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The use of tissue culture method for the study of lichenized fungi (Lobariaceae Chevall., Parmeliaceae Zenker.) of the European part of Russia

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Summary. Lichenized fungi as a multicomponent biological system are an interesting but difficult research object. In view of the complex interaction of the myco- and photobiont, the slow growth of natural thalli, their exceptional exactingness to environmental factors, the study of the biological characteristics of lichens is significantly difficult. This article discusses the problem of studying the anatomical and morphological structure of the myco- and photobiont of lichenized fungi using the tissue culture method on the example of rare species (*Lobaria pulmonaria*) and species with pharmaceutical potential (*Usnea dasopoga*, *Cetraria islandica*). The authors proposed a method for cultivating of myco- and photobionts of these species on synthetic nutrient media for the purpose of further research on the characteristics of lichenized fungi. Fragments of thalli, apothecia (for *C. islandica*), and soredia (for *U. dasopoga* and *L. pulmonaria*) were used as donor material. For the introduction of the photobiont under sterile conditions, a homogenate of the thallus region was prepared. Pure cultures of the myco- and photobiont of *L. pulmonaria*, *U. dasopoga*, and *C. islandica* were obtained on three types of hormone-free nutrient media, namely MS nutrient medium, modified MS medium with a reduced nitrogen content, Czapek medium. The verification of the research results was carried out taking into account the microscopy of the obtained cultures of myco- and photobionts. Using microscopy, the dimensional characteristics of the obtained mycobionts were determined. The diameters of the hyphae of *L. pulmonaria*, *U. dasopoga*, and *C. islandica* were 4.3–4.4 μm, 4.6 μm, and 4.1–4.3 μm, respectively, which corresponds to the size of the hyphae in natural samples. The use of the tissue culture method makes it possible to study and analyze the biological characteristics of lichenized fungi as a multicomponent biological system, as well as to contribute to the preservation of rare species and their components in the Red Data Book; while optimizing the method of cultivating myco- and photobiont on synthetic nutrient media, to reduce the anthropogenic load on natural populations of lichens when using them as medicinal raw materials.

Использование метода культуры ткани для исследования лихенизированных грибов (Lobariaceae Chevall., Parmeliaceae Zenker.) европейской части России

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Ключевые слова: культура тканей, лихенизированные грибы, микобионт, фотобионт, *Cetraria islandica*, *Lobaria pulmonaria*, *Usnea dasopoga*.

Аннотация. Лихенизированные грибы как многокомпонентная биологическая система представляют собой интересный, но непростой в изучении объект исследования. Ввиду сложного взаимодействия мико- и фотобионта, медленного роста природных талломов, исключительной требовательности их к факторам окружающей среды, изучение биологических особенностей лишайников значительно затруднено. В данной статье рассматривается проблема изучения анатомо-морфологического строения мико- и фотобионта лихенизированных грибов при помощи использования метода культуры тканей на примере редких (*Lobaria pulmonaria* и обладающих фармацевтическим потенциалом (*Usnea dasopoga*, *Cetraria islandica* видов. Авторами предложена методика культивирования мико- и фотобионта данных видов на синтетических питательных средах для целей дальнейшего исследования особенностей лихенизированных грибов. В качестве донорного материала использовали фрагменты талломов, апотеции (для *C. islandica*), соредии (для *U. dasypoga* и *L. pulmonaria*). Для введения фотобионта в стерильных условиях был приготовлен гомогенат участка таллома. Получены чистые культуры мико- и фотобионта *L. pulmonaria*, *U. dasopoga* и *C. islandica* на трех типах безгормональных питательных сред (питательная среда MS, модифицированная среда MS с пониженным содержанием азота, среда Чапека). Верификация результатов исследования произведена с учетом микроскопии полученных культур мико- и фотобионта. При помощи микроскопии определены размерные характеристики полученного микобионта (диаметры гифов *L. pulmonaria*, *U. dasypoga* и *C. islandica* составили 4,3–4,4 мкм, 4,6 мкм и 4,1–4,3 мкм соответственно), что соответствует размерам гифов в природных образцах. Использование метода культуры ткани позволяет исследовать и анализировать биологические особенности лихенизированных грибов как многокомпонентной биологической системы, а также внести вклад в сохранение редких краснокнижных видов и их компонентов, при оптимизации методики культивирования мико- и фотобионта на синтетических питательных средах снизить антропогенную нагрузку на природные популяции лишайников при использовании их в качестве лекарственного сырья.

Introduction

Lichens, or lichenized fungi, are multicomponent symbiotic systems consisting of a heterotrophic fungal component (mycobiont) and a phototrophic component (photobiont), which are green algal, cyanobacteria (Ahmadjian, 1973; Lobakova et al., 2008), as well as other symbiotic bacteria (Alphaproteobacteria, Burkholderia, etc.) (Cardinale et al., 2012). The study of associations of myco- and photobionts can reveal the evolutionary aspects of the biology of symbiotic organisms, as well as serve as the basis for the search and creation of new biologically active substances. In addition, the production of biologically active substances using tissue culture will reduce the anthropogenic load on natural lichen populations, which are actively used as medicinal raw materials. Lichens produce a wide range of secondary metabolites that are unique and form under lichenizing conditions. Secondary metabolites of lichens showed significant inhibition of various biological activities against pathogenic microorganisms at concentrations of 2–16 μg/ml against bacteria (*Staphylococcus, Streptococcus, Clostridium, Bacteroides*), up to 30 μg/ml against influenza B, H5N1, H3N2 viruses, up to 250 μg /ml against fungi (*Candida, Aspergillus, Microsporum*) (Verma et al., 2015; Luzina et al., 2016).

