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Antibacterial effect of *Ishige okamurae* extract against cutaneous bacterial pathogens and its synergistic antibacterial effect against *Pseudomonas aeruginosa*

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Abstract

Background: Cutaneous bacterial pathogens including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Propionibacterium acnes* are often involved in acne vulgaris. The currently available therapeutic option for these skin pathogens is an antibiotic treatment, resulting in the emergence of antibiotic-resistant bacteria. The objective of this study was to discover an alternative antibacterial agent with lower side effect from marine algae.

Results: The ethanolic extract of edible brown algae *Ishige okamurae* exhibits potent antibacterial activity against cutaneous bacterial pathogens. Among the ethanol soluble fractions, the *n*-hexane (Hexane)-soluble fraction exhibited the strongest antibacterial activity against the pathogens with MIC values ranging 64 to 512 μg/mL and with minimum bactericidal concentration values ranging 256 to 2048 μg/mL. Furthermore, the combination with Hexane fraction and antibiotics (ceftazidime, ciprofloxacin, and meropenem) exhibited synergistic effect.

Conclusion: This study revealed that the *I. okamurae* extract exhibited a synergistic antibacterial effect against acne-related cutaneous bacterial pathogens acquired antibiotic resistant. Thus, the results of the present study suggested that the edible seaweed extract will be a promising antibacterial therapeutic agent against antibiotic-human skin pathogens and its infections.

Keywords: Antibacterial effect, Cutaneous pathogens, *Ishige okamurae*, *Pseudomonas aeruginosa*

Background

Opportunistic bacterial pathogens such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Propionibacterium acnes* are often associated with cutaneous pathogens (Kim et al. 2017b, a, c). These pathogenic bacteria are often involved in the development of abnormal follicular keratinization and inflammation known acne vulgaris (Yamaguchi et al. 2009; Kim et al. 2017b, a, c). Generally, topical therapeutic option for these cutaneous pathogens is antibiotic treatment to destroy the microbes. However, irrational use of such antibiotics resulted in antibiotic-resistant bacteria causing treatment failures and fatal outcomes in various infectious diseases (Lee et al. 2014; Kim et al. 2017b, a, c).

Surveillance of *P. aeruginosa* infections has revealed trends of increasing antibacterial resistance, including carbapenem resistance and multidrug resistance (Driscoll et al. 2007). Ceftazidime, carbapenem, or piperacillin resistance rates increased up to 50% in intensive care unit patients and the multidrug resistance rate, which is resistant to three or more antibiotics, reached 30% (El Solh and Alhajhusain 2009; Song and Joo 2010). Because of the lack of antibiotics effective against multidrug-resistant *P. aeruginosa*, colistin that has not been used in clinical practice for some time due to toxicity has reappeared and started to be used again (Li et al. 2006; Michalopoulos and Karatza 2010). However, new toxicity occurs in more than

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3% of patients with the use of colistin, and because of the incompleteness of the drug, multidrug-resistant *P. aeruginosa* has emerged as a clinically significant problem (Paul et al. 2010; Song and Joo 2010).

With increasing the multiple drug-resistant bacteria, it urgently requires a search for new antibacterial substances (Eom et al. 2016a; Eom et al. 2017; Eom et al. 2014). Recently, marine algae have been received attraction as an important source of novel bioactive substances. It has been revealed that marine algae originated compounds exhibiting various biological activities (Wijesekara and Kim 2010; Eom et al. 2016b). Although there are various physiologically active substances in marine algae, there is not much research on the influence of marine algae against cutaneous pathogens. Hence, the present study was aimed at exploiting bioactive compounds of edible marine algae *Ishige okamurae* against cutaneous pathogens.

I. okamurae, a kind of brown algae with narrow fronds, thick cortical layer, and acute apices, belongs to the family of Ishigeaceae and grows on rocks in the upper and middle intertidal zone on rough open coasts (Cho et al. 2005). The extract of *okamurae* is known to possess various biological activities such as antioxidant activity (Kim et al. 2008), anti-diabetic activity (Min et al. 2011), anti-obesitic activity (Park et al., 2013), anti-cancer activity (Heo et al. 2012), anti-matrix metalloproteinase activity (Bae et al. 2015), and anti-inflammation (Vo et al. 2011). However, few report is only available on antimicrobial activity of this edible marine algae. The object of this study is to evaluate an antibacterial activity of *I. okamurae* against cutaneous pathogens.

