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Biocidal effect of thymol and carvacrol on aquatic organisms: Possible application in ballast water management systems

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ABSTRACT

Ballast water is essential for maintaining the balance and integrity of a ship. However, exchanging ballast water resulted in discharging water of different origins in vessel recipient ports, and this may have caused ecosystem disturbance or aquatic pollution. The ballast water management (BWM) system is essential for the purification and disinfection of the ballast water that is taken up. Because current BWM systems widely use biocides for the treatment of aquatic organisms, the biocides may result in unintended toxicity of the discharged ballast water. In this study, we suggested thymol and carvacrol as chemical biocides for BWM systems and investigated their effectiveness using *Artemia salina* and *Escherichia coli*. Thymol and carvacrol showed biocidal effects in our study. A combination of these substances showed a synergistic increase in the biocidal effects. Moreover, carvacrol naturally degrades after disinfection, which indicates that natural substances may be promising candidates to increase the efficacy and reduce unwanted side effects of the BWM system.

1. Introduction

Ballast water regulates the roll of a ship during loading or unloading of cargo by maintaining the stability, balance, and structural integrity of the ship (Hua and Liu, 2007). As the weight of moving ballast water has to correspond to that of cargo, the annual amount of ballast water moved is approximately 3.1 billion tons (David and Gollasch, 2015). Ballast water is taken up by a ship from the sea adjacent to the departing harbor and discharged to the sea adjacent to the arriving harbor. This process results in mixing of seawater from different seas, which sometimes leads to ecosystem disturbance or marine pollution (Werschkun et al., 2014). Therefore, the International Maritime Organization (IMO) developed regulations for ballast water management (BWM) systems and required minimal functions of such systems. The regulations focus not only on the capacity to eliminate aquatic organisms but also on the environmental safety of the discharged ballast water, which concerns the protection of aquatic environments (Čulin and Mustać, 2015; Tsolaki and Diamadopoulos, 2010).

Currently, BWM systems are of two types. One type employs physical methods of high energy, such as electricity and irradiation, to filter solid particles from seawater and eliminate aquatic organisms (Tsolaki and Diamadopoulos, 2010). However, the filters in such systems need to be changed after damage and deterioration during the filtering step, and the high operational cost of these filters lowers the disinfection efficacy of electricity and irradiation systems (Nanayakkara et al., 2011). The other type employs chemical methods to eliminate aquatic organisms, namely, treatment with a chemical biocide. Although various chemical compounds with high reactivity are known to show cytotoxicity at a low concentration, the excessive toxicity of these chemical compounds causes secondary problems when ballast water is discharged to the sea (Cañizares et al., 2009). Although recent studies have focused on the natural degradation of active substances and preventing secondary cytotoxicity, optimization of the concentration of the biocide and the duration of the biocidal effect remains to be investigated (La Carbona et al., 2010; Perrins et al., 2006; Werschkun et al., 2014).

"Natural product" is the general term for derivatives from natural organisms including plants, fungi, and animals. Natural products

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usually show remarkable biological effects through their high activity and selectivity (Wright et al., 2007). The other outstanding characteristic of natural products is that they are easily degraded in the natural environment because of their eco-friendly structure, derived from biosynthesis processes (Nicolaou and Snyder, 2005). Recently, many biological and engineering studies have suggested the usefulness of natural products as novel compounds for the control of biological events and disease (Bauer and Bronstrup, 2014; Koehn and Carter, 2005). Thymol and carvacrol are phenolic monoterpenes of the essential oil from thyme and oregano, respectively (Castillo et al., 2014). Although they have a simple molecular structure and few reactive groups, the antimicrobial, antifungal, and antiviral effects of these compounds have been reported (Mechergui et al., 2016). However, the application of thymol and carvacrol in ballast water disinfection has never been attempted. Investigation of these products has great potential for revealing useful novel compounds.

