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Development and evaluation of a plant-based air filter system for bacterial growth control

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We investigated a novel plant-based air filter system for bacterial growth control. The volatile components released from the experimental plant (*Cupressus macrocarpa*) were used as the basis of the bacterial growth control and inhibition. We monitored the effect of light on the gas exhausted from the system, and we found that the presence of light induced an increase in the O_2 concentration and a decrease in the CO_2 concentration in the exhaust gas. A variety of Gram-positive and -negative bacteria was used to elucidate the effect of the exhaust gas on bacterial growth. In the *Bacillus subtilis* cultivation aerated with the exhaust gas (batch mode), we observed a decrease in the specific growth rate (μ = 0.227 h^{-1}) compared with the control experiments (0.257 h^{-1}). The same result was observed for the *Staphylococcus aureus* cultivation aerated with the exhaust gas. In the case of Gram-negative bacterial cultivation aerated with the gas, no significant inhibitory effect of the exhaust gas on the bacterial growth was observed. When the number of bacteria (*B. subtilis*) in a continuous culture was varied at different aeration rates (between 50 to 200 mL/min) using the exhaust gas, a prominent inhibitory effect was observed. Preliminary gas analysis showed that the major inhibitory factors in the exhaust gas are α- and β-pinene and linalool. The results show that the air filter system used in this study could be applied not only as a methodological aspect for estimating antibacterial activity but also for bacteria control in a given system.

Key words: Plant-based biofilter, *Cupressus macrocarpa*, *Bacillus subtilis*, *Staphylococcus aureus*, α-pinene, β-pinene.

INTRODUCTION

The antimicrobial activity of plant oils and extracts has been recognized since antiquity, while scientific investigations to characterize their properties in the laboratory date back only to the early 1990s (Dorman and Deans, 2000). In particular, the antimicrobial activity of plant oils and extracts has formed the basis of many applications,

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including raw and processed food preservation, pharmaceuticals, alternative medicine, and natural therapies (Hammer et al., 1999; Cowan, 1999). The antimicrobial activities of plant extracts from a wide variety of plants have been examined and reviewed (Cowan, 1999). Previous investigations showed that plant secondary metabolites, including terpenoids, are the chemicals responseble for the antimicrobial activity of plant oils and/or extracts. Because of these efforts, methods for evaluating the antimicrobial activity of plant oils and extracts have been effectively established (Dorman and Deans, 2000; Hammer et al., 1999; Cowan, 1999). Several methods such as the agar dilution method, broth dilution method, and disk diffusion method have been used to determine antimicrobial activity of a given sample (Cowan, 1999). However, most studies on the antimicrobial activity of plant extracts and their components have been conducted *in vitro*, and little information is available regarding the antimicrobial activity of plants as "active components" for bacteria removal from the atmosphere (air).

Recently, attempts were made to verify the practical role of plants in woodland as a gigantic "biofilter" or "air-purifier" for air pollutants (Grime, 1998; Betts et al., 2008; Smith et al., 2008). Indeed, it has been proved that indoor gaseous toxic substances such as benzene, formaldehyde, and trichloroethylene are efficiently removed by both physical and biological actions of plants (Wolverton and Wolverton, 1993). In addition, interior plants can significantly reduce airborne microbes and mold spore number (Wolverton and Wolverton, 1996). These findings indicate that interior plants influence the microbial level in air where large numbers of interior plants are grown. Previous results show that various volatile compounds from plants are likely the main factor underlying their antiseptic /antibacterial properties (Dorman and Deans, 2000). In these cases, however, conventional techniques for the measurement of antimicrobial activity, such as the disk diffusion method or minimum bacterial inhibition method, are adopted for evaluating the antimicrobial activity of a sample; thus, an enumeration and/or assessment method for accurately determining antimicrobial activity is required to allow for a more effective estimation of the antimicrobial activity of volatile components released by plants.

The aim of this investigation was to develop an assessment method for the estimation of antimicrobial activity of volatile components from plants. The present study also deals with the construction of a plant-based biofilter system for bacterial growth control. As an experimental plant species, *Cupressus macrocarpa* (Monterey Cypress "Goldcrest") was used. Previous investigations showed that the Cupressaceae family plants, including *C. macrocarpa*, release antimicrobial volatile components from their leaves (Mazari et al., 2010). However, these studies were performed using components extracted from leaves, and the direct antimicrobial activity of the volatile components based on the direct interaction between bacteria and the components in a given system was not examined. Therefore, the practical configuration of the biofilter system and the effects of plants on bacterial growth were essential elements of our present study.

