Development of toxicity test using a brown alga *Undaria pinnatifida*

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Introduction

Macroalgae play an important role in coastal ecosystems, such as being primary production site, as well as a place for spawning and growth of fish and crustaceans and other organisms. It is important to assess the impact of chemicals on seaweed to conserve coastal ecosystems. Brown seaweed *Undaria pinnatifida* grows in coastal areas of Asia, the Mediterranean Sea and so on. For the test organisms of toxicity test, it is required to be easily obtain, so *U. pinnatifida* is suitable test organism. But toxicity test methods using *U. pinnatifida* have not been standardized. In this study, toxicity test conditions were evaluated using four antifouling agents to develop the toxicity test method using *U. pinnatifida*.

Method

The gametophyte of the algae *U. pinnatifida* (KU-1630) was got from the Kobe University Macro-Algal Culture Collection (KU-MACC). Algae were cultured in a flat culture flask in an artificial seawater medium supplemented with nutrient salts. Four nutrient salts (KNO_3, KH_2PO_4, $FeCl_3 \cdot 6H_2O$, and $NaHCO_3$) were added to 1 L of artificial seawater and stirred well then filtered through a 0.2 µm filter was used as the toxicity test medium (SW-ASW : ISO 10710)

The biomass was determined as *in vivo* Chlorophyll-a measured by fluorescence microplate reader, and toxicity was evaluated from reduction in RGR.

$$\text{RGR (Relative Growth Rate)} = \frac{1}{7} \ln \frac{\text{Chl-a fluorescence at 7 days exposure}}{\text{Chl-a fluorescence at exposure}}$$

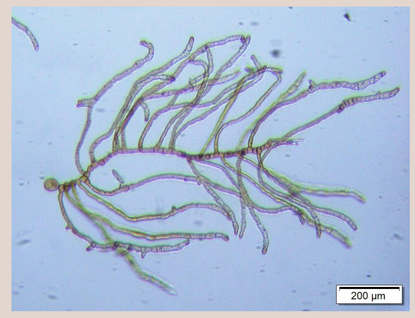

Fig.1 Gametophyte of the algae *U. pinnatifida* (KU-1630)

-examined test conditions-

1. Initial biomass at the start of preculture / 2. Cutting method to create a uniform concentration algae solution / 3. Days of adhesive period / 4. Illuminance during culture

-toxicity test of antifouling agent-

The chemicals tested are Cybutryne, Diuron, $CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$, which are used as antifouling agents for ship bottom. Cybutryne was independently tested 7 times to assess reproducibility.

Results

-Test condition-

temperature : 20℃ ,
Light and dark cycle : 14 h / 10 h ,
light source : LED light for plant culture
Illuminance : 100 lux

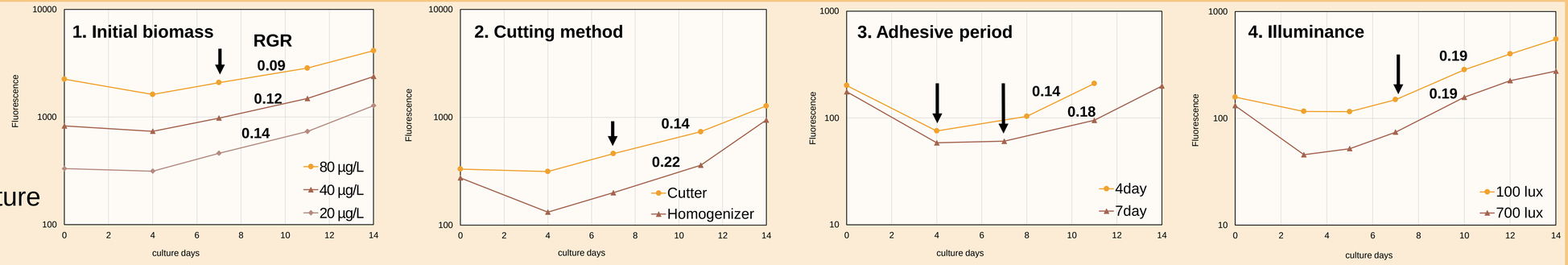

Fig.2 Change in *in vivo* Chl-a fluorescence over time. Arrows indicate media exchange dates