Escherichia coli is widely known as a colonic bacillus, gram-negative microorganism, and commensal in the human intestine (Baker, 2014). *E. coli* can survive in various environments, and it induces pathogenicity by decomposing substances that surround it, including food, living organisms, and even seawater. Because of its pathogenicity, *E. coli* is classified as a microorganism that must be disinfected by BWM systems. *Artemia salina* is a plankton commonly found in the saline environment, and its size satisfies that suggested by IMO BWM guidelines (MEPC.279(70)). These two species were chosen in previous studies as models for validating the efficiency of BWM systems (Chen et al., 2016; Wright et al., 2009). Following those studies, we utilized the two species for validating the biocidal effects of thymol and carvacrol in this research.

In this study, we searched biocidal natural product pools for materials with high potential to be used in BWM systems. As a result, we proposed novel natural products, and their high biocidal effects were shown through biological investigations using plankton and an aquatic microorganism. Moreover, the potential for utilizing these substances in BWM systems was evaluated.

2. Materials and methods

2.1. Measurement of seawater characteristics

To analyze the chemical characteristics of natural seawater and synthetic seawater, we collected natural seawater samples from adjacent sea around Songdo Beach, Busan, Republic of Korea, and prepared synthetic seawater by mixing sea salt (Sigma-Aldrich, St. Louis, MO) to distilled water (DW). The large particles in natural seawater samples were eliminated by filtering with a 0.45-μm polyethersulfone (PES) membrane filter. We compared samples of the two types of seawater to analyze four characteristics including dissolved oxygen (DO), salinity, total dissolved solids (TDS), and conductivity. DO was calibrated and measured using a DO-300 L DO meter (Istek, Seoul, Republic of Korea). Salinity, TDS, and conductivity were assessed using a YSI Pro30 conductivity meter (YSI Inc., Yellow Springs, OH).

2.2. Microorganism cultivation and treatment

For investigating the biocidal effect of natural products on aquatic microorganisms, *E. coli* was used for experiments. The DH5α strain of *E. coli* was purchased from Bethesda Research Laboratories Inc. (Rockville, MD). The stock solution of *E. coli* was made by adding glycerol (20% of v/v) to the medium with *E. coli,* and this was stored in deep freezer (−70 °C) until use in experiments. The cells were thawed on ice for 10 min, transferred to fresh lysogeny broth (LB) media, and incubated at 37 °C in a shaking incubator (Vision Scientific Co. Ltd., Daejeon, Republic of Korea). To mimic the aquatic growth environment, LB medium with 3.5% salinity was formulated (3.5% NaCl, 1% Bacto Tryptone, and 1% Bacto Yeast extract in DW). For investigating the biocidal effect of thymol and carvacrol, 1×10^5 cells were inoculated to fresh LB media and treated with thymol or carvacrol. Thymol and carvacrol were purchased from Sigma-Aldrich.

2.3. Plankton cultivation and treatment

For investigating the biocidal effect of natural products in planktons, *A. salina* was used for experiments. The eggs of *A. salina* were purchased from Artemia International LLC. (Houston, TX) and were incubated in seawater at 25 °C. The eggs hatched after 3 days, and *A. salina* larvae of length 1–3 mm were used for further studies. The *A. salina* larvae were treated with thymol or carvacrol in a 100-mL beaker.

2.4. Measurement of the survival of E. coli and A. salina

To measure the number of the *E. coli* grown in LB media treated with natural products, a UV spectrophotometer (UV-1800, Shimadzu Corp., Tokyo, Japan) was used. One milliliter of the LB medium containing *E. coli* was sampled, and the absorbance at a 600-nm wavelength was analyzed every 1 h. To measure the survival of *A. salina* upon treatment of natural products, approximately 50 planktons were sampled from the seawater treated with natural products, and the survival of planktons was checked through microscopic observation with an Olympus IX71 microscope (Olympus Optical Co. Ltd., Tokyo, Japan) every 2 h.