The interaction of the components of lichenized fungi, as well as factors affecting symbiotic associations, remains one of the little studied issues in fundamental lichenology (Kono et al., 2020).

Many species of lichens that have prospects for use are rare and need protection, in particular *Lobaria pulmonaria* (L.) Hoffm. (Istomina, 2008; Karakus et al., 2009; Atalay et al., 2015). Some species, e. g. *Usnea dasopoga* (Ach.) Nyl. and *Cetraria islandica* (L.) Ach. are widely distributed in Russia (Urbanavichus, 2010) and actively used as plant materials, that reduces the population size and their ability to recover.

The scientific community is just beginning to form an idea about the study of lichenized fungi, the features of the interaction of their components using the tissue culture method – obtaining non-tissue structures of the lichen thallus and its components *in vitro* (Lobakova et al., 2008). There is information about the successful co-cultivation of myco- and photobiont species of the genus *Usnea* Dill. ex Adans.: *U. ghattensis* G. Awasthi, *U. hakonensis* Asahina, *U. articulata* (L.) Hoffm., *U. ciliifera* Motyka, *U. inermis* Motyka, *U. pusilla* Räsänen, *U. torulosa* (Müll. Arg.) Zahlbr., and *U. xanthopoga* Nyl. (Behera et al., 2006; Rafat et al., 2015; Kono et al., 2020). Cornejo et al. (2015) provided data on an attempt to cultivate the mycobiont *Lobaria pulmonaria*. Experience in creating model associations of potentially interacting components of myco- and photobiont has been described (Lobakova et al., 2008). Data on the use of donor parts, components of nutrient media in the experience of cultivating lichenized fungi and their components described in the scientific literature is given in Table 1.

Table 1

Experience in cultivation of lichenized fungi *in vitro*

Species	Component	Donor part	Nutrient medium			Source
			Medium type, composition	Carbon source	Other	
Usnea ghattensis	mycobiont, photobiont together	thallus	MYE, malt yeast extract	sucrose 4 %	polyethylene glycol 4 %	Behera et al., 2006
Usnea hakonensis	mycobiont, photobiont separately	thallus	1) MYE, malt extract 2 %, yeast extract 0.2 %; 2) $Ca(NO_3)_2$ 15 mg, KNO_3 10 mg, β-glycerophosphate 5 mg, $MgSO_4$ 4 mg, $FeCl_3$ 19.6 mg, $MnCl_2$ 3.6 mg, $ZnCl_2$ 1.04 mg, $CoCl_2$ 0.4 mg, B12 0.01 µg, biotin 0.01 µg, thiamine 1 µg	carbon-containing components in the composition of Tris buffer 50 mg	–	Kono et al., 2020
Usnea ciliifera, *Usnea inermis*, *Usnea pusilla*, *Usnea torulosa*, *Usnea xanthopoga*	mycobiont, photobiont together	thallus	Bold's VVM medium, Lilly-Barnett medium	sorbose	–	Rafat et al., 2015
Lobaria pulmonaria	mycobiont	apothecaries	Germination medium (Denison, 2003), Lichen medium BBM (Honegger, 1985), BBM (Deason, Bold, 1960)	glucose 0.9375 g	agar 1.5 %	Cornejo et al., 2015

Note: A dash corresponds to the absence of data.

However, at the current time, the issue of cultivation of both individual components and their associations, such species as *U. dasopoga* and *C. islandica*, which are widespread in Russia, remains unattended, the interaction of components and the process of lichenization of *L. pulmonaria* on nutrient environments. The study of the biological characteristics of lichen components, as well as their interaction under *in vitro* conditions, has not been considered. The prospect of obtaining primary and secondary metabolites by the method of tissue culture of the designated species is not covered.

There are no data on the joint cultivation of myco- and photobiont of the rare species *L. pulmonaria*, the study of the interaction of isolated components. There is no experience in obtaining cultures of ascomycetes, lichenized fungi *U. dasopoga* and *C. islandica*, common in the European part of Russia, whose thalli are widely used as medicinal raw materials. Due to slow growth in natural conditions and the limited availability of natural raw materials (Brunauer et al., 2005), an alternative is the method of culturing cell mass in the laboratory for the production of primary and secondary metabolites.

The purpose of the study is development and optimization of a technique for obtaining a culture of a non-tissue structure and associations of photo- and mycobiont of some medicinal and rare lichenized fungi of the European part of Russia for further study of their components and symbiotic associations, as well as for further use of cell culture as a source of primary and secondary metabolites.

Objects and methods of research

The objects of study were species of lichenized fungi that are widely used as medicinal raw materials in the European part of Russia, as well as a species included in the Red Data Book of the Russian Federation.

