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Tohoku Journal of Agricultural Research
Vol. 38 No. 1-4, March 1988
Printed in Japan

Genetic Differences between Natural and Cultured Populations of *Porphyra yezoensis*

Patricia Liliana GIL KODAKA, Motoyuki HARA*,
Kazuo AKIYAMA*, and Yoshihisa FUJIO

Deparment of Fishery Science, Faculty of Agriculture, Tohoku University, Sendai 980, Japan.
**Tohoku Regional Fisheries Research Laboratory, Shiogama 985, Japan.*

(Received, August 3. 1987)

Summary

To characterize genetically, both natural and cultured populations of the haploid laver *Porphyra yezoensis*, eleven enzyme loci were analyzed by starch electrophoresis. Allelic frequencies were calculated for 1 location of a natural population and for 2 locations of cultured populations.

Proportion of polymorphic loci was 0.788 on average for natural population and 0.029 for cultured population. Heterozygosity over 11 loci was 0.239 on average for natural population and 0.006 for cultured population. Low levels of genetic variability in cultured populations can be explained by their system of breeding.

Only one allelic combination at a linkage group of *Aat-Cat-Dia-Gpi Mpi-6Pgd-G6pd* loci was dominant in cultured populations and the allelic combination was one of the observed combinations in the natural population.

There are many kinds of seaweeds that are utilized as food in Japan. Among them, *Porphyra* (nori) is the pioneering species. They were originally natural populations occuring in the nori cultivation grounds. Recently, the *Porphyra* cultivators have selected one useful line from *Porphyra yezoensis* populations by means of cultured conchocelis-filaments and artificial seeding techniques. This species has been adapted to warm and cold water temperature, and higher and lower water salinity throughout its whole life cycle. The cultivation of *Porphyra yezoensis* ("susabi-nori" or "narawasusabi-nori") have been selected in many cultivation grounds. On the other hand, natural *Porphyra tenera* distributed widely in waters of lower salinity around the estuaries within bays and inland seas under the influenece of both cold and warm currents. (1)

Fujio *et al.* (2) demonstrated enzyme polymorphism in haploid laver of natural *Porphyra yezoensis* and high levels of genetic differentiation and genetic variability. Fujio *et al.* (3) examined the natural *Porphyra yezoensis* in 1 loca-

tion and demonstrated that the population structure had a remarkable tendency to consist of a number of patches and that the observed number of allelic combination at one linkage group of 7 loci was significantly lesser than the expected number.

The purpose of the present work is to estimate the amount of genetic variability in natural and cultured populations of *Porphyra yezoensis* and to characterize both populations in distribution of allelic combinations at seven linked group

Materials and Methods

Natural and cultured populations of *Porphyra yezoensis*, were collected from 3 locations in Miyagi Prefecture of Japan. Natural thalli were collected in 3 sites from Tomarihama in February 1985; geographic distances between sites varied from 3 to 200 m. Cultured thalli were collected at 11 sites from Shichigahama in February 1986 and 3 sites from Matsushima in December 1986 and March 1987; geographic distances among sites in the location varied from 3 to 500 m.

Seaweed thalli were sandwiched in filter papers to remove free water, then wrapped and stored at $-80°C$ for electrophoretic analysis. Each thallus was weighted and grounded with a little quartz sand in 2 volumes of 0.5 M sucrose solution using a glass homogenizer. Homogenates were frozen for a day, and then thawed and centrifuged at 3,000 rpm for 25 minutes. The supernatants were analyzed by horizontal starch gel electrophoresis for 4 hours under a constant voltage of 300 V. The electrophoresis and procedures were described by Fujio (4).

Eleven enzymes were scored to detect genetic variation. Nomenclature of alleles was identical to the Tomarihama collection used by Fujio *et al.* (3). Allele frequency was calculated directly from the phenotype frequency in each population. Heterozygosity was calculated as $H=1-\sum p_i^2$, where p_i is the frequency of the ith allele at a locus; this value is the expected heterozygosity obtained when 2 haploid gametes pair at random. A locus was considered polymorphic, if the frequency of the most allele was no greater than 0.950.

