J. Microbiol. Biotechnol. (2007), **17**(11), 1797–1804

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Damage to the Cytoplasmic Membrane and Cell Death Caused by Lycopene in *Candida albicans*

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Received: April 4, 2007 Accepted: July 1, 2007

Abstract Lycopene, an acyclic carotenoid found in tomatoes (*Lycopersicon esculentum*) and a number of fruits, has shown various biological properties, but its antifungal effects remain poorly understood. The current study investigated the antifungal activity of lycopene and its mode of action. Lycopene showed potent antifungal effects toward pathogenic fungi, tested in an energy-independent manner, with low hemolytic effects against human erythrocytes. To confirm the antifungal effects of lycopene, its effects on the dimorphism of *Candida albicans* induced by fetal bovine serum (FBS), which plays a key role in the pathogenesis of a host invasion, were investigated. The results showed that lycopene exerted potent antifungal activity on the serum-induced mycelia of *C. albicans*. To understand the antifungal mode of action of lycopene, the action of lycopene against fungal cell membranes was examined by FACScan analysis and glucose and trehalose-release test. The results indicated that lycopene caused significant membrane damage and inhibited the normal budding process, resulting from the destruction of membrane integrity. The present study indicates that lycopene has considerable antifungal activity, deserving further investigation for clinical applications.

Keywords: Lycopene, phytochemical, antifungal activity, membrane disruption

Rapid increase of microbes that are resistant to conventionally used antibiotics has recently been observed [6]. About 2 million people in the United States acquire bacterial and fungal infections each year, and 65% of patients show resistance to at least one drug [28]. Furthermore, adequate treatment of mycotic infections is difficult, since fungi are eukaryotic organisms with a structure and metabolism that are similar to those of eukaryotic hosts. Therefore, there is an inevitable and urgent medical need for antibiotics with novel antifungal mechanisms.

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The isolation and development of new drugs from plants is not a new phenomenon. Plants and plant-derived agents have a long history of clinical relevance as a source of potential chemotherapeutic agents [7]. About 25% of drugs prescribed worldwide come from plants; 121 such active compounds are in current use. Of the 252 drugs considered basic and essential by the World Health Organization (WHO), 11% come exclusively from plants, and a significant number are synthetic drugs obtained from natural precursors [27].

Lycopene is a red pigment found in red fruits and vegetables, including tomatoes, watermelons, pink-grapefruits, apricots, and pink-guavas, and it is a natural pigment synthesized by plants and microorganisms, but not by animals. It is a carotenoid, an acyclic isomer of beta-carotene, and does not contain any vitamin A activity. It is a highly unsaturated, straight-chain hydrocarbon containing 11 conjugated and two nonconjugated double bonds [30]. In several studies, lycopene has been found to possess antioxidative activity, exhibiting higher singlet oxygen quenching ability, as compared with beta-carotene or alpha-tocopherol [8]. Recently, there has been growing interest in lycopene as a cancer-preventive agent. Some studies have suggested that lycopene decreases the risk of several types of human malignancies, including breast, lung, and prostate cancers [2]. Although various biological activities of lycopene are known, its antifungal effects and mode of action toward pathogenic fungi still remain largely unknown. In this study, we studied the antifungal effects of lycopene toward various human pathogens and its mode of action regarding antifungal activity against fungal pathogen.

Materials and Methods

Materials

Lycopene, propionic acid, amphotericin B, trehalase, and RNase A were purchased from the Sigma Chemical Co. (St. Louis, MO, U.S.A.). Stock solutions of lycopene and

amphotericn B were prepared in dimethyl sulfoxide (DMSO), and that of propionic acid was prepared in sterile distilled water and stored at -20°C. For all the following experiments, a final concentration of 2% DMSO was used as the solvent carrier.

Microorganisms and Culture Conditions

Staphylococcus aureus (KCTC 1621), *Saccharomyces cerevisiae* (KCTC 7296), *Trichosporon beigelii* (KCTC 7707), and *Malassezia furfur* (KCTC 7744) were obtained from the Korean Collection for Type Cultures (KCTC) of the Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon, Korea. *Escherichia coli* O-157 (ATCC 43895) was obtained from the American Type Culture Collection (ATCC) (Manassas, VA, U.S.A.). *Candida albicans* (TIMM 1768) was obtained from the Center for Academic Societies, Osaka, Japan. Bacterial cells were cultured in a Mueller-Hinton broth (Difco, Sparks, MD, U.S.A.) containing beef extract powder, acid digest of casein, and soluble starch (21 g/l), with aeration at 37°C, and fungal cells were cultured in an yeast extract/ peptone/dextrose (YPD) broth (Difco) containing yeast extract, peptone, and dextrose (50 g/l), with aeration at 28°C. *Malassezia furfur* was cultured in modified Bacto yeast extract/malt extract (YM) broth (Difco) containing yeast extract, malt extract, peptone, dextrose (21g/l), and 1% olive oil, at 32°C.

