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Coralline algae also contain feeding stimulant glycerolipids for marine gastropods

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The presence of feeding-stimulant glycerolipids digalactosyldiacylgycerol (DGDG) and 6-sulfoquinovosyldiacylglycerol (SQDG), and no feeding inhibitors was confirmed on four species of coralline algae (one genicuiate species *Amphiroa beauvoisii* and 3 nongeniculate species:*Lithophyllum okamurae, L. yessoense* and *Mesophyllum cystocarpideum*) using Avicel Plate Method for *Haliotis discus, Omphalius pfeifferi* and *Turbo cornutus* and TLC analysis.

Key words; feeding stimulant, *Amphiroa beauvoisii*, coralline algae, glycerolipids, *Mesophyllum cystocarpideum, Lithophyllum okamurae, Lithophyllum yessoense.*

Recently, feeding stimulant glycerolipids against marine herbivores were found in green (Sakata *et al.*, 1988), brown (Sakata and Ina 1985), and red algae (Fujita *et al.*, 1990). In our further survey, these substances were also detected in the methanol extracts of coralline algae, a calcified red algal group (Rhodophyta, Corallinaceae).

Materials & Methods

One geniculate species, *Amphiroa beauvoisii* (=*A. zonata*) and nongeniculate species *Mesophyllum cystocarpideum* were collected in Toyama Bay, and two nongeniculate species *Lithophyllum okamurae* and *L. yessoense* were from Southwestern Hokkaido in July of 1990. Each air-dried coralline alga was extracted with MeOH (Table 1).

The test Avicel plates (5×20cm with 0.25mm thickness) were made in the same way as previously reported (Sakata *et al.* 1984). Each algal extract (40−80 $\mu\ell$, equivalent to *ca.* 50 and 100mg of air-dried alga) was applied with a microsyringe as evenly as possible on a sample zone (23mm in diameter) made on the test plate.

The test animals were juvenile abalone *Haliotis discus* (shell length≒15mm, number of shells 〈N〉=30) and top shells *Turbo* (*Batillus*) *cornutus* (shell height≒15mm, N=20) and *Omphalius pfeifferi* (SH≒30mm, N=9). These animals were seperately kept in three

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Table 1. MeOH extraction from coralline algae.

Species	Weight of materials (g)	MeOH volume (mℓ×times)	Weight ratio of extracts (%)
Amphiroa beauvoisii	500	200×3	3.8
Mesophyllum cystocarpideum	100	300×3	2.1
Lithophyllum okamurae	500	800×2	1.1
Lithophyllum yessoense	500	600×2	0.7

polyvinyl-chloride aquarium compartments (12×35×15cm), and was set in a box of styrofoam (Sakata *et al.*, 1991a). Water depth and temperature were maintained by circulation at 5cm and 22℃, respectively. As maintenance food, *Laminaria angustata* var. *longissima* was given to these animals once every a few days, and starved for a day before the assay.

The test plate for the assay was set in each compartment of the aquarium after sunset. The next morning, grazing patterns on the test plates were checked for feeding activity.

Results

Typical assay plate is shown in Fig. 1, and results are summerized in Table 2. On Avicel plates, every species of gastropods (*H. discus, T. corunutus* and *O. pfeifferi*) grazed off almost all the sample zone of all examined extracts (100mg eqivalent of the air-dried algae) and DGDG (40μg =minimum effective dose, See Sakata *et al.* 1988), but little within blank zones. The presence of feeding inhibitors was not suggested from these results.

Each algal methanol extract was subjected to thin-layer chromatographic (TLC) analysis. The conditions of TLC analysis were as follows: silica gel 60 F_{254} (Merck); $CHCL_3$: MeOH : 28% ammonia=65:35:5, and $CHCL_3$: EtOAc : MeOH : H_2O=10:4:2:2. Each chromatogram was very similar to each other. A typical result (*M. cystocarpideum*) was shown in Fig. 2. Co-chromatography of the extract confirmed the presence of previously known feeding stimulants, digalactosyldiacylglycerol (DGDG, Sakata and Ina 1985) and 6-sulfoquinovosyldiacylglycerol (SQDG, Sakata *et al.* 1988). Results of the TLC analysis clearly showed that all the algal extracts contain at least two potent feeding stimulant glycerolipids, DGDG and SQDG.

Discussion

Coralline algae are positioned at the most grazing-difficult level among seaweed because of their toughness (Steneck and Watling 1982). The presence of feeding stimulants and no (or little) feeding inhibitors suggest that coralline algae (both geniculate and non-

