

Toxic Effects of Triorganotins on the Adhesion and Germination—Growth of Conchospores of *Porphyra yezoensis*, Red Arga

T. MARUYAMA*, D. SUN†, S. HASHIMOTO* and A. MIURA‡

*Tokyo University of Fisheries, Department of Marine Science and Technology, Konan 4, Minato, Tokyo, 108 Japan

†National Bureau of Oceanography, Institute of Marine Environmental Protection, P.O. Box 303, Dalian, China

‡Tokyo University of Fisheries, Department of Aquatic Biosciences, Konan 4, Minato, Tokyo, 108 Japan

Triorganotins (TOTs) have been widely used as antifoulants for ships and fishing nets, and the toxic effects of TOTs on marine organisms are becoming a serious problem. In this study, nori (*Porphyra yezoensis*) was used as an organism for the bioassay of TOTs. Nori is understood to be one of the most suitable organisms for the bioassay of hazardous chemicals for the following reasons: (1) Nori is widely cultivated along the coast of Japan. (2) Even though other seaweeds have become extinct due to the pollution of coastal water, nori has survived. (3) The material of any stage in the life cycle can be easily obtained in the laboratory.

The objectives of this study are to clarify the effective concentrations (EC) of TOTs depending on the ratios of adhesion of conchospores on the glass plate and the germination (upper portion of conchospores standing up-right and lower portion of conchospores projecting a rhizoidal filament)-growth (commencing cell division) of conchospores of *Porphyra yezoensis*.

Materials and Methods

(1) **Materials:** The conchospores were obtained through the free-living conchocelis of *Porphyra yezoensis* Ueda (strain ZGRW) which were maintained in the laboratory. A 1/20PES culture medium (1/20 Provasoli's enriched seawater) was prepared using aged seawater autoclaved at 120°C for 20 min. followed by filtering with 0.45µm Millipore filter. Four kinds of tributyltin [bis(tri-n-butyltin) oxide (TBTO), tributyltin chloride (TBTCI), tributyltin acetate (TBTAc) and tributyltin hydroxide (TBTH)] and two kinds of triphenyltin [triphenyltin chloride (TPTCI) and triphenyltin acetate (TPTAc)] were examined. Prepared TBTCI and TPTCI solutions were analysed by the capillary gas chromatography FPD method (Hewlett Packard 5980). The analysed concentrations of TBTCI and TPTCI were 99% and 101% of the designed concentration, respectively.

(2) **Methods:** Adhesion tests and germination-growth tests of conchospores were conducted by means of a culture of conchospores. All cultures were performed by incubation in a growth chamber at a light intensity of 7Klx, at 15°C and with a 10-14h light-dark cycle.

(a) **Adhesion tests:** 0.04ml of TOT solution was dropped onto a glass plate and 0.04ml of the centrifuged conchospore suspension containing 300-500 conchospores was dropped onto the TOT solution. The concentrations of each TOT and the period exposing conchospores to TOT were from 0 to 1,600µg/l and 48h, respectively. The glass plate was put in sealed petri dishes containing a small volume of 1/20PES culture medium to prevent evaporation. The number of adhered conchospores was counted with a microscope after exposing the conchospores to TOT for 48h.

(b) **Germination-growth tests:** After the adhesion test, the glass plate on which conchospores adhered were transferred to the petri dish containing 1/20PES culture medium with or without TOTs. Concentrations of TOT and the period exposing adhered conchospores to a TOT were from 0 to 280µg/l

and 96h, respectively. The culture media were changed every two days. The number of germlings was counted with a microscope after exposing conchospores to each TOT for 48 or 96h.

Results and discussion

(1) Effects of TOTs on the adhesion of conchospores: Figure 1 shows the adhesion ratios of conchospores on the glass plate as a function of TOT concentration. The 50% EC of TBIO, TBICl, TBIAc, TBTH, TPTCl and TPTAc on the adhesion of conchospores was estimated to be 120, 120, 120, 820, 30 and 60 $\mu\text{g}/\text{l}$, respectively. The minimum EC (48-h $\text{EC}_{\text{Min.,Ad.}}$ in Table 1) of the six TOTs on the adhesion of conchospores on the glass plate was estimated to be 50, 50, 50, 500, 5 and 28 $\mu\text{g}/\text{l}$, respectively. There is a large difference between the 50% EC and the minimum EC values depending on the kinds of TOT. When the conchospores were cultured in 1/20PES culture medium with TPTCl of 28 $\mu\text{g}/\text{l}$, the conchospores were deteriorated.