Determining of Antimicrobial Susceptibility

Bacterial cells (2×10^7/ml) were inoculated into a Mueller-Hinton broth and 0.1 ml/well was dispensed in 96-well microtiter plates. MICs were determined by a serial two-fold dilution of test compounds, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [5]. After 24 h of incubation at 37°C, the minimal concentration of compound to prevent the growth of a given test organism was determined and was defined as MIC. Growth was assayed with a microtiter ELISA Reader (Molecular Devices Emax, California, U.S.A.) by monitoring absorption at 620 nm. Fungal cells (2×10^4/ml) were inoculated into a YPD or YM broth, and 0.1 ml/well was dispensed into microtiter plates. MICs were determined by a serial two-fold dilution of test compounds, following a microdilution method [4, 19, 22] and MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide] assay [21]. After 48 h of incubation at 28°C or 32°C, the minimal concentration of compound to prevent the growth of a given test organism was determined and was defined as MIC. Growth was assayed with a microtiter ELISA Reader by monitoring absorption at 580 nm. MIC values were determined by three independent assays.

Kinetics of the Killing of Bacteria and Fungi

The kinetics of the antimicrobial action of lycopene were evaluated with *S. aureus* and *C. albicans*. Log-phase bacterial cells (2×10^7 CFU/ml) were incubated with 10 μg/ml of lycopene or 20 μg/ml of propionic acid (at the MIC), which was used as a positive control. Cells were obtained and spread on a Mueller-Hinton agar plate, and then the CFUs were counted after incubation for 16 h at 37°C. In addition, log-phase fungal cells (2×10^4 CFU/ml) were incubated with 5 μg/ml of lycopene or 2.5 μg/ml of amphotericin B (at the MIC), which was used as a positive control. The culture was obtained and spread on a YPD agar plate, and the CFUs were then counted after incubation for 24 h at 28°C [18]. The values represented the average of measurements conducted in triplicate of three independent assays.

Hemolytic Activity Against Human Erythrocytes

To assess the cytotoxicity of lycopene against human erythrocytes, hemolytic activity was evaluated by the percentage of hemolysis of a 4% suspension of human red blood cells (hRBCs) in various concentrations (from 0.625 to 10 μg/ml) of lycopene. The hRBCs were washed three times with a phosphate-buffered saline (PBS: 35 mM phosphate buffer/150 mM NaCl, pH 7.4). One-hundred μl aliquots of a hRBC suspension were added to 96-well microtiter plates, and then 100 ml of the lycopene solution in PBS was mixed into each well. After incubating the mixtures for 1 h at 37°C, the mixtures were centrifuged at 1,500 rpm for 10 min, and aliquots were transferred to new 96-well microtiter plates. The absorbance of aliquots was measured at 414 nm by using a microtiter ELISA Reader. Hemolytic rates of 0 and 100% were determined in PBS and 0.1% Triton X-100, respectively [16, 29, 31]. The percentage of hemolysis was calculated by employing the following equation: Percentage hemolysis=[($Abs_{414\,nm}$ in the compound solution-$Abs_{414\,nm}$ in PBS)/($Abs_{414\,nm}$ in 0.1% Triton X-100-$Abs_{414\,nm}$ in PBS)]×100.

Effect of Lycopene on Dimorphic Transition

C. albicans were maintained by periodic subculturing in a liquid YPD medium. Cultures of yeast cells (blastoconidia) were maintained in a liquid YPD medium at 37°C. To induce mycelial formation, cultures were directly supplemented with 20% of FBS. The dimorphic transition in *C. albicans* was investigated from cultures containing 10 or 20 μg/ml of lycopene (at 2 and 4 times the MIC), which were incubated for 48 h at 37°C [17, 20]. The dimorphic transition to mycelial forms was detected by phase-contrast light microscopy (NIKON, ECLIPSETE300, Tokyo, Japan).

Effects of Sodium Azide (NaN_3) on Antifungal Activity

To determine whether the antifungal activity of lycopene is dependent on the metabolic activity of fungal cells, killing assays were performed in the presence of NaN_3, which blocks mitochondrial respiration. Thus, *C. albicans* were seeded on a 96-well microtiter plate at a density of 2×10^4

cells (100 μl per well). Five μg of lycopene (at the MIC), with and without 0.002% NaN_3, a well-known metabolic inhibitor, was added to these fungal cells at final desired concentrations [20]. A MTT assay was performed in the same way as in that of an antifungal activity test. The results represent the average of measurements taken in triplicate of three independent assays.

Flow Cytometric Analysis for Plasma Membrane Potential

To elucidate the mode of action of lycopene, we investigated its ability to dissipate the membrane potential of *C. albicans*. Thus, log-phased cells of *C. albicans* (1×10^8 cells), cultured in a YPD medium, were harvested and resuspended with 1 ml of fresh YPD medium containing 80 μg/ml of lycopene (at 16 times the MIC). After incubation for 3 h, the cells were washed three times with PBS. To detect depolarization of the cell membrane, 1 ml of PBS, containing 50 μg of bis-(1,3-dibutylbarbituric acid) trimethine oxonol [$DiBAC_4(3)$] (Molecular Probes Inc., Eugene, OR, U.S.A.), was added, and the samples were incubated for 1 h at 4°C in the dark [23]. Flow cytometric analysis was performed *via* a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, U.S.A.).