2.5. High-performance liquid chromatography (HPLC)

To assess the amount of carvacrol in LB media, a Waters 1525 Binary HPLC pump (Waters, Milford, MA) and a Waters 2489 UV/Visible detector (Waters) were used for analysis by reversed-phase high-performance liquid chromatography. Ten microliters from an aliquot of LB medium were injected and separated on a Sunfire C18 column (4.6 × 250 mm; Waters). The mobile phase was an isocratic combination of acetonitrile:H_2O (50:50) with a flow rate of 1 mL/min. The effect of carvacrol was verified by measuring the absorbance at a 274-nm wavelength. The amount of carvacrol was calculated with Breeze™ HPLC software (Waters) by quantifying the area of the carvacrol peak.

3. Results

3.1. Selection of the natural biocide for BWM systems

For the development of a BWM system with natural products, we first selected natural products to be utilized in the system. Because large aquatic organisms including fish, shells, and aquatic plants are easily filtered by the initial management system, the objective of the research was to eliminate aquatic organisms of size approximately 50 μm. We used plant-derived monocyclic monoterpenes for analysis, which previously were suggested to have high antibacterial functions (Koziol et al., 2014). Among the monoterpenes with similar molecular structures, we noticed that thymol and carvacrol have hydroxyl groups in a monocyclic structure, which plays a crucial role in their bioactivity (Fig. 1A and B) (Veldhuizen et al., 2006). Despite the previously reported biocidal effects of these two natural products, their utilization in the treatment of ballast water has not yet been reported; therefore, we selected thymol and carvacrol for further investigation (Botelho et al., 2007; Kordali et al., 2008).

Further, we established an experimental system for verification of the biocidal effect of the natural products in the BWM system. The IMO set the standards for BWM systems regarding planktons and microorganisms according to their size (MEPC.279(70)). In addition, *E. coli* and toxic *Vibrio cholerae* were selected as specific target species for BWM. *A. salina* and *E. coli* also were widely used in studies covering BWM (Holm et al., 2008; Tsolaki and Diamadopoulos, 2010; Tsolaki et al., 2010). According to the IMO regulations and previous studies, we

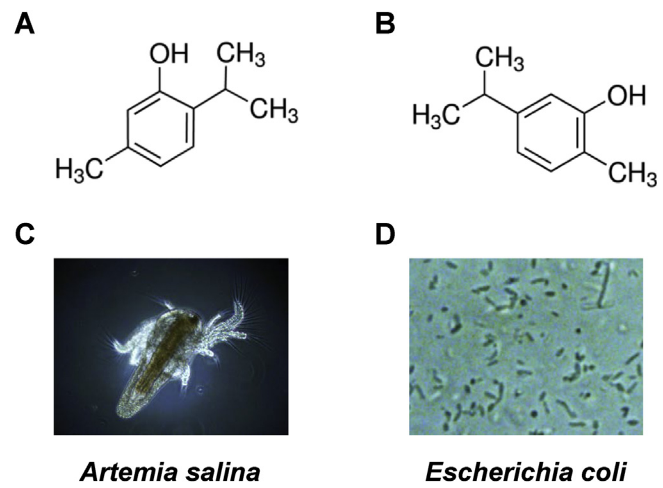

Fig. 1. Selection of the natural biocide for the BWM system.
(A and B) The molecular structure of the target natural product. (A) Corresponds to thymol and (B) corresponds to carvacrol. (C and D) The photographs of the target aquatic organisms. (C) Corresponds to *Artemia salina* and (D) corresponds to *Escherichia coli*.

Table 1
Characteristics of synthetic and natural seawater.

	Conductivity (ms/cm)	DO[a] (mg/L)	Salinity (mg/L)	Temperature (°C)	TDS[b] (g/L)
Synthetic Seawater	45.11	5.57	34.7	18.4	33.57
Natural Seawater-[b]	47.27	4.49	35.6	18.6	34.97

[a] DO: Dissolved oxygen.
[b] TDS: Total dissolved solids.

selected *A. salina* as the marine plankton target and *E. coli* as the aquatic microorganism target of BWM (Fig. 1C and D). We then compared the chemical characteristics of natural seawater and synthetic seawater to verify the utility of using synthetic seawater for further experiments. We selected DO, TDS, salinity, and conductivity as parameters that affect zooplankton growth following a previous study (Gaikwad et al., 2008). As shown in Table 1, there were no significant differences between the types of seawater; hence, we used synthetic seawater for further study.

