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PROTEIN HETEROGENEITY OF RED ALGA PALMARIA PALMATA IN THE BARENTS SEA

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Electrophoretic protein patterns in the red alga Palmaria palmata were analyzed from plants collected from three different habitats along the Barents Sea coast. Significant heterogeneity on protein components characterized by distinct molecular weight was observed. Noted differences were related to variates found among plants from settlements unique with regard to wave exposure, distance from each other and separation by natural barriers. Since these dissimilarities were slight in absolute value, the settlements appear to belong to the same population. There are some differences between sporophytes and male gametophytes in areas of extreme wave exposure, but not in semi-exposed habitats. Based on protein fractions, plants from upper and lower parts of littoral belt within one settlement are only slightly distinguishable from each other.

***Key words**: general electrophoretic protein patterns, red alga, different settlements.*

Introduction. Palmaria palmata (L.) Kuntze (=Rhodymenia palmata) is a large and abundant red alga which is widely distributed over boreal and arctic areas of the Atlantic and Arctic oceans. The species is distinguished by possessing significant morphological variability in the North Atlantic. Crosses between plants of Palmaria palmata from different geographical regions suggest that this species is in the process of splitting into several sibling species (Meer, 1987). Palmaria palmata has a peculiar life cycle with macroscopic sporophyte, male gametophyte and microscopic female gametophyte which are considered to be a new type of life cycle amongst red algae (Meer, Chen, 1979; Meer, Todd, 1980). Palmaria palmata is harvested and used for food in some countries (Morgan et al., 1980).

Palmaria palmata is a typical representative of algal vegetation on Murman coast of the Barents Sea. It forms independent associations or occurs as a subdominant within fucoid assemblages on open and semi-exposed coastal areas within the intertidal, where its average biomass is 2-3 kg/m^2.

Our observation in different settlements of Palmaria palmata demonstrated the presence of significant variability of both population parameters (biomass, quantity and correlation between age groups) and species specific parameters (total size and weight of the whole thallus and that of its individual blades). Ranges of variability depend on environmental factors, mainly on extend of exposure to wave action.

In this paper, we present data on heterogeneity of Palmaria palmata based on samples collected in the littoral zone at three locations in the Barents Sea. At each site sporophyte and male gametophyte from upper and lower parts of Palmaria palmata belt were analyzed separately. Electrophoretic techniques are widely used nowadays not only for solution of taxonomic problems (Cheney, Babbel, 1978; Blair, Mathieson, Cheney, 1982; Harnedy, FitzGerald, 2013), but also for estimation of the genetic differences between ecotypes and populations of individual alga species (Innes, 1984, 1987; Feng Liu, Shao Jun Pang, 2010).

Materials and methods. Three settlements of Palmaria palmata from Priboynaya, Dalnezelenetskaya and Yarnyshnaya Inlets, all located within 1-3 km of each other, but with distinct differences in wave exposure were chosen for investigation. The Priboynaya Inlet is an open exposed site on the Barents Sea coast. The level of wave action is defined as I-II (Guryanova, Zax, Ushakov, 1930). There, Palmaria palmata occupied small patches of substrate (1-2 m^2) in rock fissures at mid tide level. In the Dalnezelenetskaya Inlet, Palmaria palmata was collected on Probnyi Cape, a moderately exposed site, where the level of wave action is defined as II-III. There, the species is abundant and occupies vast areas of intertidal zone, predominantly at mid and low tide levels, attached to rocks and fronds of Fucus species. It is associated with several different species assemblages and encompasses a belt about 25 m wide. The Yarnyshnaya Inlet settlement is least exposed of the three sites. The site is situated in the middle part of the inlet in an area defined as wave exposure III-IV. Distribution of Palmaria palmata is similar to that of Dalnezelenetskaya Inlet. However, the steeper inclination of shore slope at Yarnyshnaya Inlet results in Palmaria palmata belt only about 7 m wide. Apical segments of a few sporophytes and male gametophytes (1+ year old) collected from upper and lower zone of its distributional extent in the littoral zone were used for analysis.

