Scientific Papers, UASVM Bucharest, Series A, Vol. LIII, 2010, ISSN 1222-5339

ANTIFUNGAL ACTIVITY OF MACROALGAE EXTRACTS

ANDREEA COŞOVEANU, OANA AXÎNE, BEATRICE IACOMI

University of Agronomic Sciences and Veterinary Medicine of Bucharest

Keywords: *algae, antifungal activity, plant pathogens*

Abstract

Macroalgae are ubiquitous organisms, they inhabit almost everywhere. They are a renewable living resources which are also used as food, feed, pharmaceuticals, wastewater treatment or for the industrial production of phycocolloids. Biostimulant properties of seaweeds are explored for use in agriculture (as fertilizer).

In our study, the biological activity of Alaria esculenta, Fucus vesiculosus, Fucus sp. *(Bioalgua®), Spirulina platensis, and Ecklonia maxima (as Kelpak®) was tested in vitro against Fusarium roseum, F. oxysporum, Alternaria alternata, A. dauci, A. longipes, Trichoderma viride, Botrytis cinerea, Aspergillus niger, Penicillium expansum. Their potential toxic effects were evaluated on mycelial growth. Results are presented as effective concentration which inhibits mycelial growth by 50% and 90%. Almost all the algal extracts tested showed an antifungal activity, as ethanol extracts.*

To our knowledge, this is the first report in Romania providing data on the antifungal activity of algal extracts. Macroalgae are an attractive and natural source of bioactive molecules. Such natural products may have potential for the management of fungal diseases in sustainable agriculture such as organic farming. Further research is needed regarding such alternative (seed treatment, foliar applications) in an integrated crop disease management program.

INTRODUCTION

Red and brown algae are mainly used as human healthy food sources, due to their high concentration in polysaccharides, natural richness in minerals, polyunsaturated fatty acids and vitamins. Macroalgae (seaweeds) are rich sources of structurally new and biologically active metabolites. In recent years, there have been many reports of macro algae derived compounds that have a broad range of biological activities, such as antibacterial, antiviral, antioxidant, anti-inflammatory, cytotoxic and antimitotic activities [2]. Special attention has been reported for antiviral, antibacterial and/or antifungal activities against human pathogens [8, 3, 6, 7, 10, and 11] and biostimulant properties of seaweeds are explored for use in agriculture.

The present study was undertaken to investigate the antifungal activities of seven green and brown algae species. The tested algae were: *Spirulina platensis* - green algae (*Chlorophyta*); *Fucus vesiculosus* and other members of the *Fucaceae*

family; *Alaria esculenta* and *Ecklonia maxima* as Kelpak® - brown algae (*Phaeophyta*).

MATERIAL AND METHODS

Algal extracts. Extracts of 5 algal species (listed in Table 1) were tested *in vitro* for their antifungal activities against 9 fungal isolates. Algal dried samples (*Alaria esculenta* and the mixture of *Fucaceae* family – as Bioalgua®) were cut into small pieces and ground in an electric coffee mill. The other algal species were formulated as powder, except *E. maxima* – as Kelpack® (liquid formulation). Ten grams of each algal powdered sample were submitted to lipid-soluble extraction with ethanol by soaking for overnight at room temperature. The extracts were filtered through bacterial filters (Millipore, 0.45μm). Stock solutions were prepared for each algal species at 10% concentration. **Fungal isolates.** *In vitro* tests were conducted using 9 fungal isolates belonging to the genera *Alternaria*, *Aspergillus*, *Botrytis*, *Fusarium*, *Penicillium* and *Trichoderma*. All fungal strains used in this study are listed in Table 2. All strains were purified by monospore isolation and maintained on malt agar (MA) medium (2% malt, 2% agar, w/v) at 4^0C. **Assay on mycelium.** The effect of algal hydro alcoholic extracts (HAE) on mycelial growth was tested *in vitro*. Growth of fungal isolates was scored after 7 days of incubation at 24°C. Agar disks were cut from the margin of a 7-day-old fungal colony and were transferred to MA medium supplemented with the algal extracts (aliquots of stock solutions were incorporated to medium at 45-50^0C to provide concentrations of 0.25, 0.5, 1.0 and 2.0%). Only for *E. maxima* the tested concentrations were 1% and 2%, recommended for Kelpak® application. Three replicates were used per treatment. For each algal extract and concentration, the percentage of growth inhibition in treated samples compared to the control was calculated. The results are expressed as the effective concentration EC_{50}/EC_{90} (the concentration which reduced mycelial growth by 50%/90%) determined by regressing the inhibition of radial growth values (% control) against the values of the algal extracts tested concentrations.