Determination of Released Glucose and Trehalose

Fungal strains were grown at 28°C in a YPD medium. *C. albicans* were washed three times with PBS, and then 1 ml of *C. albicans* suspension (1×10^8 cells), containing 100 μg/ml of lycopene (at 20 times the MIC), was incubated for 2 h at 28°C in PBS. The negative control was incubated without lycopene, and a positive control was incubated with 50 μg/ml of amphotericin B (at 20 times the MIC). Fungal cells were settled by centrifugation (12,000 rpm for 20 min). Pellets were dried to calculate dry weight, and supernatants were transferred to a new tube. Released glucose and trehalose-containing supernatants were reacted with 0.05 units of trehalase. After allowing the enzymatic reaction for 1 h at 37°C, the reaction suspension was mixed with water, and 16% DNS reagent (3,5-dinitrosalicylic acid 1%, NaOH 2%, sodium potassium tartrate 20%) was added. For the reaction of glucose with the DNS reagent, the mixture was boiled for 5 min and cooled. Color formed was measured at 525 nm. The results represent the average of measurements conducted in triplicate of three independent assays.

Flow Cytometric Analysis for Fungal Cell Cycle

Log-phased cells of *C. albicans* (1×10^8 cells), cultured in a YPD medium, were harvested and treated with 50 μg/ml of lycopene (at 10 times the MIC). After incubation for 6 h, the cells were washed with PBS and fixed with 70% ethanol overnight at 4°C. The cells were treated with 200 μg/ml of RNase A, and the mixture was left to react for 2 h at 37°C. For DNA staining, 50 μg/ml of propidium iodide was added, and the mixture was incubated for 1 h at 4 C in the dark [14]. Flow cytometric analysis was performed by a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, U.S.A.). The values represent the average of measurements conducted in triplicate of three independent assays.

RESULTS

***In Vitro* Antimicrobial Activity**

The antimicrobial effects of lycopene on human pathogenic bacteria and fungi were investigated and described in terms of the minimum inhibitory concentration (MIC). In the current study, propionic acid and amphotericin B were used as a positive control for bacteria and fungi, respectively; propionic acid is an antibacterial agent that is widely used as a food preservative agent [3], and amphotericin B is a fungicidal agent that is widely used to treat serious systemic infections [15].

Lycopene, in an MIC range of 5–10 μg/ml, showed significant antibacterial activity against *S. aureus* and *E. coli* O-157, and its antibacterial activity was more potent than propionic acid, showing MIC values of 10–20 μg/ml toward *S. aureus* and *E. coli* O-157. Lycopene showed remarkable potential as an antibacterial agent in the treatment of *S. aureus* and *E. coli* O-157 infectious diseases.

In a fungi test, lycopene also showed potent antifungal activity against human pathogenic fungal strains. This compound, within a concentration range of 2.5–5 μg/ml, exhibited MIC values lower than antibacterial activity. The

Table 1. Antimicrobial activity of lycopene.

Compound	MIC (μg/ml)					
	Gram-positive bacteria	Gram-negative bacteria	Fungal cells			
	S. aureus	*E. coli* O-157	*C. albicans*	*T. beigelii*	*S. cerevisiae*	*M. furfur*
Lycopene	10	5	5	5	5	2.5
Propionic acid	20	10	ND[a]	ND	ND	ND
Amphotericin B	ND	ND	2.5	5	2.5	0.3125

[a]Not done.

MIC values of this compound were approximately the same level as those of amphotericin B, showing MIC values of 0.3–5 μg/ml toward all fungal strains (Table 1).

To assess the killing potency of lycopene, a killing-curve assay against *S. aureus* and *C. albicans* was conducted, and the results showed that lycopene has antimicrobial activity through biocidal effects. *S. aureus* was significantly decreased at the MIC values of lycopene in a time-dependent manner, similar to the decrease observed in the presence of propionic acid. *C. albicans*, was decreased rapidly at the MIC of lycopene, but was less than the decrease observed in the presence of amphotericin B, after 3 h (Fig. 1).

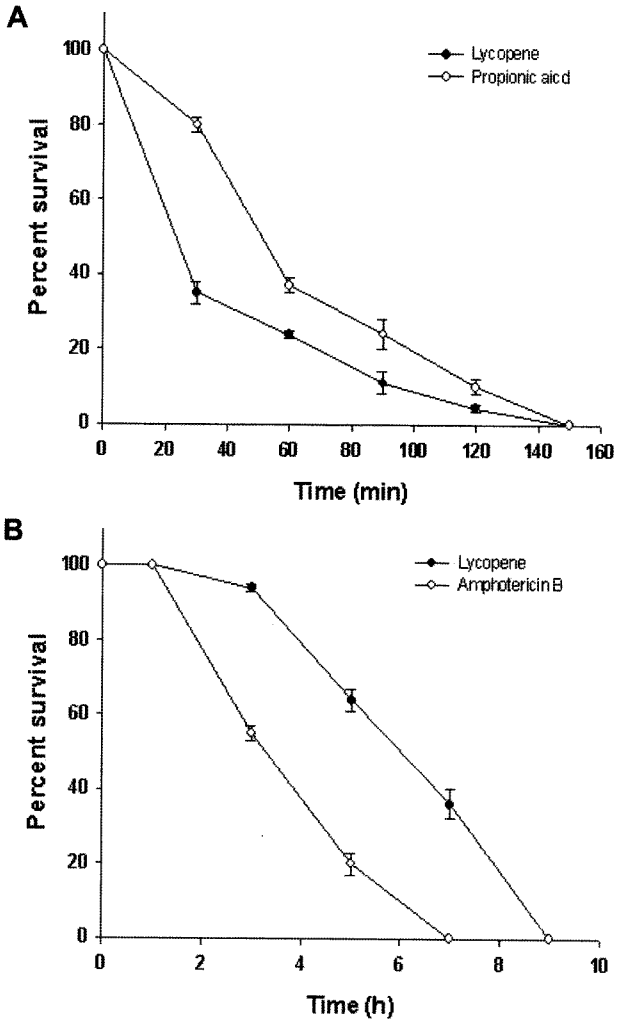

Fig. 1. Time-killing plots for *S. aureus* and *C. albicans* by lycopene.