Methods

Materials and extraction

The fresh *I. okamurae* collected from the coastal area of Jeju Island, Korea. The marine algae were washed three times with water to remove salt and foreign materials, and then dried at 50 °C. Dried *I. okamurae* was finely powdered with a blender (HMF-1000A; Hanil Electronics, Seoul, Korea). The dried sample (1.0 kg) was extracted with 70% ethanol (EtOH; 10.0 L × 3 times) at 70 °C for 3 h in triplicate. Then, the solvent was evaporated a using rotary evaporator (Eyela Co., Tokyo, Japan) at 40 °C in vacuo. The EtOH extract was suspended in 10% ethanol and fractionated with *n*-hexane (Hexane; 1.0 L × 3 times), dichloromethane (CHCl3), ethyl acetate (EtoAc; 1.0 L × 3 times), and water solution in order, according to relative polarities. Each fraction was concentrated using the evaporator under vacuum at 45 °C.

Strains and culture conditions

Type bacterial strains used in this study were from the Korean Collection for Type Cultures (KCTC; Daejeon, Korea) and the American Type Culture Collection (ATCC; Manassas, VA, USA): *S. aureus* KCTC 1927, *S. epidermidis* ATCC 14990, *P. aeruginosa* KCTC 1637, and *P. acnes* KCTC 3314. *P. aeruginosa* clinical isolates were kindly provided by the Gyeongsang National University Hospital (Jinju, Korea), a member of the National Biobank of Korea. *S. aureus*, *S. epidermidis*, and *P. aeruginosa* strains were grown aerobically at 37 °C in tryptic soy broth (TSB; Difco Inc., Detroit, MI). *P. acnes* strain was anaerobically cultivated in brain heart infusion broth (BHI; Difco Inc.) supplemented with 1.0% glucose at 37 °C for 24 h in a CO_2 incubator (NAPCO 5400; General Laboratory Supply, Pasadena, TX, USA) that was modified with a 10% CO_2 humidified atmosphere.

Measurement of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The assay of minimum inhibitory concentration (MIC) was followed by the guideline of Clinical and Laboratory Standards Institute (CLSI) (2015). The MIC assay was performed using serial twofold dilution method with Mueller–Hinton broth (MHB; Difco Inc.) and 96-well microtiter plates (with clear flat bottoms). MIC values were determined by reading the plates visually. This test was repeated three times.

The assay of minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agent required to kill a particular bacterium (Eom et al. 2017). MBC values were determined by subculturing to agar plates that do not contain the test agent using MIC tested broths. MBC is defined as the lowest concentration of antibacterial agent that reduces the viability of the initial bacterial inoculum by ≥ 99.9%.

Antibiotic susceptibility test (AST)

AST is used to determine whether an organism is susceptible or resistant to an antimicrobial agent (Jenkins and Schuetz 2012; Kim et al. 2017b, a, c). The antibiotic resistance of test strains was confirmed against four kinds of commercial antibiotics (amikacin, ceftazidime, ciprofloxacin, meropenem; Sigma-Aldrich Co., St. Louis, MO) by MIC assay. An antibiotic was serially diluted and then the bacterial growth was visually checked.

Fractional inhibitory concentration (FIC) assay

Fractional inhibitory concentration (FIC) assay has been used to evaluate in vitro synergy effect in combination of multiple agents (Hsieh et al. 1993; Odds 2003). In this study, the synergy effect between the Hexane-soluble fraction and antibiotics against *P. aeruginosa* strains exhibiting antibiotic resistance was evaluated. FIC index was calculated using the formula as previously reported (Norden et al. 1979). The FIC was calculated as the minimum inhibitory concentration (MIC) of an antibiotic or the Hexane fraction in combination

divided by the MIC of the antibiotic or the Hexane fraction A alone.