Lobaria pulmonaria (L.) Hoffm. (Ascomycota, Peltigerales W. Watson, Lobariaceae Chevoll.) is a large-leaved epiphytic lichen that has a wide but local distribution in the European part of Russia and is included in the Red Data Book of the Russian Federation under category 2 (a vulnerable species that is declining in numbers as a result of changing living conditions, habitat destruction and collection) (Istomina, 2008; Ivanova et al., 2012; Golovko et al., 2018). A three-component lichen whose thallus contains as a phototrophic component: the nitrogen-fixing cyanobacterium of the genus *Nostoc* and the green algal *Dictyochloropsis reticulata* (Ignatenko et al., 2020); A number of sources also indicate the presence of bacterial communities with a predominance of Proteobacteria and Archaea (Schneider et al., 2011; Cardinale et al., 2012).

Usnea dasopoga (Ascomycota, Lecanorales Nannf., Parmeliaceae Zenker) is a fruticose epiphytic lichen whose phototrophic component is the free-living green alga *Trebouxia* (recently classified as *Asterochloris* sp.) (Balarinova et al., 2013; Rafat et al., 2015; Gagarina et al., 2017). The thalli of the genus *Usnea* accumulate usnic acid, the content of which reaches 13 % of the mass of air-dry raw material; this compound has a wide spectrum of biological activity (Guo et al., 2008).

Cetraria islandica (Ascomycota, Lecanorales Nannf., Parmeliaceae Zenker) is a fruticose epigean lichen containing green algalalgal of the genus *Trebouxia* as a photobiont (Grujicic et al., 2014; Maonian et al., 2020). Thalli are widely used due to the content of usnic acid (up to 1.2 mg/g of air-dry raw materials), the presence of polysaccharides (up to 73 g/100 g of air-dry raw materials), tannins (up to 6.1 g/100 g of air-dry raw materials) (Burkin et al., 2013; Kravchenko et al., 2015).

The donor material of *U. dasypoga* and *C. islandica* for introduction into culture *in vitro* was collected on the territory of the pine forest of the Krasnoselsky district of the Kostroma Region; thalli of *L. pulmonaria* were provided by the State Natural Reserve “Kologrivsky Forest” named after M. G. Sinitsyna (Kologrivsky area, Kostroma Region). Fresh thalli were used for introduction into culture. Fragments of thalli (for all objects), apothecia (for *C. islandica*), and soredia (for *U. dasopoga* and *L. pulmonaria*) were used as donor material. To introduce the photobiont, a homogenate of a thallus segment was also prepared under sterile conditions as follows: a 0.5 cm^2 thallus segment was placed in an Eppendorf microtube, 1.0 ml of sterile distilled water was added, and the thallus was brought to a homogeneous mass using a Potter homogenizer.

Sterilization of the donor material was carried out according to the following scheme in a laminar box: after the initial washing of the thalli in distilled water, they were placed in a 70 % aqueous solution of ethanol for 30 seconds, after which the thalli were transferred to a 3 % aqueous solution of sodium hypochlorite (exposure 10 minutes). Then the thalli were kept twice in flasks with sterile distilled water (exposure for at least 5 minutes).

Sterile donor explants were passivated into several types of nutrient media, the composition of which is described in Table 2.

Incubation was carried out at an air temperature of 20–22 °C, a light intensity of 1200 lux on culture racks with a light regime of 16 h (day) / 8 h (night).

The contamination of explants with lichenophilic microorganisms was assessed on the 5^{th} day of cultivation, the growth of pure cultures of myco- and photobiont was assessed on the 15^{th} day. The cultures obtained on nutrient media were examined using microscopy: the morphology of the mycobiont, the diameter of its hyphae, and the size of the cells of the photobiont of isolated cultures were studied. Microscopy of samples was carried out on the 25^{th} day of cultivation using a Biomed-3 light microscope at a total magnification of ×400–1000; the sizes of mycobiont structures and photobiont cells were estimated using an eyepiece micrometer with a division value at a total magnification of ×40–160 as 0.02 mm and 0.002 mm, respectively. In order to compare the morphology of myco- and photobiont in natural samples and in culture (in lichenized and non-lichenized state), the main morphological characteristics of myco- and photobiont in natural lichen samples were studied using light microscopy: the size and shape of photobiont cells, the diameter of mycobiont hyphae. Photography under light microscopy was carried out on a Samsung MX 10 camera.

