Enhancement of Seaweed Rhizoid and Blade Formations by the Chlorophyte Codium fragile Extract

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Living organisms can maintain or extend their territories by producing allelochemicals that influence the growth, survival, and reproduction of other organisms. To identify natural biostimulants of positive allelochemicals, we screened 18 common seaweed extracts for enhancement of rhizoid and blade production in a convenient Porphyra suborbiculata monospore assay. By addition of methanolic extract from the most potent green seaweed, Codium fragile, 100% and 50% enhancement doses reflecting the amount of C. fragile extract required to enhance rhizoid formation (in terms of numbers of spores with rhizoids per total spores tested) were approximately 100 and 50 μg/ml, respectively, in the P. suborbiculata monospore culture. The C. fragile extract quickly enhanced rhizoid formation, rhizoid numbers per rhizoid-holding spore, rhizoid length, blade formation (in number of spores with blade per total spores tested), and blade length from most monospores at early culture days. The extract enhanced rhizoid formation after 2 days of culture significantly, rhizoid numbers per rhizoid-holding spore after 3 days, rhizoid length after 3 days, blade formation after 2 days, and blade length after 1 day, respectively, from most monospores. The allelochemicals that enhanced favorite seaweed species may be efficacious for new seaweed management technologies, including the development of bio-stimulant agents based on natural products.

Key words: Allelochemicals, Codium fragile, enhancement, monospore, rhizoid

Introduction

The marine ecosystem is a community of living organisms with severe competition, cooperation, and regulation occurring between them. For competition or cooperation, some plants produce allelopathic substances—biochemicals that influence the growth, survival, and reproduction of other organisms—that facilitate growth of the producing or receiving organism [21]. Allelochemicals are either beneficial (positive allelopathy) or detrimental (negative allelopathy) to the target organisms and play an important role in plant induction or defense against herbivory [18]. Allelochemicals that suppress or eliminate competing plant species have received special attention due to their potential as natural herbicides in agriculture [9, 20]. This focus has shifted attention to alternative seaweed control technologies, such as algicidal or growth-stimulating agent development based on selective natural products. For example, the red seaweed Ceramium rubrum has anti-germination activity in Sargassum muticum, Enteromorpha intestinalis, and Ulva lactuca [8]. The green seaweed Monostroma nitidum produces a microalgal growth-enhancer levoglucosan [13]. The green seaweed Monostroma arcticum has a positive effect on the growth and photosynthetic activity of Porphyra yezoensis in co-cultures, cultured medium filtrate, and dry powder assays [22]. Foliar application of eckol, from the brown seaweed Ecklonia maxima, enhances shoot and root length, leaf area and number, and aphid resistance capacity in cabbage plant [16]. For the development of environmentally friendly biostimulant products for seaweed growth, natural compounds from marine plant and animal sources are the best candidates.

The red seaweed Porphyra suborbiculata is a common wild seaweed that uses a discoidal holdfast to grow on rocks in the higher intertidal zone [1]. Monospores (blade archesporos) from juvenile blades can be produced year-round

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Fig. 1. Micrographs of monospores released from the juvenile blade of Porphyra suborbiculata (A) and their rhizoids and blades formed from monospores (B). The bars in A and B indicate 100 μm, respectively.
Results

To search for positive allelopathic or growth-enhancing agents in seaweeds, common seaweed extracts were tested for their ability to enhance rhizoid and blade production in a *P. suborbicularata* monospore assay. The 18 seaweed species tested included green seaweed (*Codium fragile*, *Monostroma nitidum*, *Ulva linza*, *Ulva pertusa*), brown seaweed (*Ecklonia cava*, *Eisenia bicyclis*, *Hizikia fusiformis*, *Ishige sinicola*, *Saccharina japonica*, *Sargassum fulvellum*, *Sargassum hemiphylllum*, *Sargassum hornei*, *Sargassum thunbergii*, *Scytosiphon lomentaria*, *Undaria pinnatifida*), and red seaweed (*Chondrus ocellatus*, *Corallina pilulifera*, *Pachymeniopsis elliptica*). Most seaweed extracts at 200 μg/ml suppressed the rhizoid and blade production (Table 1). Among the seaweed extracts tested, *C. fragile* exhibited significant enhancement, with 63% of monospores producing rhizoids compared with 41% of the control PES (p<0.01). Rhizoid numbers per rhizoid-holding spore also increased. *C. ocellatus* and *C. fragile*, rhizoid length was enhanced significantly, with average rhizoid lengths of 40.2 and 34.6 μm, respectively, compared with 19.5 μm for the control (p<0.01). Regarding germinated spores, *E. bicyclis* extract enhanced blade formation significantly, with 27% of monospores germinating to juvenile blades in 1 week compared with 14% for the control (p<0.05). *C. fragile* showed blade formation similarly to the control. *E. cava, U. linza*, and *U. pertusa* extracts enhanced blade growth significantly, to an average of 14.3, 10.9, 11.9 μm, respectively, compared with 7.1 μm for the control (p<0.01). *C. fragile* increased blade length a little. Thus, the *C. fragile* extract was selected for further evaluation, based on overall enhancing activities of rhizoid and blade formation.

