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Polymorphism of Catalase Isozyme in *Porphyra*

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Summary

The wild and cultured populations of *Porphyra yezoensis* were analyzed by catalase isozyme. The wild populations were classified into three groups; the first group was the populations possessing only Cat^A gene, the second group the populations possessing only Cat^B gene, and the last group the populations possessing both Cat^A and Cat^B genes. It suggests that the population of *Porphyra* differentiates genetically to local populations.

Although the wild populations showing both Cat^A and Cat^B genes, the cultured populations of *P. yezoensis* and its variety, *P. yezoensis f. narawaensis*, showed almost all Cat^A gene, suggesting that the cultured populations of *Porphyra* had been selected for the Cat^A gene.

The thalli possessing Cat^A gene were more wider in width and smooth or undulate on the margins, and the thalli possessing Cat^B gene were narrower in width and warped or curled on the margins. From the above facts, it is suggestible that there is the relation between catalase genotype and morphology in *P. yezoensis*. The mean weight of the thallus with Cat^A gene was more heavier than that with Cat^B gene.

Development of the culture of "Nori", species of *Porphyra* can be expected to continue in coastal area through Japan. There also exist many species and races of *Porphyra* in suitable coastal areas through Japan. Perhaps, they have the great array of adaptive genotype in the population, the carriers of which are tolerably well adapted to survive and to reproduce in the range of environments which the species or races encounter in its habitats. Since there is a great potential for genetic improvement of stocks for culture, it is necessary to analyze the adaptive genotype of the populations in *Porphyra*. For survey of such an adaptive genotype, the use of allelic isozymes would be especially advantageous in the analysis of relationship between genotype and morphological characters. Genetic variations of isozymes have been reported in higher plants such as rice (1–3), wheat (4, 5), and maize (6), but not in seaweeds such as *Porphyra*, because it was very difficult to obtain the clear soluble supernatants from thallus of *Porphyra* species. In the previous paper (7), the isozymes of *Porphyra* species were detected and the genetic variation of catalase isozymes was found in the cultured populations of *P. yezoensis*.

In the present work, the comparison of wild population with cultured population in *P. yezoensis* was done by gene frequency of catalase isozyme, and the relation between genotype and morphology of thallus was discussed.

Materials and Methods

Wild thalli of *P. yezoensis* were collected at Matsumae in Hokkaido, at 7 localities in Miyagi and at Haragama in Fukushima Prefecture. Seven localities in Miyagi were shown in Fig. 1. Cultured thalli of *P. yezoensis* and *P. yezoensis f. narawaensis* were obtained from Prefectural Fishery Experimental Stations of Miyagi, Chiba, Aichi, Mie, Okayama, Fukushima, Saga, and Nagasaki. *P. yezoensis f. narawaensis* is a variety of *P. yezoensis* and cultured race.

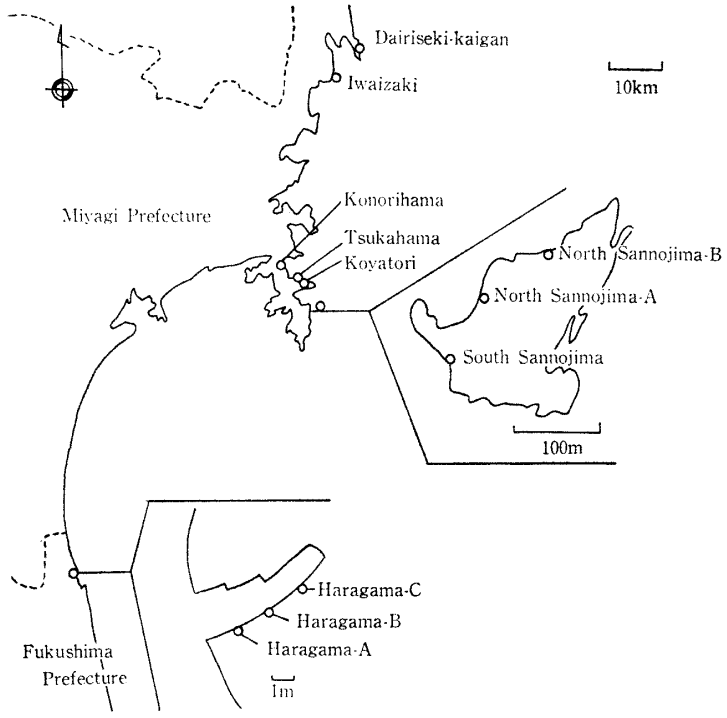

FIG. 1. Showing the localities of the samples collected in Miyagi and Fukushima Prefectrue.