MATERIALS AND METHODS

Plant materials and bacterial strains

The bacterial strains used in this study, *Bacillus subtilis* KCTC 1021, *Staphylococcus aureus* KCTC 1621, *Escherichia coli* KCTC 1682, and *Pseudomonas aeruginosa* KCTC 2004 were obtained from the Korean Collection for Type Culture (KCTC; Daejeon, Korea). The microorganisms were maintained on Luria broth agar media, which were kept at 4°C after growth had occurred at 30°C, and the strains were subcultured weekly. *C. macrocarpa* plants were bought from the public market of Seoul, Korea (pot size: diameter 7 cm, height 10 cm). The plants were cultivated by adding of 50 mL tap water to the pots every day and acclimated for several weeks to approximately the same experimental conditions of lighting and temperature to minimize any stress resulting from the closed filter system. The apparent surface area of the leaves of each plant used in the experimental stage was about 400 m^2 (Sher-Kaul et al., 1995).

Filter system construction and operation

Figure 1 shows a schematic diagram of the plant-based air filter system employed in this study. A clear, cubical, air-sealed, polyacrylic plastic chamber with a volume of approximately 300 L was used to maintain the plants (20 pots) in a sealed environment during the experimental periods. The chamber lid was removable; it was fitted with a watering pipe that was incorporated from the lid to the pots. The watering pipe for the pot was placed at a depth of 3 cm below the pot soil surface. To minimize air contamination by the soil bacteria, the soil compartments of the pots were wrapped with aluminum foil (18 m thickness; Daihan Eunpakgy Co., Korea). A water drain trap was installed at the bottom of the chamber. Filtered (0.2 µm PTFE filter; PALL Corporation, USA) compressed air (N_2: 75.6%, O_2: 24.2%, CO_2: 0.2% in w/w; Dong-Ah Special Gas, Korea) was supplied to the filter system via the air inlet of the system, and the exhaust gas was collected via the air outlet. The same air filter was installed at the outlet of the system to remove the filtered particles and microorganisms from the system. The system was operated in a fluorescence light incubator (light intensity of 81 µmol/m^2 s) at a constant temperature of 27°C. To investigate the CO_2 gas exchange capability of the system, the compressed air was fed to the system at a rate of 200 mL/min, and the exhaust gas was collected using an air sample bag (model 253-10; SKC, USA). The collected gas was stored for the further analysis.

To investigate the inhibition activity of the gas exhausted from the plant based filter system on bacterial growth in aqueous media, the compressed air was fed to the system at a rate of 200 mL/min, and the exhaust gas from the system was supplied to a bacterial culture. In this experiment, we attempted to verify the inhibition activity of the gas on bacterial growth in aqueous media, as well as the actual components in the exhaust gas as bacterial growth inhibition factors. Approximately, 220 mL Luria broth (containing 0.05 mL silicon oil as an antifoam agent; Shin-Etsu, Japan) in a 1000 mL conical flask was inoculated with 20 mL of an overnight bacterial grown culture and aerated with the exhaust gas (gas flow rate: 200 mL/min) via a small sparger. The growth temperature was 37°C. For the control experiment, direct air connection was established from the compressed air tank to the bacterial cultivation flask (Figure 1). To monitor the effect of the flow rate of the exhaust gas on bacterial growth inhibition, continuous cultivation of bacteria (chemostat) was performed using the same medium. The continuous culture was carried