3.2. Biocidal effect of thymol on A. salina and E. coli

To validate the biocidal potential of thymol, we first treated *A. salina* or *E. coli* with 5 mg/L to 30 mg/L and with 5 mg/L to 10 mg/L thymol, respectively. The concentration of the treatment was selected on the basis of previous studies that reported natural products exerted biocidal effects toward planktons and microorganisms at the μg/L to mg/L scales (Wright et al., 2007). In addition, the concentrations are in accord with IMO guidelines that describe the accumulation possibility of active substances needs to be reduced in BWM systems (MEPC.169(57)). The results of thymol treatment showed that the minimum biocidal effect of thymol was observed at a concentration of 5 mg/L in 2 h (Fig. 2A). In addition, the viability of *A. salina* constantly decreased in a time- and dose-dependent manner. Finally, > 90% of the *A. salina* died with thymol treatment at a concentration of 30 mg/L for 3 h. However, treatment with thymol did not induce significant changes in the viability and proliferation of *E. coli*, even at a concentration of 10 mg/L (Fig. 2B). Taken together, these findings indicate that thymol was effective for the treatment of planktons in ballast water, but it was not suitable for the treatment of microorganisms.

3.3. Biocidal effect of carvacrol on A. salina and E. coli

We also treated *A. salina* or *E. coli* with carvacrol at concentrations of 5 mg/L to 30 mg/L and with 5 mg/L to 10 mg/L, respectively. Unlike thymol treatment, carvacrol did not exert a biocidal effect on *A. salina*, at concentrations of up to 10 mg/L (Fig. 3A). However, carvacrol resulted in a drastic decrease in the viability of *E. coli*. In particular, treatment with carvacrol at a concentration of 5 mg/L prevented *E. coli* from growing and caused death in a time-dependent manner (Fig. 3). Moreover, *E. coli* did not proliferate in response to treatment with 10 mg/L carvacrol. These results showed that carvacrol had a selective biocidal effect on *E. coli* but a less effect on *A. salina*.

3.4. Synergistic effect of thymol and carvacrol on A. salina and E. coli

As described above, we observed that thymol and carvacrol showed selective biocidal effects toward *A. salina* and *E. coli*, respectively (Figs. 2 and 3). Further, we treated each target organism with both thymol and carvacrol to investigate the synergistic effect of thymol and carvacrol. In particular, *A. salina* was treated with a 1:1 mixture of thymol and carvacrol at a concentration of 5 mg/L to 30 mg/L and *E. coli* at 1 mg/L to 10 mg/L. The results revealed that combined treatment with thymol and carvacrol led to a more drastic decrease in the viability of *A. salina* (Fig. 4A). Unlike with thymol-alone treatment, 5 mg/L of combined treatment showed complete eradication of *A. salina* in 5 h. Moreover, combined treatment at a concentration of 20 mg/L exerted the maximum biocidal effect in 2 h. As shown in Fig. 4B, combined treatment with carvacrol and thymol exerted a biocidal effect on the viability of *E. coli* at a concentration of 5 mg/L. As shown in Figs. 2B and 3B, treatment with 5 mg/L thymol did not exert a biocidal effect on *E. coli*, but treatment with 5 mg/L carvacrol showed stagnation of *E. coli* growth. These results suggest that thymol and carvacrol exerted synergistic effects on the survival and growth of *A. salina* and *E. coli*.

3.5. Natural degradation of carvacrol

The elimination of chemical agents from seawater after treatment has been identified as a problem that must be solved. For example, chlorine used for the disinfection of saline wastewater results in the unwanted generation of disinfection byproducts (Yang and Zhang, 2014). By contrast, natural products often can be converted into non-toxic compounds through biotransformation by organisms including microbes (Kumaran and Paruchuri, 1997). Biotransformation is a biological reaction that converts reactants into products with less toxicity toward the organisms, and this is usually observed during the degradation of bioactive reactants. Therefore, we conducted experiments using seawater and media for microorganisms that were treated with thymol or carvacrol to validate the degradation of these compounds in the BWM system.