The thalli weighed more than 50 mg were used for the extraction. The extracts were prepared by the procedure reported previously (7). Namely, the thallus was grinded with four volumes of distilled water in a glass homogenizer, placed at −20°C for 6–16 hours, and then thawed. The clear supernatants were obtained by centrifuging at 3,500 rpm for 10 min. Horizontal starch gel electrophoresis was carried out with 11% starch (Connaught Laboratories, Toront, Canada) in 15.5 m*M* Tris and 4.3 m*M* citrate (pH 7.0) by imposition of 200 V for 5 hours at 4°C. The catalase isozyme was detected by the following procedure. The staining solutions were prepared; Solution A was composed of 5 ml of 3% H_2O_2

solution, 10 ml of 0.1 *M* phosphate buffer (pH 7.0), 7 ml of 0.06 *M* $Na_2S_2O_3$ and 78 ml of distilled water, and Solution B was 50 ml of 0.09 *M* KI and 50 ml of distilled water. The gel was placed in solution A at room temperature for 15 min. The solution A porued off and the gel was rinsed with water. Then the solution B was added and let stand until the gel was dark blue with white catalase bands.

Results

1) *Gene frequency of catalase isozyme in the wild and cultured populations*

Catalase isozyme showed only a single band in each thallus and two different bands were observed in mobility. The slower anodal band was always designated as "A" and the other band as "B", as shown in Fig. 2. Since it is well known that the thallus of *Porphyra* is haploid chromosomally, the genotype of catalase isozyme could be directly identified with phenotype. Thus, two bands, A and B were coded by Cat^A and Cat^B gene located in catalase locus, respectively.

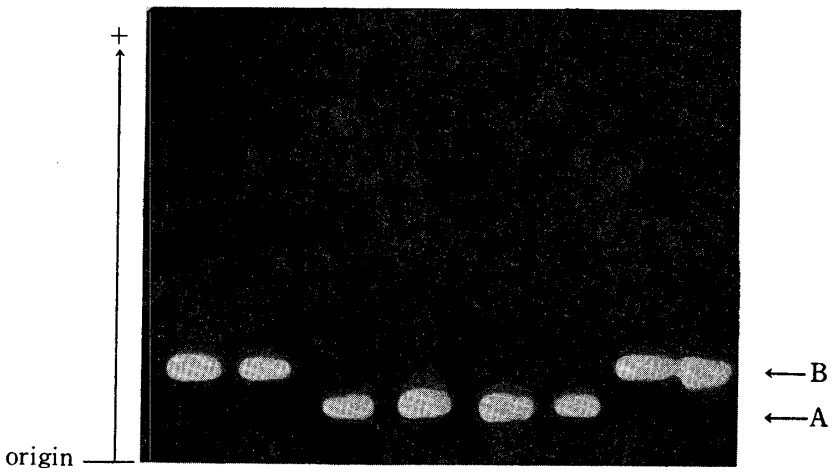

FIG. 2. Catalase isozyme detected in *Porphyra yezoensis*.

The gene frequencies of catalase isozyme were calculated in 12 wild populations of *P. yezoensis*. As shown in Table 1, the wild populations of *P. yezoensis* showed from 0 to 1.00 in gene frequency of Cat^A. The populations at Dairiseki-kaigan and North Sannojima-A possessed only Cat^A gene and the population at Iwaizaki possessed only Cat^B gene. The remained 9 populations possessed both Cat^A and Cat^B genes. Geographical cline from nothern to southern locality was not observed in Cat^A gene frequency, but the different gene frequencies were observed among local populations. The gene frequency showed the same value in different points within a locality such as North Sannojima and Haragama. It suggests that the population of *Porphyra* differentiates genetically to local populations. In Koyatori population, the frequency of Cat^A was 0.5 at January and 0.9 at March and in Haragama, it was 0.25 at the middle of March and 1.00 at the end of March (Table 2). It suggested that the

