J. Microbiol. Biotechnol. (2007), **17**(11), 1848–1855

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Antibacterial Activity of Essential Oils on the Growth of *Staphylococcus aureus* and Measurement of their Binding Interaction Using Optical Biosensor

CHUNG, KYONG-HWAN[1], KI-SOOK YANG[2], JIN KIM[3], JIN-CHUL KIM[4], AND KI-YOUNG LEE[1,5]*

[1]*Center for Functional Nano Fine Chemicals, Chonnam National University, Gwangju 500-757, Korea*
[2]*Interdisciplinary Program of Perfume and Cosmetics Graduate School, Chonnam National University, Gwangju 500-757, Korea*
[3]*Department of Advanced Chemicals, Chonnam National University, Gwangju 500-757, Korea*
[4]*School of Biotechnology & Bioengineering, Kangwon National University, Kangwon-do 200-701, Korea*
[5]*Department of Applied Chemical Engineering & The Research Institute for Catalysis, Chonnam National University, Gwangju 500-757, Korea*

Received: June 8, 2007 Accepted: August 6, 2007

Abstract Antibacterial activity of essential oils (Tea tree, Chamomile, Eucalyptus) on *Staphylococcus aureus* growth was evaluated as well as the essential oil-loaded alginate beads. The binding interactions between the cell and the essential oils were measured using an optical biosensor. The antibacterial activity of the essential oils to the cell was evaluated with their binding interaction and affinity. The antibacterial activity appeared in the order of Tea Tree> Chamomile>Eucalyptus, in comparison of the inhibition effects of the cell growth to the essential oils. The association rate constant and affinity of the cell binding on Tea Tree essential oil were 5.0×10^{-13} ml/(CFU·s) and 5.0×10^{5} ml/CFU, respectively. The affinity of the cell binding on Tea Tree was about twice higher than those on the other essential oils. It might be possible that an effective antibacterial activity of Tea Tree essential oil was derived from its strong adhesive ability to the cell, more so than those of the other essential oils.

Keywords: Antibacterial activity, essential oil, *Staphylococcus aureus*, growth inhibition, binding interaction

The essential oils are considered as important antimicrobial agents present in plants, and may also have antioxidant and antiinflammatory activities. Essential oils are a complex mixture of compounds, mainly monoterpenes, sesquiterpenes, and their oxygenated derivatives such as alcohols, aldehydes, esters, ethers, ketones, phenols, and so on. Generally, the oil composition is a balance of various compounds, although in many species, one constituent may prevail over all others [12]. Antimicrobial plant products have gained special interest because of the resistance to antibiotics that some microorganisms have acquired [18].

*Corresponding author
Phone: 82-62-530-1843; Fax: 82-62-530-1819;
E-mail: kilee@chonnam.ac.kr

Essential oils from spices, medicinal plants, and herbs have been shown to possess antimicrobial activities and could serve as a source of antimicrobial agents against some pathogenic bacteria [15, 28]. Essential oils and their components are known to be active against a wide variety of microorganisms, including Gram-negative [25, 32, 44] and Gram-positive bacteria [28]. It was explained that Gram-negative bacteria were shown to be generally more resistant than Gram-positive ones to the antagonistic effects of essential oils because of the lipopolysaccharide present in the outer membrane [39].

Atopic dermatitis is a chronic inflammatory skin disease with hypersensitivity reaction against common environmental allergens [50]. A bacterial flora on the skin of atopic dermatitis patients is unique, and *Staphylococcus aureus* is commonly detected on patients with atopic dermatitis [1, 9, 22–24, 33]. It has been known that patients with atopic dermatitis often experience various ocular diseases, such as atopic keratoconjunctivitis, keratoconus, cataract, and retinal detachment [5–7, 11, 46].

Tea Tree, Eucalyptus, and other essential oils have gained recent acceptance as safe and effective antiseptics [13, 40]. *In vitro* [2, 14, 23, 35] and clinical trials [41, 42] have demonstrated the potent antibacterial activity of these oils. These oils have been used as a therapeutic agent for atopic dermatitis. Tea Tree oil has been reported to have powerful antibacterial activity [48, 49]. It was found that undiluted Tea Tree oil possessed antibacterial activity against *Staphylococcus aureus*, *in vitro* [17]. However, it is not clear why the Tea Tree essential oil exhibits a superior antibacterial activity to *Staphylococcus aureus*.

Surface plasmon resonance (SPR) biosensors have a range of versatile applications, from target characterization, compound screening, and lead optimization to supporting clinical trials, regulatory approval, and biopharmaceutical manufacturing [4, 36, 37]. SPR biosensors also allow direct real-

time detection of the binding interactions of macromolecules without any chemical alteration of the ligands for signal generation. A captured molecule or ligand is covalently immobilized on the sensor chip, and a binding molecule captured by the immobilized ligand in a continuous flow system [19, 20, 26]. In addition, a quantitative analysis of the molecular interaction and kinetic parameters can also be obtained from SPR biosensor systems [3, 27]. On the other hand, no studies have been reported on the evaluation of the antibacterial activity of essential oils on cells on the scope of binding interaction using SPR biosensor.