On addition of *A. salina* to seawater samples, *A. salina* was eliminated after treatment with thymol at a concentration of 10 mg/L. However, *A. salina* could not survive even 5 days after thymol treatment (data not shown), thereby suggesting that thymol did not naturally degrade. *E. coli* was inoculated into the LB medium that was treated with 10 mg/L carvacrol and then incubated at 37 °C, after which samples were inoculated daily with additional *E. coli*. We found that *E. coli* could survive and proliferate in the medium that was treated with carvacrol and incubated for at least 4 days, thus suggesting that carvacrol was naturally degraded (Fig. 5A). Further, we conducted HPLC analysis to validate carvacrol degradation. Following a previous study, we confirmed the condition for analyzing carvacrol by applying pure carvacrol (Fig. 5B, upper) (Hajimehdipoor et al., 2010). The LB medium containing *E. coli* and carvacrol (10 mg/L) was immediately analyzed by HPLC (Fig. 5B, middle). The LB medium with degraded carvacrol

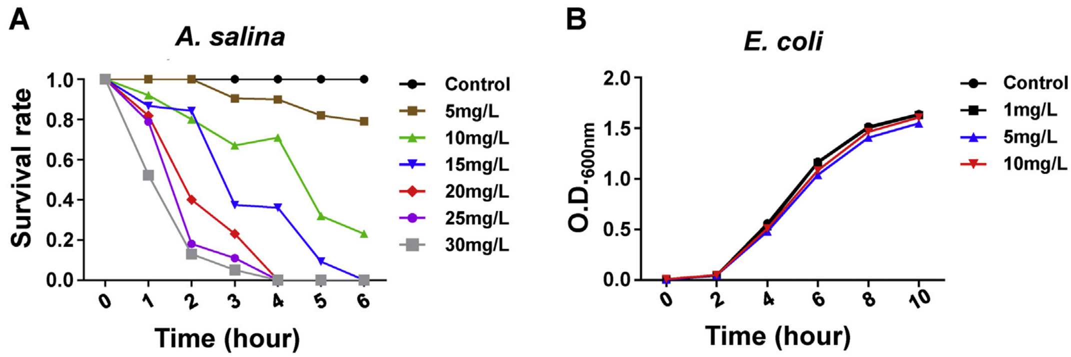

Fig. 2. Biocidal effect of thymol on *Artemia salina* and *Escherichia coli*.
(A) The biocidal effect of thymol on *A. salina* was assessed every 1 h by counting the number of surviving *A. salina* upon treatment with thymol. Thymol was treated at a concentration from 5 mg/L to 30 mg/L at the start of the experiment. (B) The biocidal effect of thymol on *E. coli* was assessed every 2 h by measuring the absorbance at a wavelength of 600 nm of media containing *E. coli*. Thymol was treated at concentrations of 1, 5, or 30 mg/L at the start of the experiment.