Table 2

Nutrient medium compositions used for the cultivation of components of lichenized fungi *Lobaria pulmonaria*, *Usnea dasypoga*, and *Cetraria islandica*

Medium type	Macroelement composition	Microelement composition, vitamins	Carbon sources	Agar	pH
MS (Murashige, Skoog, 1962) (option 1)	KNO_3 – 1900 mg/l, NH_4NO_3– 1650 mg/l, $MgSO_4$ – 180 mg/l, $CaCl_2$ – 330 mg/l, KH_2PO_4 – 170 mg/l	$FeSO_4$ – 27.8 mg/l, $MnSO_4$ – 22.3 mg/l, $ZnSO_4$ – 8.6 mg/l, H_3BO_3 – 6.2 mg/l, $CoCl_2$ – 0.025 mg/l, Thiamine – 0.5 mg/l, Pyridoxine – 0.5 mg/l	Sucrose – 20 g/l	6 g/l	5.5
MS modification for photobiont (option 2)	KNO_3 – 250 mg/l, KH_2PO_4 – 175 mg/l, $MgSO_4$ – 75 mg/l, $CaCl_2$ – 25 mg/l, NaCl – 25 mg/l	$FeSO_4$ – 5 mg/l, H_3BO_3 – 11 mg/l, $ZnSO_4$ – 8 mg/l, $Co(NO_3)_2$ – 0.5 mg/l	Maltose – 10 g/l, glucose – 10 g/l	4–6 g/l	5.3
Czapek environment (Czapek, 1903) (option 3)	KH_2PO_4 – 1 g/l, $MgSO_4$ – 0.5 g/l	$ZnSO_4$ – 0.5 mg/l, Thiamine – 1 mg/l	Maltose – 10 g/l,	6 g/l	5.0

Results and its discussion

In accordance with the described procedure, pure cultures of the *L. pulmonaria* photobiont, a green alga of the genus *Dictyochloropsis* and a cyanobacterium of the genus *Nostoc*, were obtained on the MS nutrient medium modified by us (Fig. 1). The cell culture of cyanobacteria was obtained by using medial fragments of the thallus with complete immersion of donor explants in the thickness of the nutrient medium. This is probably explained by the localization of *Nostoc* cells in the cephalodia of the core layer of the thallus, where algal cells are covered by layers of mycobiont hyphae (Pystina et al., 2010). Growth of green algalalgal cells was observed on the medium both when thallus sites and sorals were used as donor explants. In the latter case, a smaller number of *Dictyochloropsis* cells on the nutrient medium was noted, which may be the reason for the inhibition of algal growth by the mycobiont. The macroelemental component composition of the MS nutrient medium modified by us (option 2), indicated in Table 2, is characterized by a poorer content of nitrogen, magnesium sulfate and calcium chloride, which probably inhibits the growth of lichenophilic microorganisms. In addition, this medium contains a combination of maltose and glucose (10 g/l each) as a source of carbon nutrition; is characterized by a lower content of agar, which provides a less dense medium and a more optimal arrangement of photobiont cells in culture. This is the reason for the successful use of the nutrient medium of this composition to obtain a cell culture of the photobiont.

The *L. pulmonaria* mycobiont culture was successfully obtained on Czapek's medium (option 3) (Fig. 1, right). The growth of the mycobiont was characterized by slower growth compared to lichenophilic fungi that form contamination of donor explants (growth began on the 15th and 5th day of incubation, respectively). The mycobiont formed on Czapek's medium was characterized by rare branching of hyphae in dense and randomly arranged strands in the nutrient medium.

During the subsequent joint cultivation of the obtained components of *L. pulmonaria*, lichenization of the cells of the green alga *Dictyochloropsis* was observed on the 20th day of subculturing. At the same time, the formation of a conglomerate of algal cells was noted, and then their entanglement with mycobiont hyphae (Fig. 2).