Results and discussion

Antibacterial effect of *I. okamurae* extracts against cutaneous bacterial pathogens

As shown in Table 1, the ethanolic extract of *I. okamurae* exhibited an antibacterial activity against cutaneous bacterial pathogens in ranging of MIC with 256–512 μg/mL. These results suggested that the extract contains an antibacterial substance against human skin bacterial pathogens such as *S. aureus*, *S. epidermidis*, *P. aeruginosa*, and *P. acnes*. To further investigate the antibacterial activity against the bacterial pathogens, the ethanolic extract was fractioned into Hexane-, CHCl3-, EtoAc-, and H_2O-soluble fraction as described in the "Methods" section. The antibacterial activity of solvent fractions was then quantitatively evaluated by the MIC assay (Table 2). The MIC values of *I. okamurae*-soluble fractions were in the range of 64 to 2048 μg/mL against cutaneous pathogens tested in this study. Among these, the Hexane fraction showed the strongest antibacterial activity with the MICs range of 64 to 512 μg/mL, followed by the EtoAc fraction, suggesting that an antibacterial substance against the pathogens will abundantly present in the Hexane-soluble fraction. In contrary to other reports that the strongest antibacterial activity of marine algae extracts observed in EtOAc fraction, the Hexane-soluble fraction in *I. okamurae* extracts exhibited superior antibacterial activity against cutaneous pathogens (Lee et al. 2014; Kim et al. 2016; Kim et al. 2017a).

Table 1 Minimum inhibitory concentration (MIC) of *Ishige okamurae* extracts against cutaneous bacterial pathogens

Strains	MIC (μg/mL)				
	EtOH	Hexane	CHCl3	EtoAc	H_2O
Staphylococcus aureus KCTC 1927	512	512	512	256	1024
Staphylococcus epidermidis ATCC 14990	512	512	1024	128	512
Propionibacterium acnes KCTC 3314	512	256	1024	512	2048
Pseudomonas aeruginosa KCTC 1637	256	64	256	128	1024
P. aeruginosa isolate 366	256	128	512	128	1024
P. aeruginosa isolate 4068	256	64	256	128	1024
P. aeruginosa isolate 4135	512	128	512	128	1024
P. aeruginosa isolate 4561	512	128	512	512	1024

EtOH ethanol extracts, *Hexane* *n*-hexane-soluble fraction, *CHCl3* dichloromethane, *EtoAc* ethyl acetate-soluble fraction, *H_2O* water-soluble fraction

Table 2 Minimum bactericidal concentration (MBC) of *Ishige okamurae* extracts against cutaneous bacterial pathogens

Strains	MBC (μg/mL)				
	EtOH	Hexane	CHCl3	EtoAc	H_2O
Staphylococcus aureus KCTC 1927	2048	1024	2048	512	> 2048
Staphylococcus epidermidis ATCC 14990	2048	2048	2048	512	> 2048
Propionibacterium acnes KCTC 3314	2048	1024	> 2048	2048	> 2048
Pseudomonas aeruginosa KCTC 1637	1024	256	2048	512	> 2048
P. aeruginosa isolate 366	1024	256	2048	1024	> 2048
P. aeruginosa isolate 4068	1024	256	1024	1024	> 2048
P. aeruginosa isolate 4135	2048	512	2048	1024	> 2048
P. aeruginosa isolate 4561	2048	512	2048	2048	> 2048

EtOH ethanol extracts, *Hexane* *n*-hexane-soluble fraction, *CHCl3* dichloromethane *EtoAc* ethyl acetate-soluble fraction, *H_2O* water-soluble fraction

In order to evaluate the bactericidal effect of *I. okamurae* extracts, the MBC assay was performed (Table 2). The MBC values of *I. okamurae* fractions were in the range of 512 to above 2048 μg/mL against cutaneous pathogens. Similar to results obtained in the MIC assay, the Hexane-soluble fraction showed superior bactericidal activity in ranges of MBC with 256 to 1024 μg/mL. The MBC values were twofold increased compared to those of MIC values. Similar patterns between MIC and MBC values were reported by several studies (Eom et al. 2014; Kim et al. 2016). Considering both MIC and MBC results, it was concluded that the Hexane-soluble fraction of *I. okamurae* possess the highest antibacterial activity against cutaneous bacterial pathogens tested in this study. Interestingly, the fraction exhibited strong antibacterial activity against *P. aeruginosa* strains. As mentioned above, *P. aeruginosa* infection is serious problems mainly due to its antibiotic resistance (Driscoll et al. 2007; Michalopoulos and Karatza 2010). In addition, there are few studies on natural substances having antibacterial activity against *P. aeruginosa* strains. Thus, the results obtained in this study strongly indicated that *I. okamurae* will be a potential candidate to develop an alternative therapeutic agent for the treatment against cutaneous pathogens, specially *P. aeruginosa* infection.

