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RESEARCH ARTICLE

Cytotoxic effect of biosurfactants produced by novel thermophillic Geobacillus thermoleovorans (JQ 912239).

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Abstract

In recent years, interest has been growing in the cytotoxic effect of biosurfactant. The aim of this study was to investigate the cytotoxic effect of biosurfactant on breast cancer (MCF7) cell line. *Geobacillus thermoleovorans* (JQ 912239) gave good result for biosurfactant production with emulsification index and surface tension reduction. MCF7 cell line was exposed to different concentration of biosurfactant and apoptotic death was assessed by monitoring cell accompanied by caused a significant decrease of cell count, with significant increase in cell permeability, cytochrome C releasing from mitochondria, and nucleus intensity and a significant reduction in mitochondrial membrane potential of MCF7 cells.

Introduction

Hot environments are between the supporting life extreme niches that appear to have maintained some degree of pristine quality and of special biotechnological interest. These habitats have attracted broad interest because of the unique thermophilic properties of the organisms thriving in these biotopes and the description of an increasing number of new thermophilic species (Satyanarayan *et al*., 2005; Tomova *et al*., 2010).

The genus *Geobacillus* was established in 2001 with the following key characteristics: Rod shaped cells producing one endospore per cell, cells may be single or in short chains and may have peritrichous flagella. Cells have a gram-positive cell wall structure but the gram stain may vary. Chemo-organotrophs, which are aerobic or facultatively anaerobic using oxygen as the terminal electron acceptor, replaced by nitrate in some species (Marchent *et al*., 2002).

An extremely thermophillic and alkane degrading *Geobacillus thermoleovorans* B23 was previously isolated from deep subsurface oil reservoir in Japan (Kato *et al.,* 2001; Nazina *et al.,* 2001), this strain effectively degraded alkanes at 70^{o}C with the carbon chain longer than twelve, dodecane. Since tetradecanoate and hexadecanoate or pentadecanoate and heptadecanoate were accumulated as degradation intermediates of hexadecane or heptadecane respectively (Kato *et al.,* 2009).

Biosurfactant are amphiphilic compounds. Contain hydrophobic and hydrophilic moieties that reduce surface tension and interfacial tension between individual molecules at the surface and interface respectively (Mulligan, 2005).Biosurfactant produced by a wide variety of microorganisms such as bacteria, yeasts and fungi as membrane components or secondary metabolites (Gautam and Tyagi, 2006). The major classes of biosurfactant include

glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants and particulate surfactants (Cameotra and Makkar, 2004; Salihu *et al.*, 2009).

Many researcher elucidated on the wide range of applications of biosurfactant in medicine such as antibacterial, antifungal and antiviral activities make them relevant molecules for applications in combating many diseases and as therapeutic agents (Banat *et al.*, 2010).

The problems of systemic toxicity and drug resistance in cancer chemotherapy urge the continuing discovery of new anticancer agents. It is explored the specific anticancer activity from microbial metabolites to find new compound.

One of the most thrilling results that have been recently reported for biosurfactant is their potential to act as anti-tumor agents interfering with some cancer progression processes (Rodrigues, 2011; Fracchia *et al.*, 2012). Biosurfactant induce apoptosis and differentiation of the many cancerous cells line.

Screening potential drugs for toxicity is an essential aspect of the drug discovery process. In vitro toxicity assessments performed early in drug discovery are cost-effective and fast. Cytotoxicity is a complex process affecting multiple parameters and pathways. After toxic insult, cells often undergo either apoptosis or necrosis (Taylor *et al.*, 2007).

Furthermore the serratamolide AT514, cyclic depsipeptide from *S. marcescens,* belonging to the group of serrawettins, has also been reported to be a potent inducer of apoptosis of several cell lines derived from various human tumors and B-chronic lymphocytic leukemia cells, primarily involving the mitochondria-mediated apoptotic pathway and interference with Akt/NF-kB survival signals (Matsuyama *et al.*, 2010).

According to the great importance of biosurfactant, this study aimed to investigate the cytotoxic effect of microbial biosurfactant produce by *Geobacillus thermoleovorans* (JQ 912239) against MCF7 cell line.

2. Materials and methods:

Bacterial strain:

Geobacillus thermoleovorans (JQ 912239) was isolated in a previous study (AL- Jailawi *et al.*, 2013). The strain was optimized for good productivity of biosurfactant (AL-Jailawi *et al*., 2015), and this biosurfactant was extracted and purified according to Jara *et al.*, (2013) to use it.

