional deconvolution microscopy. This observation suggests that the bulk of Min protein dynamics occurs along spiral tracks within the cell (Shih et al., 2003).

2.2.7 MinCD during sporulation

Deletions in the MinCD locus cause a decrease in sporulation of nearly ten fold (Barak et al., 1998) although the relative sporulation efficiency is nearly identical to wild-type. The cause of the decrease is unclear as MinCD's role in sporulation has not been well examined. *B. subtilis* with null mutations in MinCD form thin, sporulation like septa at the midcell, suggesting that MinCD is helpful to inhibit sporulation septa from forming at the midcell. Since its role during vegetative growth is to prevent FtsZ ring formation at the poles, MinCD may also trigger the switch from midcell division to polar division in conjunction with spo0A. It's also been suggested that MinCD confers polarity to SpoIIIE dependent chromosome translocation by inhibiting the assembly of forespore expressed SpoIIIE (Sharp et al., 2002). In the absence of MinCD, SpoIIIE can assemble on the forespore side of the septum and can reverse translocate DNA into the mother cell leading to anucleate forespores.

2.2.8 Conservation of Min proteins

MinD is well conserved among many bacteria and archaea and is also found in number of eukaryotic chloroplasts (Gérard et al., 1998). Interestingly, this high degree of conservation is not shared with MinC and/or MinE suggesting that the mechanism by which MinD homologues lack MinC and/or MinE regulates division site placement is not conserved in all organisms (Margolin, 2000; Rothfield et al., 1999). For example, *B. subtilis* has both MinC and MinD but lacks MinE. In *B. subtilis*, DivIVA imparts topological specificity to MinCD-mediated division inhibition in a manner very different from that of MinE (Cha & Stewart, 1997). DivIVA, which stably associates with cell poles, recruits MinCD to the poles and thereby, spatially restricts the inhibitory activity of the complex to the polar potential division sites (Marston & Errington, 1999b; Marston et al., 1998). Therefore, the Min protein oscillation is not required to spatially regulate the Z-ring assembly in all organisms.

2.2.9 Minicells in cancer treatment

Molecularly targeted drugs such as cell cycle inhibitors are being developed to achieve a higher degree of tumor cell specificity and reduce toxic side effects. Unfortunately, relative to the cytotoxics, many of the molecularly targeted drugs are less potent and the target protein is expressed only at certain stages of the cell cycle thus necessitating regimens like continuous infusion therapy to arrest a significant number of tumor cells in a heterogeneous tumor mass (Jennifer et al., 2007). Therefore, trend to produce minicells or nanocells from non-pathogen bacteria is essential. The minicells were made from bacteria and contained pieces of genetic material, known as short interference RNA (siRNA), which knockout or "silence" the drug-resistant genes of tumours. Bacterial membranes might be quite different because they have protein channels (in their membrane) through which siRNAs can enter (Jennifer et al., 2009). By means of the receptors in the tumor surface, the minicell will specify and deliver the drugs to the tumors. Minicells are produce by minicell producing parent cells. These parent cells undergo cell division in an abnormal manner that produces a chromosomal-containing cell and a minicell lacking a copy of the parental chromosome. Minicells are often smaller than their parent cells. For example, minicells produced from E. coli are generally spherical in shape and are about 0.1 to about 0.3 μm, whereas whole E. coli cells are about 2 to about 10μm in length. The minicell can be tagged with siRNA that is also play a role in disease prevention and drug resistant reduce. It was discovered that an

unprecedented concentration of one million to ten million drug molecules can be packaged within a minicell. In contrast, other nanovectors such as liposomes have been shown to package ~10,000 molecules of drug within each liposome. Similarly, armed antibodies can conjugate only four to ten drug molecules per antibody. The potency of observed anti-tumor effects may depend on the concentration of a drug that is delivered intracellularly within cancer cells. At present, the cancer treatment is the big pressure in the world because of the high cost for treatment such as the high price of monoclonal antibodies. This is the difficulties of the pharmaceutical companies which did not supply enough demands wheras minicell will made the cost reduce and delivers the small amounts of many drugs intracellularly and then decrease the drug resistance of the cell (Jennifer et al., 2007). To get improvement of minicells, the research of *min* gene encoding for cell division will be done to understand about the new approach for drug treatment. The *min* gene was cloned and studied for the function. The generated minicells or nanocells during gene expression will be used to study as the drug delivery to cancer cell.