Table 1

Algal extracts

Algal extract	Algal type	Extract type	Origin
Alaria esculenta	*Phaeophyta* (brown algae)	HAE	Dried seawed, Naturalia
Fucus vesiculosus		HAE	Laboratoires Vitarmonyl
Fucus sp. (mixture)		HAE	Bioalgua®
Ecklonia maxima		-	Kelpak®
Spirulina platensis	*Chlorophyta* (green algae)	HAE	Hofigal

Table 2

Fungal species and isolates

No.	Fungal species	Isolate	Host	Geographic origin
1	*Alternaria dauci*	Ad P2	*Daucus carota/seeds*	France
2	*Alternaria longipes*	Al 2207	*Capsicum annuum/seeds*	Bulgaria
3	*Alternaria alternata*	Aa 100	*Trichocereus pachanoi/stem*	Romania
4	*Aspergillus niger*	As 205	*Allium cepa/onions*	Romania
5	*Botrytis cinerea*	Bc 2107	*Vitis vinifera/grapes*	Romania
6	*Fusarium oxysporum*	Fo 809	*Cucumis sativus/fruits*	Romania
7	*Fusarium roseum*	Fr 101	*Nerium oleander/seeds*	Romania
8	*Penicillium expansum*	Pe 2712	*Malus domestica/ fruits*	Romania
9	*Trichoderma viride*	Tv 65	*Malus domestica/fruits*	Romania

RESULTS AND DISCUSSION

The antifungal activities of five algal extracts were screened against important fungal species, 8 important plant pathogens and one well known antagonist. Variable response patterns to *Alaria esculenta* extracts were obtained (Figure 1a). For example, isolates An 205 (*Aspergillus niger*) and Aa 100 (*A. alternata*) appeared to be the least and the most sensitive isolates. All *Alternaria* tested isolates were sensitive (EC_{50} values ranging from 0.67 to 0.98%). The others isolates exhibited values of EC_{50} ranging from 1.27% (*F. oxysporum* Fo 809) to 1.54% (*P. expansum* 2712). Fungal mycelium growth was strongly inhibited at concentrations from 1.67% (*A. longipes* 2207) to 2.46% (A. *niger* 205).

The fungitoxic effects of *Fucus vesiculosus* extracts on the mycelial growth of fungal isolates are shown in Figure 1b. Like in the presence of *Alaria esculenta* extract, the most sensitive isolates were those belonging to *Alternaria* genus (mean EC_{50} values: 0.85% – *A. dauci* Ad P2, 0.81% *A. longipes* Al 2207; EC_{90} values below 2%). The other tested isolates exhibited similar sensitivity, EC_{50} values ranging from 5.66% - *B. cinerea* Bc 2107 and *T. viride* Tv65 to 6.33 – *P. expansum* Pe 2712; EC_{90} ranging from 6.46% - Bc 2107 and Tv 65 to 7.13% – Pe 2712. The less sensitive isolate was *Penicillium expansum* Pe 2712.

In vitro effects of *Fucaceae* sp. extracts (as Bioalgua®) on the mycelial growth of tested fungal isolates is presented in Figure 1c. The most sensitive isolate was *Botrytis cinerea* Bc 2107, the mycelial growth being 50% inhibited at 0.5% and 90% inhibited at 2.02%. The response of *Penicillium expansum* Pe 2107 isolate was different in the presence of Bioalgua®, compared to the two previously extracts, the mycelial growth being strongly inhibited at 2.20%. The response of the three *Alternaria* tested isolates was similar, with EC_{90} values ranging from 2.13% (*A. alternata* Aa 100) to 2.74% (*A. dauci* Ad P2). The least sensitive isolate was *Aspergillus niger* An 205 (mean $EC_{50/90}$ values 5.75 and 10.75, respectively).