Total proteins were extracted as follows. All extractions were carried out at 2^0 C. Thallus samples were washed in distilled water, blotted dry and homogenized with a motor-driven stainless steel knife homogenizer in an extraction buffer. The

extraction buffer contained 50 mol m^{-3} TRIS-HC1 pH 7.6; 100 mol m^{-3} NaCl; 2 mol m^{-3} EDTA; 100 mol m^{-3} L-ascorbic acid-Na salt; 5 mol m^{-3} DTT; 2 kg m^{-3} $CaCl_2$; 100 kg m^{-3} Polyclar AT (treated according to Loomis (1974) and prehydrated overnight with extraction buffer). The extraction buffer to sample ratio was 10 to 1 (v/w). The resulting mixture was expressed through 2 layers of Miracloth, then centrifuged for 10 min at 7000 x g.

Ammonium sulfate was added to the supernatant to 80 % saturation. Insoluble material was recovered by centrifugation for 60 min at 16000 x g. The pellet was resuspended in buffer containing 62.5 mol m^{-3} TRIS-HCl Ph 6.8; 20 kg m^{-3} SDS; 50 kg m^{-3} 2-mercaptoethanol; 100 kg m^{-3} glycerol.

SDS polyacrylamide gel electrophoresis was carried out according to King, Laemmli (1971) (T=12.5 %; C=4.5 %). Calculation of relative molecular weight and area of discrete protein components at the electrophoretic gels were carried out using a DU-8 Beckman spectrophotometer. Lysozyme (29 kD), ovalbumin (46 kD), bovine serum albumin (68 kD) and phosphorylase B (92.5 kD) were used as a molecular weight markers.

Experiments were performed in laboratory of phycology in Murmansk Marine Biological Institute of Russian Academy of Sciences (Dalnie Zelentsy).

Results and discussion. Significant heterogeneity in general protein molecular weight patterns were observed in the red alga Palmaria palmata. There were forty discrete components identified within the range of 15-120 kD (Fig.1). The general e1ectrophoretic protein pattern of Palmaria palmata is characterized by a high level (about 10 % and more) of components with molecular weights less than 40 kD and a large prevalence (about 50 %) of low molecular weight (less than 20 kD) components.

We found no qualitative differences in general electrophoretic protein patterns of seaweeds harvested from the three investigated settlements (the Pribovnaya, the Dalnezelenetskaya and Yarnyshnaya Inlets; Table 1). They differ from each other only in relative amounts of individual components (these differences account for 1-2 % or up to 5 % of total). Gametophytes components from Dalnezelenetskaya Inlet constituted an exception. A component with a molecular weight 115 kD was identified in the gametophytes but was absent in sporophytes of the same settlements and in other variants.

Confirmed differences between plants harvested from the Priboynaya and Dalnezelenetskava Inlets were found only in protein components with molecular weights of 20-30 kD. These two settlements are closest spatially and the gap in the occurence of Palmaria palmata between them is not wide. Habitat and wave exposure differences are not so important and do not produce a substantial dissimilarity among protein fractions between sites. The lack of major differences could also be attributed that in Priboynaya Inlet Palmaria palmata grows in rock

fissures and is protected against direct effect of waves. This also lessens the chance for potential protein differences among sites.

In two other variants – between plants taken from the Dalnezelenetskaya and Yarnyshnaya Inlets and between plants from Priboynaya and the Yarnyshnaya

Table 1

Relative amount (%) of discrete molecular weight protein fractions in Palmaria palmata from different settlements

Molecular weight (kD)	Relative amount of protein (%)		
	Priboynaya Inlet n=6	Dalnezelenetskaya Inlet n=12	Yarnyshnaya Inlet n=12
90	3,02 ± 0,15	3,60 ± 0,56	3,09 ± 0,21
90-80	1,53 ± 0,11	1,76 ± 0,15	2,60 ± 0,28
80-70	1,42 ± 0,13	1,46 ± 0,08	1,62 ± 0,10
70-60	2,30 ± 0,15	2,27 ± 0,24	4,12 ± 0,18
60-50	7,27 ± 0,20	6,81 ± 0,27	4,84 ± 0,25
50-40	4,96 ± 0,28	5,76 ± 0,27	5,28 ± 0,18
40-30	14,12 ± 0,75	12,79 ± 0,56	15,29 ± 0,46
30-20	7,74 ± 0,89	10,07 ± 0,21	10,90 ± 0,21
20	57,64 ± 1,69	55,48 ± 0,66	52,26 ± 0,56

Inlets – quite large differences were found in similar segments of general electrophoretic protein patterns (Table 1). Relative amount is unlike mostly in predominating components that are characterized by high content in p1ants.