Antibiotic resistance of cutaneous bacterial pathogens

Antibiotics have been extensively used to treat diseases caused by cutaneous pathogens (Kim et al., 2017b, a, c). However, continued use of these antibiotics caused antibiotic resistance and the emergence of multidrug-resistant bacteria (Unemo and Nicholas 2012; Kim et al., 2017b, a, c). In an effort to discover an alternative therapy for antibiotic-resistant bacteria, we evaluated the

antibiotic susceptibility of cutaneous bacterial pathogens tested in this study. For kinds of antibiotics belong to aminoglycosides, cephalosporins, quinolones, and carbapenems were used for antibiotic susceptibility test (AST). Antibiotic susceptibility or resistance was determined based on the MIC breakpoint values (European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2018).

Among the stains tested in this study, MICs of all strains against amikacin were in the range of the acceptable MIC breakpoint values ranging from 4 to 8 μg/mL indicating susceptibility to the test agent (Table 3). In addition, the MIC of *S. aureus*, *S. epidermidis*, and *P. acnes* were showed susceptibility against all four antibiotics used in this study. However, some *P. aeruginosa* strains (KCTC1637, isolate 4068, isolate 4135, and isolate 4561 strain) exhibited resistant exceeding the MIC breakpoint against ceftazidime, ciprofloxacin, and meropenemm suggesting that the *P. aeruginosa* strains are multidrug-resistant bacteria (Kim et al., 2017b, a, c).

Synergistic effect between the Hexane-soluble fraction and antibiotics against cutaneous bacterial pathogens

Since many antibiotic-resistant and multidrug-resistant bacteria have been reported, it has been studied not only the development of new antibiotics or therapies but also the development of alternative therapies in combination with using antibacterial materials derived from natural product (Eom et al. 2013; Eom et al. 2016a). As shown in Tables 1 and 2, the Hexane-soluble fraction of *I. okamurae* was presented the highest antibacterial activity against *P. aeruginosa* among the solvent-soluble fractions. Therefore, the Hexane fraction was chosen for investigating a synergy effect with antibiotics in an effort to develop an alternative therapy against multidrug-resistant *P. aeruginosa*. The synergistic interaction of Hexane fraction of *I. okamurae* and antibiotics was tested against *P. aeruginosa* strains by the checkerboard method using FIC assay as stated in the "Methods" section (Hsieh et al. 1993; Odds 2003).

Table 3 Minimum inhibitory concentration (MIC) of antibiotics against cutaneous bacterial pathogens

Strains	MIC (μg/mL)			
	Amikacin	Ceftazidime	Ciprofloxacin	Meropenem
Staphylococcus aureus KCTC 1927	1	2	0.5	1
Staphylococcus epidermidis ACTC 14990	1	4	4	1
Propionibacterium acnes KCTC 3314	4	0.5	4	4
Pseudomonas aeruginosa KCTC1637	1	8	8	16
P. aeruginosa isolate 366	2	4	4	8
P. aeruginosa isolate 4068	1	16	16	16
P. aeruginosa isolate 4135	1	8	16	8
P. aeruginosa isolate 4561	2	16	16	16
MIC breakpoint[a]	4–8	1–4	2–8	8

[a]*EUCAST* European committee on antimicrobial susceptibility testing

As shown in Table 3, the MICs of ceftazidime, ciprofloxacin, and meropenem against the multidrug-resistant *P. aeruginosa* strains (KCTC1637, isolate 4068, isolate 4135, and isolate 4561 strain) ranged from 8 to 16 μg/mL exceeding the MIC breakpoint. However, the MICs were dramatically decreased in combination with the Hexane-soluble fraction of *I. okamurae*. The MICs of ceftazidime against *P. aeruginosa* KCTC 1637 and isolate 4135 strains were reduced, up to 2 μg/mL when applied in combination with the Hexane fraction. In the same way, *P. aeruginosa* isolate 4068 and 4561 strains were reduced up to 4 μg/mL. Thus, the MICs decreased twofold in the combination of the Hexane fraction. In addition, the MICs of ciprofloxacin against *P. aeruginosa* strains were reduced two- to threefold in combination with the Hexane fraction. The MICs of meropenem decreased twofold in combination with the Hexane fraction. The median ∑FIC of the Hexane fraction-antibiotic combinations against *P. aeruginosa* strains were ranged from 0.50 to 0.75, suggesting that a weak synergistic antimicrobial effect in combination with the Hexane fraction (Eom et al. 2016a; Kim et al., 2017b, a, c) (Table 4).