A. Bacteria cells were incubated with 10 μg/ml of lycopene or 20 μg/ml of propionic acid, which was used as a positive control. The viability was determined every 30 min by using colony forming units (CFUs) and expressed as the percent of survivals. **B.** Fungal cells were incubated with 5 μg/ml of lycopene or 2.5 μg/ml of amphotericin B, which was used as a positive control. The viability was determined every 2 h by using colony forming units (CFUs) and expressed as the percent of survivals. The error bars represent the standard deviation (SD) for three independent experiments, performed in triplicate.

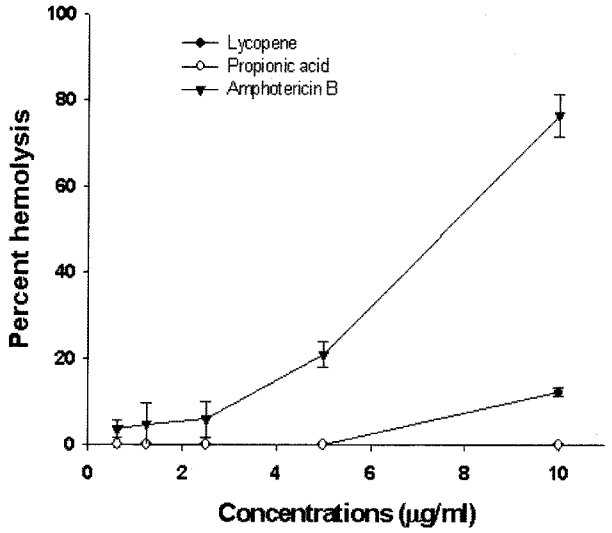

Fig. 2. The hemolytic effects of lycopene on human erythrocyte cells.

The error bars represent the standard deviation (SD) for three independent experiments, performed in triplicate.

In the cytotoxicity test, lycopene exhibited no hemolytic activity, except at a relatively high concentration, whereas amphotericin B exhibited hemolytic activity at all concentration levels that could be fatal for patients when they are treated for fungal infections with this agent. On the other hand, propionic acid exhibited no hemolytic activity at any concentration (Fig. 2).

Effects of Lycopene on Dimorphic Transition of Fungal Cells

The opportunistic dimorphous pathogen *C. albicans* is of increasing importance in human medicine, since it can cause deeply invasive mycoses, including candidiasis [23]. To investigate the antifungal effects of lycopene against *C. albicans* in the human body, we performed an *in vitro* test of the antifungal effects on the mycelial forms of *C. albicans* that were induced by supplementing with 20% FBS. As shown in Fig. 3, the serum-induced mycelia were significantly inhibited from extending and forming in the presence of lycopene (Fig. 3C and 3D). The mycelia formed were normal in the absence of lycopene (Fig. 3B).

Effects of Sodium Azide (NaN_3)

To investigate the effects of cellular energy consumption on the membrane-mediated transport pathway in the presence of lycopene, we performed an energy-dependence test on antifungal activity. *C. albicans* were incubated with lycopene in the absence or presence of 0.002% NaN_3 for an energy-dependency test [20]. Sodium azide (NaN_3) is a metabolic

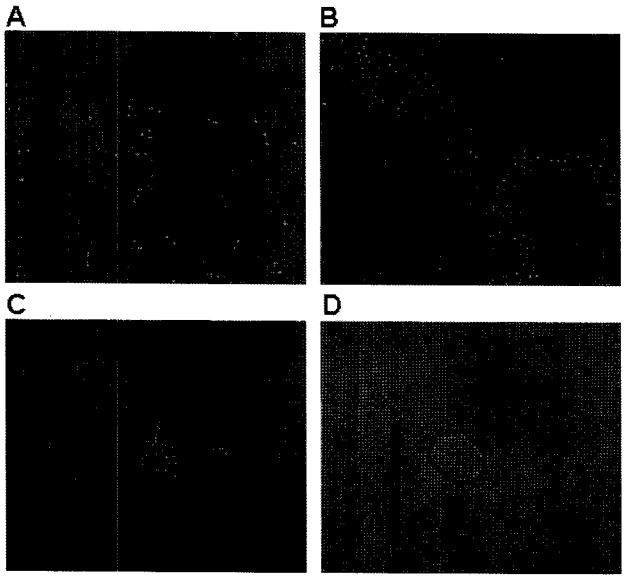

Fig. 3. The effects of lycopene on the dimorphic transition in *C. albicans*.
Each culture was incubated with various concentrations (10 or 20 μg/ml) of lycopene for 48 h in a YPD media with 20% FBS. **A**. Yeast control with no 20% FBS and lycopene; **B**. Not treated with lycopene; **C**. Treated with 10 μg/ml; **D**. 20 μg/ml of lycopene in 20% FBS.

inhibitor that blocks intracellular ATP synthesis and the action of ATPase by inhibiting cytochrome oxidase, which