-Toxicity test method for antifouling agent using *U. pinnatifida*-

1. Culture gametophytes for 5 weeks or more
2. Grind the algae with a homogenizer (300RPM, 10 min)
3. Remove large algae with a 500 µm mesh filter.
4. After standing for 1 hour, remove small algae (supernatant)
5. Dilute the precipitated algae and adjust the Chl-a concentration to 10 µg / L
6. Place 0.5 mL / well in a 48-well microplate
7. At 7 day after preculture, replace medium with fresh medium containing chemicals
8. Calculate RGR from the amount of Biomass on the 0th and 7th days of exposure to determine the inhibition rate in each concentration group.
9. Calculate EC_{50} (50% Effective concentration) and NOEC (non-observed effect concentration) using Ecotox statics

-Results of toxicity tests-

As a result of 7 independent tests, the average RGR was 0.18 day^{-1} in control and the coefficient of variation (CV) was 14 %. The EC_{50} of Cybutryne was 5.1 µg/L in average and the CV of EC_{50} was 22 %. Thus, high reproducibility was obtained. (Table 1)

As a result of evaluating the toxicity to the antifouling agents Cybutryne, Diuron, Cu and Zn, the sensitivity was higher than the method using the germination tube elongation, germination rate, and gametophyte photosystem efficiency as indicators in the references. (Table 2)

Table1 Reproducibility of RGR in control and Cybutryne toxicity on algal growth

repeats	Control		Cybutryne
	RGR (day^{-1})	CV (%)	EC50 (95% CI range, $\mu g/l^{-1}$)
1	0.19	19	5.2 (5.1-5.4)
2	0.18	18	5.8 (5.7-6.0)
3	0.19	9	4.8 (4.6-4.9)
4	0.19	12	7.2 (6.9-7.5)
5	0.19	11	4.8 (4.7-5.0)
6	0.17	11	4.0 (3.8-4.1)
7	0.15	17	3.6 (3.5-3.7)
Average	0.18	14	5.1 (4.9-5.2) (CV=22%)

Table2 Phytotoxic effect of four antifouling agents on *U. pinnatifida* (* 95% confidence intervals in paretheses, ** not reported)

Toxicants	Endpoints	exposure (day)	Culture media	NOEC ($\mu g\ l^{-1}$)	EC10* ($\mu g\ l^{-1}$)	EC50* ($\mu g\ l^{-1}$)	References
Cybutryne	Relative Growth Rate	7	SW-ASW	1.8	—	5.1 (4.9-5.2)	This study
	Female Relative fluorescence	2	PES	—**	17 (16-17)	172 (111-232)	Lee et al. (2020)
	Male Relative fluorescence	2	PES	—	19 (15-23)	96 (90-103)	Lee et al. (2020)
Diuron	Relative Growth Rate	7	SW-ASW	5.0	—	14 (13-14)	This study
	Female Relative fluorescence	2	PES	—	13 (12-14)	128 (101-154)	Lee et al. (2020)
	Male Relative fluorescence	2	PES	—	6 (6-7)	37 (34-40)	Lee et al. (2020)
Cu	Relative Growth Rate	7	SW-ASW	6.0	—	17 (16-18)	This study
	Germination	1	OTT's artificial seawater	—	11 (2-8)	185 (141-347)	Park et al. (2016)
	Germ-tube elongation	2	OTT's artificial seawater	—	8 (5-25)	45 (31-76)	Park et al. (2016)
Zn	Relative Growth Rate	7	SW-ASW	250	—	1500 (1400-1600)	This study
	Germination	1	OTT's artificial seawater	—	2700 (450-8400)	>10000	Park et al. (2016)
	Germ-tube elongation	2	OTT's artificial seawater	—	>10000	>10000	Park et al. (2016)

Conclusions

The test gave high reproducibility and sensitivity to from antifouling agents. We developed a week toxicity test method using gametophytes of seaweed *U. pinnatifida*.

References

ISO 10710 (2010), Water quality Growth inhibition test with the marine and brackish water macroalga *Ceramium tenuicorne*
Lee et al (2020), Ecotoxicology, 29, 559-570, DOI : 10.1007/s10646-020-02207-2
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