2.2.10 Role of *min* gene from *Streptomyces lavendulae* in minicell formation for therapy

There are many cell division genes in bacteria summarized as above. However, only one *minD* gene exists in the human chromosome. To understand the role of minicell in drug delivery in human, the study tried to find the diversity of *minD* gene in the bacteria which have the linear structure as human chromosome that belong to *Streptomyces. Streptomyces* have a complex morphological differentiation and are well-known for their ability to produce an enormous variety of bioactive secondary metabolites, such as antibiotics and immunosuppressant drugs (Wright et al., 1976b).The *minD* gene *Streptomyces coelicolor* and *Streptomyces avernitilis* genome have been sequenced completely (Ikeda et al., 2003; Betley et al., 2002). In *Streptomyces*, there are *minD1, minD2* or *minD3* depending on the these strains. Clearly, the *min* gene cluster has also tried to obtain in *Streptomyces lavendulae*. Because *Streptomyces coelicolor* and *Streptomyces avernitilis* are well studied, the aim of the *minD* gene study focused on *Streptomyces lavendulae*. The cloned *min* gene was expressed in many way to analyze the functional regulations of cell division. The goal is to develop the effects of Min homolog harbored by *Streptomyces* in morphological change, especially the minicell and filamentous morphology in *Escherichia coli*. The minicells generated by *min* gene expression gave many hopes for cancer- targeted drug delivery in cancer therapy while the chemotherapeutic therapy used nowadays are not specific but also toxic to cancer cell.

The introduction of *min* gene with the sequence containing 49 nucleotides at upstream of *min* gene gave the apparent morphological alteration into minicells when using the pET 28a(+) as expression vector while the introduction of *min* gene gave the filamentous under the pET 21a(+). To construct *E. coli* expressing the *S. lavendulae min* gene, a PCR with primers as 5'- CAC*CATATG*ACCACTCGAATCCTCCCCGC- 3' and 5'- CAC*CTCGAG*GCC CTCCCGGCCGCGC- 3' was performed. The product was treated with *NdeI* and *XhoI* (italic sites), and inserted into pET 21a(+) to create pET 21a(+)/*min*. To amplify upregulated-*min* gene (*min* containing 49-bp upstream of *min*), PCR primers (5'- GGA*ATTCCA*GTCCCACCGACCGCACGTAC-3'and 5'-CCG*CTCGAG*CGGGCCACGAGC AGCAGGATC-3) were designed. The resulting amplified product was digested with *EcoRI* and *XhoI* (italic sites) and inserted into pET 28a(+) to create pET 28a(+)/upregulated-*minDSL*.