Results and Discussion

A total of 11 enzyme loci controlling 11 enzymes were detected by electrophoresis. The eleven enzymes were: Aspartate aminotransferase (AAT), Catalase (CAT), Diaphorase (DIA), Glutamate dehydrogenase (GDH), Glucosephosphate isomerase (GPI), Glucose 6 phosphate (G6PD), Malate dehydrogenase (MDH), Mannosephosphate isomerase (MPI), Phosphoglucomutase (PGM), 6-Phosphogluconate dehydrogenase (6PGD) and Superoxide dismutase (SOD). All of them exhibit the activities in the anodal region and showed only a single band, indicating that the thallus of *Porphyra* is haploid. Nine of 11 enzyme loci were

polymorphic in at least 1 of natural population. The remaining 2 enzyme loci (*Pgm* and *Sod*) were monomorphic in all samples.

Table 1 shows allele frequencies at the 11 loci examined in 3 collection sites from Tomarihama, 11 collection sites from Shichigahama, and 3 collection sites from Matsushima. *Gdh*, *Mdh* and *6Pgd* indicated the presence of 2 alleles; *Aat*, *Cat*, *Dia*, *Gpi*, *G6pd* and *Mpi*, the presence of 3 alleles. *A* allele at the *Gpi*, *G6pd*, *Mdh* and *Pgm* was not observed in the present work, and both *A* and *B* alleles at the *Mpi* were also not observed. This can be explained by the difference of collection sites which form clones, and a particular allele can be fixed or disappear in each site.

Proportion of polymorphic loci in natural populations collected at Tomarihama varied from 0.727 to 0.817 with a mean of 0.788, and average of heterozygosity varied from 0.198 to 0.274 with a mean of 0.239. The genetic variability of the present natural population was the same level as the result ($P=0.583$, $H=0.197$) of Fujio *et al.* (3). On the other hand, in cultured populations the proportion of polymorphic loci varied from 0.000 to 0.058 with mean of 0.029, and average of heterozygosity varied from 0.001 to 0.010 with a mean of 0.006. It indicates that genetic variability of cultured population is significantly lower than natural population of *Porphyra yezoensis*. Furthermore, in cultured populations, nine of the 14 site collections were monomorphic at all loci, the remaining 5 sites showing variation at 2 to 4 loci with a low frequency. Variation in the 4 collection sites may be explained by a contamination of conchospores from natural or cultured populations of *Porphyra yezoensis*. Since we cannot find out that cultured collections were monomorphic at other alleles, we supposed a contamination from natural population. If the supposed contamination from a natural population is true, we can say that cultured population is generally fixed. The absence of genetic variation in cultured population can be explained by their source of cultured conchocelis-filaments. Since the cultured conchocelis-filaments originated from a selected thallus, the cultivation of selected population have become popular. Indeed, some nori cultivators of the Narawa Fishermen's Cooperative Association in Chiba Prefecture first tried out the isolation and breeding of large and vigorous fronds from a *Porphyra yezoensis* population in 1969. Repeated selective breedings have been carried out by them, year after year, resulting in the establishment of the present algal form, "narawasusabi-nori" as a cultivar (6).