Confirmed differences between plants from Dalnezelenetskaya and Yarnyshnaya Inlets were obtained in protein with molecular weights within intervals of 90-80, 70-50, 40-30 and less than 20 kD. Comparison of plants taken from the Priboynaya and Yarnyshnaya Inlets shows the differences in relative amount of protein components within intervals of 90-80, 70-50 and less than 30 kD. These settlements are the most distant from each other and differ significantly in habitat and level of wave action.

There were some variations in protein components with different molecular weight in diverse life history forms of Palmaria palmata (macroscopic sporophytes and male gametophytes from each investigated settlements, Table 2). As noted above, Palmaria palmata has a unique life cycle among the algae. Impregnation of microscopic female gametophytes of one to several months old is effected by spermatia of macroscopic male qametophytes of more than one year old. A perennial macroscopic sporophyte arises from the impregnated female gametophyte and the spores then develop into gametophytes (Meer, Chen, 1979; Meer, Todd, 1980). Due to such life cycle the related sporophytes and male gametophytes belong to different generations and develop under somewhat various conditions in different years.

Differences exist between sporophytes and male gametophytes in the Priboynaya and Dalnezelenetskaya Inlets. These two places are characterized by rather high level of wave action. The plants in these settlements undergo significant elimination during severe storms and frosts. In the Priboynaya Inlet, differences are marked by proteins with molecular weight of less than 50 kD. In the Dalnezelenetskaya Inlet, sporophytes and male gametophytes differ in protein components with molecular weight 50-30 and less than 20 kD, as in Priboynaya Inlet, and possess a high molecular weight components interval (more than 90 kD). In the Yarnyshnaya Inlet, characterized by the lowest level of wave action for Palmaria palmata, confirmed varieties between two macroscopic forms were obtained only in high molecular weight components (more than 90 kD).

Comparative analysis of the relative amount of proteins with various molecular weights was carried out for littoral forms from the upper and lower parts of Palmaria belt. As shown in Table 3 for the Dalnezelenetskaya and Yarnyshnaya Inlets (where Palmaria palmata was occured in various associations between the upper and lower littoral), differences in the relative amount of protein fraction within definite molecular weight are slight (1-2.5 %). In the first inlet, differences in the relative amount of protein fraction within definite molecular weights are noted at interval of 50-40 kD and more than 90 kD; in the second inlet – 60-50 and

Table 2

Relative amount (%) of discrete molecular weight protein fractions in Palmaria palmata sporophytes and male gametophytes

Molecular weight (kD)	Relative amount of protein (%)					
	Priboynaya Inlet		Dalnezelenetskaya Inlet		Yarnyshnaya Inlet	
	sporophytes n=3	male gametophytes n=3	sporophytes n=6	male gametophytes n=6	sporophytes n=6	male gametophytes n=6
90	2,78 ± 0,13	3,25 ± 0,21	2,33 ± 0,41	4,87 ± 0,75	3,59 ± 0,16	2,59 ± 0,20
90-80	1,52 ± 0,20	1,54 ± 0,12	2,03 ± 0,20	1,49 ± 0,17	2,58 ± 0,22	2,62 ± 0,15
80-70	1,23 ± 0,12	1,61 ± 0,18	1,52 ± 0,11	1,39 ± 0,12	1,72 ± 0,15	1,53 ± 0,13
70-60	2,50 ± 0,18	2,10 ± 0,20	2,47 ± 0,29	2,07 ± 0,38	4,20 ± 0,29	4,04 ± 0,23
60-50	6,93 ± 0,20	7,61 ± 0,23	7,10 ± 0,24	6,52 ± 0,47	4,92 ± 0,38	4,75 ± 0,51
50-40	5,46 ± 0,20	4,45 ± 0,29	6,43 ± 0,36	5,10 ± 0,16	4,95 ± 0,27	5,61 ± 0,13
40-30	15,75 ± 0,28	12,50 ± 0,28	14,53 ± 0,14	11,04 ± 0,37	14,75 ± 0,85	15,83 ± 0,22
30-20	9,71 ± 0,25	5,77 ± 0,17	10,14 ± 0,33	10,00 ± 0,28	10,53 ± 0,26	11,27 ± 0,24
20	54,12 ± 0,95	61,17 ± 0,94	53,45 ± 0,45	57,52 ± 0,27	52,76 ± 0,84	51,76 ± 0,75

