海藻類における酵素の耐熱性変異

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著者	奥村, 誠一 藤野, 和男
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Thermostability Variations of Enzymes in Sea Algae*1

Sei-ichi Okumura and Kazuo Fujino*2

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Intraspecific thermostability variations of enzymes and hemoglobin have been reported in several species of insect, marine molluscs, and man. The observations on correlations between such variations in wild populations of the pacific abalone, *Haliotis discus hannai*, and water temperatures in habitat, and on heart rate responses against thermal stresses strongly suggested a probable association between such variations and temperature tolerance of the animals. No such work, however, has been reported on aquatic plants or algae. The present paper describes such variations of enzymes in the two species of sea algae, *Porphyra yezoensis* and *Undaria pinnatifida*, and analyses the relationships between water temperature in the habitat and the variations in the former species.

Sampled were a total of 83 specimens of *P. yezoensis* from waters of Yamada, Iwate and Iwaki, Fukushima and of 22 specimens of *U. pinnatifida* from Sanriku, Iwate. Thallus of each specimen was analysed for thermostability variations in the three enzymes according to the method described before. Variations were observed at the three loci coding phosphoglucomutase, phosphoglucose isomerase, and glutamate dehydrogenase in *P. yezoensis* and those at phosphoglucose isomerase in *U. pinnatifida*. Close associations between water temperatures in habitat and frequencies of thermostability alleles at the three loci strongly suggested existence of temperature-related differential viabilities among these variations.

Intraspecific thermostability variations of enzymes and hemoglobin have been reported in several species of animals, such as drosophila,[1–9] marine molluscs,[10–12] and man.[13] The observations on correlations between water-temperatures in habitat and frequencies of alleles coding such variations in wild populations of marine animals,[14] including the pacific abalone *Haliotis discus hannai* and on heart-rate responses[15] against thermal stresses strongly suggested probable association between such variations and temperature tolerance of the animals. No such work has been reported on plant or algae. The present paper describes the thermostability variations of enzymes in the two species of sea algae, *Porphyra yezoensis* and *Undaria pinnatifida*, and analyses the relationships between water-temperature in the habitat and frequencies of alleles coding such variations of enzymes in the former species.

Materials and Methods

Specimens of Sea Algae

Sampled were a total of 83 specimens of *Porphyra yezoensis* from waters of Yamada, Iwate and Iwaki, Fukushima and of 22 specimens of *Undaria pinnatifida* from Sanriku, Iwate (Table 1). Thalli of each specimen were packed in a plastic bag and were kept frozen. After thawing, pieces of thallus sample were homogenized with approximately an equal weight of 10% glycerol solution. The homogenate was centrifuged at 3,000 rpm for 20 min at 2°C. The supernatant was kept frozen at −20°C until electrophoretic analysis was performed.

Electrophoresis and Heat-treatment

Horizontal starch gel electrophoresis were performed for phosphoglucomutase (EC 2.7.5.1, PGM), phosphoglucose isomerase (EC 5.3.1.9, PGI) and glutamate dehydrogenase (EC 1.4.1.3, GDH) according to Shaw *et al.*[16] Heat-treatment of gel after electrophoresis, described by Wilkins *et al.*,[12] was followed with some modifications in temperature and length of time of heating as follows. The conditions were 15 min at 57°C±1°C for PGM, 13.5 min at 50°C±1°C or 14 min at 51°C±1°C for PGI, and 10 min at 41°C±1°C, 44°C±1°C, or 46°C±1°C for GDH of *Porphyra yezoensis* and 10 min at 48°C±1°C for PGI of *Undaria pinnatifida*. A mixture of an

*1 Supported in part by the grant from the Ministry of Education, Science and Culture, Japan.

*2 Kitasato University School of Fisheries Sciences, Sanriku, Kesen, Iwate 022-01, Japan (奥村誠一, 藤野和男: 北里大学水産学部).

Table 1. Specimens of the sea algae used for analyses of electrophoretic and thermostability variations

Species	Localities*	Number of specimens analysed	Month and year sampled
Porphyra yezoensis	Yamada, Iwate	53	Mar. 1984
〃 〃	Iwaki, Fukushima	30	Mar. 1984
Undaria pinnatifida	Sanriku, Iwate	22	Apr. 1983

* Latitudes at Yamada, Iwaki, and Sanriku are 39°30′N, 37°06′N, and 39°06′N respectively.