In this study, the antibacterial activity on *Staphylococcus aureus* growth focused on evaluation of the essential oils (Tea Tree, Chamomile, Eucalyptus) and the essential oil-loaded alginate beads. The binding interactions between the cell and the essential oils were determined from kinetic study of the binding from the SPR. The antibacterial activity of the essential oils on the cells is discussed along with their binding interaction and affinity.

MATERIALS AND METHOD

Microorganism

Staphylococcus aureus (KCTC 1928) was purchased from the Korean Collection for Type Culture (KCTC). The cell was grown for 18 h at 37°C in nutrient broth.

Reagents

Tea Tree (*Melaleuce alternifolia*, 99.9%), Chamomile (*Chamaemelum nobile*, 99.9%), and Eucalyptus (*Eucalyptus globulus*, 99.9%) oils were purchased from Monet Co. (France). Alginate (medium viscosity, 3,500 cps at 2% concentration) and calcium chloride were purchased from Sigma (St. Louis, MO, U.S.A.). Bipheynl-4-4'-dithiol (95%, Aldrich) and other chemicals were used as reagent grade without further purification.

Preparation of Alginate Bead and Essential Oil-loaded Alginate Bead

The alginate beads were prepared by dripping of alginate solution (1% w/v) by a microfeeder into a calcium chloride solution (4% w/v) with vigorous stirring. The prepared beads were washed and dried at room temperature.

The alginate solution (1% w/v) and the pure essential oils (400 μl) were mixed and homogenized at 13,500 rpm for 2 min. The mixture was dropped into a calcium chloride solution (4% w/v) using a microfeeder. The prepared essential oil-loaded alginate beads were washed and dried.

Antimicrobial Screening

The agar disc-diffusion method was employed to determine the antimicrobial activity of the essential oils. The suspension of the *Staphylococcus aureus* (1×10^8 CFU/ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were individually impregnated with 5 μl of the essential oils and placed on the incubation plates. The plates were placed at 4°C for 2 h, followed by incubation at 37°C for 24 h. The diameters of the inhibition zones were measured and expressed in millimeters. Each test was performed in three replicates and repeated twice.

Test of the Growth Inhibition of the Essential Oils in Nutrient Broth

The nutrient broth used in the growth inhibition test of the cells consisted of peptone (5 g), beef extract (3 g), agar (15 g), and distilled water (1 l). *Staphylococcus aureus* (1×10^8 CFU/ml) was suspended in each nutrient broth (100 ml), to which the pure essential oils at different contents were added. The cell concentration was controlled as 1×10^6 CFU/ml when the essential oil-loaded beads were injected in the nutrient broth. The culture medium was maintained at 37°C with shaking. Growth of the cells was estimated by measuring the absorbance of the culture medium at 600 nm using a UV/VIS spectrophotometer (Specgene, Techne Inc., U.K.). Growth of the cells was evaluated by dry cell weight (DCW) determined from a calibration curve of DCW versus absorbance at 600 nm. The percent of growth inhibition was defined as the following:

$$\% \text{ Inhibition} = \left(\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{essential}}}{\text{Abs}_{\text{control}}} \right) \times 100$$

where $\text{Abs}_{\text{control}}$ and $\text{Abs}_{\text{essential}}$ are the absorbance of control and essential oils at 600 nm, respectively. In this study, "control" means the experimental result of the cell growth with injection of the cell only.

Analysis of Loading Amount of Essential Oils Loaded into the Beads

The content of essential oils in the beads was analyzed by a gas chromatograph (Donam Co., Korea) equipped with a FID detector and HP1 capillary column (i.d.: 0.32 mm; length: 50 m; film thickness: 0.17 μm). At first, we drew up the calibration curves of the essential oils from a characterized peak area for the essential oils with various concentrations of essential oils in ethanol (99%, Aldrich). The essential oil-loaded beads were dissolved in ethanol for 3 days using an ultrasonic cleaner, and then the solution was analyzed by gas chromatography. Operating conditions of the gas chromatograph were as follows; injector temperature, 290°C; FID temperature, 250°C; carrier gas, helium. Oven temperature rose from 40°C to 250°C with a ramping rate of 5°C/min, and was held at the highest temperature for 5 min.

Estimation of *Staphylococcus aureus* Binding to the Essential Oils by SPR Assay

The gold surface of the SPR sensor chip was chemically activated with "piranha" solution that consisted of sulfuric

acid and NaOH solution, and then immersed in a 0.2% (w/v) aqueous solution of cysteamine for 2 h. The resultant gold surface was rinsed with distilled water and dried with nitrogen. After immersing the gold surface in 1.0% of biphenyl-4-4'-dithiol solution for 1 h, it was washed with distilled water to remove the adhesive biphenyl-4-4'-dithiol on the surface of the chip. Essential oils without dilution were coated on the sensor chip layer, and then dried at room temperature. The essential oil layer formed on the gold surface of the chip was characterized through measurement of SPR responses.

Staphylococcus aureus was harvested by centrifugation after incubation in Luria-Bertani broth medium for 18 h. The cell was washed once and resuspended to ~1×10^{10} CFU/ml. The cell suspension was injected to the essential oil layer of the SPR assay (Spreeta 5, Texas Instruments Inc., U.S.A.) with a flow rate of 20 µl/min for 30 min. The specific interaction between the cell and the essential oils on the sensor chip surface was determined by monitoring of refractive unit (RU) values [10, 31].