TABLE 1. *Gene frequency of Catalase isozyme in the Wild populations of P. yezoensis.*

Locality	Date of sampling	No. of thalli tested	Phenotype		Gene Frequency	
			A	B	*qA*	*qB*
Hokkaido (Matsumae)	Mar., '78	33	19	14	0.58	0.42
Miyagi (Dairiseki-kaigan)	Jan., '78	49	49	0	1.00	0
Miyagi (Iwaizaki)	Jan., '78	21	0	21	0	1.00
Miyagi (North Sannojima-A)	Feb., '78	27	27	0	1.00	0
Miyagi (North Sannojima-B)	Feb., '78	35	34	1	0.97	0.03
Miyagi (South Sannojima)	Feb., '78	75	48	27	0.64	0.36
Miyagi (Konorihama)	Jan., '78	24	17	7	0.71	0.29
Miyagi (Tsukahama)	Jan., '78	22	3	19	0.14	0.86
Miyagi (Koyatori)	Jan., '78	146	75	71	0.51	0.49
Fukushima (Haragama-A)	Mar., '78	101	25	76	0.25	0.75
Fukushima (Haragama-B)	Mar., '78	114	26	88	0.23	0.77
Fukushima (Haragama-C)	Mar., '78	102	14	88	0.14	0.86

Large letter in parenthesis presents different point within one locality.

TABLE 2. *Change of Gene frequency in Time.*

Locality	Date of sampling	No. of thalli tested	Phenotype		Gene Frequency	
			A	B	*qA*	*qB*
Miyagi (Koyatori)	15th, Jan.	146	75	71	0.51	0.49
Miyagi (Koyatori)	3rd, Mar.	100	94	6	0.94	0.06
Miyagi (Koyatori)	31st, Mar.	92	83	9	0.90	0.10
Fukushima (Haragama)	15th, Mar.	101	25	76	0.25	0.75
Fukushima (Haragama)	30th, Mar.	49	49	0	1.00	0

gene frequency might be changeable by time, though the cause was unknown.

The gene frequencies in cultured populations of *P. yezoensis* and *P. yezoensis f. narawaensis* were shown in Table 3. The cultured populations possessed only Cat^A gene or almost all Cat^A gene except Ariake-B population in which there was observed more Cat^B than Cat^A gene. The Ariake-B population was wild origin and the mother thalli were taken from wild population. So, the population could be classified into the wild population. Comparison of cultured populations with wild populations suggests that cultured populations of *Porphyra* have been selected for the Cat^A gene.

2. *Relation between catalase genotype and morphological characters*

The attempt was done to find the relation between catalase genotype and morphology of thallus in the wild population, in which a segregation of catalase gene was observed. In the populations, the different thalli were observed in morphology as shown in Fig. 3. One of them, designated as type I, was wider in width and the margin was smooth or undulate. The other designated as type II was

TABLE 3. *Gene frequency of Catalase isozyme in the Cultured populations of P. yezoensis and P. yezoensis f. narawaensis.*

Species	Locality	Date of sampling	No. of thalli tested	Phenotype		Gene Frequency	
				A	B	*qA*	*qB*
P. yezoensis	Mie (Shiroko)	Nov., '76	48	45	3	0.94	0.06
	Mie (Momotori)	Feb., '77	19	19	0	1.00	0
	Saga (Ariake-A)	Feb., '77	107	29	3	0.91	0.09
	Saga (Ariake-B)	Feb., '77	32	8	25	0.24	0.76
	Saga (Ariake-C)	Feb., '77	107	103	4	0.96	0.04
P. yezoensis f. narawaensis	Chiba (Ushigome)	Jan., '77	12	12	0	1.00	0
	Chiba (Aohori)	Jan., '77	20	20	0	1.00	0
	Chiba (Futtsu-A)	Jan., '77	22	21	1	0.96	0.04
	Chiba (Futtsu-B)	Mar., '78	38	38	0	1.00	0
	Aichi (Oshima-A)	Feb., '77	28	26	2	0.93	0.07
	Aichi (Oshima-B)	Feb., '77	23	23	0	1.00	0
	Fukuoka(Yanagawa)	Feb., '77	20	20	0	1.00	0
	Saga (Ariake-A)	Feb., '77	10	10	0	1.00	0
	Saga (Ariake-B)	Feb., '77	16	16	0	1.00	0
	Saga (Ariake-C)	Feb., '77	30	28	2	0.93	0.07

narrower in width and the margin was warped or curled. However, some of the thalli were difficult to classify into the type I or II for an intermediate between them. They were designated as "Unclassified".