Table 3

Relative amount (%) of discrete molecular weight protein fractions in Palmaria palmata from lower and upper part of belt

Molecular weight (kD)	Relative amount of protein (%)			
	Dalnezelenetskaya Inlet n=6		Yarnyshnaya Inlet n=6	
	lower part	upper part	lower part	upper part
90	2,34 ± 0,41	4,85 ± 0,76	2,94 ± 0,42	3,24 ± 0,12
90-80	2,11 ± 0,17	1,41 ± 0,14	2,97 ± 0,39	2,23 ± 0,37
80-70	1,48 ± 0,13	1,43 ± 0,11	1,64 ± 0,16	1,61 ± 0,13
70-60	2,13 ± 0,42	2,41 ± 0,26	4,17 ± 0,30	4,07 ± 0,22
60-50	7,09 ± 0,23	6,53 ± 0,48	5,37 ± 0,25	4,32 ± 0,31
50-40	6,24 ± 0,44	5,28 ± 0,22	5,12 ± 0,34	5,44 ± 0,13
40-30	13,15 ± 0,62	12,43 ± 0,96	14,18 ± 0,63	16,41 ± 0,22
30-20	10,02 ± 0,29	10,13 ± 0,32	11,35 ± 0,23	10,44 ± 0,22
20	55,44 ± 0,98	55,53 ± 0,99	52,26 ± 0,91	52,24 ± 0,74

40-20 kD. In Priboynaya Inlet Palmaria palmata does not form a belt and grows only in patches at mid tide level, and therefore such a comparison could not be made.

Conclusion. The conducted investigation is an attempt to use the method of general electrophoretic protein patterns for estimating differences between settlements of red alga Palmaria palmata. The investigations showed the existence of relative dissimilarities in separate protein components in plants from various settlements. More significant varieties were found between plants from settlements that differed in exposure to wave action and separated by natural obstacles, such as capes and inlets with high wave activity. Thus, our data show that exposure to wave action may determine not only morphological variability, but stipulates differentiation of Palmaria palmata settlements as reflected in relative amount of protein fractions of discrete molecular weight. Since these dissimilarities are slight in absolute value, the studied settlements can be considered to belong to the same population. Less significant differences are marked between sporophytes and gametophytes. There are more differences between various life history forms of Palmaria palmata in areas of high wave action that at semi-exposed sites. There are no significant differences between plants collected from upper and lower parts of the littoral belt in the relative amount of protein fractions of definite molecular weight. This study confirms earlier works (Innes, 1984, 1987) concerning the existence of genetic differences between settlements of seaweeds of the same species from various habitats.

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References

1. Blair S.M., Mathieson A.C., Cheney D.P. Morphological and electrophoretic investigations of selected species of Chaetomorpha (Chlorophyta, Cladophorales). // Phycologia. – 1982. – V.21. – P.164-172.

2. Cheney D.P., Babbel G.R. Biosystematic studies of the red algae genus Eucheuma. I. Electrophoretic variation among Florida populations. // Mar. Biol. – 1978. – V.47. – P.251-264.

3. Feng Liu, Shao Jun Pang Stress tolerance and antioxidant enzymatic activities in the metabolisms of the reactive oxygen species in two intertidal red algae Grateloupia turuturu and Palmaria palmata. // J. Exp. Mar. Biol. and Ecol. – 2010. – V.382. – P.82-87.

4. Guryanova E.F., Zax I.G., Ushakov P.V. The intertidal zone of Kola Bay. // Trudy Leningradskogo obshestva estestvoispytateley. – 1930. – V.60(2). – P.17-107(in Russian).