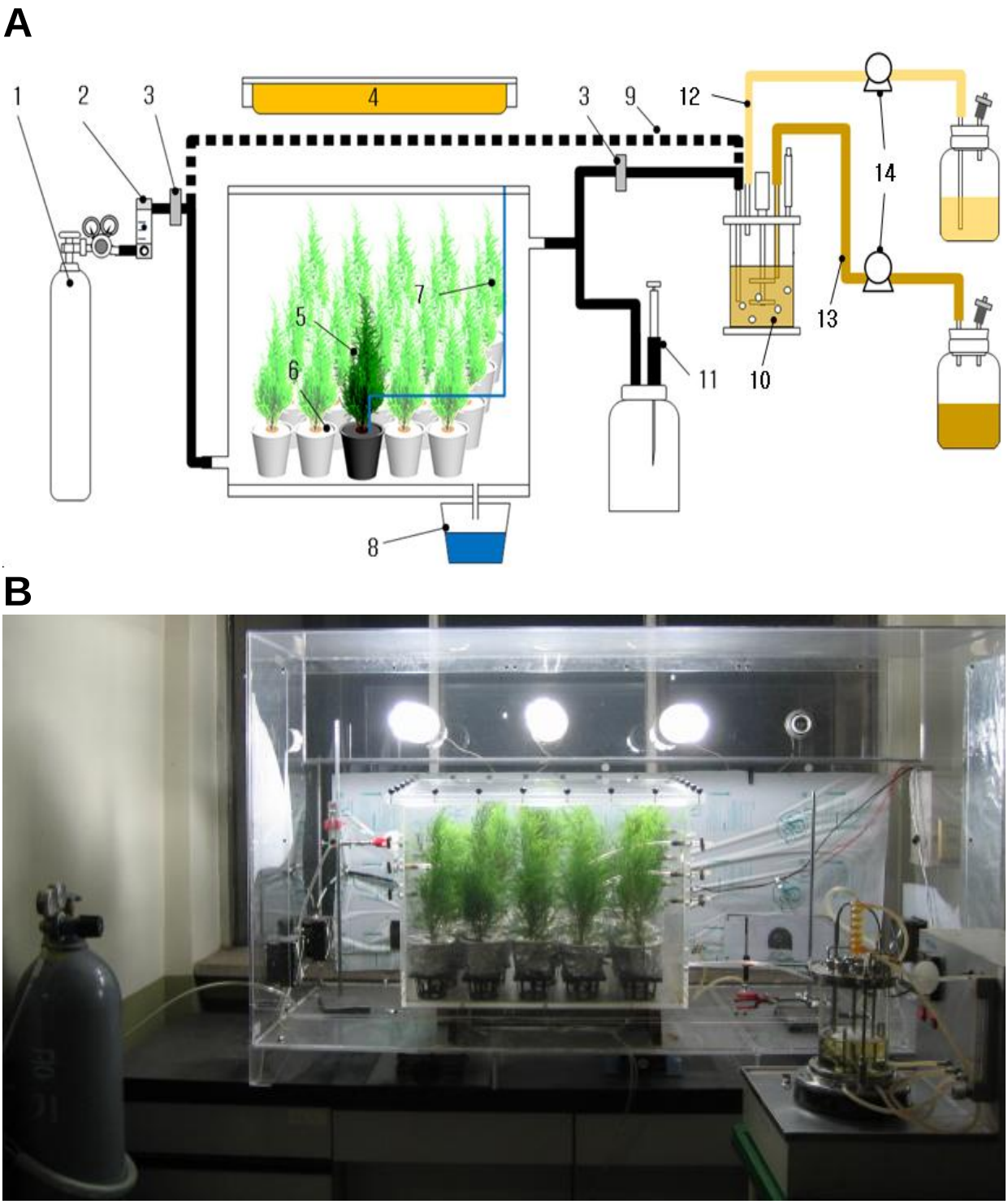

Figure 1. Schematic diagram (A) and photo (B) of the plant-based air filter system: 1, compressed air; 2, gas flow meter; 3, air filter; 4, fluorescent lamp; 5, *C. macrocarpa*; 6, aluminum foil; 7, watering pipe; 8, water drain; 9, air bypass line; 10, bioreactor (flask or fermentor); 11, solid-phase microextraction; 12, medium in (only available in the chemostatic culture); 13, medium out (only available in the chemostatic culture); 14, peristaltic pump.

out in a 2.5 L (total volume) tabletop continuous culture vessel (Model Marado A, CNS Co., Korea) with a working volume of 1 L, which was inoculated with 50 mL of an overnight bacterial grown culture. The pH of the cultivation broth was maintained at 7.0 ± 0.1 by using a pH control system (Model F695; Sechang, Korea). The culture was aerated with various airflow rates of the gas exhausted