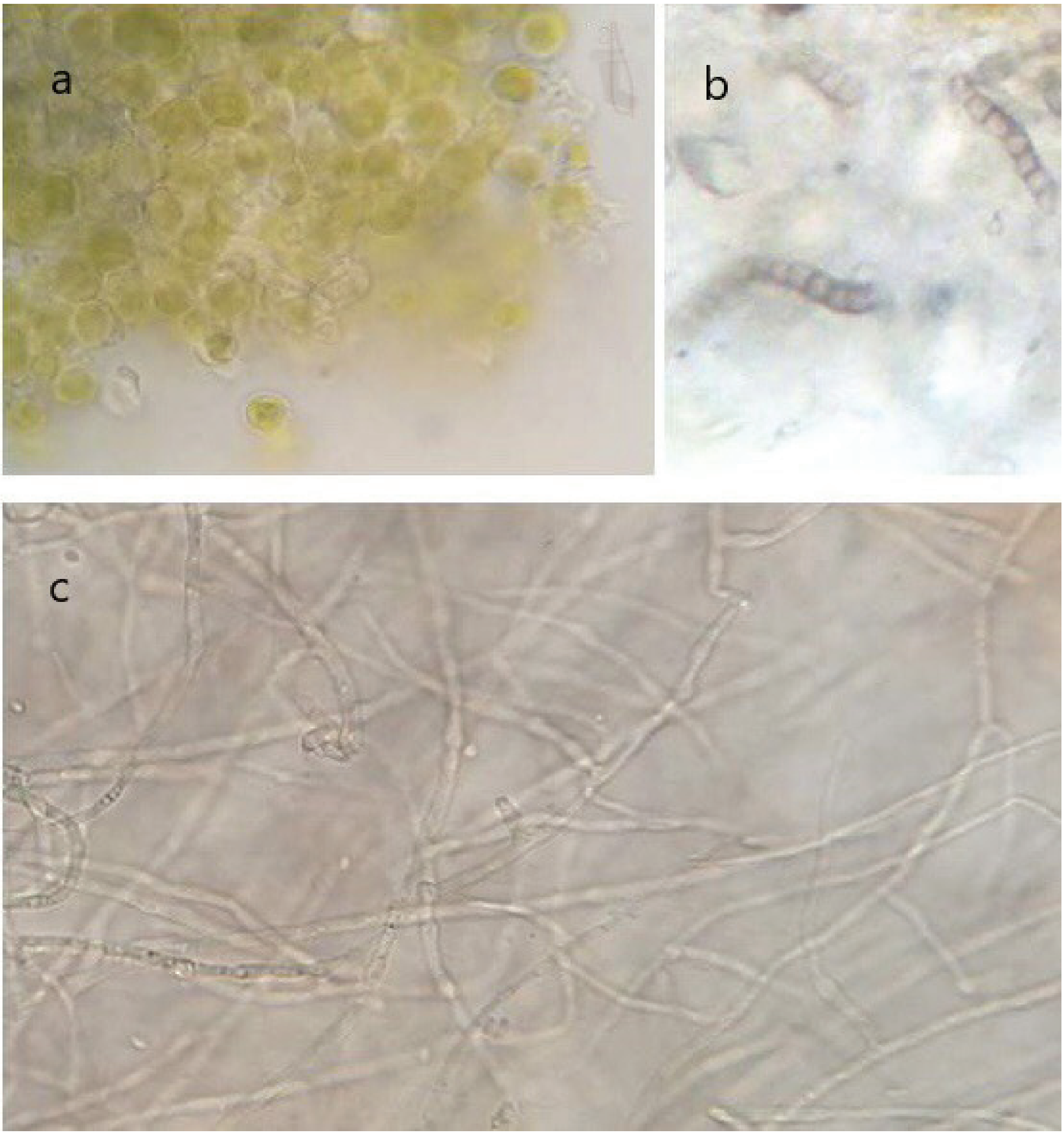

Fig. 1. Pure tissue cultures of *Lobaria pulmonaria*: **a** – *Dictyochloropsis* photobiont; **b** – *Nostoc*; **c** – mycobiont. Total magnification ×400, ×400, and ×100, respectively.

The hyphae of the mycobiont obtained in culture *in vitro* correspond morphologically to the hyphae of the natural sample of *L. pulmonaria*; the hyphae were 4.3–4.4 and 4.6 μm in diameter, respectively. The diameter of the hyphae of the natural *L. pulmonaria* specimen growing in the northern part of the Kostroma Region corresponds to the data described for mature thalli of this species in the European northeast of Russia (Golovko et al., 2018). Young mycobiont hyphae formed on a nutrient medium are, on average, somewhat thinner than the hyphae of natural specimens and do not exceed the diameter of the hyphae of young thalli. The cells of the *Dictyochloropsis* culture under *in vitro* conditions in a non-lichenized form were dispersed in the thickness of the nutrient medium. At the same time, growth points of green alga colonies from morphogenic (viable) passivated photobiont cells, freely located in a weakly agarized medium closer to its surface, were observed at first; the size of the cells in diameter did not exceed 4 μm, the shape of the cells is round, spherical, visually the chloroplast looks like a homogeneous mass that fills the entire volume of the cell. These data are generally consistent with the data described in the literature for both natural lichenized samples of species of the genus *Dictyochloropsis* and free-living ones, however, there are smaller sizes of cells introduced into the culture from the lichen thallus (up to 4 μm) compared to natural lichenized ones (about 5 μm) (Golovko et al., 2018) and with free-living samples (7–40 μm) (Skaloud et al., 2005). Samples 4 μm, collected in a complex and surrounded by strands of the hymicobiont. At the initial stage of lichenization, no changes in the shape of green algalalgal cells were found.