The fungitoxic effects of *Spirulina platensis* extracts on the mycelial growth of fungal isolates are shown in Figure 1f. All the tested isolates were recorded as sensitive, the mycelial growth being strongly inhibited at concentrations below 2%. The most sensitive isolate was *F. roseum* Fr 101 (EC_{50} 0.5% and EC_{90} 1%). Less sensitive were *A. dauci* Ad P2, *P. expansum* Pe 2712 and *T. viride* Tv 65, with EC_{90} values of 1.95, 1.85 and 1.95% respectively.

No variation in response was obtained with the tested isolates towards Kelpak®, with no inhibition of mycelial growth, except *Alternaria longipes* Al 2207 isolate. In this later case, a slight inhibition of mycelial growth was recorded (13.5% at 1% and 21.6% at 2%). Previous reports have shown that seaweed extracts can reduce disease and promote plant growth. This lack of direct action of Kelpak®could be explained by the fact that, like other products (Iodus® and Vacciplant® - two plant activators with laminarine as bioactive molecule; SW product [5] based on *Ascophyllum nodosum*) is a well known plant growth stimulator, which improves general plant health and enhances plant resistance to nematodes, pests and fungal diseases.

Our results highlighted the strongly *in vitro* antifungal activity of the tested algal extracts, although in recent years, most of the compounds of marine algae were reported as antibacterial in human medecine. It is expected that the antifungal activity found by us to be done in the the presence of bioactive molecules, as phenolic compounds (phlorotannins, terpenes, alkaloids), polysaccharides or fatty acids, many of these structures being identified as antimicrobials [1].

Using organic solvents which are able to extract a large quantity of lipophilic compounds (glycolipid, phenolic-terpenoidsds, unsaturated-fatty acids and hydroxylated unsaturated-fatty acids), the higher antifungal activity found in ethanol extracts, compared to water extracts (data not shown) could be explained [9, 4]. Biochemical analysis are currently undertaken to determine the structure and nature of these compounds.

Current research is under different field cropping systems to assess the plant protective role of seaweed extract and impact on plant diseases. It will be interesting to study the potential elicitor and disease suppressive activities of the algal extracts.

These compounds could be an environmentally friendly means of plant disease control and could be utilized in organic farming and for vegetable cropping systems where application of synthetic fungicides or chemicals needs to be avoided.