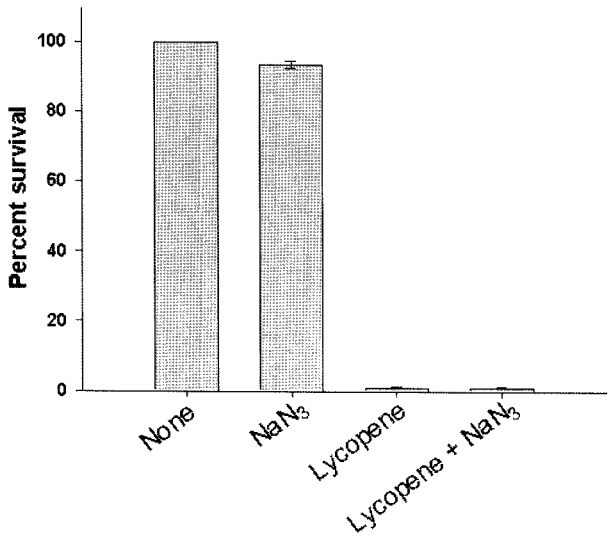

Fig. 4. The effects of sodium azide (NaN_3) on the antifungal activity of lycopene.
Exponential-phase *C. albicans* were treated with 5 μg/ml of lycopene. The cells were incubated at 28°C for 19 h in the presence of sodium azide (0.002% NaN_3 per well). The cells incubated in the absence of sodium azide were used as positive controls. The cell population was examined by MTT assay. The turbidity of each well was measured by absorbance at 580 nm using a Microtiter ELISA Reader, and the error bars represent the standard deviation (SD) for three independent experiments, performed in triplicate.

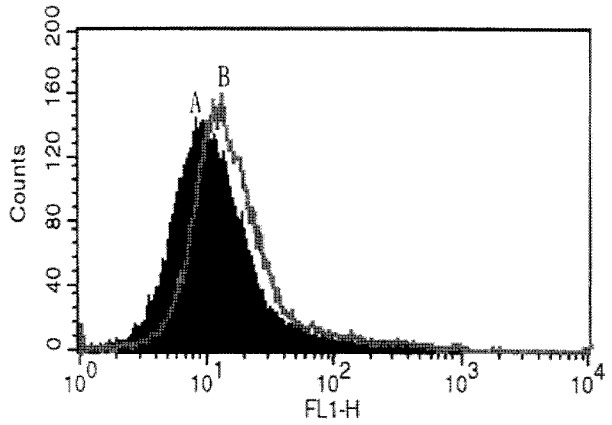

Fig. 5. The effects of lycopene on the membrane of *C. albicans*.
C. albicans were mixed with 80 μg/ml of lycopene and incubated at 28°C for 3 h under constant shaking. After washing the cells with PBS, the cells were stained with 50 μg/ml of $DiBAC_4(3)$ at 4°C for 1 h. **A**. Not treated with any compound; **B**. Treated with lycopene.

further prevents membrane active transport [33]. The results showed that the viability of the cells was not affected by the presence of 0.002% NaN_3; however, cell viability disappeared in the presence of lycopene, regardless of the presence of NaN_3 (Fig. 4).

Dissipation of Plasma Membrane Potential

To assess whether lycopene can affect the function of fungal plasma membrane, the dissipation of fungal plasma membrane potential was investigated by staining with $DiBAC_4(3)$. $DiBAC_4(3)$ has high voltage sensitivity and enters depolarized cells, where it binds to lipid-rich intracellular components [23]. Therefore, the increased fluorescence of $DiBAC_4(3)$ indicates membrane depolarization. The results showed that addition of lycopene to *C. albicans* increased the fluorescence intensity, indicating membrane depolarization (Fig. 5).

Intracellular Glucose and Trehalose Release

The ability of lycopene to disturb the integrity of the plasma membrane of fungal cells was also assessed by measuring glucose and trehalose released in cell suspensions that were exposed to this compound. Thus, *C. albicans* were cultured in the presence of lycopene or amphotericin B, and the amounts of glucose and trehalose released were investigated. Amphotericin B, used as a positive control, binds to membrane sterols and forms transmembrane pores, thereby causing a leakage of cell constituents and eventually cell death [11]. The results showed that both lycopene- and amphotericin-B-treated cells released more glucose and trehalose than the compound-untreated cells. Glucose and trehalose released as a result of amphotericin B were 148.8 μg per 1 mg of fungal dry mass. However, when induced by lycopene, the amounts of released

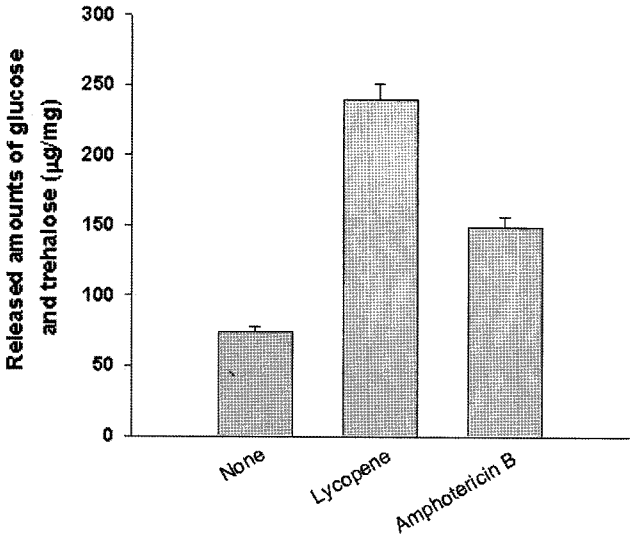

Fig. 6. The concentration of trehalose and glucose released from *C. albicans* by lycopene and amphotericin B.
C. albicans were incubated with 100 μg/ml of lycopene or 50 μg/ml of amphotericin B at 28°C for 2 h. After incubation, the cell supernatants were examined for the levels of trehalose and glucose released, and the fungal cell dry mass was determined. The error bars represent the standard deviation (SD) for three independent experiments, performed in triplicate.