Theory of the Kinetic Analysis

The association and dissociation rate constants are calculated by curve-fitting to the SPR sensorgrams as described in our previous study [10]. The general rate equation for binary complex formation, $[A]+[B]\underset{k_{dis}}{\overset{k_{ass}}{\rightleftharpoons}}[AB]$, is written as

$$\frac{d[AB]}{dt}=k_{ass}[A]B-k_{dis}[AB] \quad (1)$$

where [A] and [B] are the analyte and surface bound ligand, respectively. k_{ass} is the association rate constant and k_{dis} is the dissociation rate constant in Eq. (1). The solution equation for the rate equation of complex formation is as follows:

$$\frac{dR}{dt}=k_{ass}CR_{max}-(k_{ass}C+k_{dis})R \quad (2)$$

where dR/dt is the rate of surface complex formation, R is the RU value reflecting the amount of analyte bound to the immobilized ligand on the sensorchip, R_{max} is the RU value when the binding sites of the immobilized ligand have been saturated by the analyte, and C is the concentration of analyte in a free solution.

Equation (2) is rewritten as

$$\frac{dR}{dt}=k_{ass}CR_{max}\left(1-\frac{k_{ass}C+k_{dis}}{k_{ass}CR_{max}}R\right) \quad (3)$$

Equation (3) can be expressed as an integral equation, like Eq. (4). The integral equation can be solved with variation of R as a function of t, like Eq. (5)

$$\int_0^R \frac{1}{1-\frac{k_{ass}C+k_{dis}}{k_{ass}CR_{max}}R}dR=k_{ass}CR_{max}\int_0^t dt \quad (4)$$

$$R=\frac{k_{ass}CR_{max}}{k_{ass}C+k_{dis}}[1-\exp(-k_{ass}C-k_{dis})t] \quad (5)$$

where k_{ass}, k_{dis}, C, and R_{max} are constants. The analyte concentration (C) is determined before measuring the SPR sensorgram, and R_{max} can be obtained from the result after estimation of the SPR sensorgram. The rate constants k_{ass} and k_{dis} are determined from the results of the best curve-fitting to the experimental SPR sensorgrams. Affinity (K_A) is determined from k_{ass}/k_{dis}.

Results and Discussion

Essential Oil-loaded Alginate Beads

Essential oil-loaded alginate beads were prepared to enhance their utilities and to reduce unnecessary dissipation of the essential oils. The morphology of the beads was a spherical shape with white color. The bead size estimated from the photograph was *ca.* 4 mm uniformly. Table 1 lists the amount of essential oils loaded into the beads, resulting from GC analysis. The loading amount of Chamomile and Eucalyptus essential oils was much almost twice than that of Tea Tree essential oil. The loading amount of Chamomile essential oil was a little more than that of Eucalyptus essential oil.

Antimicrobial Disc-Diffusion Assay of Essential Oils on *Staphylococcus aureus*

Results from the antimicrobial disc-diffusion assay are shown in Fig. 1. The essential oils showed a moderate inhibiting activity against the cells. The broadest spectrum revealed was for Tea Tree essential oil. The halo zones determined from antimicrobial disc-diffusion assay of Eucalyptus, Chamomile, and Tea Tree essential oils on the cells were 12.0 mm, 12.5 mm, and 13.0 mm, respectively. The inhibitory ability of Tea Tree essential oil was higher than those of the other essential oils.

Inhibition of *Staphylococcus aureus* Growth with Injection of the Essential Oils

Variations of the cell concentration with injection of the essential oils in nutrient broth are represented in Fig. 2. Inhibition of the cell growth with various injection amounts of the essential oils was evaluated from the results, which was compared with the cell concentration.

Table 1. Amount of essential oil loaded on the alginate beads.

Essential oil	Amount of essential oil loaded on the beads (µl/g of bead)
Tea Tree	5.7
Chamomile	10
Eucalyptus	9.5

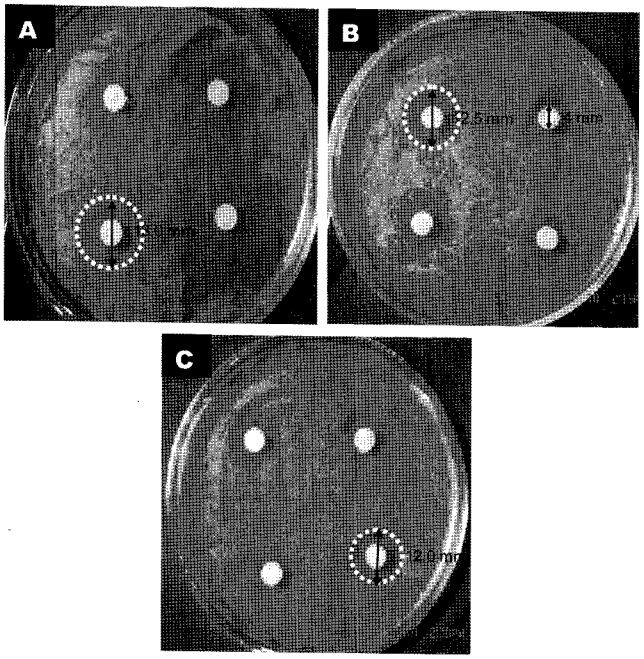

Fig. 1. Photos of halo zones formed by the antibacterial effect of essential oils tested with the disc-diffusion method.
(**A**) Tea Tree, (**B**) Chamomile, (**C**) Eucalyptus essential oils.

