ISSN 0026-2617, Microbiology, 2018, Vol. 87, No. 1, pp. 79–88. © Pleiades Publishing, Ltd., 2018.
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EXPERIMENTAL ARTICLES

Bacterial Complexes of Khibiny Mountains Lichens Revealed in *Cladonia uncialis, C. portentosa, Alectoria ochroleuca,* and *Nephroma arcticum*

T. A. Pankratov

Lomonosov Moscow State University, Moscow, Russia
e-mail: tpankratov@mail.ru

Received April 26, 2017

Abstract—Fluorescence in situ hybridization was used to investigate microbial communities of four lichen species collected in the Murmansk province. The maximal bacterial abundance was shown to depend on both the lichen species and the part of the thallus. Predominant groups of bacteria were revealed: *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*. Criteria for assessment the facultative and obligatory presence of bacteria in lichen microbial communities were established, and approaches to classification of the lichen microbial communities were proposed based on the interdependence of various bacterial groups.

Keywords: lichens, endophytic and epiphytic bacteria, symbiotic bacteria, Arctic ecosystems, *Acidobacteria*
DOI: 10.1134/S0026261718010149

Lichens, of which ~25000 species are presently known, are symbiotic organisms, which are able to thrive and form biomass under extreme conditions (from low negative temperatures to high temperatures with water limitation. These are eurybiont organisms not limited to terrestrial ecosystems. They also occur in marine and freshwater environments. In the ecosystems with low levels of organic matter and biologically available nitrogen, the biomass of dead lichens, as well as of cyanobacteria and microalgae, forms the basis of the primary humus. The lichen taxonomic diversity, ecology, and physiology are well-studied. The lichen microbial communities have been investigated during the last decade. Molecular genetic and microscopic techniques were used to obtain the data on prokaryote integration into the thalli, where bacteria form biofilms, with their localization depending on the lichen anatomical structure (Cardinale et al., 2008). The taxonomic structure of the lichen bacterial complexes was shown to be similar to that of soil, bog, and freshwater ecosystems with limited supply of minerals and available nitrogen (Pankratov et al., 2017). Most works revealed quantitative predominance of members of the subclass *Alphaproteobacteria* and of the class *Acidobacteria* (Grube and Berg, 2009). In spite of the importance of investigation of the lichen microbial communities, dependence of the structure, abundance, and activity of bacterial complexes on the degree of the thallus degradation remains unclear. This may be the key issue in determination the form of lichen–bacterial coexistence. Development of criteria for determination of the obligate and facultative relations of bacterial groups and species within lichen microbiomes is also an important task.

The goal of the present work was to determine bacterial abundance, composition, and localization on the surface and inside the thalli of four species of fruticose and foliose lichens using in situ hybridization with fluorochrome-labeled probes and to carry out statistical analysis of experimental data.

MATERIALS AND METHODS

Experimental subjects. In the Khibiny Mountains (Kola Peninsula, Murmansk oblast; 67°47′45.2′′ N, 33°34′58.7′′ E), four lichen species were collected: *Cladonia uncialis*, *C. portentosa*, *Alectoria ochroleuca*, and *Nephroma arcticum*. Whole thalli of each species were separated into three parts: the apex (actively growing part of the thallus), the intermediate part (aging part of the thallus), and the lower, degrading part of the thallus. The borders between these zones were determined visually, according to the color, shape, and texture of the thallus.

Sample preparation. The samples were prepared using an ULTRA-TURRAX® Tube Drive homogenizer (IKA, Germany). Relevant parts of the thallus were cut into small (3–5 mm) fragments using sterile scissors. The sample (~500 mg) was transferred into the homogenizer cup (ST-20, rotating pestle) together with two sterile glass balls (d = 5 mm). The cells were washed off with 5 mL of phosphate buffer (NaCl, 8.0 g; KCl, 0.2 g; Na_2HPO_4, 1.44 g; NaH_2PO_4, 0.2 g;