Transformation the constructed plasmids in *E. coli* were performed. *E. coli* BL21(DE3) harboring pET 21a(+)/*min* and pET 28a(+)/upregulated-*min* was grown at 37°C in 1L of an LB medium to OD600=0.5, whereupon isopropyl-D-thiogalacto-pyranoside (IPTG) was added to culture at the final concentration of 0.5 mM to induce the expression of Min. The *E. coli* cells were grown for 5.5 h at 28°C and observed by microscope. Introduction of pET 21a(+)/*min* into *E. coli* BL21(DE3) cell caused a filamentous shape, as observed by a light microscope (Fig. 3B) and scanning electron microscope (Fig. 3D). The investigation of
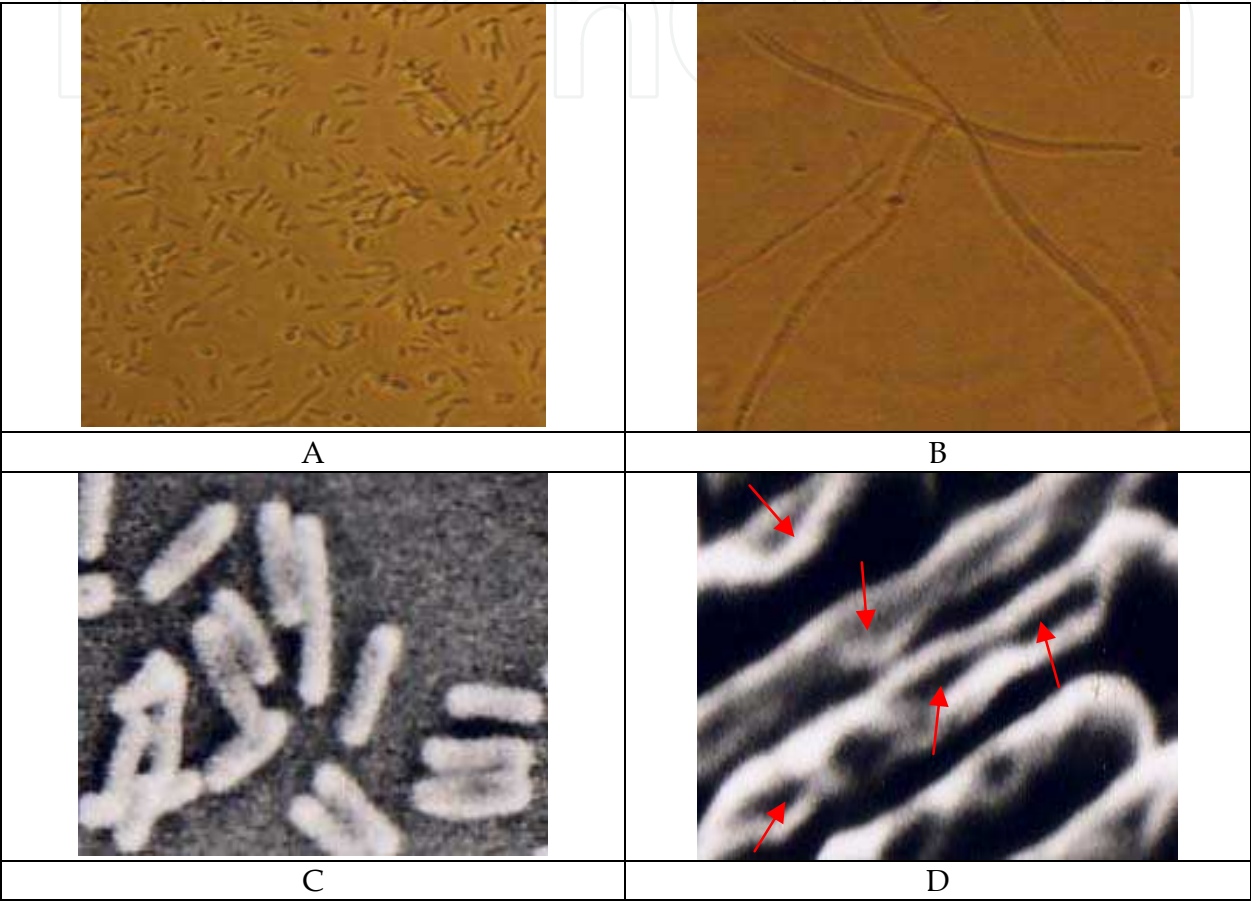
Fig. 3. The cell shapes of *E. coli* BL21 (DE3) carrying *min* gene when using pET 21a(+) as an expression vector. (A) and (B) are the pictures filmed by light microscope, but (C) and (D) are the picture filmed by scanning electron microscope. *E. coli* BL21 (DE3) transformed with pET 21a(+) is a typical rod shape, as shown in (A) and (C). The same host transformed with pET 21a(+)/*min* exhibits a filamentous phenotype, as shown in (B) and (D). Red arrows show the indentations along the cells.Photographs of light microscope with the scale bar is 5 m, and both figures are at the same magnification. Photographs of scanning electron microscope with the scale bar is 1 m.

minicell production indicates that the production of minicells is blocked when maltose was added in the culture. By the microscopic analysis, the indentations in the cell wall of *E. coli* reflect the *min* gene may be play a role in cellular mechanism. The relationship between phenotype differentiation and drug resistance was not clear. However, in the *E. coli* system with the overexpression of MinCDE, the function of *min* gene from *Streptomyces* was activated by means of the putative primary promoter upstream of *min* gene along with the

selective conditions of environment. The interaction between Min proteins in *E. coli* in the different cultured conditions maybe also affected on the cells. This hypothesis should be studied before the minicells formed by this way will be applied in treatment.

