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Seasonal variation of antibacterial activities in the green alga *Ulva pertusa* Kjellman

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Abstract

The present study was performed to screen out the extracts of algae and assess the seasonal variation in antimicrobial activity of *Ulva pertusa* against *Gardnerella vaginalis*. Seasonal variation in antibacterial activity was observed, with the extracts showing no activity during summer and autumn, and showing antibacterial activity from early winter (December) to middle spring (April). The maximum value of antimicrobial activity (6.5 mm inhibition zone at 5 mg disk^{-1}) of *U. pertusa* against *G. vaginalis* was observed in April. Otherwise, for both chlorophyll *a* and *b*, the highest content (2.87 mg g^{-1} and 1.37 mg g^{-1}) was observed in March 2009. These results may reflect variation in cellular chemical compositions such as secondary metabolite(s) rather than chlorophyll and biological activities according to season.

Key words

Antimicrobial activity, *Gardnerella vaginalis,* Seasonal variation, *Ulva pertusa*

Introduction

Ulva pertusa Kjellman is a common and abundant seaweed found in the intertidal zone along the coastline of Korea. It is a fast-growing alga that is opportunistic for space and nutrient uptake. Significant growth of *U. pertusa* is often observed in eutrophic coastal waters, and the alga proliferates in the form of green tides (Hong *et al.,* 2011; Lee *et al.,* 2011). This phenomenon often results in mass production, which could reach harmful levels in Korea.

Due to the economic and ecological importance, *U. pertusa* has been studied extensively (Robic *et al.,* 2009). Till date biomass of *U. pertusa* has been used an fertilizer and as human food for consumption (Oh *et al.,* 1990), for feedstuff production (Hong *et al.,* 2011) and for bioethanol production (Lee *et al.,* 2011). Furthermore, this species has valuable biological properties for pharmaceutical applications, such as treatment of fever, heat shock, lymphatic swellings, antipyretics, goiter, high blood pressure, dropsy and burn (Oh *et al.,* 1990). Recently, *U. pertusa* has been used as a source of ulvan, which has valuable biological properties for the agricultural, food, and pharmaceutical industries (Robic *et al.,* 2009).

Ha *et al.* (2009) found that *U. pertusa* showed considerable antimicrobial activity against *Gardnerella vaginalis* without side effects at a moderate dose. Various seaweeds show seasonal variation in antimicrobial activity (Moreau *et al.,* 1998; Stirk *et al.,* 2007; Vidyavathi and Sridhar 1991), but whether *U. pertusa* does, has yet to be examined. Therefore, in the present study, methanol extracts of *U. pertusa* was examined to observe the seasonal variation in antimicrobial activity against the human pathogen *G. vaginalis*. In addition, to ascertain the relationship between antimicrobial activity and seaweed viability, chlorophyll content of seaweed samples collected throughout the year was also estimated.

Materials and Methods

Seaweed extracts and bacterial strains : The green seaweed *U. pertusa* was collected from Chungsapo (35°16´07.07˝N, 129°19´37.95˝E) on the east coast of Busan, South Korea, during