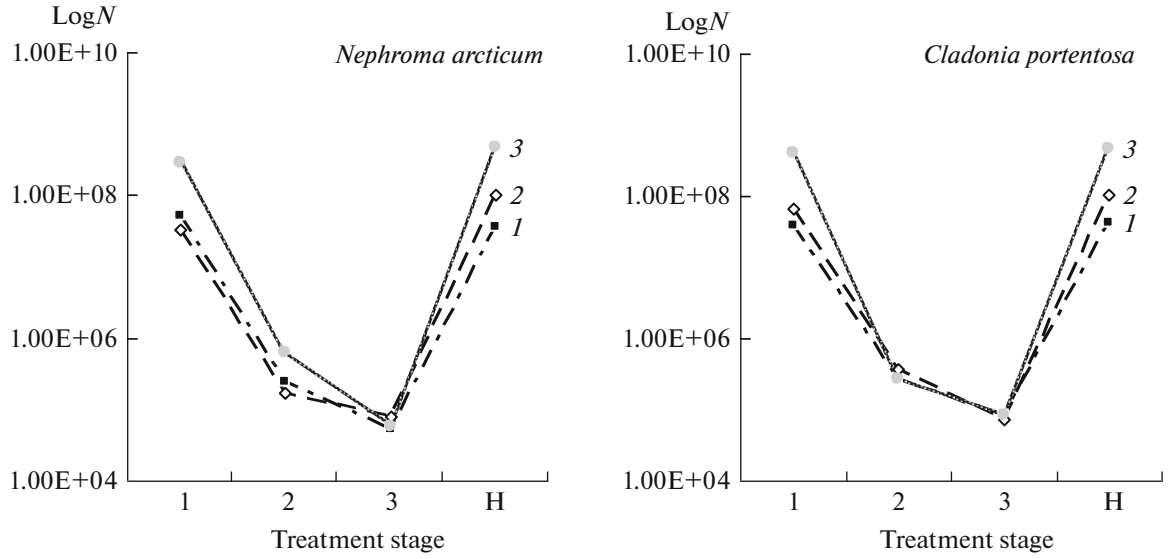

Fig. 1. Bacterial cell numbers (acridine orange staining) depending on the stage of activator treatment of the thalli. Designations on the *X* axis: treatment stages (1–3) and homogenate analysis (H). The lines relate to the upper (*1*), intermediate (*2*), and lower part of the thallus (*3*), respectively.

H_2O, 1 L; pH 7.0) for 5 min at the no. 6 mode (~4000 rpm). The suspension was then centrifuged at 9000 rpm for 3 min. The supernatant was removed, and the pellet was resuspended in 500 μL of 4% formaldehyde in the above phosphate buffer and fixed for 1.5 h at room temperature. After fixation, the cells were concentrated by centrifugation and washed with 50% ethanol. This material was then resuspended in 1 mL of 50% ethanol and stored at –20°C. Washout efficiency was ascertained by three sequential treatments with subsequent determination of bacterial cell number by epifluorescence microscopy (acridine orange staining).

The thallus material was then washed with sterile distilled water, dried on sterile filter paper, and transferred into a test tube with the rotor-stator homogenization mechanism (DT-20). Homogenization was carried out in 10 mL of phosphate buffer using two steel balls ($d = 5$ mm), for 5 min at 4700 rpm. A 2-mL aliquot of the homogenate was then fixed as described above.

For calculation of bacterial cell numbers, weighed original thallus samples were dried for 2 h at 105°C, and dry mass was determined at 20–22°C.

Hybridization was carried out using oligonucleotide probes developed for detection of members of the major phylogenetic groups (Pankratov et al., 2005).

Fluorescence microscopy and enumeration. Detection was carried out according to generally accepted procedures. Total bacterial cell numbers were determined in the preparations stained for 5 min with acridine orange in citrate buffer (NaOH, 0.1 M, 200 mL; citric acid, 21 g; 1 : 10000) and washed with distilled water for 5 min. Total bacterial abundance and the numbers of bacterial groups were determined for three parallel samples in 30 microscope fields.

Statistical analysis. Correlation analysis and calculation of the coefficients of variation and dispersion factors were carried out using the statistical package implemented in MS Excel 2003.

RESULTS AND DISCUSSION

The procedure used to separate the epibiotic and endobiotic lichen microbial communities involved two-stage treatment: mild cell desorption by an intense buffer flow (activator treatment) and homogenization using the rotor-stator technology. Efficiency of this procedure was studied in the course of preliminary investigation, which included three stages: (1) sequential threefold activator type treatment with subsequent washing of the sample with sterile phosphate buffer; (2) determination of bacterial numbers after each treatment; and (3) sample homogenization and enumeration of bacteria inside the thallus. Bacterial cell numbers at different stages of treatment are shown on Fig. 1 (the data for the thalli of *Nephroma arcticum* and *Cladonia portentosa*, two morphologically ad anatomically contrasting lichens, are presented). These data indicated that the second treatment did not result in significantly higher cell desorption from the surface of foliose and fruticose lichens. After the second and third treatments, cell numbers remained at the threshold level for direct counts, 10^5–10^4 cells per gram, i.e., one or less cells per 100 microscope fields).

Total bacterial abundance determined using acridine orange staining was similar to the values for other