To facilitate further study the function of Min protein, the *E. coli* BL21(DE3) carrying the pET 28a(+)/upregulated-*min* gene carrying plasmid was overexpressed. Under the presence of IPTG, the transformant took a minicell phenotype (Fig. 4B), whereas the same cell transformed with pET 28a(+) exhibited a normal rod-shaped morphology (Fig. 4A). The minicells are produced by a process that is similar to the normal cell division except that it occurs near one or both poles of the cell. The division process, that generates the minicells,
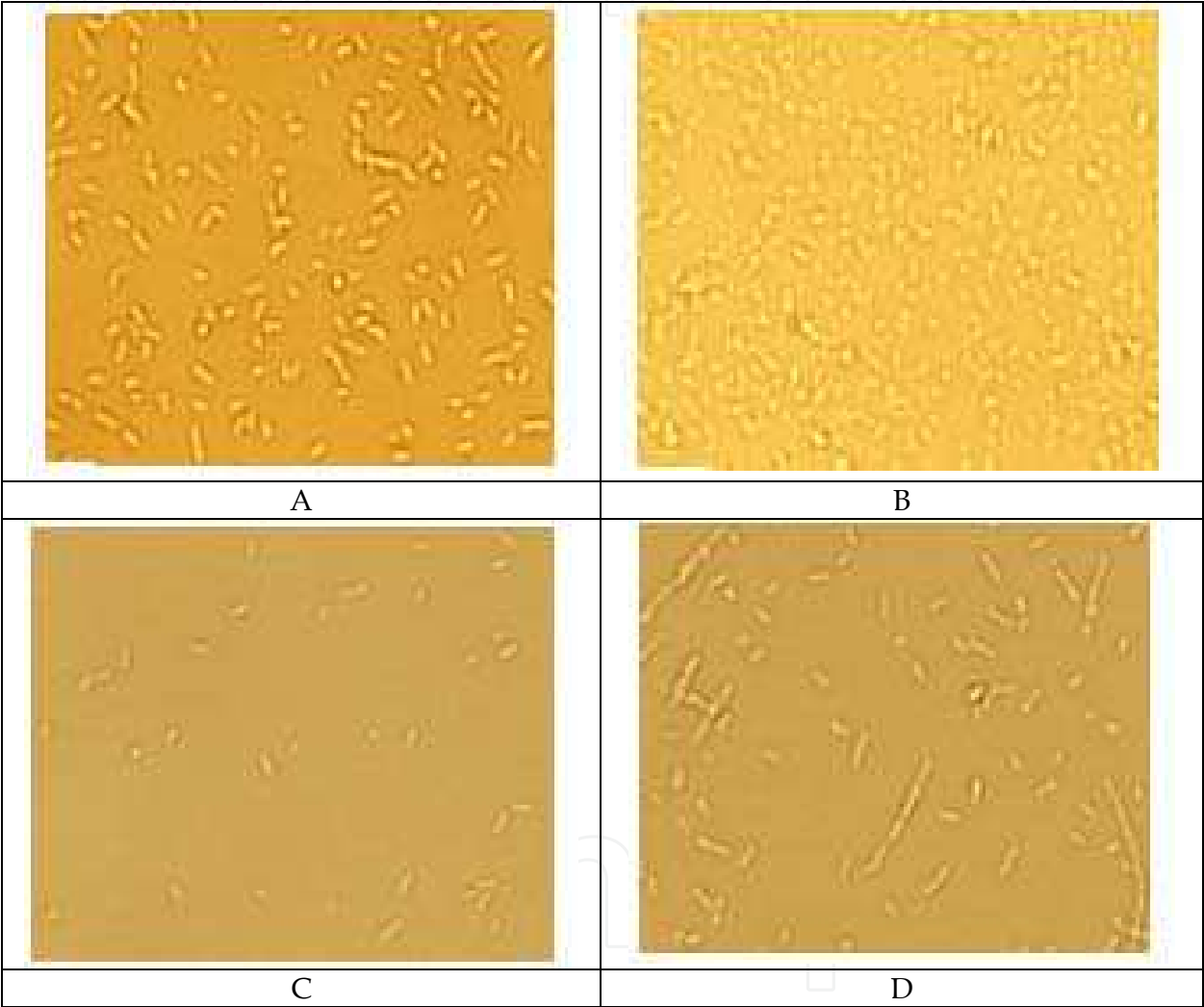

Fig. 4. The cell shapes of *E. coli* BL21 (DE3) carrying *min* gene when using pET 28a(+) as an expression vector.(A) *E.coli* BL21(DE3) cells were transformed with pET 28a(+). (B) *E. coli* BL21(DE3) cells were transformed pET 28a(+)/upregulated-*min*. (C) Phenotype of *E.coli* BL21 (DE3) cells were transformed with pET 28a(+) in LB medium supplemented with maltose (1%). (D) *E. coli* BL21(DE3) cells were transformed pET 28a(+)/ upregulated-*min* in LB medium supplemented with maltose (1%). Photographs of light microscope with the scale bar is 5m, and both figures are at the same magnification.