Various concentrations of the *C. fragile* extract were added to the monospore culture to determine enhancement activity. The enhancement dose 100 (ED100) is expressed as the concentration of *C. fragile* extract required to produce rhizoids maximally from monospores after 7 days of culture. The enhancement dose 50 (ED50) is the extract concentration required to increase rhizoid production in 50% of monospores after 7 days. The ED100 and ED50 values reflecting the amount of *C. fragile* extract required to enhance rhizoid formation (in terms of number of spores with rhizoids per total spores

Table 1. Comparison of various seaweed extracts for enhancing activities based on rhizoid and blade productions of *Porphyra suborbicularis* monospores

<table>
<thead>
<tr>
<th>Code of species</th>
<th>Rhizoid formation (%)</th>
<th>No. of rhizoids / rhizoid-holding spore</th>
<th>Rhizoid length (μm)</th>
<th>Blade formation (%)</th>
<th>Blade length (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11±9</td>
<td>1.0±0.1</td>
<td>40.2±3.2**</td>
<td>7±7</td>
<td>0.0±0.0</td>
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<tr>
<td>2</td>
<td>63±8**</td>
<td>1.2±0.0*</td>
<td>34.6±2.3**</td>
<td>11±3</td>
<td>9.5±2.5</td>
</tr>
<tr>
<td>3</td>
<td>6±4</td>
<td>1.0±0.0</td>
<td>2.7±0.4</td>
<td>15±2</td>
<td>6.9±0.3</td>
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<tr>
<td>4</td>
<td>0±0</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
<td>2±0</td>
<td>14.3±0.2**</td>
</tr>
<tr>
<td>5</td>
<td>10±4</td>
<td>1.0±0.0</td>
<td>4.5±0.3</td>
<td>27±8*</td>
<td>7.9±2.2</td>
</tr>
<tr>
<td>6</td>
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</tr>
<tr>
<td>7</td>
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<td>8.5±1.1</td>
<td>5±1</td>
<td>8.8±2.7</td>
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<tr>
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</tr>
<tr>
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<tr>
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<tr>
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<td>0.0±0.0</td>
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<td>0.0±0.0</td>
</tr>
<tr>
<td>14</td>
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<tr>
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<tr>
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<td>10.9±0.5**</td>
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<tr>
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<td>0.0±0.0</td>
<td>10±2</td>
<td>11.9±1.1**</td>
</tr>
<tr>
<td>18</td>
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<td>0.0±0.0</td>
<td></td>
</tr>
<tr>
<td>PES</td>
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<td>19.5±2.1</td>
<td>14±4</td>
<td>7.1±0.4</td>
</tr>
</tbody>
</table>

Fig. 2. Effects of different concentrations of Codium fragile extract on the rhizoid formation from Porphyra suborbiculata monospores. Enhancing activities of rhizoid formation were expressed as % of spores with rhizoid per total monospores tested. No addition of C. fragile extract is the value for PES control. *p<0.05 and **p<0.01 as compared with PES control by student t-test.