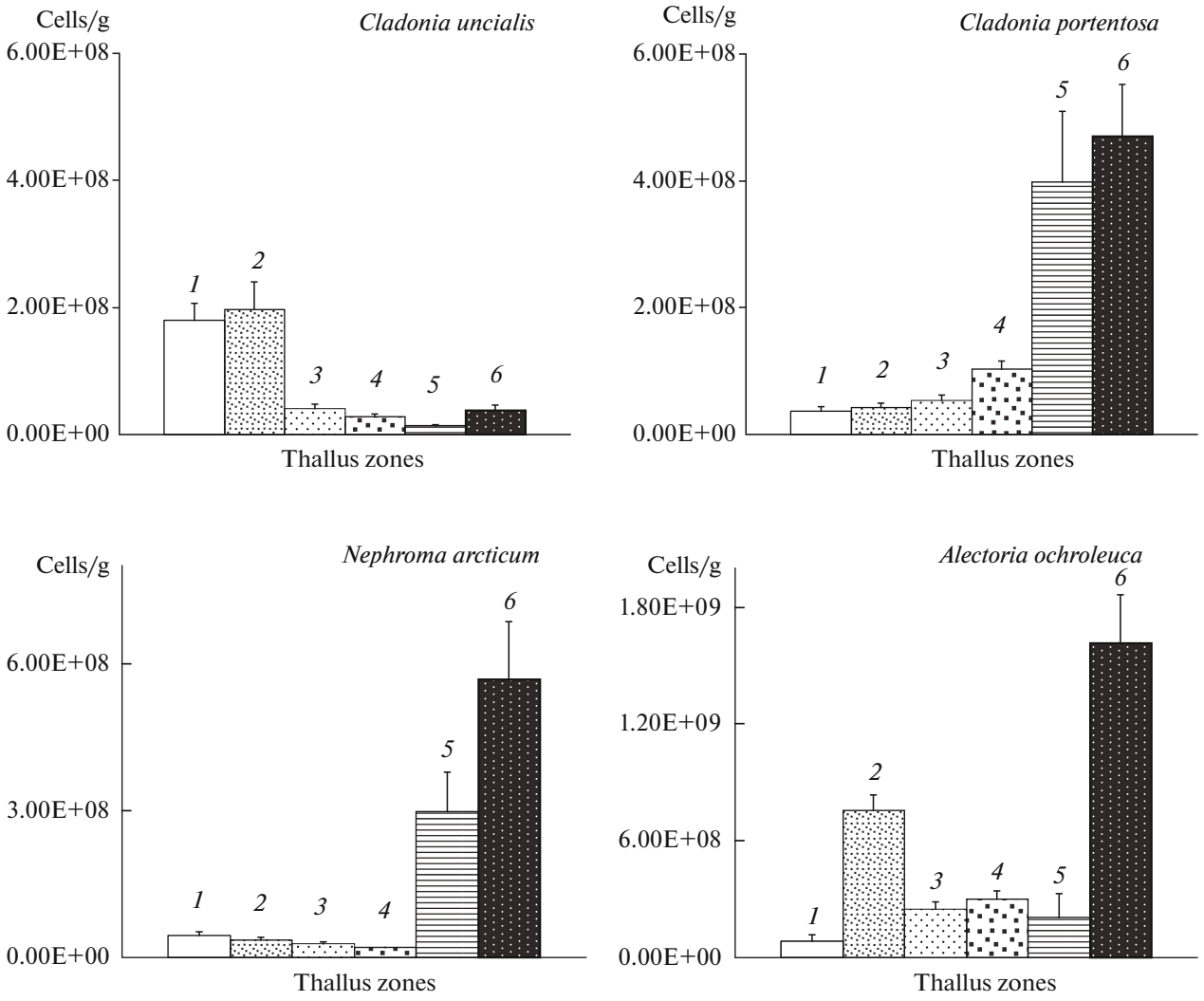

Fig. 2. Total bacterial cell numbers (acridine orange staining) in lichen samples depending on the thallus zone: washouts from the surface (*1*, *3*, *5*) and homogenates of the zones of growth, aging, and degradation, respectively (*2*, *4*, *6*). Reliability of the differences was determined as averages ($P \geq 0.95$) calculated from the standard deviation within the samples.

environments (soils and peat) and varied from 1.36×10^7 to 1.61×10^9 cells/g (Fig. 2). The numbers of bacteria washed off the thallus surface depended on the lichen species and the thallus area. The highest levels of bacterial cells were found in the homogenate of the lower thalli of *Alectoria ochroleuca* (~2×10^9), *C. portentosa* (~5×10^8), and *N. arcticum* (~6×10^8 cells/g). Higher abundance of endophytic bacteria compared to the epiphytic bacterial community was found in the thallus decomposition zone of all four lichen species (Fig. 2). The highest numbers of epiphytic bacteria were found in the growth zone of *C. uncialis* (~2×10^8 cells/g), while endophytes predominated in homogenates of *C. uncialis* (~2×10^8 cells/g) and *A. ochroleuca* (~8×10^8 cells/g). In other species bacterial numbers were below 10^8 cells/g.

In the aging zone of the studied lichens, bacterial numbers at the surface and inside the thalli were similar. The slight difference was observed in *C. portentosa* thalli, where the number of endobiotic bacteria was approximately twice higher than that of the epibiotic community.

In the zone of thallus decomposition, which contacts with the substrate (in this case, the surface soil layer), bacterial numbers at the surface and inside the thallus were 3×10^8 and $5–6 \times 10^8$ cells/g, respectively. The samples of *C. uncialis*, where the numbers of endophytic and epiphytic bacteria were the same and did not exceed 4×10^7 cells/g, and *A. ochroleuca*, where the number of endobionts was an order of magnitude higher (~2×10^9 cells/g), were exceptional in this respect. In general, a tendency to increased endophyte abundance in aging thalli was observed.

