Estimation of Antibacterial Properties of Chlorophyta, Rhodophyta and Haptophyta Microalgal Species

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Introduction

Microalgae represent a rich source of potentially bioactive compounds and are thus of great interest [1]. From the very beginning, cyanobacteria and prokaryotic algae inhabited the earth. Through their ability to synthesize oxygen as a photosynthetic byproduct, they contributed in regulating the oxygen atmosphere of our planet, enabling life as we know it [2]. Environmental tolerance and high adaptability to conditions, ranging from extreme heat to extreme cold, drought, salinity and UV exposure, have enabled microalgae to survive in a large span of different ecosystems [2]. The conditions they were forced to adapt resulted in the biosynthesis of scientifically interesting chemical compounds [3]. Furthermore, microalgae cultivation is generally easy and cost-effective which is one of their biggest advantages as compound donors [4].

Marine microalgae are a rich source of vitamins, pigments, proteins and other substances providing benefits in cosmetics and skin care product composition [5, 6]. A lot of secondary metabolites from cyanobacteria have been associated with antimicrobial effects. Among different types of antimicrobial compounds isolated from...

In this exploratory study, eight types of microalgae from different phyla (Chlamydomonas reinhardtii, Chlorella species, Haematococcus pluvialis, Porphyridium purpureum, Porphyridium cruentum, Isochrysis species, Isochrysis galbana, and Pavlova lutheri) were tested for their antibacterial activities against eight target pathogenic bacterial strains. The agar well diffusion method and broth micro dilution assay were conducted to estimate the antibacterial activity. Microalgae cell-free supernatants, exopolysaccharides (EPS), water, and organic solvent extracts were used for inhibition analysis. EPS extracted from P. lutheri showed activity against Bacillus subtilis and Pseudomonas aeruginosa. Inhibition zone diameters of 14−20 mm were recorded on agar plates, while the minimum inhibitory concentrations in the broth micro dilution assay were 0.39−25 mg ml−1. During this study, haptophyte microalgae, Isochrysis species, and P. lutheri extracts showed the highest activity against most of the tested pathogenic bacterial strains, while most of the extracts were active against the important foodborne pathogen P. aeruginosa. This study showed promising results regarding important microalgae phyla, which will further aid research related to extracts and exploitation of bioactive metabolic compounds in the food and pharmaceutical industries.

Keywords: Antibacterial activity, exopolysaccharides, microalgal extracts, natural compounds, pathogenic bacteria
microalgae, fatty acids and their derivatives have drawn special attention [7–9]. Different exometabolites and antifouling agents have also been explored from other microalgae species [8–11], including the bioactive exometabolites isolated from the filamentous marine cyanobacterium Geitlerinema sp. [12]. Microalgae such as Arthrospira and Chlorella species have been repeatedly used in cosmetics, skin care, and hair care products [13, 14]. Additionally, recent advancement in biotechnology has enabled the manufacturing of high-valued microalgae byproducts that are nearly free of contaminations.

Aqueous and organic extracts, as well as fatty acids, exopolysaccharides (EPS), and eicosapentaenoic acid (EPA) extracted from microalgae species have been reported active against several pathogenic bacteria [8, 15]. Eicosapentaenoic acid from Phaeodactylum tricornutum showed antibacterial activity against Multidrug-Resistant Staphylococcus aureus (MRSA), Listonella anguillarum, Lactococcus garvieae and Vibrio sp., [8, 16]. Pressurized liquid extracts obtained from Haematococcus pluvialis have been reported as active against Escherichia coli and S. aureus [17]. Organic extracts from Euglena viridis have shown activity against Vibrio, E. coli, Pseudomonas, Aeromonas and Edwardsiella [18]. Methanol and hexanol extracts from Chlamydomonas reinhardtii and Chlorella vulgaris were found active against S. aureus, Staphylococcus epidermidis, Bacillus subtilis, E. coli, and Salmonella typhi [19]. β-phycocerythrin extracted from Porphyridium cruentum was found active against S. aureus, Streptococcus pyogenes and Salmonella typhimurium [15].

Bacterial resistance to synthetic antibiotics emphasizes searching for the novel natural antibacterial compounds. Nevertheless, antibiotics are being discovered at a steady rate, but the consequences of this phenomenon are slow to be appreciated. At present, the paucity of the antimicrobial compounds coming into the market has led to the problem of antibiotic resistance, fast escalating into a global health crisis [20]. One approach to encounter antibiotic resistance is the discovery of novel natural antimicrobial compounds for clinical application [8]. Thus, the scientists are eager to explore the compounds and discover new drug leads [21, 22].

The use of microalgae in the medical and pharmaceutical industry is rapidly increasing, which shows the potential of this microorganism for the benefit of human-kind. Despite the availability of several antibacterial compounds yet there is a lot more space for improvement. In the present study, green and red eukaryotic microalgae species from different phyla were explored for their antibacterial properties. Conditions to extract the viable natural antibacterial substances were practiced and the extracted compounds were checked against eight, gram-positive and gram-negative pathogenic bacterial strains through the agar well diffusion method and the broth microdilution assay.

Material and Methods

Microalgae species and culture conditions

Eight microalgae species from different phyla including Chlorophyta, Rhodophyta and Haptophyta were obtained from the Korea Marine Microalgae Culture Center (KMMCC) Busan, Republic of Korea (Table 1). Stock cultures of each microalgae species were maintained on the appropriate agar slants. For antimicrobial activity analyses, non-axenic cultures were grown in 500 ml flask bioreactors with the respective medium, at 25 ± 2°C under a continuous light intensity of 150 μmol photons m⁻² s⁻¹. Aeration was provided by shaking at 110 rpm [26]. Both cell pellets and cell-free supernatants were used for sample preparation.

Bacterial strains and culture conditions

The tested bacterial strains include Micrococcus luteus KCTC 1071, Staphylococcus aureus RN 4220, Bacillus subtilis KCTC 1021, Streptococcus iniae FP 5228, Escherichia coli KCTC 1116, Salmonella enterica KCTC

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Species</th>
<th>Medium</th>
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<tbody>
<tr>
<td>Chlorophyta</td>
<td><em>Chlamydomonas reinhardtii</em></td>
<td>TAP [23]</td>
</tr>
<tr>
<td></td>
<td><em>Haematococcus pluvialis</em></td>
<td>Modified BBM [24]</td>
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<tr>
<td></td>
<td><em>Chlorella species</em></td>
<td>Modified ASW [25]</td>
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<td>Haptophyta</td>
<td><em>Pavlova lutheri</em></td>
<td>Modified ASW [25]</td>
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<td></td>
<td><em>Isochrysis species</em></td>
<td>Modified ASW [25]</td>
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<td></td>
<td><em>Isochrysis galbana</em></td>
<td>Modified ASW [25]</td>
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<tr>
<td>Rhodophyta</td>
<td><em>Porphyridium purpureum</em></td>
<td>Modified ASW [25]</td>
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<td></td>
<td><em>Porphyridium cruentum</em></td>
<td>Modified ASW [25]</td>
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TAP: Tris-acetate-phosphate; BBM: Bold basal medium; ASW: Artificial seawater.

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