seems to be "normal" in many ways. In this case, DNA is not distributed to both sides of the division plane. The production of minicells is a consequence of the unusual genetic constitution and does not require any unusual manipulation of the environment. They has anticipated that the minicell phenotype will be valuable in a variety of genetic and biochemical studies. During morphological change in bacteria, the Min expression caused different solubility (Nguyen et al., 2008; Nguyen, 2010). Because the morphological change, the physiological differentiation concerning to drug resistant may also be effected. That is the why min gene is essential for cell division and viability in *E. coli*. The *E. coli* BL21(DE3) carrying the pET 28a(+)/upregulated-*min* carrying plasmid was overexpressed under the presence of maltose, the cell are longer than the *E. coli* BL21(DE3) carrying the pET 28a(+) (Fig. 4C, D). In a conclusion, the overexpression of *S. lavendulae minDSL* in *E. coli* causes the cellular shape change. That is the consequence of the *min* gene in fused construction that results in the filamentous shape, whereas the native Min corrects the minicell phenotype in *E. coli* (Fig. 4B). Since the minicell phenotype will be valuable in a variety of genetic and biochemical studies, a native Min protein was purified. After *E. coli* BL21 (DE3) carrying *min* was grown, the cell mass was collected in the inclusion body from the cell-free extract. Washing the inclusion body with 1% n-decyl-D-glucopyranoside was useful to reduce the contaminated proteins. The washed inclusion body was dissolved in a buffer containing 1% n-decyl-D-maltopyranoside. The Min protein was purified from the inclusion body, as described in the experimental procedures section. The Min protein was purified to homogeneity, as shown in Fig. 5. This procedure showed the difference of the native Min protein properties from the tagged one. The experiment pointed out the morphological differentiation as well as the different expressing ways caused by *min* gene. Probably the *min* gene of *Streptomyces* had any effects on *Escherichia coli*. The result supported that the application of minicells obtained by this procedure should be studied well before used as drug delivery.
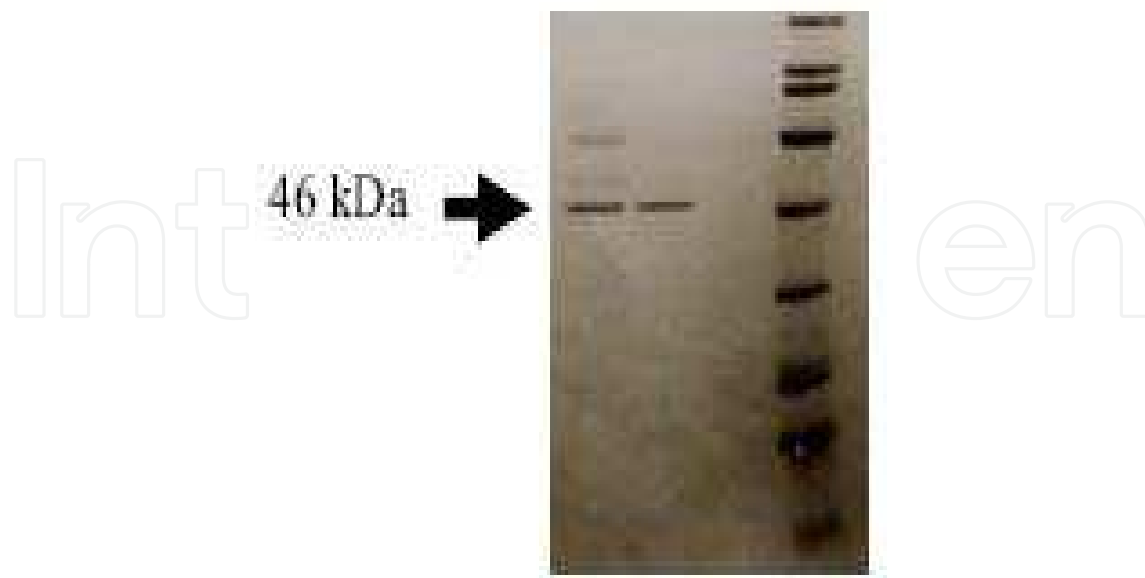

Fig. 5. Purification of Min protein was analyzed by Tricine-SDS-PAGE. The arrow showed the purified 46 kDa of Min protein after it was dissolved in a buffer containing 1% n-decyl-D-maltopyranoside.