High bacterial numbers in the podetial zone of *C. uncialis* may be explained by high numbers of apothecia and phylloclades at the time of fixation; these structures probably contained a numerous bacterial community with high abundance of hydrolytics and *Alphaproteobacteria*. High bacterial abundance in *C. portentosa* thallus, compared to that of *C. uncialis*, may be explained by predominance of actinobacteria and decreased share of alphaproteobacteria in degrading thalli. *Streptomyces uncialis*, which is specifically associated with *C. uncialis*, is known to have high antagonistic activity due to uncialamycin production (Parrot et al., 2016).

Analysis of abundance of the major bacterial groups. The initial list of group-specific probes used in this work included oligonucleotides targeting members of the *Planctomycetes*, *Verrucomicrobia*, and *Deltaproteobacteria*. Preliminary screening revealed, however, the absence or extremely low abundance (below 10^5 cells/g) of these groups. Quantitative studies were therefore limited to the following groups: *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidetes*, and *Firmicutes*.

The epiphytic (epibiotic) communities of the four studied lichen species were characterized by predominance of *Proteobacteria*. The shares of the alpha-, beta-, and gammaproteobacteria depended on the lichen species and the thallus zone (Fig. 3). The numbers and relative abundance of other studied groups varied depending on the degree of thallus degradation and, to a lesser degree, on the lichen species.

In the growth zones of three lichen species, the structure of microbial communities was similar, with the *Alphaproteobacteria* predominant in both endo- and epibiotic communities. Depending on the lichen species, the share of this group varied from 42 to 66%. *Gamma-* and *Betaproteobacteria* were the second most abundant component in microbial communities of *N. arcticum* and *C. portentosa*. Their overall share in *N. arcticum* and *C. portentosa* was 39 and 25%, respectively. In two other species, subdominant groups were *Acidobacteria* (16% *A. ochroleuca*; 11% *C. uncialis*) and *Actinobacteria* (8 and 14%, respectively).

In the aging zone of all four species, the distribution of the dominant and subdominant groups changed: the share of alphaproteobacteria increased to 66% in *C. portentosa* and decreased by 10–20% in other species. The share of acidobacteria in *A. ochroleuca* and *C. uncialis* increased to 27 and 23%, respectively. In *N. arcticum* the *Betaproteobacteria* population became predominant (48%). The share of actinobacteria remained stable in all species (9 to 19%).

In the course of active thallus degradation, *Alphaproteobacteria* become dominant once more: 43–46% in *C. portentosa* and *A. ochroleuca* and 55–63% in *C. uncialis* and *N. arcticum*. The share of *Acidobacteria* increased significantly in *C. portentosa* (up to 36%) and *N. arcticum* (up to 25%), while it decreased somewhat in *A. ochroleuca* (19%) and *C. uncialis* (10%). Interestingly, the share of actinobacteria was almost the same in all three zones of *C. uncialis* thallus (14–19%). Insignificant fluctuations of the share of this group were observed in the endophyte microflora of all studied lichen species.

While *Proteobacteria* generally predominated in the endophytic (endobiont) bacterial communities of all four lichen species, the distribution of their shares was more diverse (Fig. 4).

Among the endophytic bacteria of the growing part of the lichens, proteobacteria predominated, with the *Alphaproteobacteria* being the most numerous, except for *A. ochroleuca*, where *Acidobacteria* were the main component of the community in the growing part of the thallus (47%), while the combined share of proteobacteria was 34%. In other species the share of *Acidobacteria* was also significant: 16 to 22%. Similar to the epibiont community, the share of actinobacteria was low, varying from 4 to 12%.

Alphaproteobacteria (62 and 58%), *Gammaproteobacteria* (15 and 11%), and *Acidobacteria* (8 and 25%, respectively) predominated in the endophyte blocks of the aging thalli of *C. portentosa* and *A. ochroleuca*. In *C. uncialis* and *N. arcticum*, apart from these groups, actinobacteria were present (up to 23 and 15%, respectively).

The share of actinobacteria increased to 23–24% in degrading parts of the thalli of three lichen species, while the relative abundance of *Acidobacteria* in *C. portentosa* reached the same level. The share of the latter group increased considerably in *N. arcticum* (up to 36%).

Considering involvement of the studied bacterial groups in communities of four lichen species, the following patterns may be discerned: (1) the ratio of the groups varied depending on the degree of the thallus degradation; (2) increased degradation of the thallus resulted in increased ratios of the hydrolytic block bacteria, primarily due to *Actinobacteria*; (3) the share of invasive hydrolytic bacteria, which are capable of penetrating the thallus due to the presence of the mycelium or via active gliding motility, increased at the stage of aging of the thallus; and (4) changes in the community composition are more pronounced within its epibiont component, probably due to less pronounced action of antibacterial and bacteriostatic compounds due to decreased mycobiont activity. These conclusions confirm interpretation of lichens as miniature ecosystems following the general biological patterns (*Lichen Biology*, 2008).