Strong linkage disequilibrium in natural population indicates linkage groups at the *Aat-Cat-Dia-Gpi-Mpi-6Pgd-G6pd* loci (5). Based on linkage group, the allelic combination at the 7 loci were counted. The results are given in Table 2. The observed number of allelic combinations varied from 4 to 18 in natural population, while it varied from 1 to 5 in cultured populations. The allelic combination Aat^B-Cat^B-Dia^B-Gpi^C-Mpi^C-$6Pgd^A$-$G6pd^C$ was not only observed in

TABLE 1. *Allele frequency al 11 enzyme loci in natural*

		Natural Populations						
		Collection sites in Tomarihama			Collection			
Locus	Allele	1	2	3	1	2	3	4
Aat	N	32	74	58	125	60	55	50
	A	0.312	0.216	0.207	0	0	0	0
	B	0.688	0.757	0.793	1.000	1.000	1.000	1.000
	C	0	0.027	0	0	0	0	0
Cat	N	35	94	69	120	60	55	50
	A	0.257	0.191	0.290	0	0	0	0
	B	0.743	0.809	0.681	1.000	1.000	1.000	1.000
	C	0	0	0.029	0	0	0	0
Dia	N	20	85	43	105	35	40	35
	A	0	0.059	0.186	0	0	0	0
	B	0.950	0.906	0.814	1.000	1.000	1.000	1.000
	C	0.050	0.035	0	0	0	0	0
Gdh	N	20	90	48	75	40	40	55
	A	0.750	1.000	0.938	1.000	1.000	1.000	1.000
	B	0.250	0	0.062	0	0	0	0
Gpi	N	45	95	74	120	60	55	55
	B	0	0.042	0.203	0	0	0	0
	C	0.689	0.768	0.794	1.000	1.000	1.000	1.000
	D	0.311	0.190	0.203	0	0	0	0
G6pd	N	35	95	73	120	60	55	55
	B	0	0.042	0	0	0	0	0
	C	0.829	0.884	0.863	1.000	1.000	1.000	1.000
	D	0.171	0.074	0.137	0	0	0	0
Mdh	N	35	69	28	110	45	45	40
	B	0.800	0.826	0.929	1.000	1.000	1.000	1.000
	C	0.200	0.174	0.071	0	0	0	0
Mpi	N	31	84	38	93	45	40	40
	C	0.742	0.810	0.763	1.000	1.000	1.000	1.000
	D	0.258	0.179	0.237	0	0	0	0
	E	0	0.011	0	0	0	0	0
Pgm	N	5	24	28	105	35	30	40
	B	1.000	1.000	1.000	1.000	1.000	1.000	1.000
6Pgd	N	35	95	73	120	60	50	40
	A	0.800	0.926	0.808	1.000	1.000	1.000	1.000
	B	0.200	0.074	0.192	0	0	0	0
Sod	N	30	30	30	20	20	15	15
	A	1.000	1.000	1.000	1.000	1.000	1.000	1.000
P		0.818	0.727	0.818	0	0	0	0
H		0.274	0.198	0.246	0	0	0	0

N : Number of thalli tested, P : Proportion of polymorphic loci,

and cultured populations of Porphyra yezoensis

Cultured Populations									
sites in Shichigahama							Collection sites in Matsushima		
5	6	7	8	9	10	11	1	2	3
45	80	80	28	37	68	48	50	80	80
0	0	0	0	0	0	0	0	0	0
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0	0	0	0	0	0	0	0	0	0
55	88	108	58	90	128	43	80	80	80
0	0	0.019	0	0.022	0.086	0	0	0	0
1.000	1.000	0.981	1.000	0.978	0.914	1.000	1.000	1.000	1.000
0	0	0	0	0	0	0	0	0	0
40	100	95	33	68	88	30	50	80	80
0	0	0	0	0	0	0	0	0	0
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0	0	0	0	0	0	0	0	0	0
45	65	70	13	23	53	8	80	80	72
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0	0	0	0	0	0	0	0	0	0
60	110	115	63	107	125	43	80	80	80
0	0	0	0	0	0	0	0	0	0
1.000	0.936	0.904	1.000	0.925	0.896	1.000	1.000	0.988	1.000
0	0.064	0.096	0	0.075	0.104	0	0	0.012	0
60	110	110	48	99	118	43	80	80	80
0	0	0	0	0	0	0	0	0	0
1.000	1.000	1.000	1.000	1.000	0.966	1.000	1.000	1.000	1.000
0	0	0	0	0	0.034	0	0	0	0
50	70	85	33	39	108	38	80	80	80
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0	0	0	0	0	0	0	0	0	0
60	81	98	53	88	109	49	80	80	78
1.000	0.963	0.898	1.000	0.989	0.899	1.000	1.000	0.988	1.000
0	0.037	0.102	0	0.011	0.101	0	0	0.012	0
0	0	0	0	0	0	0	0	0	0
50	60	65	8	13	8	8	80	80	77
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
60	95	90	58	79	115	43	80	80	80
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0	0	0	0	0	0	0	0	0	0
15	30	30	15	30	30	15	30	30	30
1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0	0.091	0.182	0	0.091	0.273	0	0	0	0
0	0.017	0.021	0	0.019	0.054	0	0	0.004	0