A 100% inhibition appeared on the 100 μl injection of Eucalyptus essential oil in nutrient broth. Chamomile essential oil suppressed the growth of the cell above 50 μl of the oil injection. When the oil was injected above 50 μl in nutrient broth, the cell growth was suppressed thoroughly. Specifically, a significant inhibition effect on the cell growth appeared with Tea Tree essential oil at 10 μl injection. The %inhibition of the cells at 16 h reached nearly 100% when 10 μl of Tea Tree essential oil was injected in the nutrient broth.

Inhibition of *Staphylococcus aureus* Growth with Injection of Essential Oil-loaded Beads

The cell growth in nutrient broth was investigated with injection of the pure alginate beads without essential oils. The cells grew gradually in the nutrient broth after 4 h. The pure alginate beads could not suppress the growth of the cells. In fact, the cells grew more in the nutrient broth when more pure alginate beads was added. As the injection of the pure alginate beads increased, the cell growth became higher. It is surmised that the pure alginate beads may perhaps serve as a nutrient for the cells.

The cell growth on the essential oil-loaded beads in nutrient broth is shown in Fig. 3. Each loading amount of essential oils into the beads is represented in the parenthesis of the legend in the figure. The inhibition effects did not appear on the adding of Eucalyptus essential oil-loaded beads when the adding amount was less than 1.0 g of the beads (Fig. 3C). On the contrary, the cells grew well

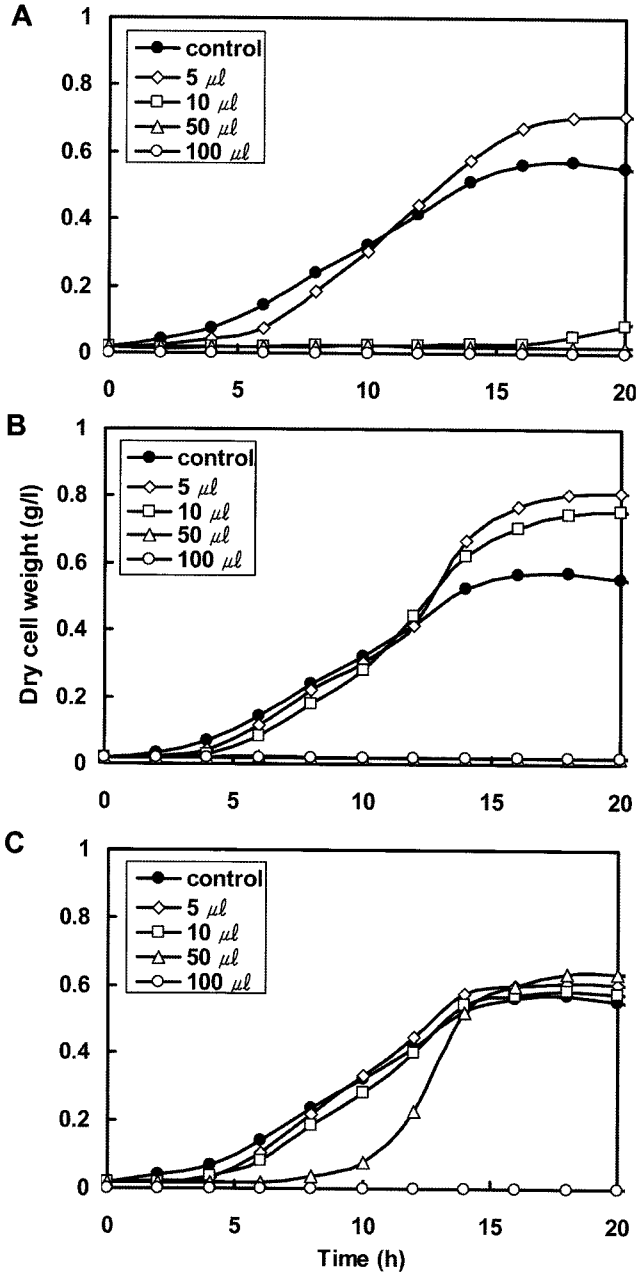

Fig. 2. Variation of *Staphylococcus aureus* concentration with injection of the pure essential oils in 100 ml of nutrient broth.
(**A**) Tea Tree, (**B**) Chamomile, (**C**) Eucalyptus essential oils.

because the alginate beads were acting as a nutrient. Growth of the cell was suppressed when the injection amount of the beads exceeded 1.5 g. A 100% inhibition of the cells occurred in the injection of 2.0 g of the beads. The loading amount of Eucalyptus essential oil was *ca.* 19 μl in the beads at the 100% inhibition.