Cytotoxic effect of biosurfactants:

Cell Preparation:

MCF7 cells were inoculated in EMEM medium containing the following supplements: 10% fetal bovine serum, sodium pyruvate, non-essential amino acids, penicillin and streptomycin. Cell was split when they reached 90% confluence at a dilution of 1:4. cells at a passage number ≤ 10 was used, then harvested by trypsinization, diluted into EMEM complete medium and cell density was determined. Cells were diluted to 7.5×10^4 cells/ml in EMEM complete medium. The cell suspension (100 µl) was added to each well of a 96-well microplate to achieve 7,500 cells/well and incubated overnight at 37°C in 5% CO_2.

Cell treatment:

A volume 25 µl of doxorobin at concentration 50 µg/ml and purified biosurfactant at concentration 50, 100 µg/ml was added separately to cells. Cells were incubated at 37°C for 24hours. Then 50 µl of Live Cell Staining Solution was added to each well and incubated at 37°C for 30 minutes. The medium and the staining solution was gently aspirated and 100 µl/well of Fixation Solution was added, plate was incubated for 20 minutes at room temperature. Then the Fixation Solution was gently aspirated and 100 µl/well of 1X Wash Buffer was added. Washing Buffer was removed and 100 µl/well of 1X permeabilization Buffer was added. Plate was incubated for 10 minutes at room temperature and protected from light. The permeabilization buffer was aspirated and plate was washed twice with 100 µl/well of 1X Wash Buffer. Wash buffer was aspirated and 100 µl of 1x blocking buffer was added and plate was incubated for 15 minutes at room temperature.

Blocking Buffer was aspirated and 50 µl/well of primary antibody solution was added. Plate was incubated for 60 minutes protected from light at room temperature. Primary antibody solution was aspirated and plate was washed three times with wash buffer. Wash buffer was aspirated and 50 µl/well of Secondary Antibody/Staining solution. Incubate for 60 minutes protected from light at room temperature. Secondary antibody/staining solution were aspirated and wash the plate three times with 100 µl/well of Wash Buffer. Finally wash buffer was added and the plate was sealed and evaluated on the Array Scan HCS Reader (Vivek *et al.*, 2008).

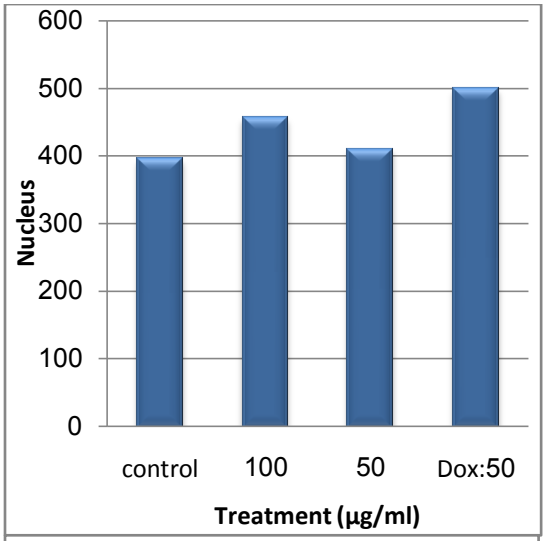
3. Results and Disscusion

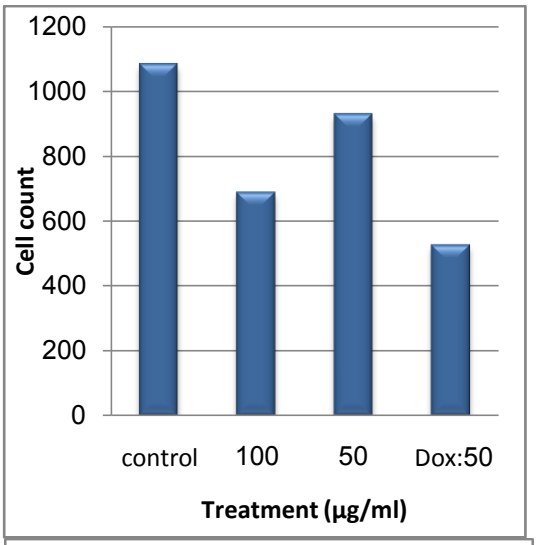
Bacterial Strain:

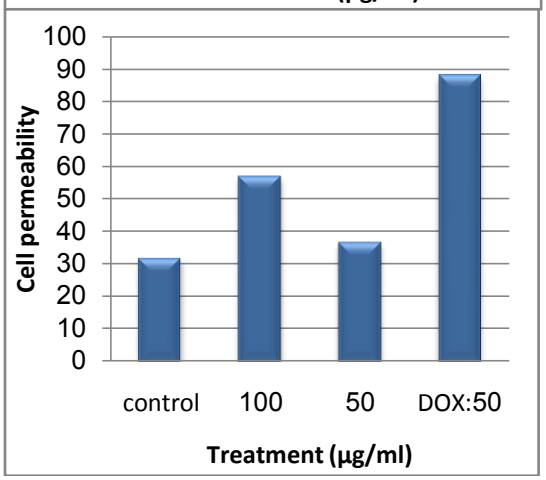
Geobacillus thermoleovorans (JQ 912239) was obtained from previous study (AL-Jailawi *et al.*, 2013). This strain showed good ability to emulsify crude oil (Unpublished data). This bacterium gave the highest emulsification index (87%) and the lower surface tension (43 mNm^{-1}) when grown under optimul conditions for biosurfactant production (Al-Jailawi *et al*., 2015).

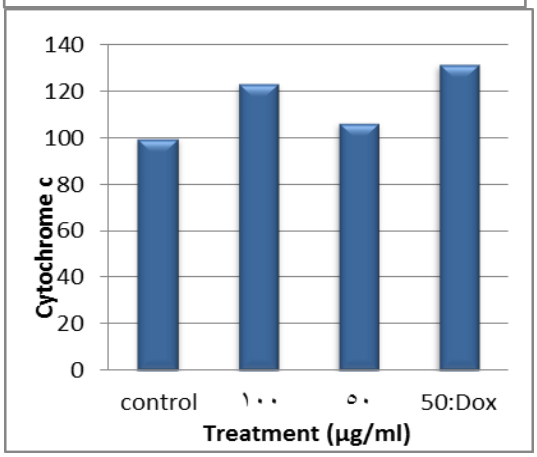
Multiparameter cytotoxic effect:

The result illustrated in figure (1) show that 50 and 100µg/ml of purified biosurfactants caused a significant decrease of cell count, significant increase in cell permeability, a significant increase in cytochrome C releasing from mitochondria , significant increase in nucleus intensity and a significant reduction in mitochndra membrane potential of MCF7 cells(figure 2). Cytotoxicity is a complex process affecting multiple parameters and pathways. After toxic insult, cells often undergo either apoptosis or necrosis accompanied by changes in nuclear morphology, cell permeability, and mitochondrial function, resulting in loss of mitochondrial membrane potential and release of cytochrome C from mitochondria. From the above result a clear cytotoxic effect was observed at 100 **µ**g/ml of purified *Geobacillus thermoleovorans* (JQ 912239) biosurfactant, so at this concentration, many changes was observed. Also Vivek *et al*. (2008) found that the nucleus changes can also cause by many oxidative stress inducer drugs and microtubule depolymerizing agents. While Kim and collaborators (2007) showed that surfactin blocks cell proliferation by inducing proapoptotic activity and arresting the cell cycle. Furthermore, surfactin strongly blocked the PI3K/Akt signaling pathway both proteins are involved in multiple cellular processes such as cell proliferation and apoptosis. change in mitochondrial membrane potential. This may be attributed to release of cytochrome c, and that is in accordance with Cao *et al.* (2011) showed that surfactin induces ROS formation, leading to mitochondrial permeability and membrane potential collapse that ultimately results in an increase of ion calcium concentration in the cytoplasm, afterwards, cytochrome c released from mitochondria to the cytoplasm activates caspase-9 eventually inducing apoptosis. The apoptotic process may be attributed to nuclear condensation and DNA fragmentation. This results was in accordance with Chiewpattanakul *et al.* (2010) who detected the anticancer activity of biosurfactants produced by the dematiaceous fungus *Exophiala dermatitidi*s SK80 against cervical cancer (HeLa) and leukemia (U937) cell lines this effect is commonly associated with the apoptotic process, in which the DNA is cleaved into fragments of 180 nucleosomal units by the endogenous endonuclease, caspase enzymes and nucleus condensation. Lemasters *et al.* (2009) mentioned that the induction of the permeability transition pore can lead to mitochondrial swelling and cell death through apoptosis or necrosis depending on the particular biological setting a sequence of apoptotic events was observed including the condensation of chromatin and DNA fragmentation, thus confirming the apoptosis-inducing potential of MELs in these cells the activity of protein kinase C (PKC) might be associated with apoptosis induced by MELs.