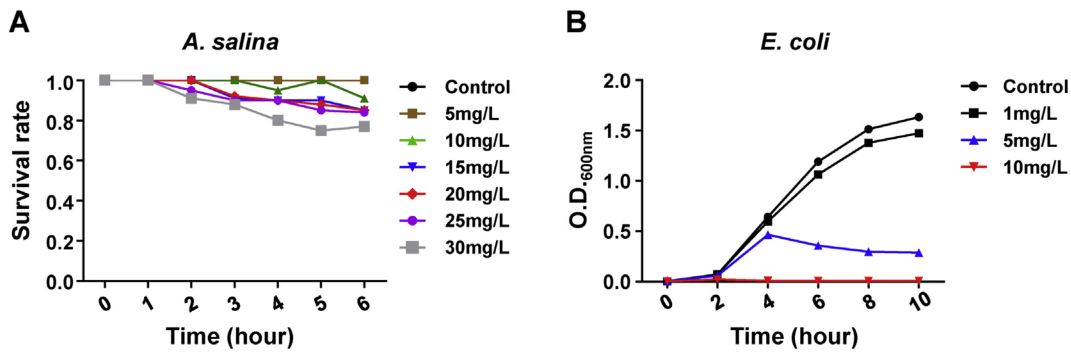

Fig. 3. Biocidal effect of carvacrol on *Artemia salina* and *Escherichia coli*.
(A) The biocidal effect of carvacrol on *A. salina* was assessed every 1 h by counting the number of surviving *A. salina* upon treatment with carvacrol. Carvacrol was treated at a concentration from 5 mg/L to 30 mg/L at the start of the experiment. (B) The biocidal effect of thymol on *E. coli* was assessed every 2 h by measuring the absorbance at a wavelength of 600 nm of media containing *E. coli*. Carvacrol was treated at concentrations of 1, 5, or 30 mg/L at the start of the experiment.

was incubated at 37 °C for 5 days, sampled, and subjected to HPLC analysis. The results revealed that the amount of carvacrol was reduced after 5 days of incubation (Fig. 5B, lower). Taken together, these findings indicated that carvacrol could be degraded naturally in media or during the eradication process, which results in a loss of its biocidal effect.

4. Discussion

BWM systems are essential for maintaining the stability and balance of ships during sailing. Because ballast water is taken up at departing ports and discharged at arriving ports, the exchange of ballast water can cause unexpected ecological problems. Therefore, equipment for treating ballast water in ships has recently become essential; however, technical advances in BWM systems are needed. In this study, we investigated whether the natural products thymol and carvacrol could be used to eliminate aquatic organisms from BWM systems. Thymol was found to influence the viability of the aquatic plankton *A. salina*, whereas carvacrol regulated the viability and growth of the aquatic microorganism *E. coli*. We observed that combined treatment with thymol and carvacrol exerted a synergistic effect, thereby leading to the elimination of both organisms. In addition, carvacrol was shown to be

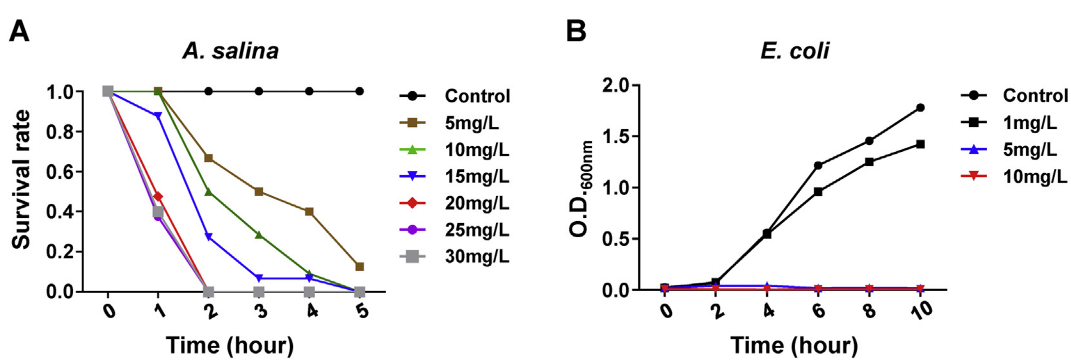

Fig. 4. Synergistic effect of thymol and carvacrol on *A. salina* and *E. coli*.
(A) The biocidal effect of the combination treatment of thymol and carvacrol on *A. salina* was assessed every 1 h by counting the number of surviving *A. salina* upon treatment with thymol and carvacrol (the combination ratio was 1:1). Thymol and carvacrol was treated at a concentration from 5 mg/L to 30 mg/L at the start of the experiment. (B) The biocidal effect of the combination treatment of thymol and carvacrol on *E. coli* was assessed by measuring the absorbance at a wavelength of 600 nm of the medium containing *E. coli*. Thymol and carvacrol was treated at concentrations of 1, 5, or 30 mg/L at the start of the experiment.

