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Antibacterial mechanism of *Myagropsis myagroides* extract on *Listeria monocytogenes*

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ABSTRACT

The ethanol extract of *Myagropsis myagroides* had antimicrobial activity against Gram-positive bacteria. The extract was fractionated through liquid–liquid extraction; the chloroform fraction had strong antimicrobial activity against *Listeria monocytogenes* (minimum inhibitory concentration (MIC) 0.063 mg/mL), and *Clostridium perfringens* (MIC 0.031 mg/mL). The chloroform fraction was separated into 22 sub-fractions using silica gel column chromatography, with the fourth fraction (CH4) possessing the strongest antimicrobial activity against Gram-positive bacteria. Leakage of 260 nm-absorbing material and ATP was observed in CH4-treated cells and morphological alterations were observed by electron microscopy. These results indicate that the cytoplasmic membrane may be a target of the CH4 fraction.

1. Introduction

Despite improvements in food hygiene and food production techniques, there has been an increase in reported cases of food-associated infections (Gould et al., 2013) and a growing incidence of bacterial resistance to conventional antimicrobials (Koluman & Dikici, 2012). It has been estimated that as many as 30% of people in industrialized countries suffer from a food-borne disease each year (Burt, 2004). Important pathogens causing food-borne diseases include *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium*, *Vibrio parahaemolyticus*, and *Clostridium perfringens*. These pathogens not only affect the quality of food but also cause serious health problems in those who consume the contaminated food.

L. monocytogenes is one of the most virulent food-borne pathogens and can cause a rare but serious disease called listeriosis. *L. monocytogenes* is more likely to cause death than other bacteria that cause food poisoning. In fact, 20–30% of food-borne listeriosis infections in high-risk individuals may be fatal (Ramaseamy et al., 2007). *L. monocytogenes* is widely distributed in the environment, and is therefore found in foods of both animal and plant origin (Gandihi & Chikindas, 2007).

Although chemical and natural preservatives, such as nisin and pediocin, are used to inhibit *L. monocytogenes* in dairy and meat products, it can become highly resistant to some preservatives (Vadyvaloo, Hastings, van der Merwe, & Rautenbach, 2002). For this reason, there is a need for new antimicrobials to control *L. monocytogenes*. Consumers are increasingly concerned about chemical preservatives in food and tend to choose food products that are natural and safe and have multiple health benefits (Sloan, 2011). To date, the development of safe and effective antimicrobial agents from natural sources has focused on terrestrial animals and plants (Jamuna & Jeevaratnam, 2009; Montes-Belmont & Carvajal, 1998; Ouattara, Simard, Holley, Piette, & Begin, 1997).

However, algae have recently been proven to be a rich source of novel bioactive compounds, as they produce a great variety of secondary metabolites that have cholesterol-lowering and hypolipidemic (Awad, Selim, Saleh, & Matloub, 2003), antioxidative (Kuda, Kunii, Goto, Suzuki, & Yano, 2007), anti-inflammatory (Kang et al., 2008), antiviral (Iwashima et al., 2005), immunomodulatory (Liu, Yoshida, Wang, Okai, & Yamachita, 1997), and antimicrobial activities (Nagayama, Iwamura, Shibara, Hirayama, & Nakamura, 2002). Interestingly, these bioactive compounds have primarily been discovered from phaeophyta and rhodophyta.

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Myagropsis myagroides belongs to the Sargassaceae family in Phaeophyta and inhabits the subtidal zone of the coasts of Japan and Korea. Members of the Sargassaceae family produce structurally unique secondary metabolites, such as plastoquinones (Segawa & Shirahama, 1987), chromanols (Kato, Kumanireng, Ichinose, Kitahara, & Kato, 1975), cyclopentenone (Nakayama, Fukuoka, Nozaki, Matsuo, & Hayashi, 1980), and polysaccharides (Hoshino et al., 1998). These compounds have a range of biological activities due to their unique structures. Because *M. myagroides* is a member of the Sargassaceae family, it may produce a similar range of biological compounds. However, although *M. myagroides* can be easily found along the coast of Korea, there have been few studies of its biological activities and application and no studies assessing inhibition of food spoilage and food poisoning microbes. Thus, the aim of this work was to investigate the antimicrobial effect of *M. myagroides* on major food-related microbes and to determine the mode of action of an antimicrobial substance from *M. myagroides* on *L. monocytogenes*.

2. Materials and methods

2.1. Media and reagent

Brain heart infusion (BHI), yeast peptone glucose broth (YPG), reinforced clostridial medium (RCM), Muller Hinton broth (MHB), and agar were purchased from Difco (Detroit, MI, USA). DMSO was purchased from Sigma (St. Louis, MO, UAS). Nutrient broth (NB) was purchased from Accumedia (Lansing, MI, USA). All solvents for liquid–liquid extraction and silica gel column chromatography were purchased from J.T Baker (Phillipsburg, NJ, USA).