Because there were the shape changes in *E. coli* transformed by these constructions as pET 21a(+), pET 28a(+), pET 21a(+)/ *min*, pET 28a(+)/upregulated-*min*, the drug resistance assay of minicells was also performed curiously. Interestingly, there was also drug resistance. The test was measured the turbidity after the *E. coli* were cultured with D-cycloserine at the interval time. To investigate the role of *min* gene in antibiotic resistance, the D-cycloserine resistant assay was performed. The results were in fig. 6. Remarkably, the resistance in minicells carrying pET 28a(+)/upregulated-*min* was the highest than the filament cells pET 21a(+)/ *min* and other controls. As the result, the minicell formation will give a mission in drug delivery and drug resistance in tuberculosis but in cancer diseases. More studies will be done so far to explain the mechanisms and to give more design for drug delivery in cancer therapy.
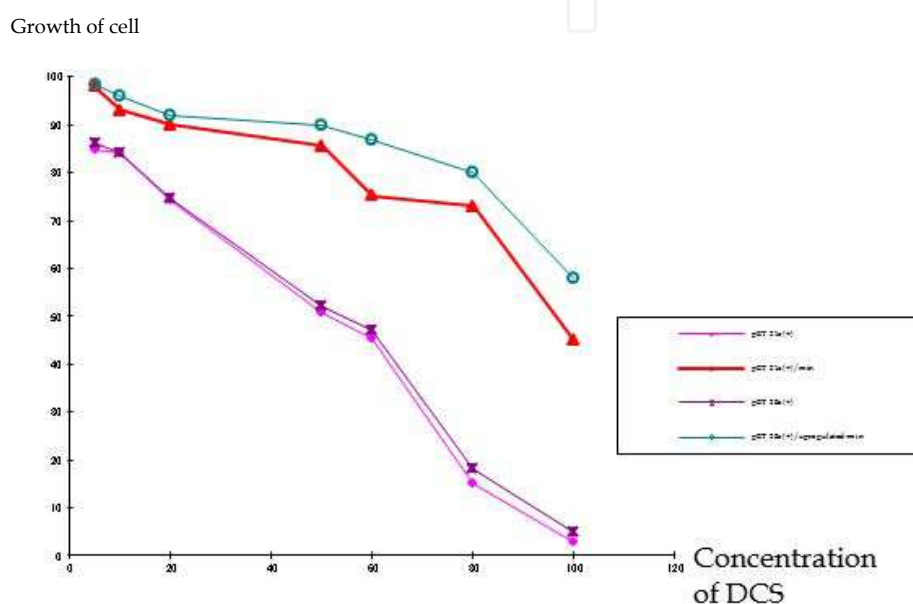

Fig. 6. D-cycloserine resistance assay in *E. coli*. the resistance in minicells carrying pET 28a(+)/upregulated-*min* was the highest than the filament cells pET 21a(+)/ *min* and the controls.

3. Conclusion

The phenotype differentiation and drug resistance in *Escherichia coli* were not clear. However, by the microscopic analysis, the indentations in the cell wall reflect that *min* may play a role in cellular mechanism. Our experiment suggests the considerations that bacterial shapes are not accidental but are biologically important. In the *E. coli* system with the overexpression of MinCDE, the function of Min protein from *S. lavendulae* was activated by means of the putative primary promoter upstream of *min* gene along with the selective conditions of environment. The chapter showed the effects of *min* gene of *Streptomyces* in *E. coli* in the formation of minicell phenotype which may participate in drug resistance that is interested in cancer treatment and vaccine preparation. The minicells produced by the expression of *min* gene with the upstream gave the high resistance of D-cycloserine, an antibiotic for tuberculosis treatment. This chapter provided the benefit of bacterial minicells which play in drug delivery and resistant to the drug used in cancer treatment. In the other hands, the *min* gene in human containing F1-ATPase is also similar to *min* genes bacteria. The purified Min protein (Fig. 5) showed the activity of an ATPase (data not shown). The

molecular dynamics of the F1-ATPase at the molecular level will be studied to develop the biomolecular devices as nanomolecular machines for therapy.

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