It should be noted that, as was shown previously (Pankratov et al., 2008), acidobacteria may be involved in polysaccharide hydrolysis. They may be therefore assigned to the hydrolytic component) of the microbial community. Their activity occurs, however, in suboptimal conditions, which results in significant

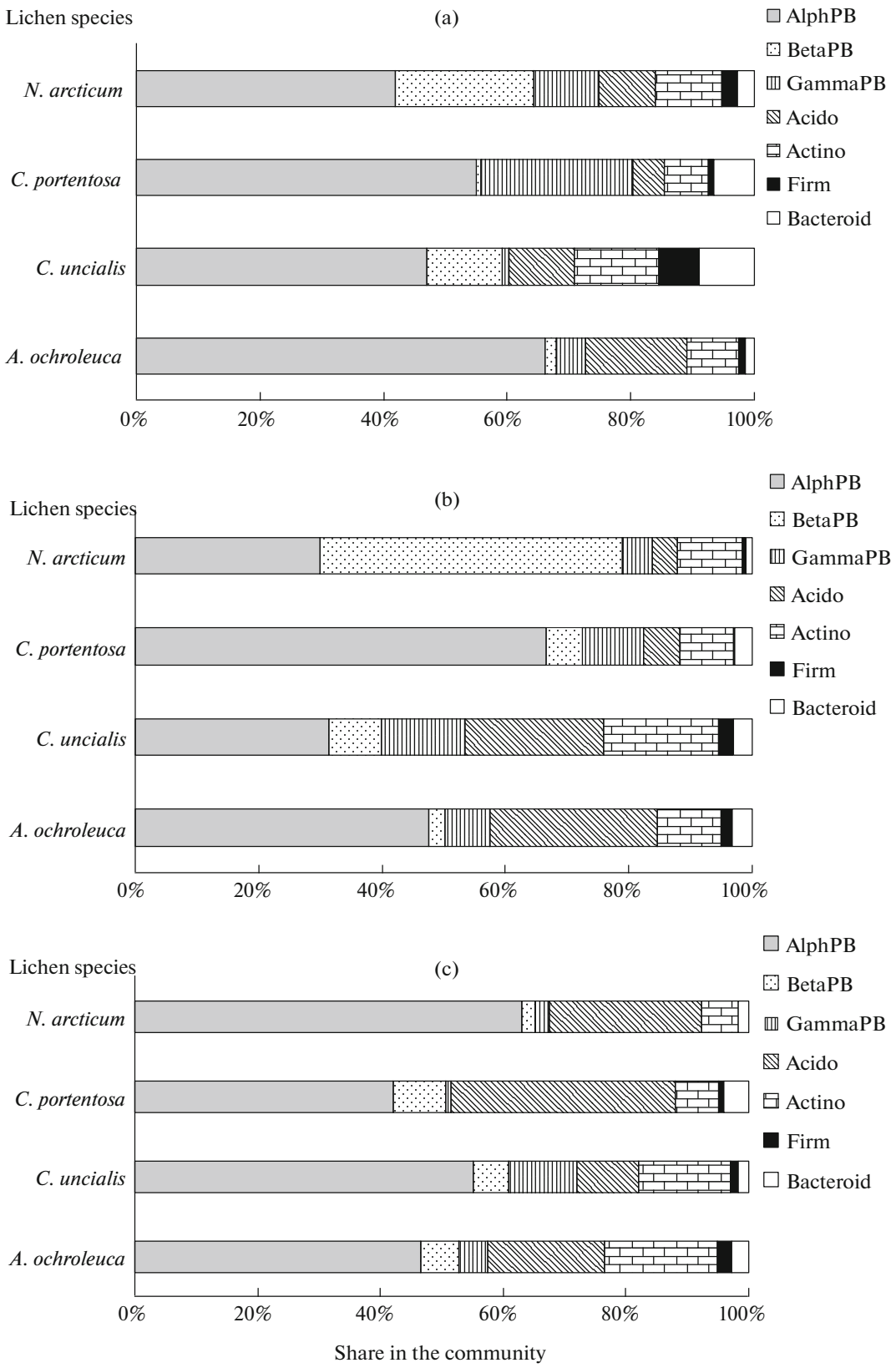

Fig. 3. Distribution of the shares of identified bacterial groups in the epiphytic bacterial communities of four lichen species depending on localization. Designations: AlphBP—*Alphaproteobacteria*, BetaPB—*Betaproteobacteria*, GammaPB—*Gammaproteobacteria*, Acido—*Acidobacteria*, Actino—*Actinobacteria*, Firm—*Firmicutes*, Bacteroid—*Bacteroidetes*, (a) growth zone, (b) zone of aging, (c) degradation area.