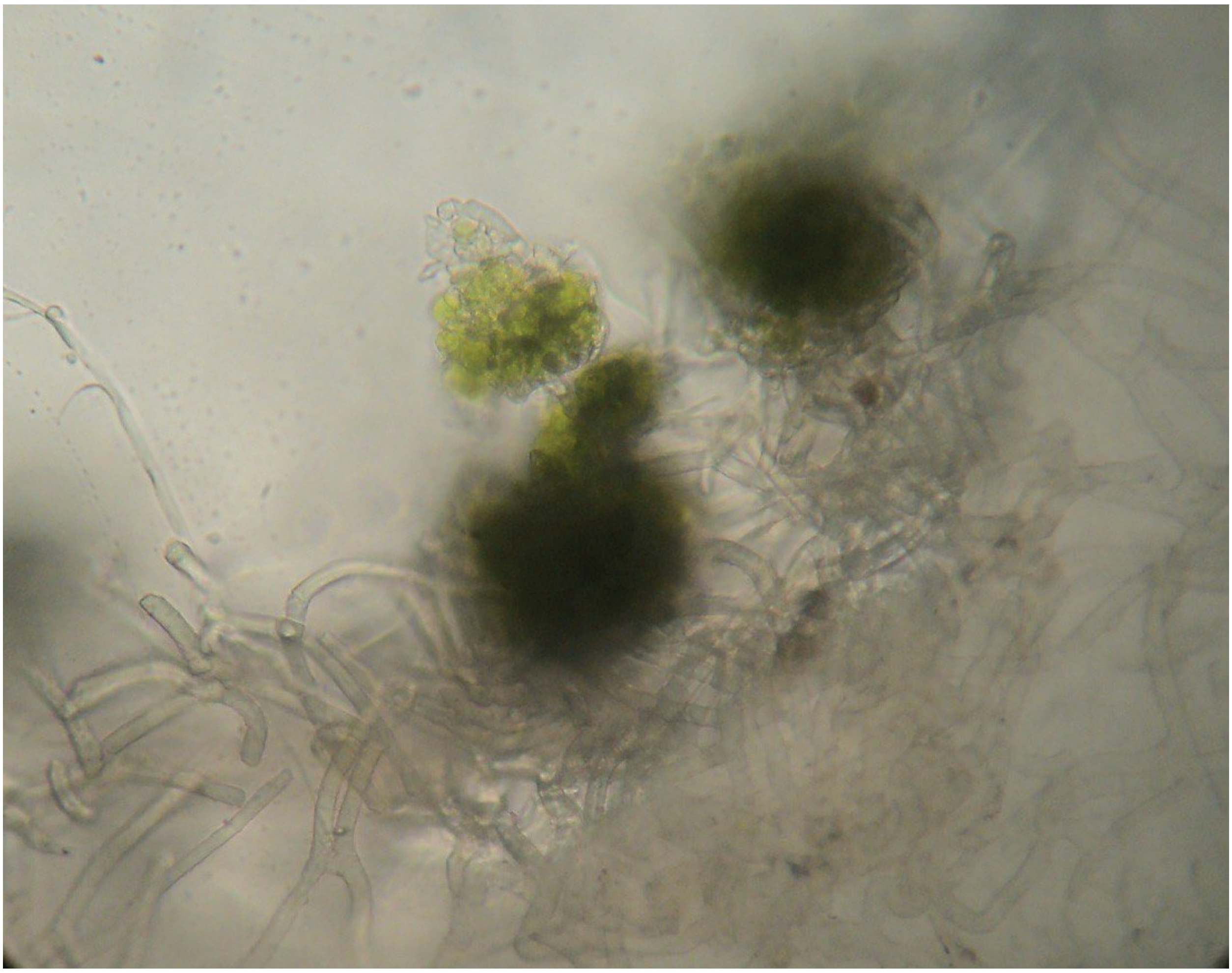

Fig. 2. Lichenization of green algal cells by the mycobiont *Lobaria pulmonaria* during their co-cultivation.

Success in obtaining the joint growth of cultures of the photo- and mycobiont *U. dasopoga* was achieved by introducing the apical parts of the thallus of this lichen species onto a modified MS nutrient medium (option 2). The soredia passivated on the medium mainly gave rise to the growth of the mycobiont, while the algal cells were not detected. This probably happened due to the suppression of photobiont cells by the mycobiont, which is reflected in the studies of a number of authors (Lobakova et al., 2008). Externally, the growth of the culture of the ascomycete *U. dasopoga* manifested itself in the form of a densely located layer of white mycelium above the surface of the medium, which corresponds to the data of foreign colleagues when cultivating another species of the genus *Usnea*, *Usnea ghattensis* (Behera et al., 2006); at the same time, the nutrient medium acquired a pronounced yellow color. Light microscopy of the samples revealed strands of weakly branched hyphamycobiont with mosaic interspersed groups of photobiont cells (green algal of the genus *Trebouxia*) (Fig. 3, left). The diameter of the hyphae of the mycobiont *U. dasopoga* on the nutrient medium did not exceed 4.6 μm, which generally corresponds to the diameter of the hyphagonidial layer of young thalli in natural samples. At the same time, green algal cells, when co-cultivated with a mycobiont on a nutrient medium, were located in small clusters (5–8 cells each) between the hyphamicobiont strands and had a size of up to 5 μm, the shape of the algal cells was round, spherical. Compared to the natural sample in the thallus of *U. dasopoga*, the cells of the green alga of the genus *Trebouxia* are somewhat larger (5–7 μm).

A pure culture of the mycobiont *C. islandica* was obtained on Czapek's medium (variant 3) using the distal parts of the lobes of the thallus and apothecia as donor explants. At the same time, the growth of the mycobiont centrifugally from the explant with dark-colored threads in the thickness of the nutrient medium was noted. Light microscopy revealed thin weakly branching mycobiont hyphae arranged in strands (Fig. 3, right).