(2) Effects of TOTs on the germination-growth of the conchospores: Figure 2 shows the germination-growth ratios of conchospores adhered onto the glass plate as a function of TOT concentration. The 50% EC of TBIO, TBICl, TBIAc, TBTH, TPTCl and TPTAc on the germination-growth of conchospores was estimated to be 8.0, 27, 33, 90, 3.6 and 6.3 $\mu\text{g}/\text{l}$, respectively. The minimum EC (48-h $\text{EC}_{\text{Min.,Ger.}}$ * in Table 1) on the germination-growth of conchospores was estimated to be 1-2, 9, 8-16, 30-5-, 1-1.6 and 2 $\mu\text{g}/\text{l}$, respectively. Figure 3 shows the germination-growth ratios of conchospores adhered onto the glass plate as a function of TBIO and TPTCl concentration. The 50% EC of TBIO and TPTCl on the germination-growth of conchospores was estimated to be 4.0 and 1.3 $\mu\text{g}/\text{l}$, respectively. Figure 4, which was obtained from the same experiment as Figure 3, shows the ratios of the number of 2-celled and 4-celled germlings of conchospores as a function of TBIO and TPTCl concentration. When the conchospores were cultured in 1/20PES with TBIO or TPTCl for 96h, the minimum EC (96-h $\text{EC}_{\text{Min.,Ger.}}$ in Table 1) of TBIO and TPTCl was estimated to be 2 and <1 $\mu\text{g}/\text{l}$, respectively, from Figure 4. The ratios of the number of 4-celled germlings decreased and those of 2-celled germlings increased accompanying the increase of concentration of TBIO or TPTCl. The phenomena shown in Figure 4 indicate that TBIO and TPTCl retarded the growth of conchospore germlings. Table 1 shows the minimum EC of each TOT on the adhesion of conchospores onto the glass plate and germination-growth of conchospores adhered onto the glass plate. Toxic effects of TOTs were much more effective in the germination-growth of conchospores (48-h $\text{EC}_{\text{Min.,Ger.}}$) than in the adhesion of conchospores (48-h $\text{EC}_{\text{Min.,Ad.}}$), as shown in Table 1. Nori was very resistive to TOTs in the adhesion of conchospores, but not resistive to TOTs in the germination-growth of adhered conchospores. The examined TPTs showed stronger toxicity than the TBIs to the adhesion and the germination-growth of conchospores. When the conchospores were cultured in 1/20PES culture medium with TPTCl of 5 $\mu\text{g}/\text{l}$, the germlings became deformed.

Conclusions

(a) Six kinds of examined TOTs hindered the adhesion of conchospores on the glass plate and retarded the germination-growth of adhered conchospores. (b) The EC of TOTs on the germination-growth of adhered conchospores was much lower than the EC on the adhesion of conchospores. (c) The toxicity of TOTs examined was estimated to be in the order of TPTCl > TPTAc \approx TBIO > TBIAc \approx TBICl >> TBTH on the adhesion of conchospores onto the glass plate and the germination-growth of adhered conchospores. (d) The minimum EC of TPTCl and TBIO on the germination-growth of adhered conchospores was estimated to be 1 and 2 $\mu\text{g}/\text{l}$, respectively. (e) Nonobservable effective concentration level of TPTCl on the germination-growth of conchospores may be <0.1 $\mu\text{g}/\text{l}$, when the safety factor of 1/10 is adopted. (f) When the concentration of TOTs in culture medium increased, the conchospores were deteriorated and the germlings were deformed.