A

E. coli inoculation (time after treatment)	1 day after	2 day after	3 day after	4 day after	5 day after
1 day	0.010	0.008	0.008	0.007	0.008
2 day		0.008	0.006	0.007	0.007
3 day			0.008	0.007	0.010
4 day				0.047	1.931
5 day					0.137

B

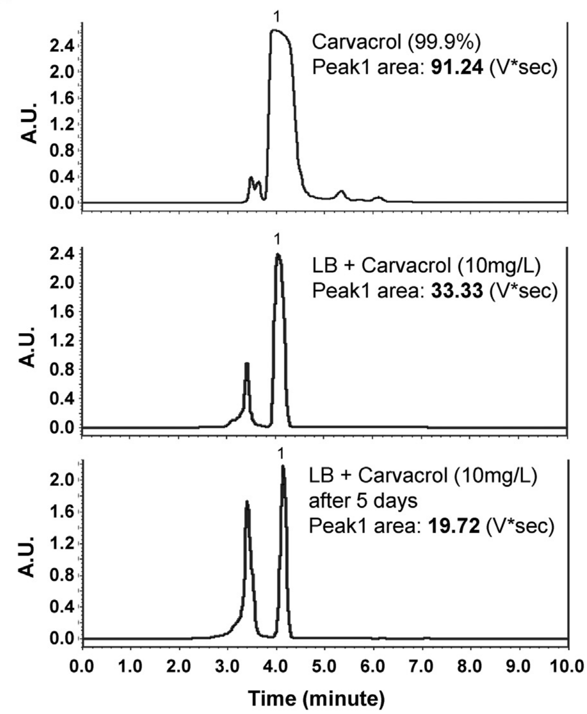

Fig. 5. Natural degradation of carvacrol.
(A) The survival of *E. coli* upon the natural degradation of carvacrol was assessed by measuring the absorbance at a wavelength of 600 nm. The *E. coli* in the LB medium was eliminated by carvacrol treatment, and additional *E. coli* inoculation was performed (left column). After inoculation, the 600-nm absorbance of the LB medium was measured every day. (B) The degradation of carvacrol in the LB medium incubated with *E. coli* was analyzed by HPLC. Ten microliters of the LB medium were subjected to HPLC, and separation was done for 10 min. Pure carvacrol was analyzed for searching the carvacrol peak (upper). The LB medium containing carvacrol (10 mg/L) was analyzed (middle) and incubated at room temperature for 5 days for degradation analysis (lower). Peak 1 was specified as the peak of carvacrol, and the area below the peak was quantified.

degraded naturally and hence lost its biocidal function in 4 days, thereby suggesting the promising application of natural products for an eco-friendly BWM system.

Choosing an appropriate target organism is a major concern in investigations of BWM systems. In this study, we used *A. salina* and *E. coli* to investigate BWM systems, as these organisms have been used in previous studies of such systems. Indeed, *A. salina* showed a relatively high resistance to a test biocide in a previous study, thus suggesting that it can be an adequate model to validate the biocidal potential of a substance for BWM (Wright et al., 2009). *E. coli* is an abundant microorganism in the human digestive system as well as in various ecosystems including seawater. In addition, *E. coli* is listed in the IMO regulations regarding BWM systems as a specific target to be eliminated because of its unique pathogenesis. Actually, the chemical compounds that exerted cytotoxicity to these model species also showed high potential as biocides for BWM systems in a previous study (Tsolaki and Diamadopoulos, 2010). Therefore, the biocidal effects of thymol and carvacrol on *A. salina* and *E. coli* shown in this study suggested their potential for universal use in BWM strategies.

Natural products have been widely investigated because of their various biological effects (Koehn and Carter, 2005). In particular, these compounds usually show antiviral, antibacterial, antifungal, and even antitumor effects (Butler et al., 2014; Kang et al., 2013). Although each natural product has a unique mechanism of action that leads to their biological effects, it is widely accepted that these effects are derived from their chemical structures based on what is known as the "structure–activity relationship" theory (Kimbaris et al., 2017). Thymol and carvacrol are phenolic monoterpenes, which are a kind of botanic derivative, and many monoterpenic compounds previously have been reported to have biocidal effects (Marchese et al., 2017). However, the structures determining the biological effects were not fully investigated. A previous study suggested that the hydroxyl group in phenolic compounds determines the bioactivity and that these hydroxyl groups function to inactivate enzymes (Singh and Singh, 2012). We can find similar results in a study covering the structural analysis of carvacrol. It was suggested that the hydroxyl group in the carvacrol principally determined the antimicrobial effects of carvacrol by comparing them with those of *p*-cymene or 2-amino-*p*-cymene, which are structurally related compounds (Veldhuizen et al., 2006). These results showed that analysis of the molecular structure of natural products is a novel way to identify new biocides.