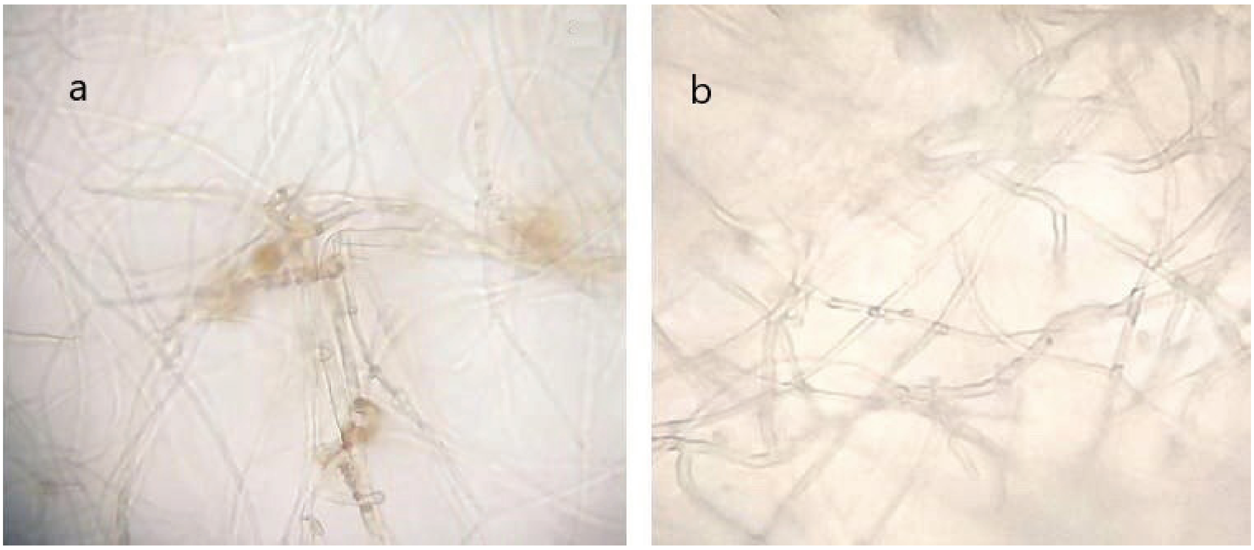

Fig. 3. Culture of mycobiont *Usnea dasopoga* (together with *Trebouxia*) (left) and *Cetraria islandica* (right).

In a number of specimens of the *C. islandica* mycobiont culture, clusters of bacteria of a spherical shape were found, with a diameter not exceeding the thickness of the hyphae located along them.

The size of the *C. islandica* hyphaemicobiont obtained *in vitro* culture averaged 4.1–4.3 μm in cross section (in diameter); in natural samples of thallus, this value corresponded to 4.6–4.7 μm. Morphologically, the hyphae of a natural sample of mycobiont and mycobiont in culture are identical.

Summing up the results of the study, we designate the main parameters of successful tissue culture of myco- and photobiont of lichenized fungi *L. pulmonaria*, *U. dasypoga*, and *C. islandica*. Key aspects of the study are summarized in Table 3.

Morphological characteristics of the mycobiont obtained in culture *in vitro* on nutrient media are summarized in Table 4 and shown in Figure 4.

Table 3

Conditions for obtaining a culture of myco- and photobiont of lichenized fungi *Lobaria pulmonaria*, *Usnea dasypoga*, and *Cetraria islandica* on nutrient media

Species	Component	Donor part	Type of nutrient medium
L. pulmonaria	Photobiont *Dictyochloropsis*	soralia, fragments of thallus	modified ½ MS
	Photobiont *Nostoc*	medial fragments of the thallus	modified ½ MS
	mycobiont	soralia, fragments of thallus	medium Czapek
U. dasypoga	Photobiont *Trebouxia*	distal thallus fragments, homogenate	modified ½ MS
	mycobiont	soralia, fragments of thallus	modified ½ MS
C. islandica	mycobiont	apothecia, thallus fragments	medium Czapek

In addition, we studied the morphological features of the photobiont on the nutrient medium. In accordance with the results obtained, at the stage of lichenization during the co-cultivation of components, we can note a pronounced effect of the mycobiont on the morphology of photobiont (green algal) cells: during lichenization, their sizes become smaller and do not exceed 3–4 μm (for *Dictyochloropsis*) and 5 μm (for *Trebouxia*). In addition, photobiont cell reproduction is inhibited: in comparison with the free dispersed arrangement of green algal cells on a nutrient medium during lichenization, photobiont complexes between mycobiont hyphal cords include no more than 5–8 algal cells (for *U. dasypoga*) and within 10–15 cells of algal (for *L. pulmonaria*).

Table 4

Morphological features of the mycobiont of lichenized fungi *Lobaria pulmonaria*, *Usnea dasypoga*, and *Cetraria islandica* on nutrient media

Species	Location of mycobiont on the medium	Diameter of hyphae, μm	
		minimum value	maximum value
L. pulmonaria	Hyphae weakly branching, forming dense chaotic clusters on the medium	4.3	4.4
U. dasypoga	The hyphae are slightly branched, forming dense clusters on the nutrient medium	4.5	4.6
C. islandica	Thin, weakly branching hyphae are located in strands in the thickness of the nutrient medium	4.1	4.3