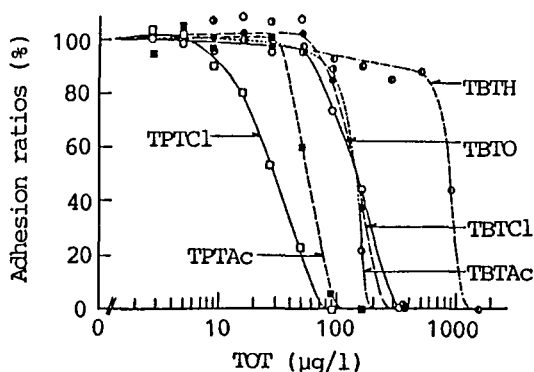


Fig. 1 Effects of TOTs on the adhesion of conchospores of *Porphyra yezoensis* onto the glass plate when the conchospores were exposed to each TOT for 48h.

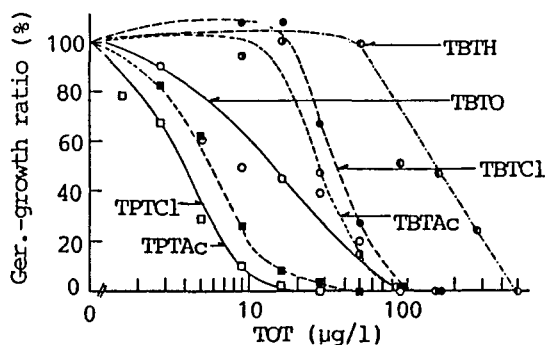


Fig. 2 Effects of TOTs on the germination-growth of conchospores of *Porphyra yezoensis* when the conchospores which adhered on the glass plate were cultured in 1/20PES medium for 48h without a TOT followed by exposing the conchospores to each TOT for 48h.

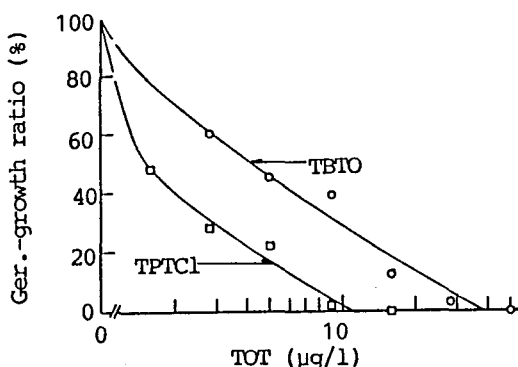


Fig. 3 Effects of TBTO and TPTC1 on the germination-growth of conchospores of *Porphyra yezoensis* when the conchospores were cultured in 1/20PES medium with TBTO or TPTC1 for 96h.

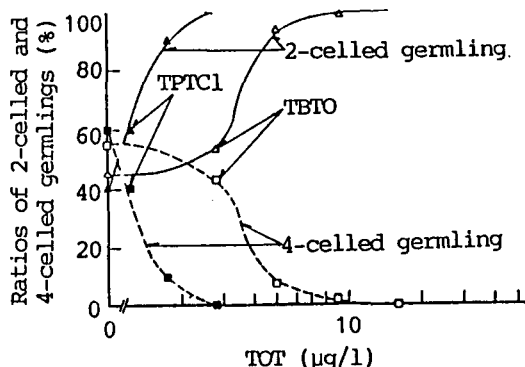


Fig. 4 Effects of TBTO and TPTC1 on the ratios of number of 2-celled germlings and 4-celled germlings of conchospores of *Porphyra yezoensis* when the conchospores were cultured in 1/20PES medium with TBTO or TPTC1 for 96h.

Table 1 The minimum effective concentrations of TOTs on the adhesion and germination-growth of conchospores of *Porphyra yezoensis*
units: µg/L

	48-h EC ₅₀ (Exp. 1)	48-h EC ₁₀ (Exp. 1)	48-h EC ₅₀ * (Exp. 2)	96-h EC ₅₀ (Exp. 3)
TBTO	50	2	1-2	2
TBTC1	50	9	9	-
TBTAc	50	16	8-16	-
TBTH	500	30	30-50	-

TPTC1	5	< 1.6	1-1.6	< 1
TPTAc	28	< 2.8	2	-

48-h EC₅₀*: EC₅₀ of TOTs on the germination-growth of conchospores when the adhered conchospores on the glass plate were cultured in 1/20PES medium without a TOT followed by exposing conchospores to each TOT for 48h.