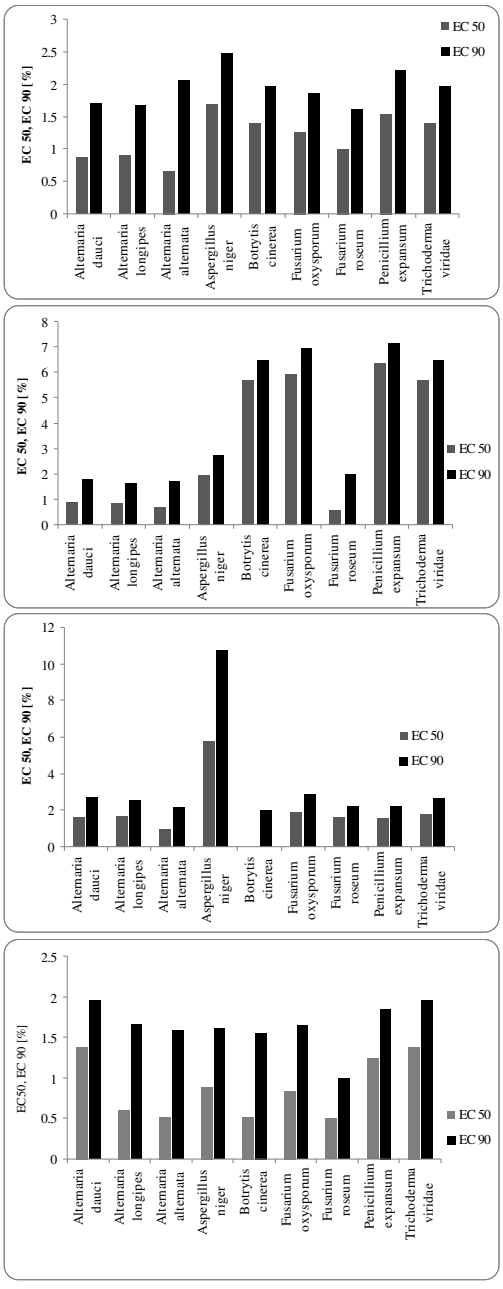

Fig. 1. ***In vitro*** **effects of algal extracts on fungal mycelial growth: a.** ***Alaria esculenta*****; b.** ***Fucus vesiculosus*****; c.** ***Fucus*** **sp. (Bioalgua®); d.*****Spirulina platensis***

CONCLUSIONS

1. In this study, we demonstrated that the tested ethanol macroalgal extracts exerted antifungal activity against different plant fungal species. Fungal mycelial growth was strongly inhibited generally below 2%. To our knowledge, this is the first report in Romania providing data on the antifungal activity of algal extracts against important pre-and postharvest or seed borne pathogens.
2. Further work is required to identify the bioactive molecules that are responsible for the antifungal activity (phenolic compounds, polysaccharides or fatty acids) and to assess the plant protective role of seaweed extract (as foliar or seed treatment).

REFERENCES

1. Awad N.E., 2000. *Biologically active steroid from the green alga Ulva lactuca.* Phytother. Res., 14 (pp. 641-643).
2. Demirel Z., F. Yilmaz-Koz, U. Karabay-Yavasoglu, G. Ozdemir, A. Sukatar, 2009. *Antimicrobial and antioxidant activity of brown algae from the Aegean Sea*. J. Serb. Chem. Soc. 74 (6) (pp. 619-628).
3. El-Baz F.K., A.M. Aboul-Enein, G.S. El-Baroty, A.M. Youssef, H.H. Abd El-Baky, 2002. *Anticarcinogenic activity of algal extracts.* J. Med. Sci., 2 (pp. 243-251).
4. Hanaa H. Abd El-Baky, Farouk K. El Baz, Gamal S.E. Baroty. 2008. *Evaluation of marine alga Ulva lactuca as a source of natural preservative ingredient.* American-Eurasian J. Agric. & Environ. Sci., 3 (3) (pp. 434-444).
5. Jayaraj J., A. Wan, M. Rahman, Z.K. Punja, 2008. *Seaweed extract reduces foliar fungal diseases on carrot.* Crop Protection 27 (pp. 1360-1366).
6. Kandhasamy M., K.D. Arunachalam, 2008. *Evaluation of in vitro antibacterial property of seaweeds of southeast coast of India*. African Journal of Biotechnology 7 (12), 1958-1961.
7. Li A.H., K. Cheng, C. Wong, F. King-Wai, C. Feng, J. Yue, 2007. *Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae*. Food Chem., 102 (pp. 771-776).
8. Lima-Filho J.V.M., A.F.F.U. Carvalho, S.M. Freitas, M.M. Vânia, 2002. *Antibacterial activity of extracts of six macroalgae from the northeastern brazilian coast*. Brazilian Journal of Microbiology 33 (pp. 311-313).
9. Mundt S., S. Kreitlow, R. Jansen, 2003. *Fatty acids with antibacterial activity from the cyanobacterium Oscillatoria redekei HUB 051*. J.Appl.Phycol.,15 (pp. 263-267).
10. Rajasulochana P., R. Dhamotharan, P. Krishnamoorthya, S. Murugesan, 2009. *Antibacterial activity of the extracts of marine red and brown alg*ae. Journal of American Science, 5(3) (pp. 20-25).
11. Vallinayagam K., R. Arumugam, R.R. Kannan, G. Thirumaran, P. Anantharaman, 2009. *Antibacterial activity of some selected seaweeds from Pudumadam Coastal Regions K. Vallinayagam*. Global Journal of Pharmacology 3 (1) (pp. 50-52).