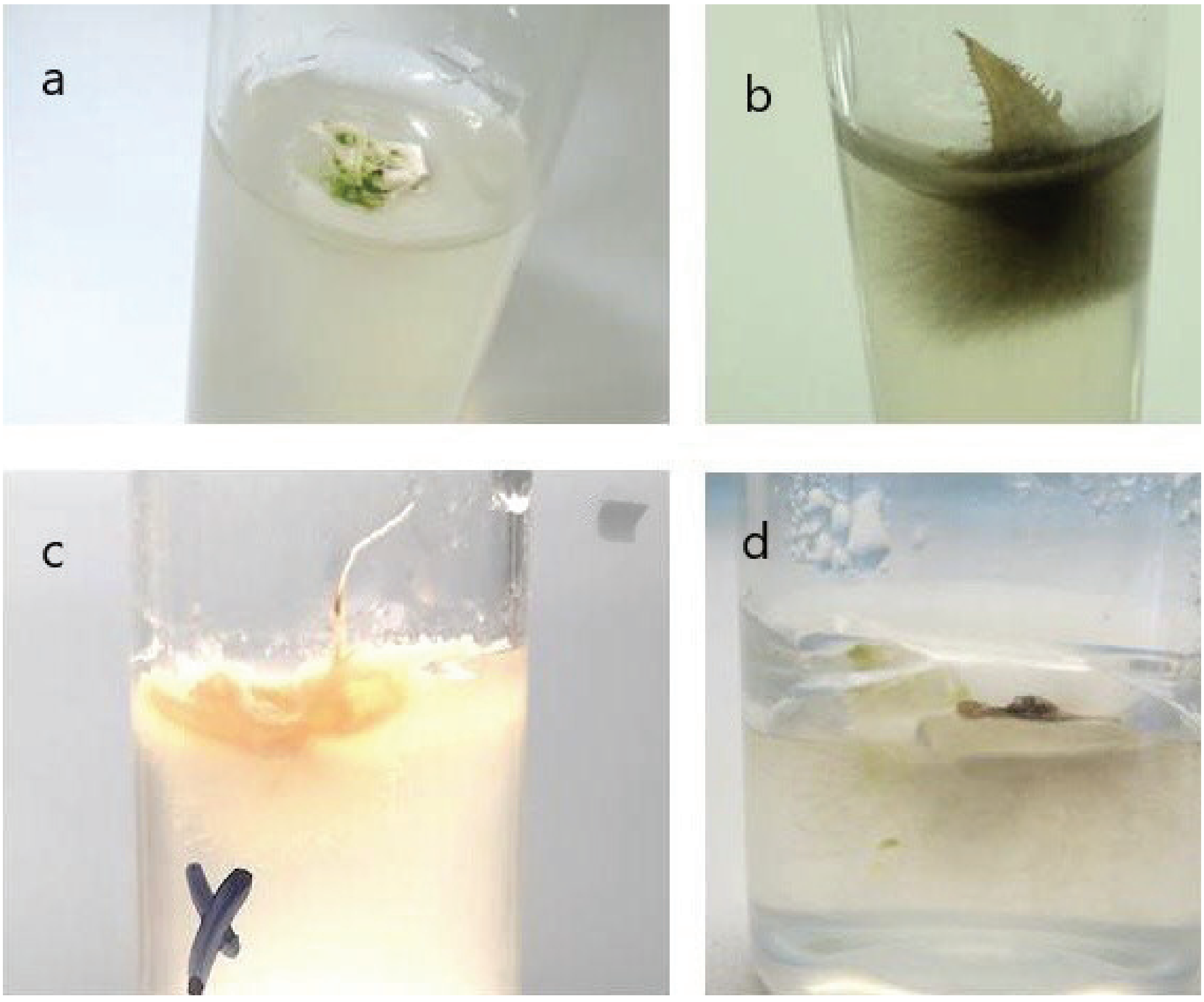

Fig. 4. Lichenized fungi on nutrient media: **a** – *Lobaria pulmonaria*; **b** – *Cetraria islandica*, *Usnea dasopoga*; **c** – resynthesis of *C. islandica* and *Trebouxia* components.

Conclusion

Thus, the data on the conditions of *in vitro* cultivation of the myco- and photobiont components of lichenized fungi make it possible to use the results obtained to study the morphology of the myco- and photobiont, as well as to reveal the features of symbiotic relationships, and currently allows us to make the process of "assembly" of two- (*U. dasopoga, C. islandica*) and three-component (*L. pulmonaria*) organisms controlled under culture conditions.

The authors proposed a method for obtaining a culture of myco- and photobiont of lichenized fungi, as well as the joint cultivation of their components. The studies of the morphological features of the photobiont and mycobiont obtained on nutrient media and in natural samples indicate slightly smaller sizes of structures on the nutrient medium, which makes it possible to use the tissue culture method to study the biological and ecological characteristics of lichenized fungi. The studied features of the components during lichenization under *in vitro* conditions allow us to conclude that the effect of the mycobiont is expressed in the inhibition of the reproduction and growth of photobiont cells on a nutrient medium.

The use of the tissue culture method is promising for solving many problems associated with lichenized fungi. One of them is the development of a methodology for the cultivation of the lichen thallus and the biosynthesis of its secondary compounds, primarily from the group of polyketides, which are of great importance for medicine. A very important problem is the features of the life cycle, its critical moments and the mechanisms of symbiotic relationships between photo- and mycobiont organisms. Also, the knowledge gained during *in vitro* cultivation on the impact of various factors and mechanisms of symbiotic relationships will solve the problem of preserving rare species of lichenized fungi.

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