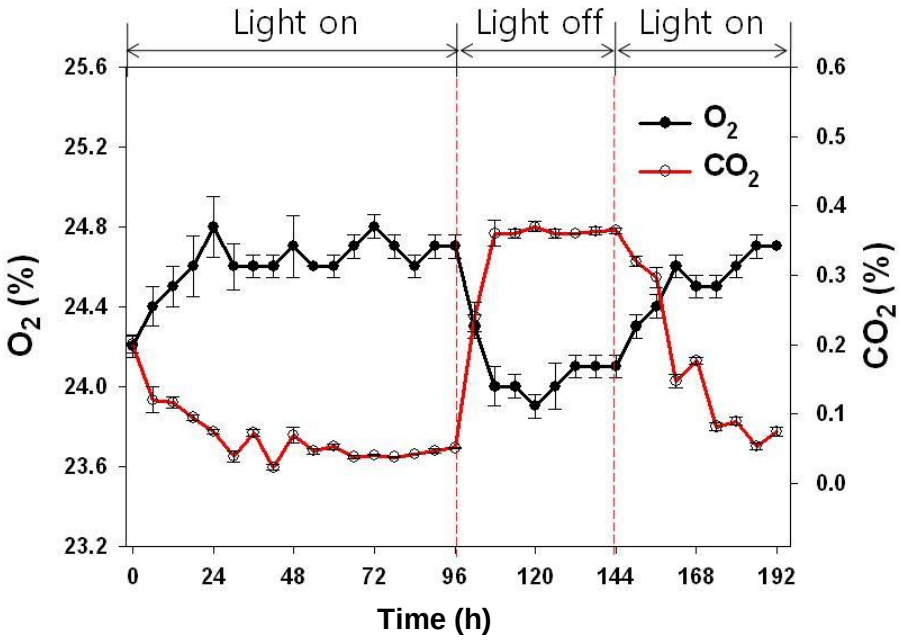

Figure 2. Effect of light on O_2 and CO_2 concentration in the exhaust gas from the biofilter system. The aeration rate was set at 200 mL/min.

from the system. The speed of agitation and medium dilution rate for the chemostat were set at 300 rpm and 0.1 h^{-1}, (fresh medium feeding and removal rates: 100 mL /h each), respectively.

For direct analysis of volatile compounds in the exhaust gas, a solid-phase microextraction (SPME) system (100 µm, polydimethylsiloxane phase fiber; Sigma, USA) was installed at the gas outlet of the system (Figure 1). The absorption time for SPME was set at 10 h with a flow rate of 200 mL/min.

Analysis

Gaseous O_2 and CO_2 collected from the plant-based air filter system were analyzed via gas chromatography, as previously described (Powell and Agrawal, 2011). For analysis of volatile compounds using SPME, a gas chromatography system (YL6100 GC; Younglin, Korea) equipped with a Supelco DB-1 column (30 m × 0.32 mm, 10-µm-thick film, Carrier gas: He) and a flame-ionized detector (Model YFID; Younglin Co., Korea) was used (Santos-Filho et al., 2011). The volatile components in the sample were identified by comparing the retention times with standard chemicals. α-Pinene, β-pinene and linalool were purchased from Sigma (USA) and used as standard chemicals.

Bacterial concentration was determined from a pre-established bacterial concentration calibration curve (colony forming units, cfu/mL vs. O.D. at 600 nm) for each strain. All experiments were performed at least in duplicate.

RESULTS AND DISCUSSION

Effect of light on the concentrations of O_2 and CO_2 in the exhaust gas

After the plant-based air filter system was set up, concentrations of O_2 and CO_2 from the system were monitored using gas chromatography. Figure 2 shows variations in O_2 and CO_2 concentrations in the exhaust gas with and without illumination for eight days. Ambient concentrations of O_2 and CO_2 in the system were 24.2 and 0.2%, respectively. In the presence of illumination, oxygen concentration in the exhaust gas was gradually increased up to 24.8%. However, a gradual decrease in the CO_2 concentration was observed. In dark conditions (after 96 h of operation under illumination), reverse phenomena in the change of O_2 and CO_2 concentrations were observed. The O_2 concentration from the exhaust gas was decreased to 24.0%; on the other hand, the CO_2 concentration in the exhaust gas was increased up to 0.38%. When the system was illuminated again, the gas concentrations in the exhaust were re-stabilized to the previous levels. It is well known that CO_2 is absorbed through the stomata of plants during the process of photosynthesis (that is, in the presence of illumination), and O_2 is absorbed during respiration (in the absence of illumination) (Aini Jasmin et al., 2012). Therefore, these results indicate that the experimental plants in the biofilter system are the active factor for the operation of the air filter system. Furthermore, they possess reliable characteristics of reversibility and stability, and the system has CO_2 exchange capacity under illuminated conditions.