The antibacterial activity of Chamomile essential oil-loaded beads appeared in injection of 0.3 g of the beads (Fig. 3B). When 0.5 g of the oil-loaded beads was injected,

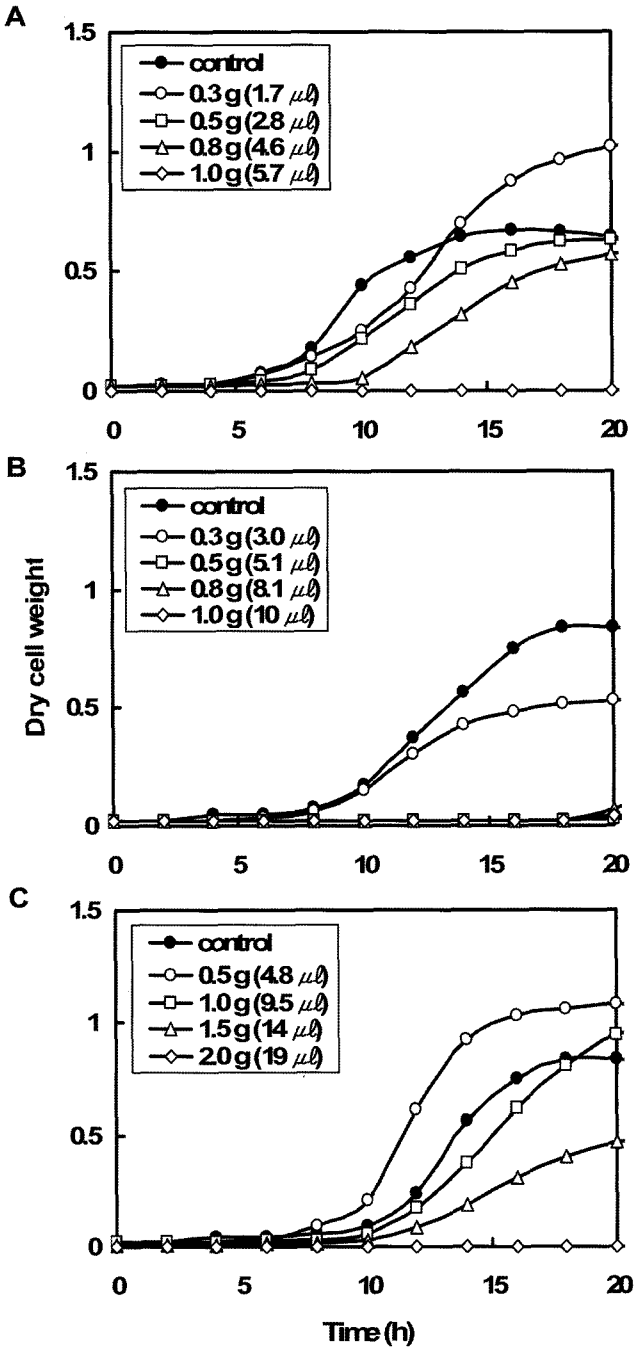

Fig. 3. Variation of *Staphylococcus aureus* concentration with injection of the essential oil-loaded beads in 100 ml of nutrient broth.
(**A**) Tea Tree, (**B**) Chamomile, (**C**) Eucalyptus essential oils.

the cell growth was suppressed before 20 h of process time. However, the cells grew gradually after 20 h. The cell growth was inhibited thoroughly on 0.8 g of the Chamomile-loaded beads injection. The amount of Chamomile essential oil loaded in the beads was *ca.* 8 μl at 100% inhibition.

Table 2. The amount of the injected beads loaded with essential oils and the amount of essential oils on the beads at 100% inhibition of *Staphylococcus aureus*.

Essential oil	Amount of essential oil-loaded beads injected (g)	Amount of essential oil loaded on the beads (μl)
Tea Tree	1.0	5.7
Chamomile	0.8	8.1
Eucalyptus	2.0	19

The inhibition effect of Tea Tree essential oil-loaded beads for the cells appeared when the beads were injected above 0.5 g into the cell solution suspended in nutrient broth (Fig. 3A). A 100% inhibition occurred with in the injection of 1.0 g of the beads. The content of Tea Tree essential oil loaded in the beads was *ca.* 5.7 μl at the 100% inhibition. It was less than the other essential oils loaded in the beads at 100% inhibition.

The amounts of essential oil loaded in the alginate beads at 100% inhibition of the cells are summarized in Table 2. The amounts of essential oils loaded on the beads at 100% inhibition were less than those of pure essential oils. On the other hand, the antibacterial activity of essential oil loaded on the beads was superior to that of pure oil. It can be suggested that cells may have adsorbed on the beads and then the adsorbed cell could react with the essential oil loaded on the beads. Therefore, the inhibition efficiency of the cell may be higher than that of the pure essential oil, because the cell concentrated on the beads can react with essential oils. In comparison of the inhibition effects of the cell growth to the essential oils, the high antibacterial activity appeared in the order of Tea Tree>Chamomile> Eucalyptus essential oils.

Binding Affinity of *Staphylococcus aureus* with the Essential Oil Ligands

The SPR sensorgrams obtained from experiment and simulation of *Staphylococcus aureus* binding to the essential oil ligands are shown in Fig. 4. The RU values in the SPR sensorgrams were rapidly increased with binding of the cells to the essential oil ligands at early binding time, and then reached to equilibrium in less then 20 min. The simulated sensorgrams were well accorded with the experimental sensorgrams.