glucose and trehalose were 239 μg per 1 mg of fungal dry mass. This rate was significantly higher than that induced in the compound-untreated cells (Fig. 6).

Arrest of Fungal Cell Cycle

To understand how lycopene affects cellular physiology, we further investigated the effects of lycopene on the cell cycle progression of *C. albicans*. The cells were cultured in the presence or absence of lycopene, and DNA content was determined *via* flow cytometry after staining with propidium iodide (PI). PI is a DNA-staining dye that intercalates between the bases of DNA or RNA molecules [32]. As shown in Fig. 7, the percentage of cells in the G_2/M phase increased by 14%, whereas that in the G_1 phase was significantly decreased by about 20% in the presence of lycopene (Fig. 7). The results showed that lycopene arrested the cell cycle of *C. albicans* at the G_2/M phase.

Discussion

Plants have developed an arsenal of chemicals that make them survive from attacks by microbial invasion. Consequently, many different antifungal phytochemicals have so far been isolated from several plants. This is because these compounds have relatively low cytotoxicity and novel antifungal mechanisms; there has been growing interest in antifungal phytochemicals [12, 13]. The present study was aimed to assess the antifungal effects of lycopene on various human

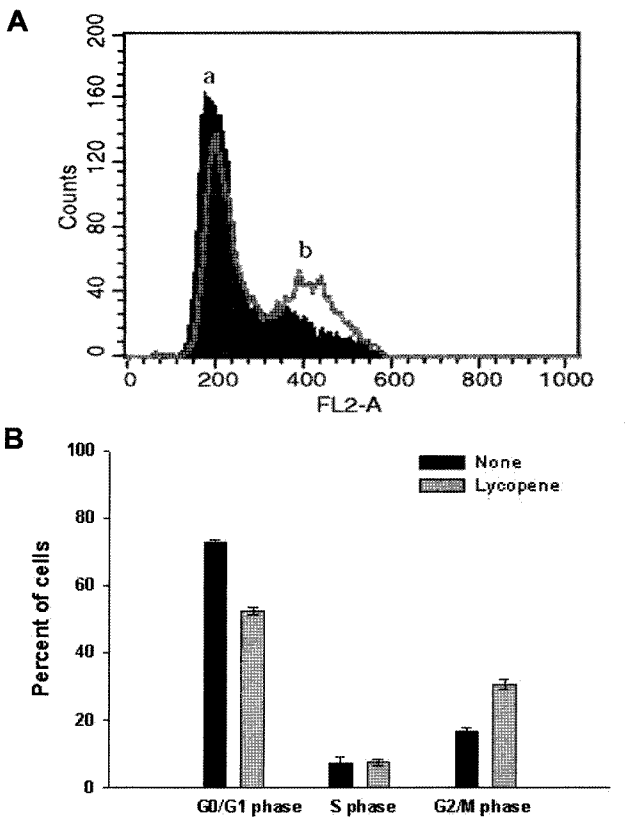

Fig. 7. The effects of lycopene on the cell cycle progression of *C. albicans*.
C. albicans were treated with 50 μg/ml of lycopene and incubated at 28°C for 6 h under constant shaking. After washing with PBS, the cells were fixed with 70% ethanol (in PBS, v/v) for 12 h, and then stained with 50 μg/ml of propidium iodide. **A.** Cell cycle histogram of *C. albicans*; **B.** graphical presentation of the percentage of cell cycle in *C. albicans*. a, Not treated with compound; b, treated with lycopene. The error bars represent the standard deviation (SD) for three independent experiments, performed in triplicate.

pathogens and its mode of action for antifungal activity against fungal pathogens.

The results of the antimicrobial test demonstrated the antimicrobial activity of lycopene against bacteria and fungi tested, which cause human infectious diseases and in turn exert more potent antibiotic activity against fungal cells than bacterial cells (Table 1). Antibiotics show antimicrobial effects by cidal or static action. Lycopene exhibited effects similar to propionic acid and amphotericin B, which has been shown to be a cidal agent toward microorganisms; the antimicrobial activity of lycopene is due to the killing action, as evidenced by the killing-curve assay with *S. aureus* and *C. albicans* (Fig. 1). The dimorphic transition of *C. albicans* from yeast form to mycelial form is responsible for pathogenicity: mycelial shapes are predominantly found during host tissue invasion, and mycelial form can be induced by temperature, pH, and serum [24]. Lycopene also exerts antifungal activity toward

serum-induced mycelial structures of *C. albicans* (Fig. 3), suggesting that lycopene is a potential therapeutic agent in the treatment of fungal infectious diseases in the human body. Many antifungal agents have limited clinical applications, because they bring about cytolysis of human erythrocytes. The results of the cytotoxicity test confirmed the antimicrobial effects of lycopene; however, it had either no effect or a weakly cytolytic effect on human erythrocytes (Fig. 2). In addition, the results of the energy-dependency test revealed that the activity of lycopene was unaffected by NaN_3, suggesting that its effects were mediated by a cellular function, which does not need energy consumption (Fig. 4).