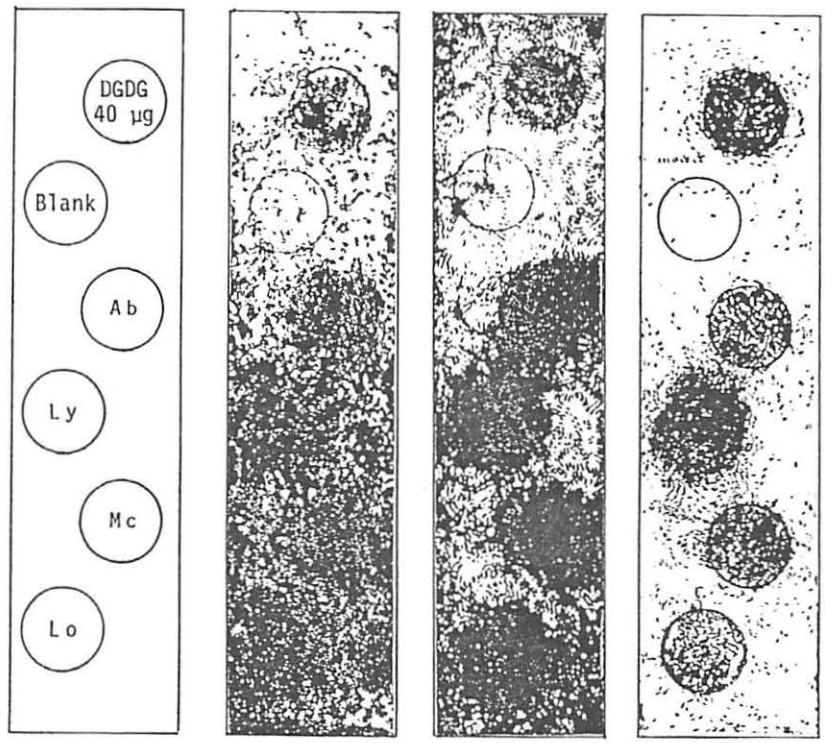

Fig. 1 Typical assay results for feeding activity of *Haliotis discus*, *Omphalius pfeifferi* and *Turbo cornutus* using Avicel plate method.

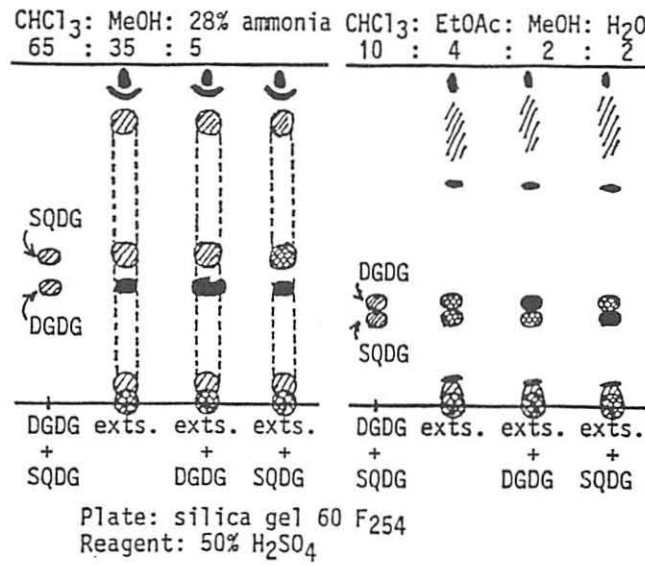

Fig. 2 Typical analysis of TLC for *Mesophyllum cystocarpideum*.
Co-chromatography with authentic DGDG or SQDG confirmed their presence in the extract of *M. cystocarpideum*.

Table 2. Feeding-stimulant assay results of algal MeOH extracts for juvenile abalone *Haliotis discus* and top shells *Turbo cornutus* and *Omphalius pfeifferi*.

Species	MeOH exts. (mg eq.*)	Feeding stimulant activity											
		Haliotis discus				*Turbo cornutus*				*Omphalius pfeifferi*			
		−	±	+	++	−	±	+	++	−	±	+	++
Amphiroa	100	0	0	0	1	0	1	1	0	0	0	0	1
beauvoisii	50	1	0	0	2	0	1	0	0	0	0	2	0
Lithophyllum	100	0	0	0	1	0	0	0	1	0	0	0	1
yessoense	50	1	0	0	2	0	0	0	2	0	0	0	2
Mesophyllum	100	0	0	0	1	0	0	0	1	0	0	0	1
cystocarpideum	50	0	0	0	3	0	0	0	2	0	0	1	1
Lithophyllum	100	0	0	0	1	0	0	0	1	0	0	0	1
okamurae	50	0	0	0	3	0	0	1	1	0	0	1	1

*: Applied sample amounts of methanol extract equivalent to each air-dried alga (mg).
−: No or nearly the same number of feeding traces are left inside the sample zone.
±: Slightly more feeding traces are observed inside the sample zone.
+: Clear difference of feeding traces are observed between inside the sample zone and outside.
++: Almost all the Avicel in the sample zone has been grazed off.

geniculate groups) defend their thalli not with secondary organic metabolites like in the case of some algae (Kurata *et al.* 1988, Sakata *et al.* 1991b) but with calcified skeleton. The crustose or protuberant form of nongeniculated coralline algae may increase the grazing-difficulty for gastropods with rhipidoglossan radula. For example, *T. cornutus* feed geniculate form well (Ino 1959), but only thin minute species or epithallial layers of thick thalli in nongeniculate group (Fujita, unpublished data).

Among the species examined, *L. yessoense* has intensively covered the bottom substrata in 'Isoyake' (urchin-dominated barren) ground (Noro *et al.* 1983, Fujita 1989). Distal part of the crust of this species is grazed by dominant herbivores like sea urchin and limpet (Fujita 1992). Some other gastropods also graze but only thin juvenile crusts and multilayered epithallium of thick crusts (Fujita, unpublished data). Because of its containing feeding stimulants, the grazing by these herbivores may induce their intense grazing activity, which therefore may reduce the germlings of other algae on and around this species.

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サンゴモ類も海産腹足類に対する摂餌刺激物質を含有している

藤田大介・岩瀬洋一郎・坂田完三

アビゼル板法及び薄層クロマトグラフィーにより，有節サンゴモのウスカワカニノテ，無節サンゴモ類のエゾイシゴロモ，ヒライボ及びクサノカキの計4種のメタノール抽出物について，エゾアワビ，バテイラ及びサザエに対する摂餌刺激活性を有する複合脂質のジガラクトシルジアシルグリセロール（DGDG）及び6－スルフォキノボシルジアシルグリセロール（SQDG）の存在を確認したが，摂餌忌避物質は認められなかった。