In four populations in which a segregation of Cat^A and Cat^B genes was observed, a majority of thalli possessed Cat^A gene showed type I and those possessed Cat^B gene showed type II (Table 4). From the fact that all thalli of cultured populations showed wider in width and undulate on the margin, the cultured thalli could be classified into type II.

Since it was supposed that the thalli of type I were heavier than those of type II, the mean weight of thalli was calculated in the classification of catalase genotype. The result of this attempt is shown in Table 5. Three populations were used. They were collected from North Sannojima and Koyatori in Miyagi Prefecture and Haragama in Fukushima Prefecture. The thalli possessed Cat^A gene were heavier in mean weight than that of the thalli possessed Cat^B gene. That is, it seems to mean that the thalli possessed Cat^A gene can grow larger than those possessed Cat^B gene.

Discussion

Differentiation of *P. yezoensis* population suggests that the population structure of *Porphyra* species has a remarkable tendency to split into a number of local populations. Such a local population may have a structure capable of being influenced by the environment which it encounters in its habitats. Such circumstances are considered responsible for the relation between genotype and morpho-

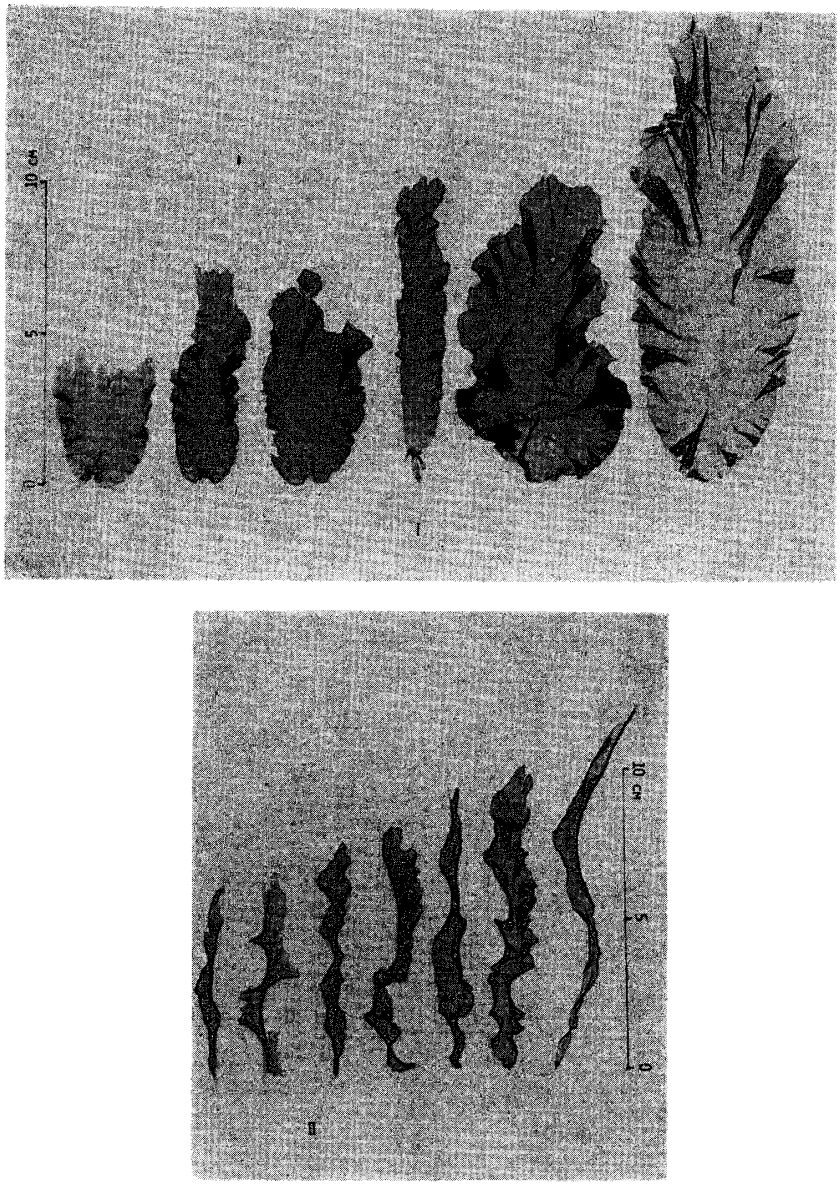

FIG. 3. Thalli designated as type I and type II.