5. Harnedy P.A., FitzGerald R.G. Extraction of protein from macroalga Palmaria palmata. // LWT – Food Science and Technology. – 2013. – V.51. – P.375-382.

6. Innes D.J. Genetic differentiation among populations of marine algae. // Helgolander wiss. Meeresunters. – 1984. – V.38. – P.401-417.

7. Innes D.J. Genetic structure of asexually reproducing Enteromorpha linza (Ulvales: Chlorophyta) in Long Is1and Sound. // Mar. Biol. – 1987. – V.94. – P.459-467.

8. King J., Laemmli U.K. Polypeptides of the tail fibres of bacteriophage T 4. // J. Mol. Biol. – 1971. – V.61. – P.465-477.

9. Loomis W.D. Overcoming problems of phenolics and quinones in the isolation of plant enzymes and organelles. // Methods in Enzymology. – 1974. – V.31. – P.528-544.

10. Marsden W.J.N., EvansL.V., Callow J.A., Keen J.N. A preliminary electrophoretic comparison of Fucus serratus and Fucus vesiculosus. // Bot. Mar. – 1984. – V.27. – P.79-83.

11. Meer J. P. van der. Experimental hybridization Palmaria palmata (Rhodophyta) from north-east and north-west Atlantic Ocean. // Can. J. Bot. – 1987. – V.65. – P.1451-1458.

12. Meer J.P. van der, Chen L.C.-M. Evidence for sexual reproduction in the red algae Palmaria palmata and Halosaccion ramentaceum. // Can. J. Bot. – 1979. – V.57. – P.2452-2459.

13. Meer J.P. van der Todd E.R. The life history of Palmaria palmata in culture. A new type for the Rhodophyta. // Can. J. Bot. – 1980. – V.58. – P.1250-1256.

14. Morgan K.C., Wright J.L.C., Sipson F.J. Review of chemical constituents of the red alga Palmaria palmata (Dulse).// Economic Botany. – 1980. – V.34(1). – P.27-50.

Анотація

Тихонов П.С., Шошина О.В. ***Гетерогенність білків червоної водорості Palmaria palmata у Баренцевому морі.*** *Проведено аналіз електрофоретичних спектрів білків червоної водорості Palmaria palmata, що була зібрана з трьох різних місцезростань на узбережжі Баренцева моря. Спостерігали значну гетерогенність білкових компонентів за молекулярною масою. Зазначені відмінності були пов'язані з варіаціями, що були знайдені серед рослин різних місцезростань на узбережжі, які різнилися за впливом хвиль, відстанню між місцезростаннями та їх поділом природними перешкодами. Оскільки ці відмінності були незначними за абсолютною величиною, можна вважати, що водорості цих місцезростань належать до однієї популяції. Є деякі відмінності між спорофітами та чоловічими гаметофітами в районах з*

екстремальним, але не помірним впливом хвиль. Рослини з верхнього і нижнього поясів літоралі в межах одного місцезростання незначно різнилися за білковими фракціями.

Ключові слова: *загальні електрофоретичні спектри білків, червона водоросты, різні місцезростання.*

Аннотация

Тихонов П.С., Шошина Е.В. ***Гетерогенность белков красной водоросли Palmaria palmata в Баренцевом море.*** *Проведен анализ электрофоретических спектров белков красной водоросли Palmaria palmata, которая была отобрана из трех разных местообитаний на побережье Баренцева моря. Наблюдали значительную гетерогенность белковых компонентов по молекулярной массе. Отмеченные различия были связаны с вариациями, обнаруженными у растений из разных местообитаний побережья, которые различались прибойностью, расстоянием между местообитаниями и разделением их природными барьерами. Поскольку эти различия были незначительными по абсолютной величине, можно полагать, что водоросли этих местообитаний относятся к одной популяции. Существуют некоторые различия между спорофитами и мужскими гаметофитами в районах с экстремальной, но не умеренной прибойностью. Растения из верхнего и нижнего поясов литорали в пределах одного местообитания незначительно различаются по белковым фракциям.*

Ключевые слова: *суммарные электрофоретические спектры белков, красная водоросль, разные местообитания.*