The results obtained in this study strongly suggested that the Hexane fraction of *I. okamurae* possess strong antibacterial activity against cutaneous bacterial pathogens. In addition, the fraction showed potent antibacterial activity against multidrug-resistant *P. aeruginosa* that is an important pathogen frequently implicated in healthcare-associated infections and often associated with multidrug resistance (Hirsch and Tam 2010). More interestingly, the fraction exhibited a synergistic effect against multidrug-resistant *P. aeruginosa* strains in combination with antibiotics losing its antibacterial activity against the infectious bacteria, suggesting the Hexane-soluble fraction is capable to restore the activity of antibiotics against the multidrug-resistant *P. aeruginosa* (Eom et al. 2016a; Kim et al. 2017b). Thus, *I. okamurae* will be a candidate to overcome multidrug resistance, especially multidrug-resistant *P. aeruginosa*. In order to progress this issue, it will be needed to isolate active compounds from the Hexane-soluble fraction and to elucidate its antibacterial action mechanism.

Table 4 Fractional inhibitory concentration (FIC) of antibiotics in combination with Hexane-soluble fraction of *Ishige okamurae* against antibiotic-resistant *Pseudomonas aeruginosa*

Strains	Test compound	MIC (μg/mL)	ΣFIC_{max}	ΣFIC_{min}	Median ΣFIC	Minimum concentration for observing synergy
P. aeruginosa KCTC 1637	Hexane	64	1.06	0.31	0.57	8
	Ceftazidime	8				2
	Hexane	64	1.06	0.52	0.63	8
	Meropenem	16				4
P. aeruginosa isolate 4068	Hexane	64	1.00	0.31	0.50	4
	Ceftazidime	16				4
	Hexane	64	1.06	0.38	0.57	8
	Ciprofloxacin	16				4
	Hexane	64	1.50	0.52	0.75	16
	Meropenem	16				4
P. aeruginosa isolate 4135	Hexane	128	1.13	0.38	0.63	16
	Ceftazidime	8				2
	Hexane	128	1.25	0.31	0.63	16
	Ciprofloxacin	16				4
P. aeruginosa isolate 4561	Hexane	128	1.00	0.38	0.52	8
	Ceftazidime	16				4
	Hexane	128	1.06	0.38	0.50	8
	Ciprofloxacin	16				2
	Hexane	128	1.13	0.50	0.75	32
	Meropenem	16				8

The FIC index indicated synergistic effect: < 0.5, marked synergy; 0.5 to < 1.0, weak synergy; 1.0, additive; > 1.0 to < 2.0, subadditivie; 2.0, indifferent; > 2.0, antagonistic

ΣFIC_{max} maximum FIC, ΣFIC_{min} minimum FIC, ΣFIC_{med} medium FIC

Conclusions

This study was conducted to discover an alternative antibacterial agent with lower side effect from marine algae against cutaneous bacterial pathogens. Edible brown algae *I. okamurae* extract exhibited antibacterial activity against acne-related bacterial pathogens. Among the solvent-soluble fractions of the ethanolic extract, the Hexane-soluble fraction exhibited the strongest antibacterial activity with the lowest MIC values ranging 64 to 512 μg/mL. In addition, the combination with Hexane fractions and antibiotics used in acne infection resulted in synergistic antibacterial effect against the antibiotic-resistant cutaneous bacteria. Thus, *I. okamurae* can be a potential source of natural product to be used as an effective therapy against skin pathogens.

Abbreviations

ΣFIC_{max}: Maximum FIC; ΣFIC_{med}: Medium FIC; ΣFIC_{min}: Minimum FIC; ACTC: The American Type Culture Collection; AST: Antibiotic susceptibility test; BHI: Brain heart infusion broth; CHCl3: Dichloromethane; EtoAc: Ethyl acetate; EtOH: Ethanol extract; FIC: Fractional inhibitory concentration; H_2O: Water; Hexane: *n*-hexane; KCTC: The Korean Collection for Type Cultures; MBC: Minimum bactericidal concentration; MHB: Mueller–Hinton broth; MIC: Minimum inhibitory concentration; TSB: Tryptic soy broth

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Authors' contributions

BK, MSK, SKP, and SCK designed this study and drafted the manuscript. SHE, WKJ, and YMK conceived and designed the study, and also revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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