H : Expected heterozygosity.

TABLE 2. *Distribution of allelic combinations in natural*

Allelic combinations							Natural population						
							Collection sites in Tomarihama			Collection			
Aat	*Cat*	*Dia*	*Gpi*	*Mpi*	*6Pgd*	*G6pd*	1	2	3	1	2	3	4
B	*B*	*B*	*C*	*C*	*A*	*C*	0.799	0.593	0.104	1.000	1.000	1.000	1.000
B	*B*	*B*	*D*	*C*	*A*	*C*	0	0	0	0	0	0	0
B	*B*	*B*	*D*	*D*	*A*	*C*	0	0	0	0	0	0	0
B	*A*	*B*	*D*	*D*	*A*	*C*	0	0	0	0	0	0	0
B	*B*	*B*	*C*	*D*	*A*	*C*	0	0.051	0	0	0	0	0
B	*B*	*B*	*D*	*D*	*A*	*D*	0	0	0	0	0	0	0
B	*A*	*B*	*C*	*C*	*A*	*C*	0	0	0.139	0	0	0	0
A	*A*	*B*	*D*	*D*	*A*	*C*	0.067	0.033	0	0	0	0	0
A	*A*	*B*	*D*	*D*	*B*	*C*	0.067	0	0.104	0	0	0	0
B	*B*	*C*	*C*	*C*	*A*	*C*	0.067	0	0	0	0	0	0
A	*A*	*B*	*D*	*D*	*B*	*D*	0	0.017	0	0	0	0	0
C	*B*	*B*	*C*	*C*	*A*	*C*	0	0.017	0	0	0	0	0
A	*A*	*A*	*D*	*D*	*B*	*D*	0	0.051	0	0	0	0	0
B	*B*	*B*	*B*	*C*	*A*	*D*	0	0.017	0	0	0	0	0
B	*B*	*C*	*C*	*D*	*A*	*C*	0	0.017	0	0	0	0	0
A	*A*	*A*	*D*	*C*	*A*	*C*	0	0.017	0	0	0	0	0
C	*A*	*C*	*B*	*D*	*A*	*C*	0	0.017	0	0	0	0	0
B	*B*	*B*	*C*	*C*	*A*	*D*	0	0.017	0.208	0	0	0	0
A	*A*	*B*	*D*	*E*	*A*	*B*	0	0.017	0	0	0	0	0
A	*A*	*B*	*D*	*D*	*A*	*B*	0	0.017	0	0	0	0	0
B	*A*	*B*	*B*	*C*	*A*	*B*	0	0.017	0	0	0	0	0
A	*A*	*B*	*D*	*C*	*A*	*C*	0	0.051	0	0	0	0	0
A	*A*	*B*	*D*	*C*	*A*	*B*	0	0.017	0	0	0	0	0
B	*A*	*B*	*D*	*D*	*B*	*C*	0	0.017	0	0	0	0	0
A	*A*	*A*	*D*	*D*	*A*	*D*	0	0.017	0	0	0	0	0
A	*A*	*B*	*D*	*D*	*A*	*D*	0	0	0.069	0	0	0	0
B	*B*	*A*	*C*	*C*	*A*	*C*	0	0	0.069	0	0	0	0
B	*B*	*A*	*B*	*C*	*A*	*C*	0	0	0.034	0	0	0	0
A	*A*	*B*	*C*	*C*	*B*	*C*	0	0	0.034	0	0	0	0
B	*B*	*B*	*C*	*C*	*B*	*C*	0	0	0.069	0	0	0	0
B	*C*	*B*	*B*	*C*	*B*	*C*	0	0	0.034	0	0	0	0
B	*A*	*A*	*D*	*C*	*B*	*C*	0	0	0.034	0	0	0	0
B	*B*	*B*	*B*	*C*	*A*	*C*	0	0	0.034	0	0	0	0
A	*B*	*B*	*C*	*D*	*A*	*C*	0	0	0.034	0	0	0	0
B	*A*	*B*	*B*	*C*	*A*	*C*	0	0	0.034	0	0	0	0
No. of thalli tested							15	59	29	78	35	35	25