Rate constants and affinity of the cell binding with the essential oil ligands are summarized in Table 3. The constants were determined from best curve-fitting to the experimental SPR sensorgrams. The kinetic model explained in the section on theory above was applied to the simulation of experimental SPR sensorgrams. Rate constants of association calculated from the kinetic model were obtained at ~10^{-13} ml/(CFU·s) to the cell (1×10^{10} CFU/ml) with the essential oil ligands.

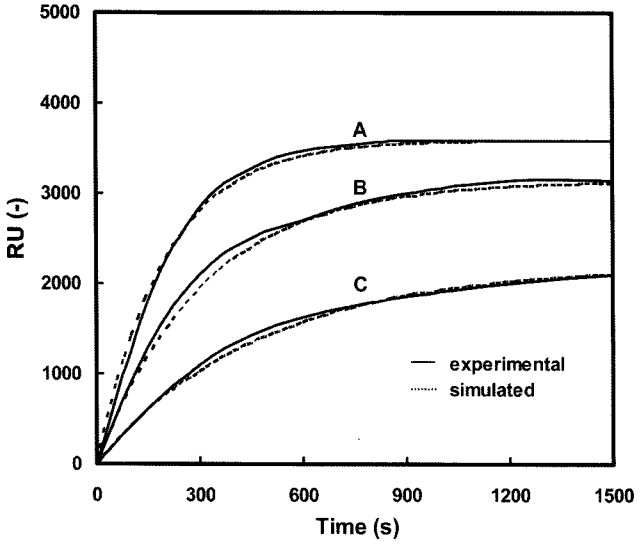

Fig. 4. Experimental and simulated SPR sensorgrams of *Staphylococcus aureus* binding with essential oil ligands.
(**A**) Tea Tree, (**B**) Chamomile, (**C**) Eucalyptus essential oils.

The association rate constant of the cell binding on Tea Tree essential oil was higher than those on the other essential oils. The affinity of cell binding with Tea Tree essential oil was about 5 times higher than that of Eucalyptus essential oil. It is evident that the cells bind on Tea Tree essential oil much more than on other essential oils. In the binding of the cells with Tea Tree essential oil ligand, not only was its association rate faster but the attached amount of the cell was much more than those on the other essential oils.

An important characteristic of essential oils and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane and mitochondria, disturbing the structures and rendering them more permeable [29, 43]. Leakage of ions and other cell contents can then occur [8, 14, 25, 30, 38, 47]. Although a certain amount of leakage from bacterial cells may be tolerated without loss of viability, extensive loss of cell contents or the exit of critical molecules and ions will lead to death [16]. There is some evidence from studies with Tea Tree essential oil and *E. coli* that cell death may occur before lysis [21]. Hydrophobicity was found to be the driving force leading to the interaction between β-amyloid (Aβ) with cells [34]. In our result, it means that the hydrophobicity of the essential oils is important in determining the binding interaction between essential oils and the cells.

Acknowledgments

This work was supported by the Research Center for BioPhotonics. We are also greatly appreciative of the financial support of the Post BK21 program.

References

1. Akiyama, H., K. Shimone, J. Tada, and J. Arata. 1993. Detection of *Staphylococcus aureus* in various skin diseases. *Jpn. J. Dermatol.* **103:** 1315–1322.
2. Allan, P. 2001. Tea Tree oil: The science behind the antimicrobial hype. *Lancet* **13:** 1245–1249.
3. Altschuh, D., M.-C. Dubs, E. Weiss, G. Zeder-Lutz, and M. H. V. van Regemmortel. 1992. Determination of kinetic constants for the interaction between a monoclonal antibody and peptides using surface plasmon resonance. *Biochemistry* **31:** 6298–6304.
4. Bae, Y. M., K.-W. Park, B.-K. Oh, and J.-W. Choi. 2006. Immunosensor for detection of *Escherichia coli* O157:H7 using imaging ellipsometry. *J. Microbiol. Biotechnol.* **16:** 1169–1173.
5. Bereston, E. S. and R. L. Baer. 1942. Keratoconus associated with atopic dermatitis. Report of two cases. *Arch. Dermatol. Syph.* **46:** 358–361.
6. Brunsting, L. A. 1936. Atopic dermatitis (disseminated neurodermatitis) of young adults. Analysis of precipitating factors in one hundred and one cases and report of ten cases with associated juvenile cataract. *Arch. Dermat. Syph.* **34:** 935–957.
7. Cameron, J. A., A. A. Al-Rajhi, and I. A. Badr. 1989. Corneal ectasia in vernal keratoconjunctivitis. *Ophthalmology* **96:** 1615–1619.
8. Carson, C. F., B. J. Mee, and T. V. Riley. 2002. Mechanism of action of *Melaleuca alternifolia* (Tea Tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. *Antimicrob. Agents Chemother.* **46:** 1914–1920.
9. Cho, J.-I., H.-J. Jung, Y.-J. Kim, S.-H. Park, S.-D. Ha, and K.-S. Kim. 2007. Detection of methicillin resistance in *Staphylococcus aureus* isolates using two-step triplex PCR and conventional methods. *J. Microbiol. Biotechnol.* **17:** 673–676.
10. Chung, K.-H., J.-S. Park, H.-S. Hwang, J.-C. Kim, and K.-Y. Lee. 2007. Detection and kinetics of mucosal pathogenic bacteria binding with polysaccharides. *J. Microbiol. Biotechnol.* **17:** [in press].
11. Coles, R. S. and J. Laval. 1952. Retinal detachments occurring in cataract associated with neurodermatitis. *Arch. Ophthalmol.* **48:** 30–39.