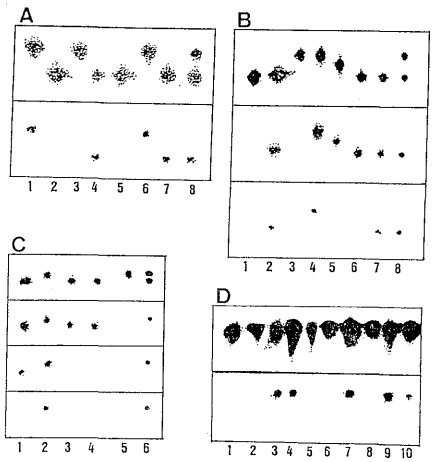

Fig. 1. Zymograms for demonstrating thermostability variations at the loci of PGM (A), PGI (B), and GDH (C) of *Porphyra yezoensis* and at the locus of PGI (D) of *Undaria pinnatifida*, each consisting of a pair of unheated (top) and heated (the other) layers of the sliced gel after electrophoresis. Double bands on the unheated gel in the zymograms of A, B, and C represent the control sample, prepared by mixing homogenates from two specimens, different both in their electrophoretic mobilities and thermostabilities from each other. Notice that some bands on the unheated layer disappeared on the heated layers. The conditions of heating gel were 15 min at 57°C for PGM, 13.5 min at 50°C or 14 min at 51°C for PGI, and 10 min at 41°C, 44°C, or 46°C for GDH of *Porphyra yezoensis* and 10 min at 48°C for PGI of *Undaria pinnatifida* as described in the text. In PGM (A), specimens of slot nos. 1, 4, 6, and 7 were typed as thermo-resistant, R, and slot nos. 2, 3, and 5 were typed as thermo-sensitive, S. In PGI (B), specimens of slot nos. 2, 4, and 7 were typed as thermo-resistant, R, and slot nos. 1, 3, 5, and 6 were typed as thermo-sensitive, *S* or *S′*. In GDH (C), specimens of slot nos. 1 and 2 were typed as thermo-resistant, *R* or *R′*, and slot nos. 3, 4, and 5 were typed as thermosensitive, *S* or *S′*. In PGI of *U. pinnatifida* (D), specimens of slot nos. 3, 4, and others were typed as thermo-resistant, *R*, and slot nos. 1, 2, and others were typed as thermo-sensitive, S.

Table 2. Frequencies of alleles coding electrophoretic variations at PGM, PGI, and GDH loci in the *Porphyra yezoensis* samples collected from the two locations*

Locus	Allele frequency	Yamada	Iwaki
PGM	p	0.811	0.367
	q	0.189	0.633
	$\overline{N}$	53	30
PGI	p	0.020	0.133
	q	0.180	0.734
	r	0.800	0.133
	$\overline{N}$	50	30
GDH	p	0.460	0.200
	q	0.520	0.767
	r	0.020	0.033
	$\overline{N}$	50	30

Remark, * Allele frequency differences between the two locations were significant at all the three loci.

equal amount of homogenates from two selected specimens, different both in electrophoretic mobilities and in thermostabilities from each other, was used as a control in starch gel electrophoresis for more accurate determination of thermostability variations, because of the haploid state of thallus in the life cycle of the sea-algae under study. The unheated and heated layers of gel were stained in normal way. Thermostability genotype of each sample was determined by comparing enzyme activities between the layers of gel as described before.[12]

Analyses of Association between Loci

Chi-square test for contingency table of genotypic frequencies was used for analysing relationships either between different loci coding electrophoretic variants or between those electrophoretic variants and thermostability variants.

Results and Discussions

Electrophoretic Variants

In *Porphyra yezoensis,* observed were two variants in electrophoretic mobilities at PGM

Table 3. Comparison of frequencies of alleles coding thermostability variations of PGM, PGI, and GDH in *Porphyra yezoensis* between the two locations sampled

Locus	Allele frequency	Yamada	Iwaki
PGM	p^R	0.868	0.900
	q^S	0.132	0.100
	$\bar{N}$	53	30
PGI	p^R	0.660	0.734
	$q^{S'}$	0.280	0.233
	r^S	0.060	0.033
	$\bar{N}$	50	30
GDH	p^R	0.060	0.067
	$q^{R'}$	0.320	0.434
	$r^{S'}$	0.380	0.333
	s^S	0.240	0.166
	$\bar{N}$	50	30

Table 4. Surface seawater temperatures (°C) at the two stations, where specimens of *Porphyra yezoensis* were sampled[19]

Stations*	Highest	Lowest
Todogasaki, Iwate	20.0 (Sep.)	5.7 (Mar.)
Shioyasaki, Fukushima	21.0 (Sep.)	7.2 (Feb.)