Next, growth of P. suborbiculata monospores was observed upon treatment with 100 μg/ml (approximate ED50 for rhizoid formation) C. fragile extract for 10 days. The C. fragile extract quickly enhanced rhizoid formation (Fig. 3A), rhizoid numbers per rhizoid-holding spore (Fig. 3B), rhizoid length (Fig. 3C), blade formation (Fig. 3D), and blade length (Fig. 3E) from most spores at early culture days in the monospore assay. The extract enhanced rhizoid formation after 2 days culture significantly, rhizoid numbers per rhizoid-holding spore after 3 days, rhizoid length after 3 days, blade formation after 2 days, and blade length after 1 day, respectively, from most P. suborbiculata monospores. However, after 10 days of culture, monospores began to reach almost maximum in rhizoid and blade formation.

Fig. 3. Effects of Codium fragile extract on the productions of rhizoids and blade from Porphyra suborbiculata monospores during 10 days of culture. Enhancing activities were measured using rhizoid formation (% of spores with rhizoids / total spores tested; A), number of rhizoids / rhizoid-holding spore (B), rhizoid length (C), blade formation (% of spores with blade / total spores tested; D), and blade length (E). ●; C. fragile extract (100 μg/ml). ○; PES control. *p<0.05 and **p<0.01 as compared with control by student t-test.
Plant interactions in ecosystems are known to be mediated by plant active compounds, referred to as allelochemicals, which are receiving increasing attention in the context of sustainable plant management [4]. It should be paid to these chemical interactions for the development of new biostimulants. To search for such positive allelopathic agents in seaweed, common seaweed extracts were tested for their ability to enhance rhizoid and blade production in a monospore assay. Among the seaweed species tested, C. fragile showed the strongest enhancement activities. C. fragile is an edible and abundant aquacultural green seaweed. The amount of C. fragile produced by farming in 2013 amounted to 2,045 t (wet weight), with an additional 132 t (wet weight) collected from natural populations in Korea [11]. Currently, it occurs in temperate regions worldwide as an invasive species [6]. In our previous study [10], C. fragile extracts showed in vivo anti-inflammatory, anti-pyretic, and analgesic activities. Siphonaxanthin having anti-angiogenic effect [7] and a sulfated galactan having immunostimulating effect [12] were also isolated from C. fragile.

The C. fragile extract quickly enhanced rhizoid formation, rhizoid numbers, rhizoid length, blade formation, and blade length at the early stages of the P. suborbiculata monospore germination. Rhodophyte Gracilaria gracilis tips and chlorophyta Ulva lactuca growth were increased by addition of a commercial kelp E. maxima extract [17]. The E. maxima triggered rooting in cucumber [14] and tomato plants [5]. Enhancing shoot length, root length, leaf area, and leaf number in cabbage plant was caused by the phlorotannin eckol compound from the E. maxima [16]. Some seaweeds contain plant growth hormones (such as auxin and cytokinin), and the additions to media increased growth of Gracilaria verniculophylla [19, 23]. Even though active compounds from the C. fragile is not proved yet, the C. fragile extract demonstrated a positive effect on the rhizoid and blade formation of P. suborbiculata monospores. Thus, there is potential for using the C. fragile or its extract in the seedling of commercial Porphyra mariculture. Such positive allelochemicals that enhanced favorite seaweed species may be efficacious for new seaweed management technologies, including the development of biostimulant agents based on marine natural products.

Acknowledgment

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References

 초록: 녹조류 청각 추출물에 의한 해조류 가근 및 유엽형성 촉진

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많은 생물체들은 다른 생물체들의 성장, 생존, 재생에 영향을 미치는 allelochemical 물질들을 생성함으로써 자신들의 영역을 유지 확장할 수 있다. 천연 생물촉진 활성을 가진 allelochemicals를 찾기 위하여 18종의 흔한 해조류를 대상으로 실험실에서 편리하게 배양가능한 둥근돌김의 중성포자들로부터 가근 및 유엽의 형성 촉진효과를 탐색하였다. 그중 가장 활성이 높은 녹조류 청각의 추출물은 약 100 및 50 µg/ml 농도에서 최대 및 반 정도의 중성포자로부터 가근 형성 촉진효과를 나타내었다. 또한 청각 추출물은 중성포자 배양 초기에 가근형성, 가근길이, 가근길이, 유엽형성, 유엽길이 등의 성장을 촉진시켰다. 이 같은 다른 해조류의 성장을 촉진시키는 allelochemicals들은 천연 해조류 촉진제의 개발 및 유용 해조류 종들의 유지관리 등에도 활용될 가능성을 지닌다.


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