Effect of the exhaust gas on bacterial growth

To investigate the action of the exhaust gas on the bacterial growth, the bacteria-inoculated cultures were aerated with the gas exhausted from the filter system. Figures 3A and B show the results of bacterial growth with aeration

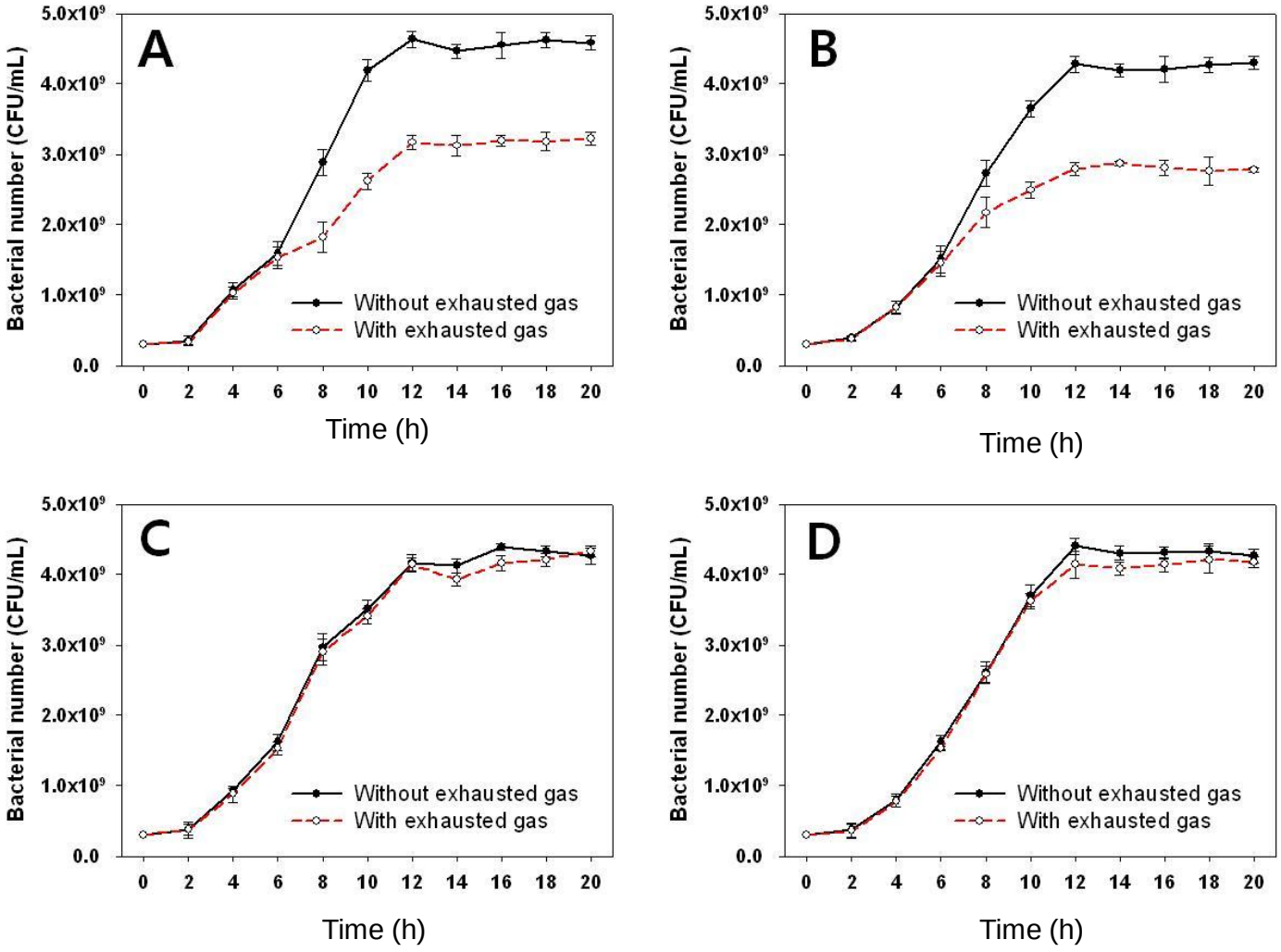

Figure 3. Effect of exhaust gas on the growth of bacteria (A, *B. subtilis*; B, *S. aureus*; C, *E. coli*; D, *P. aeruginosa*). The aeration rate for the cultivations was set at 200 mL/min