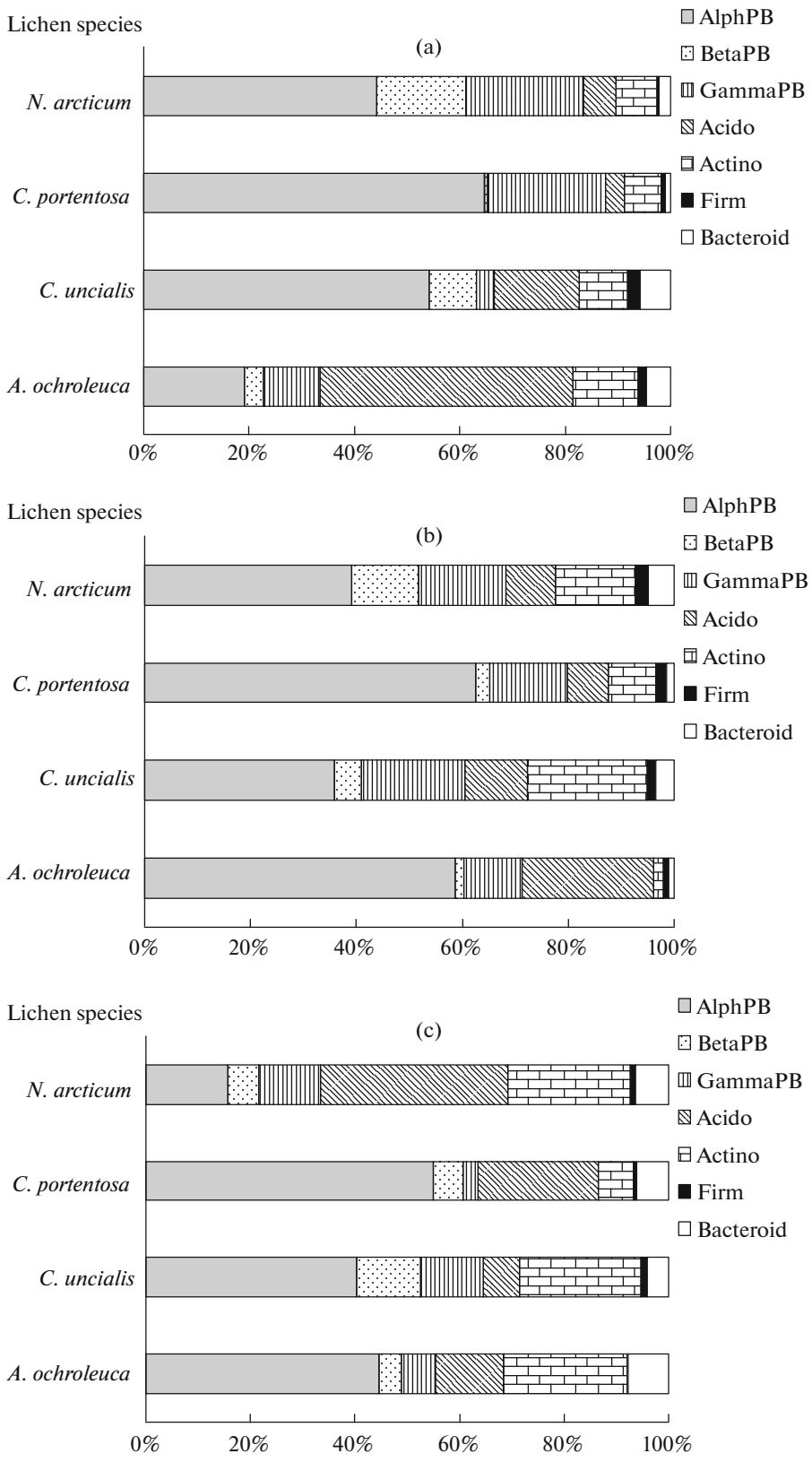

Fig. 4. Distribution of the shares of identified bacterial groups in the endophytic bacterial communities of four lichen species depending on localization. Designations: AlphBP—*Alphaproteobacteria*, BetaPB—*Betaproteobacteria*, GammaPB—*Gammaproteobacteria*, Acido—*Acidobacteria*, Actino—*Actinobacteria*, Firm—*Firmicutes*, Bacteroid—*Bacteroidetes*, (a) growth zone, (b) zone of aging, (c) degradation area.

Table 1. Assessment of dependence of cell numbers of specific bacterial groups inhabiting four lichen species using the linear correlation coefficient ($P \geq 0.95$).

Bacterial groups*	Correlation coefficient			
	Lichen species			
	Alectoria ochroleuca	*Cladonia uncialis*	*Nephroma arcticum*	*Cladonia portentosa*
AlphPB/BetPB	**0.99**	0.17	*−0.10*	**0.90**
AlphPB/GamPB	**0.98**	**0.72**	0.12	0.18
AlphPB/Acid	**0.90**	0.17	**0.79**	**0.89**
AlphPB/Actin	**0.99**	**0.67**	0.30	**0.98**
AlphPB/LGC	**0.87**	***−0.62***	*−0.33*	**0.82**
AlphPB/CFB	**0.99**	0.16	0.35	**0.99**
BetPB/GamPB	**0.99**	0.25	0.01	*−0.18*
BetPB/Acid	**0.94**	0.05	*−0.01*	**1.00**
BetPB/Actin	**1.00**	**0.52**	0.14	**0.97**
BetPB/LGC	**0.91**	*−0.12*	0.08	**0.96**
BetPB/CFB	**1.00**	0.44	0.06	**0.87**
GamPB/Acid	**0.96**	**0.56**	**0.69**	*−0.17*
GamPB/Actin	**0.99**	**0.95**	**0.96**	0.02
GamPB/LGC	**0.93**	*−0.16*	**0.81**	*−0.23*
GamPB/CFB	**0.99**	0.21	**0.96**	0.27
Acid/Actin	**0.92**	0.44	**0.82**	**0.96**
Acid/LGC	**0.99**	0.13	0.29	**0.98**
Acid/CFB	**0.93**	0.04	**0.85**	**0.85**
Actin/LGC	**0.90**	*−0.16*	**0.91**	**0.75**
Actin/CFB	**1.00**	0.33	**0.96**	**1.00**
LGC/CFB	**0.90**	0.51	**0.77**	**0.73**