The use of natural products for their biocidal effects also has been highlighted because of their high biospecificity. Indeed, this characteristic of natural products makes them effective at a low concentration (e.g., μg/L) and has aroused interest in their use as novel drugs (Wright et al., 2007). Chemical biocides usually exert biocidal effects by processing highly reactive chemical reactions including oxidation of thiol groups, free radical oxidation, and phenolic membrane penetration (Denyer and Stewart, 1998). The cytotoxicity derived from these reactions induces cell death through random internalization of the acting molecules. By contrast, the biocidal effects of natural products modulate the biological systems built into organisms. Carvacrol was reported to inhibit quorum sensing of *Pseudomonas aeruginosa* by regulating its cell proliferation and growth (Tapia-Rodriguez et al., 2017).

Thymol has been reported to disrupt the structure of the lipid monolayer and induce leakage of cellular components in many kinds of microbes (Marchese et al., 2016). However, thymol and carvacrol exerted protective effects on the human skin (Aristatile et al., 2015; Sun et al., 2017). These findings are in contrast to the cytotoxicity of chemical biocides to all living organisms. Taken together, the selective antimicrobial effects of natural products can minimize their toxicity toward unintended targets including humans, further indicating their potential for use as novel biocides for ballast water.

We found that thymol did not exert biocidal effects on *E. coli* in the present study. However, several studies on the biocidal effects of thymol treatment at a high concentration are available. One study suggested that thymol inhibited glucose metabolism of microorganisms and induced cytotoxicity at a concentration of 180 mg/L (Evans and Martin, 2000). Another report suggested that thymol affected the progress of bacterial growth at concentrations from 50 mg/L to 250 mg/L, which determined the maximum growth capacity of the microorganisms (Falcone et al., 2005). Additionally, a previous study showed that thymol at concentrations > 500 mg/L inhibits biofilm formation, which plays a critical role in biofouling (Karpanen et al., 2008). Similarly, our study revealed that thymol determined the maximum growth capacity of *A. salina* (Figs. 2 and 4). Overall, we concluded that thymol could decrease the survival of planktons at low concentrations, yet it decreased the survival of microorganisms only at high concentrations.

In this study, the biocidal effects of carvacrol were shown to degrade 4 days after treatment. However, we observed that thymol was not degraded, even after 5 days (data not shown). Previous studies have suggested that reactive natural products including thymol and carvacrol could be degraded through biotransformation (Shimoda et al., 2006). This biotransformation process takes place in all organisms including microbes, fungi, and even humans. As the chemical structures of thymol and carvacrol are similar, the biotransformation reaction generates similar products. The cultured plant *Eucalyptus perriniana* was found to induce biotransformation of thymol and carvacrol through glycosylation of its hydroxyl groups, thus inactivating their bioactivity (Shimoda et al., 2006). In another report, thymol and carvacrol were converted into many types of byproducts in a common pool of molecules by fungi (Numpaque et al., 2011). However, the biotransformation mechanism of these products by *E. coli* is currently unknown. In summary, the degradation of thymol and carvacrol in this study was determined by the target organisms, and their degradation products were inactivated. These findings strongly support the possible use of natural products as eco-friendly biocides with minimal unintended cytotoxicity in discharged seawater.

Many studies have reported novel strategies for BWM. In this study, screening biocides from natural product pools was indicated as a novel strategy for developing BWM systems. Although the identification of natural products with useful effects is not easy, the ultimate goal of naval engineering development is high economic feasibility and environmental friendliness, as in the antibiofouling field. Overall, the results of this study suggest that thymol and carvacrol could be used to develop a green BWM system.

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Conflicts of interest

The authors declare no conflict of interest to disclose.

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