The results of effects of lycopene on membrane potential indicated that this compound affected yeast cells by attacking their membranes, thereby disrupting membrane potential (Fig. 5). Fungal cells maintain membrane potential by establishing multiple ion gradients across the cytoplasmic membrane. Since the proper maintenance of intracellular components is important to fungal viability, glucose and trehalose release was measured during exposure of *C. albicans* to lycopene (Fig. 6). Trehalose can protect proteins and biological membranes from inactivation or denaturation in yeast caused by a variety of stress conditions, including desiccation, dehydration, heat, cold, oxidation, and toxic agents [1, 9]. The analysis of glucose and trehalose release during lycopene exposure suggests that it may be one of several intracellular components released during membrane disruption by lycopene. In addition, the efflux of intracellular components from *C. albicans* after treatment provides further evidence that lycopene acts on the plasma membrane by disrupting activity. As for the mechanism by which lycopene breaks down the membrane permeability barrier, it is possible that lycopene perturbs the membrane lipid bilayers, thus causing leakage of certain cellular components, as well as the dissipation of the electrical potential of the membrane.

Recent studies suggest that the inhibitory effects of lycopene on several cancer cell lines are associated with the inhibition of cell cycle progression through the G_1 phase [25]. In general, the cell cycle is of particular interest as a source of targets for drug development. It is of interest to note that one of the oldest antifungals discovered in 1939, Griseofulvin, inhibits the cell cycle [26]. To elucidate the lycopene-induced physiological changes of the fungal cells, we performed flow cytometric analysis of the cell cycle. The data suggested that lycopene inhibited some cellular processes, which are involved in normal bud growth, but it did not affect DNA replication (Fig. 7). Endo *et al.* [10] reported that the inhibition of bud growth correlates with membrane damage, and suggested that lycopene inhibits the normal budding process, probably through the destruction of membrane integrity.

In summary, lycopene exhibited potent antifungal effects on pathogenic fungi tested in an energy-independent manner, with low hemolytic effects against human erythrocytes. Although the exact mechanism of lycopene action has not yet been fully elucidated, the results reported here indicate its effect on the plasma membrane. Therefore, we conclude that lycopene has considerable antifungal activity, deserving further investigation for clinical applications.

Acknowledgment

This work was supported by the Ministry of Commerce, Industry Energy (MOCIE) through the Center for Traditional Microorganism Resources (TMR) at Keimyung University.

REFERENCES

1. Alvarez-Peral, F. J., O. Zaragoza, Y. Pedreno, and J. Argüelles. 2002. Protective role of trehalose during severe oxidative stress caused by hydrogen peroxide and the adaptive oxidative stress response in *Candida albicans*. *Microbiology* **148:** 2599–2606.
2. Amir, H., M. Karas, J. Giat, M. Danilenko, R. Levy, T. Yermiahu, J. Levy, and Y. Sharoni. 1999. Lycopene and 1,25-dihydroxyvitamin D3 cooperate in the inhibition of cell cycle progression and induction of differentiation in HL-60 leukemic cells. *Nutr. Cancer* **33:** 105–112.
3. Cha, D. S. and M. S. Chinnan. 2004. Biopolymer-based antimicrobial packaging: A review. *Crit. Rev. Food Sci. Nutr.* **44:** 223–237.
4. Cha, J.-D., M.-R. Jeong, S.-I. Jeong, and K.-Y. Lee. 2007. Antibacterial activity of Sophoraflavanone G isolated from the roots of *Sophora flavescens*. *J. Microbiol. Biotechnol.* **17:** 858–864.
5. Clinical and Laboratory Standards Institute. 2005. Performance standards for antimicrobial susceptibility testing, fifteenth informational supplement, approved standard MS100-S15, CLSI, Wayne, PA.
6. Cohen, M. L. 1992. Epidemiology of drug resistance; implications for a post-antimicrobial era. *Science* **257:** 1050–1055.
7. Cushnie, T. P. T. and A. J. Lamb. 2005. Antimicrobial activity of flavonoids. *Int. J. Antimicrob. Agents* **26:** 343–356.
8. Dimascio, P., S. Kaiser, and H. Sies. 1989. Lycopene as the most effective biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.* **274:** 532–538.
9. Elbein, A. D., Y. T. Pan, I. Pastuszak, and D. Carroll. 2003. New insights on trehalose: A multifunctional molecule. *Glycobiology* **13:** 17R–27R.
10. Endo, M., K. Takesako, I. Kato, and H. Yamaguchi. 1997. Fungicidal action of aureobasidin A, a cyclic depsipeptide antifungal antibiotic, against *Saccharomyces cerevisiae*. *Antimicrob. Agents Chemother.* **41:** 672–676.
11. Fujii, G., J. Chang, T. Coley, and B. Steere. 1997. The formation of amphotericin B ion channels in lipid bilayers. *Biochemistry* **36:** 4959–4968.