* Bacteria were enumerated using FISH. Designations: AlphPB—*Alphaproteobacteria*, BetBP—*Betaproteobacteria*, GamPB—*Gammaproteobacteria*, Acid—*Acidobacteria*, Actin—*Actinobacteria*, LGC—*Firmicutes*, CFB—*Bacteroidetes*.

fluctuations of their abundance depending on the lichen species and its developmental stage.

Correlation analysis and calculation of variation coefficients for the shares of the studied bacterial groups in lichen thalli made it possible to suggest existence of the interactions and relations between bacterial groups.

Generalized assessment of dependences between cell numbers of bacterial groups (determined by FISH) inside and on the surface of the thalli of four lichen species proved especially informative (Table 1). Individual profiles of the lichen bacterial communities were revealed. Thus, for *A. ochroleuca*, with the thalli especially rich in sugars, polysaccharides, and protein, high positive correlation was revealed between all studied bacterial groups. According to the literature date, the average content of soluble nutrient fibers (soluble polysaccharides) in this lichen is 44% of its dry mass, while it does not exceed 3% in *Cladonia* species. The levels of lichenan and glucose in this species are 300 and 2–3 times higher, respectively, than in *Cladonia* species (Svihus and Holand, 2000).

Correlation analysis for the other three lichen species revealed dependences between the numbers of the dominant proteobacteria and abundance of the hydrolytic block bacteria (actinobacteria, cytophagas, and bacilli). In *C. uncialis* abundance of most bacterial groups exhibited negative correlation with abundance of the *Firmicutes*, while in other lichen species this dependence was either absent or showed positive correlation between the groups (Table 1). These data also demonstrate close relations within the hydrolytic group, except for the *C. uncialis* community, where competition between *Actinobacteria* and *Firmicutes* occurred, as well as a weak positive correlation between other groups in the block.

Correlation between abundance of the major bacterial groups in washouts and homogenates of four lichen species was positive and high, except for the *A. ochroleuca* growth zone and *N. arcticum* aging and degradation zone (Table 2). This implies that the number of endophytic bacteria depends on the number of epiphytic bacteria, although the character of this dependence is not known. Abundance of endophytic

Table 2. Correlation (correlation coefficient, $P \geq 0.95$) between abundance of the major bacterial groups in washouts and homogenates of four lichen species

Thallus part	Correlation coefficient			
	Lichen species			
	Alectoria ochroleuca	*Cladonia uncialis*	*Nephroma arcticum*	*Cladonia portentosa*
Upper	**0.35**	0.97	0.93	0.99
Medium	0.98	0.89	**0.48**	0.99
Lower	0.97	0.94	**0.38**	0.91

Table 3. Comparison of the correlation coefficients between the numbers of individual bacterial groups in the upper and lower layers of the thalli of four lichen species

Thallus part	Correlation coefficient			
	Lichen species			
	Alectoria ochroleuca	*Cladonia uncialis*	*Nephroma arcticum*	*Cladonia portentosa*
Upper/medium (washout)	0.95	0.73	0.74	0.94
Upper/lower (washout)	0.96	0.94	0.82	**0.57**
Upper/medium (homogenate)	**0.51**	0.72	0.96	0.99
Upper/lower (homogenate)	**0.31**	0.85	**0.04**	0.85

bacteria is probably controlled both by the major components of the symbiosis (myco- and photobionts) and by bacteria inhabiting the thallus surface. Importantly, the differences were observed in the parts of lichens where due to their anatomical features the chemical composition changed. The growth zone of *A. ochroleuca* contains brown pycnidia with the chemical composition different from that of the lower thallus layers and high content of usnic acid. The *N. arcticum* thallus contains two species of photobionts, cyanobacteria *Nostoc* sp. and green algae *Coccomyxa* sp. In the course of aging, the ratio of these components changes and the density of the mycobiont hyphae increases. In this case, the ratio of the numbers of all bacterial groups together is considered, indicating a common pattern for four lichen species.