* Latitudes at Todogasaki and Shioyasaki are 39°30′N and 37°00′N respectively.

locus, three variants at PGI locus, and three variants at GDH locus in the specimens examined, in compared with three variants each at PGM, PGI, and GDH loci reported by Miura *et al.*[17] No direct comparison was conducted for identification of each variant at the three loci, described by the two independent studies. Miura *et al.*[18] reported linkage relationships among the loci coding PGM, IDH (isocitrate dehydrogenase, EC 1.1.1.42), and GDH. Our analysis, however, were not able to demonstrate such association between loci coding PGM and GDH. In *Undaria pinnatifida,* no variant was observed at PGI locus in electrophoretic mobilities (see Fig. 1D) in the 22 specimens examined. No activity of enzyme was detected on PGM, GDH, MDH (malate dehydrogenase, EC 1.1.1.37), and ES (esterase, EC 3.1.1.1) in this species. In *P. yezoensis,* significant geographical differences were observed in allele frequency distributions at the three loci between the two localities sampled (see Table 2).

Thermostability Variants

Fig. 1 presents the zymograms, demonstrating thermostability variants as prepared after the procedures of heat-treatment of gel as described before. In *P. yezoensis,* two variants in PGM, namely R (slot nos. 1, 4, 6, and 7) and S (slot nos. 2, 3, and 5) were observed. In PGI, three variants, R (slot nos. 2 and 4), S′ (slot nos. 5 and 6), and S (slot nos. 1 and 3) were observed. In GDH, four variants, R (slot no. 2), R′ (slot no. 1), S′ (slot nos. 3 and 4), and S (slot no. 5) were observed. In *U. pinnatifida,* similar thermostability variants in PGI were observed. In *P. yezoensis,* results of statistical analyses indicated the association between electrophoretic variants and thermostability variants of PGI as well as GHD, suggesting the similar relationships observed in the pacific abalone.[12] Due to scantiness of data, however, no such association was observed in PGM.

Frequencies of alleles coding thermostability variants in *P. yezoensis* are summarized by locus in Table 3. To examine probable associations

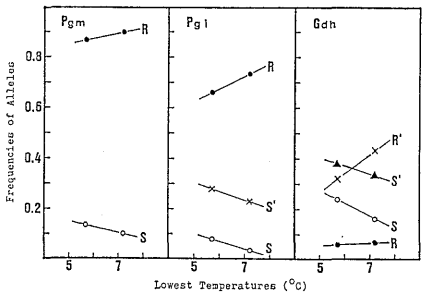

Fig. 2. Correlations between the annual lowest temperatures and frequencies of alleles coding thermostability variations of PGM, PGI, and GDH in *Porphyra yezoensis.*

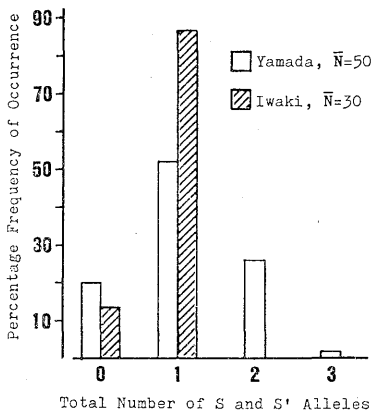

Fig. 3. Geographical comparison of frequency distributions of total number of *S* and *S′* alleles, at the three loci in *P. yezoensis* specimens sampled from the two localities.

between frequencies of thermostability alleles and water temperatures in habitat, temperature data were obtained from monthly records made by the prefectural fisheries experimental stations nearby the locations sampled (see Table 4).[19] Generally the lowest and the highest monthly-average temperature appeared in February/March and August/September respectively in the locations. Associations between frequencies of thermo-sensitive alleles and the lowest monthly average temperatures were seen more distinctly than those between frequencies of thermo-resistant alleles and the highest temperatures, commonly at the three loci, suggesting probable temperature tolerance among such variants similarly to those in the pacific abalone proposed before[12,14] (see Fig. 2).

In the foregoing paragraph, analysis on temperature association of thermostability variations was made by locus separately. A total of three alleles coding thermostability variants reside at the three loci (because thallus of *P. yezoensis* is in the haploid state in its life cycle) in each specimen. Then numbers of thermo-sensitive (S and S′) alleles, that appeared to be associated with the lowest temperatures in Fig. 2, among the possible three alleles can be counted, permitting comparison of frequency distribution of such numbers of thermo-sensitive alleles between the two localities as shown in Fig. 3. In Yamada, specimens which have two or more thermo-sensitive allels (S and S′) occurred, while no such specimen was observed in Iwaki. Thus, higher frequencies of occurrence of thermo-sensitive alleles in the specimens collected from the locality of lower water temperature (Yamada) are obvious (see Fig. 3). The result of these observations suggested strongly that the three loci coding thermostability variations act as a polygenic system but with varying degrees of contributions to temperature tolerance of intact organisms. In 22 specimens of *U. pinnatifida* sampled from Sanriku, frequencies of PGI thermo-resistant (R) and thermo-sensitive (S) alleles were 0.295 and 0.705 respectively. No further analysis was conducted in this species.

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