of the exhaust gas: the growth of both Gram-positive bacterial strains (*B. subtilis* and *S. aureus*) was suppressed. In the case of *B. subtilis* cultivation, the initial stage of exponential growth produced no significant difference in the growth rate between aeration with the exhaust gas and the control experiment (aeration with the compressed gas). However, a noticeable difference in the specific growth rate (μ) between the experimental cultivation (0.227 h^{-1}) and the control cultivation (0.257 h^{-1}) was observed in the mid and late stages of the exponential phase of cultivation. The same result was observed for *S. aureus* cultivation. When the cultivation was aerated with the exhaust gas, a decrease in the specific growth rate of the culture (0.199 h^{-1}) was observed, as compared with the control experiment (0.239 h^{-1}). It should be noted that in this experiment, the O_2 concentration in the exhaust gas was higher than that in the control experiment. Generally, for aerobic bacteria, high O_2 concentration in the aeration gas promotes higher bacterial growth yield due to the increase in electron acceptor concentration for aerobic respiration (Chen et al., 2003). Therefore, the decrease in the growth of Gram-positive bacterial strains aerated with the exhaust gas is possibly due to the release of volatile components in the exhaust gas that effectively suppressed bacterial growth. In another pair of matching experiments carried out under identical conditions, Gram-negative bacterial strains (*E. coli* and *P. aeruginosa*) were aerated with the exhaust gas from the filter system (Figures 3C and D). As shown in the figure, no prominent bacterial growth inhibition effect was observed when the cultivations were aerated with the exhaust gas. These results indicate that the volatile components in the exhaust gas have no inhibitory activity against the Gram-negative bacteria used in this study.

To verify the inhibitory effects of the volatile components on the growth of Gram-positive bacteria, the aeration rate of the exhaust gas used for the continuous cultivation of *B. subtilis* were varied. Figure 4 shows the inhibi-

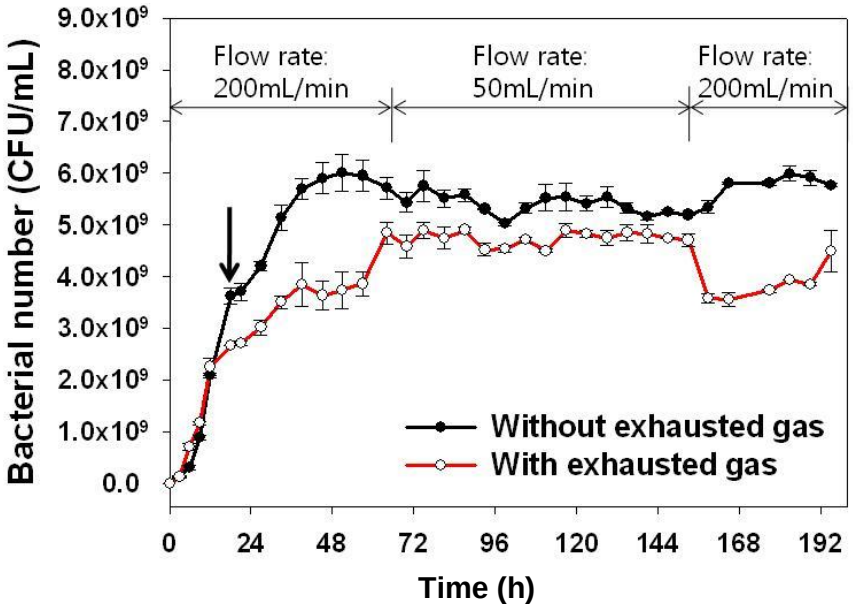

Figure 4. Effect of aeration rate for continuous culture of *B. subtilis*. The medium dilution rate for the chemostat was set at 0.1 h^{-1} (the arrow indicates the chemostat starting point: medium feeding and removal rates were set at 100 mL/h).