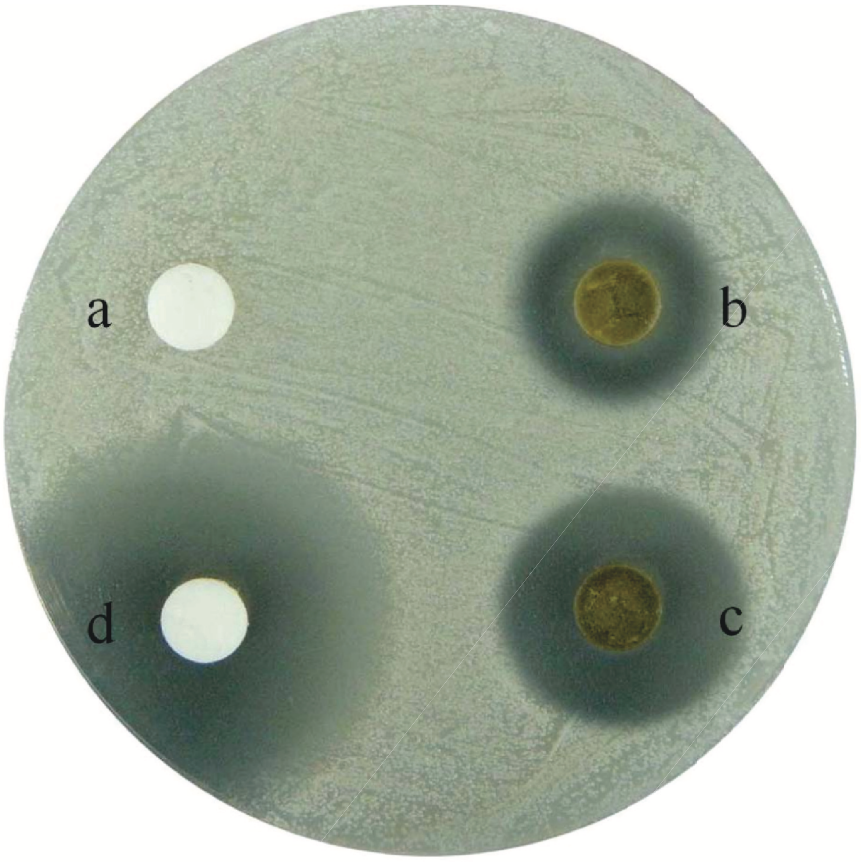

Fig. 1 : The inhibition zones of *Ulva pertusa* collected in April 2009 against *G. vaginalis*. a: Negative control; b: 3 mg disk^{-1}; c: 5 mg disk^{-1}; d: Carbenicillin as a positive control (5 µg disk^{-1})

September 2008 to August 2009. The methanol extract of *U. pertusa* was prepared as described by Jin *et al.* (1997). The microorganisms, *G. vaginalis* (KCTC 5096) were maintained and cultivated as described by Ha *et al.* (2009).

Disk diffusion assay : *In vitro* antimicrobial activity against *G. vaginalis* was determined using a disk diffusion assay following the method of Ha *et al.* (2009). The antimicrobial agent carbenicillin (Sigma C1613) was included in the assay as a positive control. All disk diffusion tests were performed independently in triplicate.

Estimation of chlorophyll *a* and *b* content : The content of chlorophyll *a* and *b* was estimated according to Sterman's method (1998) with a slight modification. Briefly, the samples (50 mg) were ground and extracted with methanol (2 ml) for 10 min on ice. After centrifugation at 1,500×g for 10 min, the absorbance of supernatant (1 ml) was measured at 664 and 647 nm using an UV/Vis spectrophotometer (UVIKON XS; SECOMAM, Alés, France). The chlorophyll *a* and *b* content in the samples were calculated using the following equation: Chlorophyll *a* = 11.93A664 – 1.93 A647; Chlorophyll *b* = 20.36A647 – 5.50 A664.

Results and Discussion

U. pertusa showed strong antimicrobial activity against *G. vaginalis*, the highest activity (6.5 mm) in April (Fig. 1). The inhibition zone of carbenicillin (Sigma C1613), as a positive control against *G. vaginalis*, was 11 mm at 5 µg disk^{-1}. As shown in Fig. 2, trace activity was present in November, May and June in the next year, 2009 and did not exhibit any activity from July to October, except for slight antimicrobial activity in August at 5 mg disk^{-1}. The *U. pertusa* showed strong antimicrobial activity against *G. vaginalis* from December (winter) to April (early spring) in the next year, 2009.

To estimate the viability of samples, chlorophyll *a* and *b* content of dried tissues of *U. pertusa* was determined (Fig. 3). The chlorophyll *a* content of *U. pertusa* ranged from 1.09 to 2.87 mg g^{-1} and chlorophyll *b* ranged from 0.45 to 1.37 mg g^{-1}, respectively. For both chlorophyll a and b, the highest quantity (2.87 and 1.37

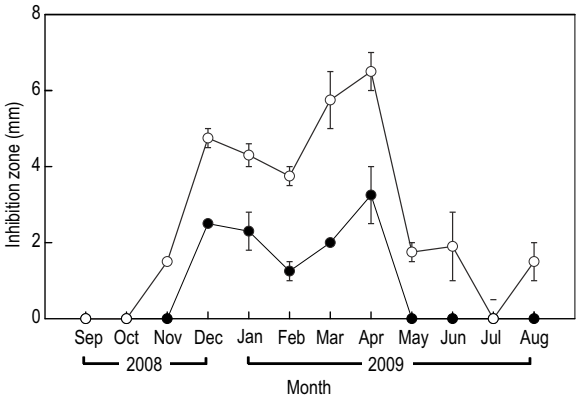

Fig. 2 : Inhibition zone (mm) presented as antimicrobial activity of a methanol extract (●, 3 mg disk^{-1}; ○, 5 mg disk^{-1}) of *Ulva pertusa* collected monthly, tested against vaginal pathogen *Gardnerella vaginalis* (KCTC 5096). Values are mean of three replicates ± SD.