12. Gordon, M. A., E. W. Lapa, and M. S. Fitter. 1980. Susceptibility of zoopathogenic fungi to phytoalexins. *Antimicrob. Agents Chemother.* **17:** 120–123.
13. Grayer, R. J. and J. B. Harborne. 1994. A survey of antifungal compounds from higher plants (1982-1993). *Phytochemistry* **37:** 19–42.
14. Green, L. J., P. Marder, L. L. Mann, L. C. Chio, and W. L. Current. 1999. LY303366 exhibits rapid and potent fungicidal activity in flow cytometric assays of yeast viability. *Antimicrob. Agents Chemother.* **43:** 830–835.
15. Hartsel, S. and J. Bolard. 1996. Amphotericin B: New life for an old drug. *Trends Pharmacol. Sci.* **17:** 445–449.
16. Jung, H. J., K. S. Choi, and D. G. Lee. 2005. Synergistic killing effect of synthetic peptide P20 and cefotaxime on methicillin-resistant nosocomial isolates of *Staphylococcus aureus*. *J. Microbiol. Biotechnol.* **15:** 1039–1046.
17. Jung, H. J., Y. B. Seu, and D. G. Lee. 2007. Candicidal action of resveratrol isolated from grapes on human pathogenic yeast *C. albicans*. *J. Microbiol. Biotechnol.* **17:** 1324–1329.
18. Klepser, M. E., E. J. Ernst, R. E. Lewis, M. E. Ernst, and M. A. Pfaller. 1998. Influence of test conditions on antifungal time-kill curve results: Proposal for standardized methods. *Antimicrob. Agents Chemother.* **42:** 1207–1212.
19. Lee, D. G., S. Y. Shin, C. Y. Maeng, Z. Z. Jin, K. L. Kim, and K.-S. Hahm. 1999. Isolation and characterization of a novel antifungal peptide from *Aspergillus niger*. *Biochem. Biophys. Res. Commun.* **263:** 646–651.
20. Lee, D. G., Y. Park, P. I. Kim, H. G. Jeong, E.-R. Woo, and K.-S. Hahm. 2002. Influence on the plasma membrane of *Candida albicans* by HP (2-9)-magainin 2 (1-12) hybrid peptide. *Biochem. Biophys. Res. Commun.* **297:** 885–889.
21. Lee, D. G., Y.-S. Chang, Y. Park, K.-S. Hahm, and E.-R. Woo. 2002. Antimicrobial effects of ocotillone isolated from stem bark of *Ailanthus altisshima*. *J. Microbiol. Biotechnol.* **12:** 854–857.
22. Lee, M. J., D. H. Bae, D. H. Lee, K. H. Jang, D. H. Oh, and S. D. Ha. 2006. Reduction of *Bacillus cereus* in cooked rice treated with sanitizers and disinfectants. *J. Microbiol. Biotechnol.* **16:** 639–642.
23. Liao, R. S., R. P. Rennie, and J. A. Talbot. 1999. Assessment of the effect of amphotericin B on the vitality of *Candida albicans*. *Antimicrob. Agents Chemother.* **43:** 1034–1041.
24. Mclain, N., R. Ascanio, C. Baker, R. A. Strohaver, and J. W. Dolan. 2000. Undecylenic acid inhibits morphogenesis of *Candida albicans*. *Antimicrob. Agents Chemother.* **44:** 2873–2875.
25. Nahum, A., K. Hirsch, M. Danilenko, C. K. Watts, O. W. Prall, J. Levy, and Y. Sharoni. 2001. Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of $p27^{kip1}$ in the cyclin E-cdk2 complexes. *Oncogene* **20:** 3428–3436.
26. Oxford, A. E., M. Raistrick, and P. Simonart. 1939. Studies in the biochemistry of microorganisms. *Biochem. J.* **33:** 240–248.
27. Rates, S. M. K. 2001. Plants as source of drugs. *Toxicon* **39:** 603–613.
28. Sathiamoorthy, B., P. Gupta, M. Kumar, A. K. Chaturvedi, P. K. Shukla, and R. Maurya. 2007. New antifungal flavonoid glycoside from *Vitex negundo*. *Bioorg. Med. Chem. Lett.* **17:** 239–242.
29. Shin, S. Y., J. H. Kang, D. G. Lee, Z. Z. Jin, S. Y. Jang, K. L. Kim, and K.-S. Hahm. 1999. Structure and antibiotic activity of fragment peptides of antifungal protein isolated from *Aspergillus giganteus*. *J. Microbiol. Biotechnol.* **9:** 276–281.
30. Stahl, W. and H. Sies. 1996. Lycopene: A biologically important carotenoid for humans? *Arch. Biochem. Biophys.* **336:** 1–9.
31. Sung, W. S., H. J. Jung, I. S. Lee, H. S. Kim, and D. G. Lee. 2006. Antimicrobial effect of furaneol against human pathogenic bacteria and fungi. *J. Microbiol. Biotechnol.* **16:** 349–354.
32. Tas, J. and G. Westerneng. 1981. Fundamental aspects of the interaction of propidium diiodide with nuclei acids studied in a model system of polyacrylamide films. *J. Histochem. Cytochem.* **29:** 929–936.
33. Wilson, D. F. and B. Chance. 1967. Azide inhibition of mitochondrial electron transport. I. The aerobic steady state of succinate oxidation. *Biochim. Biophys. Acta* **131:** 421–430.