inhibitory effect of the exhaust gas with different flow rates on the steady state of bacterial growth in the continuous culture. When the exhaust gas was supplied to the culture at 200 mL/min, the growth rate was decreased compared to the control culture (direct aeration with the compressed air). However, a further decrease of the aeration rate of the exhaust gas (50 mL/min) induced an increase in the bacterial concentration of the culture. In the control experiment, a decrease of the aeration rate from 200 to 50 mL/min induced a decrease in the bacterial concentration. When the aeration rate using the exhaust gas was restored at 200 mL/min, the bacterial concentration in the steady state decreased. In the control experiment, however, the rapid aeration rate induced the increased steady state bacterial concentration. These results also suggest that the exhaust gas from the filter system contains bacterial growth inhibition components and the concentrations of the components can be controlled by the aeration rate. In view of these observations, further experiments were carried out to analyze the volatile components that have bacterial inhibitory activity in the gas exhausted from the filter system.

Volatile components analysis

When the exhaust gas was analyzed by solid phase microextraction (SPME) and gas chromatography, we found that the gas sample contained various components, including α-pinene, β-pinene, and linalool (Figure 5). Previous reports have shown that various volatile components from plants, including α-pinene and β-pinene, have inhibitory effects on the growth of Gram-positive bacteria (Leite et al., 2007). In addition, *Cupressus* species produce various components that have antibacterial activity (Cheraif et al., 2007). It should be noted that the water solubility of linalool is higher (1.336 g/l) than α-pinene (0.018 g/L), β-pinene (0.023 g/L) (Cal, 2006). Therefore, the inhibition of bacterial growth in both batch and continuous cultivation aerated with the exhaust gas could be attributed to the presence of antibacterial components such as linalool in the gas. In the case of Gram-negative bacteria species, the inhibition and control of growth using such a plant-based air filter system was not possible. However, substitution with a different plant species in the filter system may be effective in controlling Gram-negative bacteria.

In conclusion, this study reveals the potentiality of an air filter to control bacterial growth, particularly by exploiting plants that release volatile antibacterial components. In addition, this study also describes a novel direct method to estimate the antibacterial activity of plant-based volatile components. Minimum inhibitory concentration and agar diffusion methods using plant extracts have been widely used to estimate the antibacterial activity of plant extracts. A major drawback associated with these methods is the time required for the step in which bacteria are incubated with the extract. Therefore, real-time estimation of the antibacterial activity of given volatile components from plants is possible by employing the methods used in this study.

Previous reports indicate that the indoor atmosphere is an ecological unit that affects public health (Tringe et al., 2008; Wolverton, 1997). Therefore, the plant-based air filter system used in this study could be applied not only

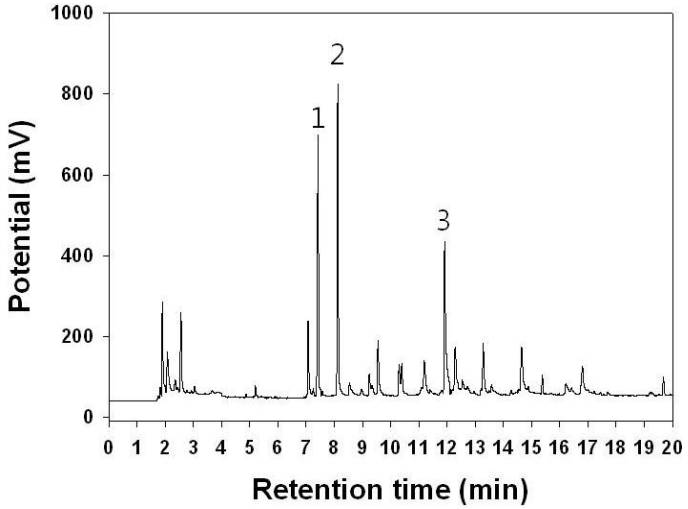

Figure 5. Analysis of volatile compounds in the exhaust gas by using GC-FID (1, α-pinene; 2, β-pinene; 3, linalool).

as a methodological aspect for estimating antibacterial activity but also for indoor air quality control from the public health perspective. To the best of our knowledge, this is the first report regarding the application of a plant-based biofilter system designed for controlling bacterial growth. As further research in this area continues, different species of plants will be used for their ability to inhibit and/or effect the removal of bacteria and other indoor pollutants. Therefore, the current research by our group is focused on the optimization of plant-based air filter systems for various species and conditions of bacteria and improving their air pollutant-removing activity for indoor air quality control.

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