Correlation analysis of overall numbers of all bacterial groups in the upper, medium, and lower parts of the thallus was carried out to determine the character of relationships between abundance of the studied bacterial groups in washouts and homogenates of different parts of the thalli (Table 3). Based on these data we can conclude that the relationship between endophytic bacterial communities is weakened from the growth zone to the zone of decomposition for *A. ochroleuca* and *N. arcticum*, with the correlation coefficients of 0.51, 0.31, and 0.04. In other cases, high positive correlation was observed. The distribution of the correlation coefficients in this case indicated that abundance of the studied bacterial groups varied depending on the individual lichen species.

Analysis of the distribution of the coefficients of variation of the shares of the studied bacterial groups in the microbiomes of the three major thallus zones may be useful for assessment of the stable or facultative occurrence of a given bacterial group in the lichen microbial communities. In mathematical statistics, the value of this coefficient below 20% indicates low data scattering, values from 20 to 33% indicate its significant level, and above 33% the set may be considered heterogeneous. In the case of relative contribution of a phylogenetic group to the composition of a bacterial community, low coefficient of variation (up to 20%) may indicate that this component is constitutive in the community. Intermediate values (20 to 30%) indicate high mobility, while the coefficient exceeding 31% indicates the facultative presence of this group or high heterogeneity of the data. Comparison of abundance of the studied bacterial groups depending on the thallus activity zones revealed individual patterns for each group and for each lichen species (Table 4). In the epiphytic microbiome of *A. ochroleuca*, *Alphaproteobacteria* and *Gammaproteobacteria* were the constitutive groups, while in the endobiont cenosis they were joined by the *Gammaproteobacteria*. Other groups were in most cases facultative. *Acidobacteria* were exceptional in this respect, occupying an intermediate position within the epibiotic community of *A. ochroleuca* (K_v = 22.47%). According to these data, the *Alphaproteobacteria* were the constitutive groups in endobiont communities of *C. uncialis* and *C. portentosa* and in the epibiont community of *C. portentosa*, while *Actinobacteria* were

Table 4. Coefficients of variation (%) for the shares of individual bacterial groups (identified by FISH) in lichen bacterial communities.

Lichen sample	Coefficient of variation, %					
	Bacterial groups*					
	AlphaBP	BetPB	GamPB	Acid	Actin	Bacteroid
A. ochroleuca						
washout	**17.63**	46.35	**16.64**	22.47	38.79	40.41
homogenate	40.00	40.82	**19.51**	49.69	44.19	61.45
C. uncialis						
washout	22.50	27.22	62.99	40.27	**13.50**	66.24
homogenate	**19.19**	33.09	59.52	31.56	36.00	28.78
N. arcticum						
washout	30.31	78.47	58.23	45.55	25.25	40.82
homogenate	37.44	37.88	24.01	79.36	39.97	39.22
C. portentosa						
washout	**17.29**	58.88	82.50	91.81	**12.30**	36.42
homogenate	**7.20**	61.64	58.84	70.10	**12.30**	88.39

* AlphPB—*Alphaproteobacteria*, BetBP—*Betaproteobacteria*, GamPB—*Gammaproteobacteria*, Acid—*Acidobacteria*, Actin—*Actinobacteria*, Bacteroid—*Bacteroidetes*.

constitutive in the epiphytic microbiome of *C. uncialis* and *C. portentosa* and in the endobiome of *C. portentosa*. Such groups as *Betaproteobacteria*, *Acidobacteria*, and *Bacteroidetes* were found to form a highly variable component of the endophyte and epiphyte microflora of the studied lichen species.

These results make it possible to suggest the absence of specific association of bacteria (at the level of large taxonomic groups) in the lichen microbial communities. Predominance of individual groups can be seen as dependence of their abundance on the stages of thallus development or degradation. As a result of the changes in the chemical composition and anatomical structure of the lichen thallus, hydrolytic bacteria affect all the bacterial pool and the ratio of bacterial groups. As in other ecosystems, group abundance and ratios in bacterial communities depends on availability of the substrates, ambient pH, concentration of oxygen, and the presence of inhibitors of the enzymatic activity. Bacterial abundance may be controlled by such biotic factors as competition for the substrates, antibiotics, and activity of protozoan predators.

The obtained data do not provide sufficient information as to which members of different groups of bacteria demonstrate the obligate or facultative lifestyle in specific lichen species. More detailed analysis of these communities using specific (at the genus or family level) fluorochrome-labeled probes is probably required for the purpose. This work requires, however, isolation of pure cultures of the dominant species, their identification, and primer design. This may be the next stage of the research.

The most important result of the work is determination of criteria for the facultative and obligatory association, i.e., the coefficient of variation for the shares of members of various bacterial taxonomic groups in the epi- and endomicrobiomes, as well as determination of the correlation between abundance of bacterial groups within an statistically homogeneous sample. These criteria may also be used for primary classification and differentiation of microbial communities of various lichen species.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research, project no. 04-16-00966a. The author is grateful to I.A. Timashev for sample collection and transportation and to Yu.Yu. Berestovskaya (Research Center of Biotechnology, Russian Academy of Sciences) for providing the relevant equipment.

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Translated by P. Sigalevich