2.2. Bacterial strains

For the antimicrobial evaluation, *Alicyclobacillus acidoterretris* KCTC 3458 (YPG), *Bacillus subtilis* KCTC 1107(NB), *S. aureus* ATCC 6538 (NB), *C. perfringens* KCTC 5014 (RCM), *L. monocytogenes* KCTC 3569 (BHI), *S. typhimurium* ATCC 14028 (NB), and *E. coli* ATCC 25922 (NB) were employed and purchased from the Korean Collection for Type Culture (KCTC, Daejeon, Korea) and the American Type Culture Collection (ATCC, Manassas, VA, USA). Each microbial culture was activated by transferring a loopful of the slant culture into the appropriate broth medium. *C. perfringens* were incubated at 37 °C for 24 h under anaerobic conditions using an anaerobic container system with a Gas-Pak (BBL; Becton-Dickinson, Franklin Lakes, NJ, USA) and anaerobic indicator. The other bacteria were incubated at 37 °C for 24 h under aerobic conditions.

2.3. Preparation of the CH4 fraction from M. myagroides

M. myagroides was collected from the Busan coast in Korea. Salt, epiphytes, and sand were removed using tap water. *M. myagroides* was dried at room temperature and then ground. The *M. myagroides* was extracted with 99.9% ethanol (using ten times the sample volume) for 24 h at room temperature. The extract was centrifuged (UNION 32R; Hanil Co., Korea) for 10 min at 2090× *g* and the supernatants collected. The supernatant was filtered over Whatman No. 5 paper, and the filtrate was evaporated using a rotary evaporator (RE 200; Yamato Co., Japan). The concentrate was dried at 37 °C and stored at −20 °C. The ethanol extract was resuspended in 10 × w/w distilled water and partitioned with an equal volume of *n*-hexane, with shaking at 180 rpm for 1 h. After settling for 1 h, the *n*-hexane fraction was collected. Using the same method, chloroform, ethyl acetate, *n*-butanol, and water fractions were obtained. The chloroform fraction was loaded into a silica gel (230–400 mesh; Merck Co., Germany) column and subjected to step-wise elution with chloroform:methanol (10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 9:1, and 0:10; 200 mL of each). One-hundred milliliters of each eluant was collected and 22 sub-fractions were obtained (Fig. 1). The fourth fraction having the highest antimicrobial activity among sub-fractions was designated by the CH4 fraction, and investigated for antibacterial mechanisms on *L. monocytogenes*.

2.4. Antibacterial evaluation

Two-fold serial dilutions of *M. myagroides* extract and its fractions were prepared in melted MHA (MHB + 1.0% agar), RCMA (RCM + 1.0% agar; for *C. perfringens*), or YPGA (YPG+1.0% agar; for *Alicyclobacillus acidoterrestris*) to a final volume of 1 mL in sterile test tubes. The final concentrations of the samples ranged from 2 to 0.0039 mg/mL. The test tubes were inoculated with 50 μL of the test bacterial suspension to obtain 10^6 CFU/mL. The mixtures were poured into plates, which were then dried for 5 min. The minimum inhibitory concentration (MIC) was reported as the lowest concentration of the samples inhibiting microbial growth. The *M. myagroides* extract and its fractions were dissolved in DMSO as a control; this solution showed no inhibitory effect when tested against bacteria.

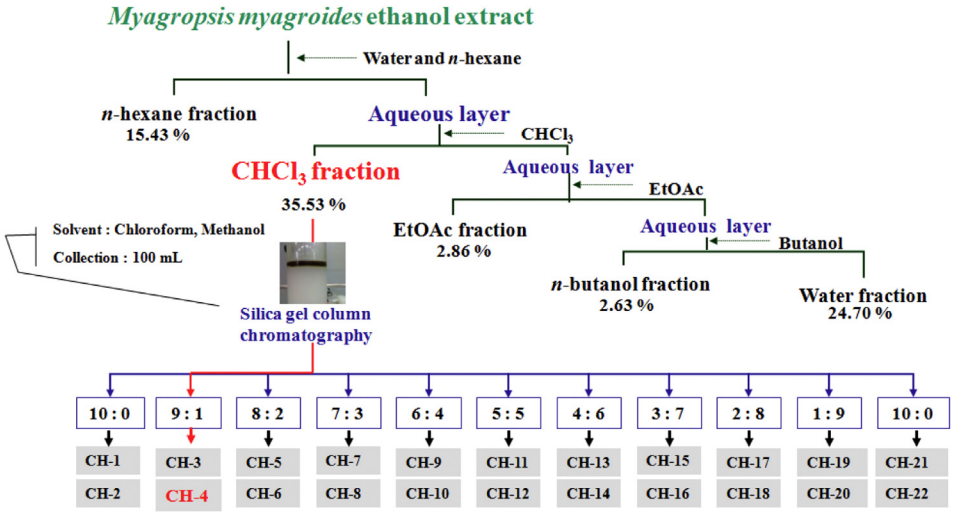

Fig. 1. The procedure to obtain solvent fractions from the ethanol extract of *Myagropsis myagroides*.