Table 3. Rate constants and affinity of *Staphylococcus aureus* binding with the essential oil ligands.

Essential oil ligand	Concentration of analyte (CFU/ml)	k_{ass} (ml/(CFU·s))	k_{dis} (s^{-1})	K_A (ml/CFU)
Tea Tree	1×10^{10}	5.0×10^{-13}	1.0×10^{-18}	5.0×10^{5}
Chamomile	1×10^{10}	3.2×10^{-13}	1.5×10^{-18}	2.1×10^{5}
Eucalyptus	1×10^{10}	2.1×10^{-13}	1.7×10^{-18}	1.2×10^{5}

12. Cowan, M. M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12:** 564–582.
13. Cox, S. D., C. M. Mann, J. L. Markham, H. C. Bell, J. E. Gustafson, J. R. Warmington, and S. G. Wyllie. 2000. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (Tea Tree oil). *J. Appl. Microbiol.* **88:** 170–175.
14. Cox, S. D., J. E. Gustafson, C. M. Mann, J. L. Markham, Y. C. Liew, R. P. Hartland, H. C. Bell, J. R. Warmington, and S. G. Wyllie. 1998. Tea Tree oil causes K+ leakage and inhibits respiration in *Escherichia coli*. *Lett. Appl. Microbiol.* **26:** 355–358.
15. Deans, S. G. and G. Ritchie. 1987. Antibacterial properties of plant essential oils. *Int. J. Food Microbiol.* **5:** 165–180.
16. Denyer, S. P. and W. B. Hugo, 1991. Biocide-induced damage to the bacterial cytoplasmic membrane, pp. 171–188. *In* S. P. Denyer and W. B. Hugo (eds.), *Mechanisms of Action of Chemical Biocides*. The Society for Applied Bacteriology, Technical Series No 27. Oxford Blackwell Scientific Publication, Oxford.
17. Donoyama, N. and Y. Ichman. 2006. Which essential oil is better for hygienic massage practice ? *Int. J. Aromather.* **16:** 175–179.
18. Essawi, T. and M. Srour. 2000. Screening of some Palestinian medicinal plants for antibacterial activity. *J. Ethnopharmacol.* **70:** 343–349.
19. Fagerstram, L. G. 1991. A non-label technology for real-time biospecific interaction analysis, pp. 65–71. *In* J. J. Villafranca (ed.), *Techniques in Protein Chemistry*. Vol. II. Academic Press, New York, U.S.A.
20. Fagerstram, L. G. and R. Karlsson. 1991. Biosensor techniques, pp. 949–970. *In* F. Scheller and R. D. Schmid (eds.), *Biosensors: Fundamentals, Techniques, Applications*. GBF Monographs, Vol. 17. VCH Publishers, Cambridge, England.
21. Gustafson, J. E., Y. C. Liew, S. Chew, J. L. Markham, H. C. Bell, S. G. Wyllie, and J. R. Warmington. 1998. Effects of Tea Tree oil on *Escherichia coli*. *Lett. Appl. Microbiol.* **26:** 194–198.
22. Hanifin, J. M. and J. L. Rogge. 1977. Staphylococcal infections in patients with atopic dermatitis. *Arch. Dermatol.* **113:** 1383–1386.
23. Harkenthal, M., J. Reichling, H. K. Geiss, and R. Saller. 1999. Comparative study on the *in vitro* antibacterial activity of Australian Tea Tree oil, Cajuput oil, Niaouli oil, Manuka oil, Kanuka oil, and Eucalyptus oil. *Pharmazie* **54:** 460–463.
24. Hauser, C., B. Wuethrich, and L. Matter. 1985. *Staphylococcus aureus* skin colonization in atopic dermatitis patients. *Dermatologica* **170:** 35–39.
25. Helander, I. M., H.-L. Alakomi, K. Latva-Kala, T. Mattila-Sandholm, I. Pol, E. J. Smid, L. G. M. Gorris, and A. Von Wright. 1998. Characterization of the action of selected essential oil components on Gram-negative bacteria. *J. Agric. Food Chem.* **46:** 3590–3595.
26. Jonsson, U. 1991. Real-time BIA: A new biosensor based technology for the direct measurement of biomolecular interactions, pp. 467–476. *In* F. Scheller and R. D. Schmid (eds.), *Biosensors: Fundamentals, Techniques, Applications*. GBF Monograph, Vol. 17. VCH Publishers, Cambridge, England.
27. Karlsson, R., A. Michaelsson, and L. Mattersson. 1991. Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor-based analytical system. *J. Immunol. Methods* **145:** 229–240.
28. Kim, J., M. R. Marshall, and C. Vei. 1995. Antimicrobial activity of some essential oil components against five foodborne pathogens. *J. Agric. Food Chem.* **43:** 2839–2845.
29. Knobloch, K., H. Weigand, N. Weis, H.-M. Schwarm, and H. Vigenschow. 1986. Action of terpenoids on energy metabolism, pp. 429–445. *In* E. J. Brunke (ed.), *Progress in Essential Oil Research*. 16th International Symposium on Essential Oils. De Gruyter, Berlin.
30. Lambert, R. J. W., P. N. Skandamis, P. Coote, and G.-J. E. Nychas. 2001. A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.* **91:** 453–462.
31. Lee, C.-M., J.-S. Park, and K.-Y. Lee. 2007. A surface plasmon resonance biosensor for detecting *Pseudomonas aeruginosa* cells with self-assembled chitosan-alginate multilayers. *Talanta* **72:** 859–862.
32. Lee, Y. H., J. H. Lee, H. J. Cho, E. J. Shin, J. H. Park, and J. H. Park. 1999. Charaterization of campylobacters newly isolated from swine gastric mucosa. *J. Microbiol. Biotechnol.* **9:** 778–783.
33. Leyden, J. J., R. R. Marples, and A. M. Kligman. 1974. *Staphylococcus aureus* in the lesions of atopic dermatitis. *Br. J. Dermatol.* **90:** 525–530.
34. Lin, M.-S., H.-M. Chiu, F.-J. Fan, H.-T. Tsai, S.-S. Wang, Y. Chang, and W.-Y. Chen. 2007. Kinetics and enthalpy measurements of interaction between β-amyloid and liposomes by surface plasmon resonance and isothermal titration microcalorimetry. *Colloids Surf. B Biointerfaces* **58:** 231–236.
35. May, J., C. H. Chan, A. King, L. Williams, and G. L. French. 2000. Time-kill studies of Tea Tree oils on clinical isolates. *J. Antimicrob. Chemother.* **45:** 639–643.
36. Myszka, D. G. and R. L. Rich. 2000. Implementing surface plasmon resonance biosensors in drug discovery. *Pharm. Sci. Technol. Today* **3:** 310–317.
37. Nam, Y. S. and J.-W. Choi. 2006. Fabrication and electrical characteristics of ferredoxin self-assembled layer for biomolecular electronic device application. *J. Microbiol. Biotechnol.* **16:** 15–19.
38. Oosterhaven, K., B. Poolman, and E. J. Smid. 1995. S-carvone as a natural potato sprout inhibiting, fungistatic and bacteristatic compound. *Industr. Crops Products* **4:** 23–31.
39. Russel, A. D. 1991. Mechanisms of bacterial resistance to non-antibiotics: Food additives and food pharmaceutical preservatives. *J. Appl. Bacteriol.* **71:** 191–201.
40. Saller, R., T. Berger, J. Reichling, and M. Harkenthal. 1998. Pharmaceutical and medicinal aspects of Australian Tea Tree oil. *Phytomedicine* **5:** 489–495.
41. Sherry, E. and P. H. Warnke. 2004. Successful use of an inhalational phytochemical to treat pulmonary tuberculosis: A case report. *Phytomedicine* **11:** 95–97.

