Short Paper

Nucleotide Sequence Variation in the Ribosomal Internal Transcribed Spacer Regions of Cultivated (Cultivars) and Field-collected Thalli of *Porphyra yezoensis*

Yuzuru Mizukami,*¹ Hitoshi Kito,*¹ Yoshio Kaminishi,*²
Noboru Murase,*¹ and Masahiko Kunimoto*²

*¹Department of Applied Aquabiology, National Fisheries University, Shimonoseki, Yamaguchi 759-6595, Japan

*²Department of Food Science and Technology, National Fisheries University, Shimonoseki, Yamaguchi 759-6595, Japan

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Many cultivars (cultivated strains) of laver (*Porphyra yezoensis* and *P. tenera*) have been isolated, stocked and used for culture in coastal farms. However, the genetic relationships between these cultivars have not been clarified on the level of nucleotide sequence. In addition, new algal breeding techniques such as artificial crossing¹⁾ and cell fusion^{2,3)} are soon to be tested to produce commercially important laver stocks. Therefore, knowledge of the extent of intraspecific variation of certain genes in *P. yezoensis* and *P. tenera* is necessary to rearrange these cultivars on the basis of genetic polymorphisms. The development of convenient and reliable genetic markers is also important for the discrimination of these cultivars.

We have previously reported the partial nucleotide sequences in the coding regions of nuclear-encoded small subunit ribosomal RNA genes from nine *Porphyra* species and eight laver cultivars and demonstrated that although considerable nucleotide differences were found among the nine species, the nucleotide sequences of the eight cultivars were identical.⁴⁾ These findings suggested that the nucleotide sequences in the coding region of the genus were conserved at the intraspecific level but not at the interspecific level. The internal transcribed spacer (ITS) regions of ribosomal RNA genes are known to evolve faster than the coding regions and, therefore, to vary among subspecific and populations levels within various species.⁵⁻⁷⁾

In the present study, we analyzed ITS 1 region sequences of nuclear-encoded small subunit ribosomal RNA genes in cultivars and field-collected thalli in order to determine the genetic relationship within and among laver cultivars and field-collected thalli, and to search for genetic markers to discriminate of these cultivars. Details of the algal materials, the ribosomal RNA gene nucleotide sequencing methods and DNA sequence alignment have been reported previously.⁴⁾ Table 1 shows the number of nucleotide differences (substitution and insertion/deletion) in the 371 bp ITS 1 regions among 11 cultivars. Although two cultivars exhibited one base difference (deletion), the nucleotide sequences in the ITS 1 regions were, on the whole, very similar among the cultivars. These results suggested that little or no nucleotide variation existed in the rRNA

Table 1. The numbers of different nucleotides (substitution, insertion/deletion) among 11 laver cultivars (cultivated strains of *P. yezoensis*) in the 371 nucleotides of the small subunit ribosomal RNA gene ITS 1 region (pairwise comparison)

	Cultivars (GenBank access No.)	Cultivars										
		1	2	3	4	5	6	7	8	9	10	11
1	Saga-5 (AB019187)	*	1	0	0	0	0	0	0	1	0	0
2	Saga-24 (AB019192)		*	1	1	1	1	1	1	0	1	1
3	Sasiki (AB019188)			*	0	0	0	0	0	1	0	0
4	Obagreen (AB019189)				*	0	0	0	0	1	0	0
5	Narawasusa [†] (AB017084)					*	0	0	0	1	0	0
6	Fukuoka-1 (AB017082)						*	0	0	1	0	0
7	D-18-1 (AB019190)							*	0	1	0	0
8	Ariake-1 (AB019191)								*	1	0	0
9	Shikoku (AB017087)									*	1	1
10	Noma-1 (AB017085)										*	0
11	Saga-103 (AB017086)											*

† Full name, Narawasusabimidorime.

genes among these *P. yezoensis* cultivars, and that nucleotide sequences in the ITS region could not be used as discriminating molecular markers. Whether nucleotide sequences in the ITS 1 region varied at the subspecies, population or individual *P. yezoensis* levels were then examined with field-collected thalli. Table 2 shows the numbers of base changes (substitution and insertion/deletion) in the ITS 1 regions among individual thalli of *P. yezoensis* collected from two different coastal sites (Hakodate-Nanaehama in Hokkaido and Ogatu in Miyagi Pref.). Differences in shapes, colour and microscopic forms could not be distinguished (data not shown). However, the nucleotide se-

Table 2. The numbers of different nucleotides (substitution, insertion/deletion) among 6 individual thalli of field-collected *P. yezoensis* and among cultivated strain (Saga-103) and field-collected *P. yezoensis* in the 371 nucleotides of the small subunit ribosomal RNA gene ITS 1 region (pairwise comparison)

Field-collected thalli (GenBank access No.)		Ogatu thallus			Hakodate thallus		
		1	2	3	1	2	3
Ogatu thallus 1 (AB017078)		*	4	13	14	3	4
thallus 2 (AB017079)			*	12	12	1	1
thallus 3 (AB017080)				*	3	11	12
Hakodate thallus 1 (AB017074)					*	11	12
thallus 2 (AB017075)						*	1
thallus 3 (AB017076)							*
Saga-103 (AB017086)		14	12	4	1	11	12

quences of the 371 bp ITS 1 regions were considerably different from each other among these individual thalli. This result indicates that the nucleotide sequences in the ITS 1 regions vary considerably at the intraspecific level of *P. yezoensis*, and that individuals of field-collected thalli, although they grow together on common coastal rock, are genetically more heterogeneous than that expected from their morphological similarity. The nucleotide sequences of cultivated strains (for example, Saga-103) were similar to those in two field-collected thalli, Hakodate thallus 1 and Ogatu thallus 3, while largely different from those of other field-collected thalli. For red algae, Goff *et al.*⁸⁾

reported that ITS sequences were conserved at the species level in *Gracilaria* and *Gracilariopsis*, contrasting with the results obtained for *P. yezoensis* by us.

From the data presented, we suggest that the cultivars examined are extremely biased towards a defined common sequence and therefore have lower genetic diversity than individual field-collected thalli. These cultivars are likely to have been derived originally from a common individual thallus collected from a coastal site. In order to breed new cultivars with novel properties, it is therefore necessary to use field-collected thalli, together with cultivar thalli, as *P. yezoensis* stock for artificial crossing and protoplast fusions.

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