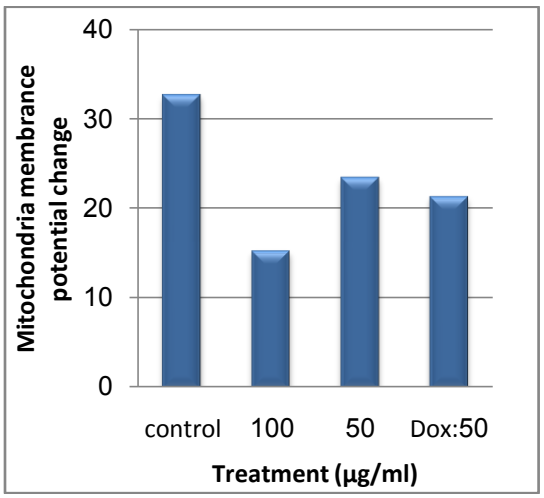
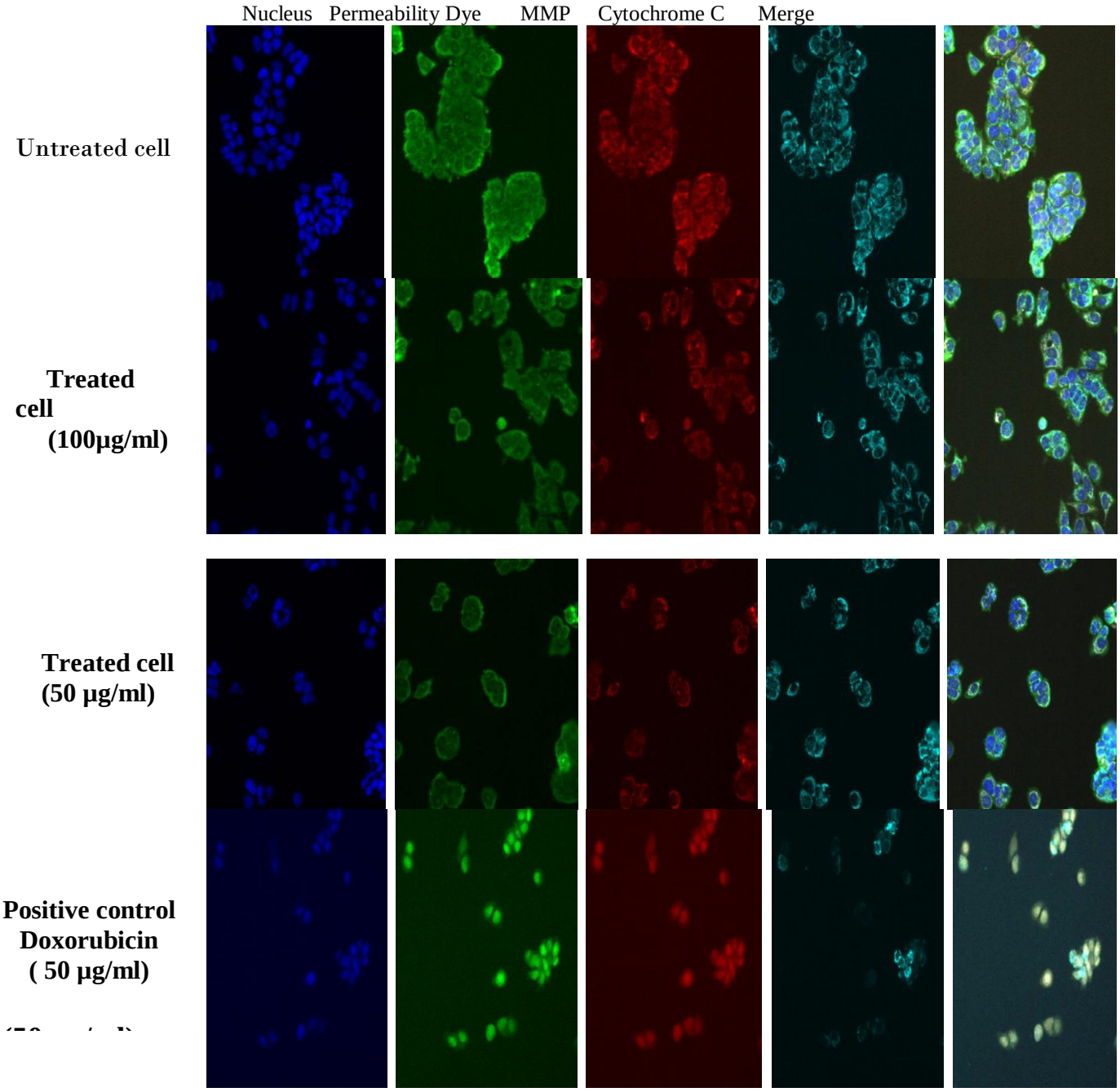


Figure (2): Effect of biosurfactant produced from *G. thermoleovorans* by different parameters against MCF7 cell line .



Figure(1)HCS image showed the cytotoxic effect of different concentrations of purified biosurfactant

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