42. Sherry, E., H. Boeck, and P. H. Warnke. 2001. Topical application of a new formulation of Eucalyptus oil phytochemical clears methicillin-resistant *Staphylococcus aureus* infection. *Am. J. Infect. Control* **29:** 346–352.
43. Sikkema, J. A., M. De Bont, and B. Poolman. 1994. Interactions of cyclic hydrocarbons with biological membranes. *J. Biol. Chem.* **269:** 8022–8028.
44. Sivropoulou, A., E. Papanikolaou, C. Nikolanou, S. Kokkini, T. Lanaras, and M. Arsenakis. 1996. Antimicrobial and cytotoxic activities of *Origanum* essential oils. *J. Agric. Food Chem.* **44:** 1202–1205.
45. Skandamis, P. N. and G.-J. E. Nychas. 2001. Effect of oregano essential oil on microbiological and physico-chemical attributes of minced meat stored in air and modified atmospheres. *J. Appl. Microbiol.* **91:** 1011 –1022.
46. Tuft, S. J., D. M. Kemeny, J. K. G. Dart, and R. J. Buckley. 1991. Clinical features of atopic keratoconjunctivitis. *Ophthalmology* **98:** 150–158.
47. Ultee, A., M. H. J. Bennink, and R. Moezelaar. 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen *Bacillus cereus*. *Appl. Environ. Microbiol.* **68:** 1561–1568.
48. Williams, L. R. 1996. Ranking antimicrobial activity. *Int. J. Aromather.* **7:** 32–35.
49. Williams, L. R. and V. Home. 1995. A comparative study of some essential oils for potential use in topical applications for the treatment of the yeast *Candida albicans*. *Aust. J. Med. Herbalism* **7:** 57–62.
50. Yim, J. H., B. H. Kim, J. K. Ku, Y. S. Kang, M.-Y. Kim, H.-O. Kim, M.-J. Chung, and Y. M. Park. 2006. Therapeutic effects of probiotics in patients with atopic dermatitis. *J. Microbiol. Biotechnol.* **16:** 1699–1705.