Short Paper

Utilization of Malic Acid by the Brown Alga *Sargassum horneri*Masaharu Ohgai,^{*1} Koji Tsujinaka,^{*2}
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It is known that some microalgae grow by utilizing carboxylic acids in dark conditions.^{1,2)} Many microalgae have autotrophic ability, while some have both autotrophic and heterotrophic abilities.^{1,2)} However, the ability to utilize carboxylic acids in the dark has not been found for macroalgae. We undertook laboratory test to determine whether the brown alga *Sargassum horneri* has the ability to utilize carboxylic acids or not.

A portion of the thallus (68 mg wet weight) was immersed in a culture medium (PESI)³⁾ containing 10 ppm of malic acid (L-[U-¹⁴C] malic acid, CEA, 200 kBq) in the dark at 20°C. After 25 min, the thallus was thoroughly rinsed with sterilized seawater, and fixed for 3 min with 0.5 ml of boiling 80% ethanol. The thallus fixed was extracted with 5 ml of 80% ethanol. The supernatant obtained by centrifugation was concentrated to 1 ml under reduced pressure. A half portion of the concentrated solution, to which was added 5 μ l of standard carboxylic acid solution, was subjected to thin-layer chromatography on cellulose (HPTLC plate F254s, Merck, Darmstadt) with *n*-butyl formate/formic acid/H₂O (30/12/3, v/v/v). The plate was visualized with 0.3% bromocresol green. The spots were scraped off and extracted with 2 ml scintisol EX-H (Dojin Chem. Ltd.). Radioactivity of each extract was measured by a liquid scintillation counter (Aloka Co. Ltd.). The other half portion, to which was added 5 μ l of standard amino acid solution, was subjected to 2D TLC on cellulose with (1) iso-butyric acid/*n*-butanol/iso-propanol/H₂O/NH₃/EDTA (100/3/14/38/4/0.05, v/v/v/v/v/g) and (2) *n*-butanol/*n* propanol/H₂O/*n*-propionic acid (100/44/93/71, v/v/v/v). The spots were scraped off and extracted with scintisol EX-H. The radioactivity was similarly measured.

Table 1. Radioactivity levels of carboxylic and amino acids isolated from the thallus of *Sargassum horneri*

(cpm)* ¹	
Organic acids	Radioactivity
Carboxylic acids	
Oxaloacetic acid	13,967 $\pm$ 132* ²
Malic acid	6,869 $\pm$ 152
Citric acid	951 $\pm$ 31
2-Ketoglutaric acid	177 $\pm$ 18
Succinic acid	154 $\pm$ 11
Lactic and fumaric acids	77 $\pm$ 9
Amino acids	
Aspartic acid	2,104 $\pm$ 106
Glutamic acid	1,400 $\pm$ 87

*¹ Organic acids with radioactivity levels of less than 50 cpm were omitted.*² Mean $\pm$ S.D.

Radioactivity levels of carboxylic acids and amino acids are shown in Table 1. The radioactivity was highest for oxaloacetic acid, and, to a lesser extent, for malic acid. Radioactivity levels of aspartic and glutamic acids were lower than those of oxaloacetic and malic acids, but were higher than those of 2-ketoglutaric and citric acids. The results may indicate that malic acid taken up by the cells of *S. horneri* was utilized directly in the TCA cycle and the intermediate products of malic acid were converted into amino acids. Therefore, it is concluded that the brown alga *S. horneri* has a heterotrophic ability to utilize carboxylic acids.

References

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