logy of thallus. The existence of two forms, laciniate and undulate on the margin of *Porphyra* thallus is referred to morphological view points (8–10). The laciniate form was found on the rock in the open coast in which the water current was swift and the waves rough. On the other hand, the undulate form was found in the place that the water movement is slow or lower water level. This tendency was observed in this work, too. Type I and type II defined in the present work may be correspond to the undulate and laciniate form, respectively.

It is claimed that the cell number of uniseriate germlings of each *Porphyra* species limits the range of variation in the form of succeeding plants; as the cell number is smaller, the plants become wider in width. Therefore, the cell number may be decided by hereditary factors (11–13).

TABLE 4. *Genotype of Catalase and the Morphology of Thalli of P. yezoensis.*

Locality	No. of thalli tested	Genotype							
		Cat^A				Cat^B			
		Type of thallus			Total	Type of thallus			Total
		I	"Unclassified"	II		I	"Unclassified"	II	
Miyagi (South Sannojima)	75	30 (63)*	13 (27)	5 (10)	48	6 (22)	5 (19)	16 (59)	27
Miyagi (Koyatori)	146	61 (82)	7 (9)	7 (9)	75	6 (8)	8 (11)	57 (81)	71
Fukushima (Haragama-A)	101	14 (56)	7 (16)	4 (28)	25	5 (7)	6 (8)	65 (85)	76
Fukushima (Haragama-B)	114	15 (58)	5 (19)	6 (23)	26	7 (8)	2 (2)	79 (90)	88
Miyagi (Dairiseki-kaigan)	49	49 (100)	0	0	49	0	0	0	0
Miyagi (North Sannojima-A)	27	27 (100)	0	0	27	0	0	0	0
Miyagi (North Sannojima-B)	35	34 (100)	0	0	34	0	0	1 (100)	1
Miyagi (Koyatori-A)	100	94 (100)	0	0	94	0	0	6 (100)	6
Miyagi (Koyatori-B)	92	83 (100)	0	0	83	0	0	9 (100)	9
Miyagi (Iwaizaki)	21	0	0	0	0	0	0	21 (100)	21
Miyagi (Tsukahama)	22	3 (100)	0	0	3	0	0	19 (100)	19

* Number in parenthesis presents precentage in catalase genotype.

TABLE 5. *Catalase genotype and Mean weight of the Thalli in P. yezoensis.*

Locality	Genotype	
	Cat^A	Cat^B
Miyagi (South Sannojima)	80±5mg (48)*	65±4mg (27)
Miyagi (Koyatori)	107±7 (75)	84±8 (71)
Fukushima (Haragma-A)	102±12 (25)	72±6 (76)
Fukushima (Haragma-B)	116±12 (26)	79±6 (88)

* Number in parenthesis presents number of thalli tested.

Concerning to the relation between catalase gene and morphology, two interpretations can be referred; one is linkage between the genes decided catalase isozyme and morphology and the other is pleiotropic effect of catalase gene against the morphology. If the relation between catalase gene and morphology results from linkage, the reverse combination would be found. Reverse combination was not observed in the present work. Thus, the pleiotropic effect of catalase gene might be accounted for the relation.

In the connection, Mathan *et al.* (14) studied on the gene *La* affecting leaf shape in the tomato and reported that the homozygous lanceolate (*La*/*La*) produced higher activity of the oxidative enzyme, tyrosinase, laccase, peroxidase and catalase than the normal leaf (La^+/La^+). They said that an increased activity of the enzymes in the homozygous lanceolate was likely to cause changes in metabolism, which, in turn, may result changes in growth and development. In the light of their work, it may be able to speculate that catalase gene may play a regulative role for characteristic metabolism and then may lead to change in growth and development. But for the support of it, further study will be needed.

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