and cultured populations of Porphyra yezoensis

Cultured population									
sites in Shichigahama							Collection sites in Matsushima		
5	6	7	8	9	10	11	1	2	3
1.000	0.932	0.803	1.000	0.879	0.815	1.000	1.000	0.987	1.000
0	0.034	0.033	0	0.091	0	0	0	0	0
0	0.034	0.033	0	0	0	0	0	0.013	0
0	0	0.115	0	0.030	0.111	0	0	0	0
0	0	0	0	0	0.018	0	0	0	0
0	0	0	0	0	0.056	0	0	0	0
0	0	0.016	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
35	58	61	23	33	54	28	47	80	73

all the collection sites of natural populations, but also in all the collection sites of cultured populations. This fixed combination was characterized in cultured populations; this fixation is possibly attributed to the way of seeding, because cultivators choose the best samples of *Porphyra* (good growth and color) to obtain the conchospores to be seeded. The Aat^{B}-Cat^{B}-Dia^{B}-Gpi^{C}-Mpi^{C}-$6Pgd^{A}$-$G6pd^{C}$ has been also observed in nine of the 11 site collections of natural populations reported by Fujio *et al.* (3). In one of the collection sites of natural population, the allelic combination Aat^{B}-Cat^{B}-Dia^{B}-Gpi^{C}-Mpi^{C}-$6Pgd^{A}$-$G6pd^{D}$ was dominant. Two allelic combinations Aat^{B}-Cat^{B}-Dia^{B}-Gpi^{C}-Mpi^{D}-$6Pgd^{A}$-$G6pd^{C}$ and Aat^{B}-Cat^{A}-Dia^{B}-Gpi^{C}-Mpi^{C}-$6Pgd^{A}$-$G6pd^{C}$, which were observed in natural population, were observed with low frequency in cultured populations. The allelic combination Aat^{B}-Cat^{B}-Dia^{B}-Gpi^{D}-Mpi^{C}-$6Pgd^{A}$-$G6pd^{C}$ which was observed only in cultured populations were observed in 1 site collection of natural populations reported by Fujio *et al.* (3).

It is supposed that the thalli having the Aat^{B}-Cat^{B}-Dia^{B}-Gpi^{C}-Mpi^{C}-$6Pgd^{A}$-$G6pd^{C}$ allelic combination grow larger than the thalli having other allelic combinations. In fact, the thalli' mean weights were heavier in the site collections of cultured populations and in two of the 3 site collections of natural population. It is also possible that the aforementioned combination might also be linked with good flavor and color characteristics. Such tendency might be made clear by surveying more sites.

From the present work, it is shown that in the population structure of *Porphyra yezoensis*, selection of conchospores plays a major role in the unification of patterns.

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