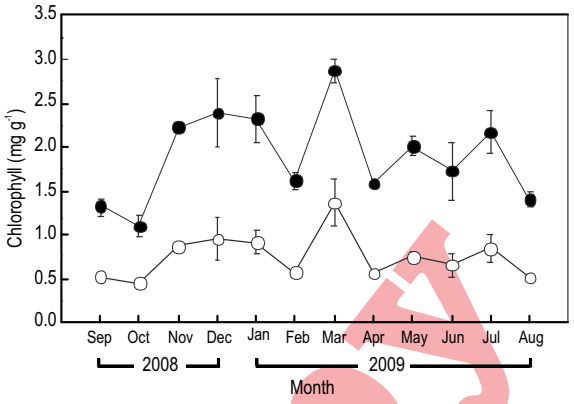

Fig. 3 : Chlorophyll content (mg) of dried tissues of Ulva pertusa (g) collected monthly (●, chlorophyll a; ○, chlorophyll b). Results are given as mean ± SD (n=3)

mg g^{-1}) was observed in March 2009, and lowest in October 2008. In April 2009, *U. pertusa* showed highest antimicrobial activity, and chlorophyll *a* and *b* content was 1.59 and 0.56 mg g^{-1}, respectively. In addition, in July 2009, *U. pertusa* showed no antimicrobial activity and chlorophyll *a* and *b* content was 2.17 and 0.85 mg g^{-1}, respectively.

U. pertusa has been reported to show considerable antimicrobial activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea*, *Escherichia coli*, *Aerobacter aerogenes*, *Proteus vulgaris* (Baik and Kang 1986), *G. vaginalis* (Ha *et al.,* 2009), *Helicobacter pylori* (Lee *et al.,* 2009), *Porphyromonas gingivalis* and *Prevotella intermedia* (Choi *et al.,* 2012). However, it was reported here for the first time that this species shows seasonal variation in its antimicrobial activity. Methanol extracts of this species showed antibacterial activity from early winter (December) to middle spring (April) but not in summer or autumn.

Several patterns of seasonal variation in the antimicrobial activity of seaweeds have been reported. Hornsey and Hide (1976) evaluated Southwest England, and observed four main patterns: uniformly active type, winter peak type, summer peak type, and spring peak type. Similar observations have been reported for the southern coast of India (Padmakumar and Ayyakkannu, 1997), the French Mediterranean coast (Moreau *et al.,* 1988), and the east coast of South Africa (Stirk *et al.,* 2007). In addition, Padmakumar and Ayyakkannu (1997) reported that Chlorophyceae are generally active throughout the year, whereas Phaeophyceae show no activity in certain seasons and Rhodophyceae exhibit clear seasonal variation but never a complete lack of activity. It was found that, although *U. pertusa* is a member of Chlorophyceae family, trace activity was not present against the tested strain during summer and autumn, excluding August. This may be due to variation in cellular chemical composition and biological activity according to season, habitat, and life stage (Lobban and Harrison, 1994).

In general, viability is an ability to live, grow and develop. Chlorophylls are essential pigments for photosynthesis, harvesting light energy. Therefore, chlorophyll content has been frequently used to assess the physiological status of seaweed. In this study, when the antibacterial activity was compared with chlorophyll content, it was found that there was no correlation between antibacterial activity and seaweed viability. This may reflect variation in cellular chemical compositions such as secondary metabolite(s) rather than chlorophylls and biological activities according to season. Similar tendency was observed in kelp *Ecklonia radiate* showing seasonal variation in relationship between growth rate and secondary metabolite(s) such as phlorotannin production (Steinberg 1995).

These preliminary results indicate that *U. pertusa* may be a source of antimicrobial compounds, and future studies should isolate the compound(s) responsible for inhibitory effects.

Acknowledgments

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