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EXPERIMENTAL ARTICLES

High Abundance of Planctomycetes in Anoxic Layers of a *Sphagnum* Peat Bog

A. O. Ivanova and S. N. Dedysh[1]

Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia

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Abstract—The depth distribution of planctomycete abundance has been examined in six different sites of the *Sphagnum* peat bog Bakchar, Tomsk oblast, Russia. In situ hybridization of peat with the fluorescently labeled oligonucleotide probes PLA46 and PLA886, reported to be group-specific for representatives of the phylum *Planctomycetes*, revealed two distinct population maxima of these bacteria in all of the profiles examined. The first population maximum was detected in the uppermost, oxic layer of the bog profile, while the second maximum was located at a depth of 30 cm below the water table level. The population sizes of planctomycetes in the uppermost layer and at a depth of 30 cm were of the same order of magnitude and comprised $0.5–1.5 \times 10^7$ and $0.4–0.7 \times 10^7$ cells per g^{-1} of wet peat, respectively. Only 25–30% of the total number of planctomycete cells in the anoxic layer could be detected if the probe PLA886, whose target specificity is restricted to taxonomically characterized aerobic planctomycetes of the genera *Gemmata, Planctomyces, Pirellula*, and *Isosphaera*, was used alone. Other planctomycete cells in this layer were detected only with the probe PLA46, which possesses a much wider scope. This suggests the affiliation of these organisms with a yet undescribed phylogenetic subgroup within the *Planctomycetes.*

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The order *Planctomycetales* (currently making up the phylum *Planctomycetes*) was proposed to accommodate aerobic budding bacteria which possess a distinctive morphology, specific cell ultrastructure, and peptidoglycan-less cell walls and form a deeply branching phylogenetic lineage within the domain *Bacteria* [1, 2]. Initial knowledge of the metabolic and physiological diversity within this group of bacteria was accumulated by studying representatives of the *Planctomycetes* that had been obtained in pure cultures. So far, all of these axenically cultured strains are aerobic chemoorganotrophic organisms. The delusion that all planctomycetes are uniform with respect to their physiology was dispelled by the sensational discovery of anaerobic autotrophic representatives of this bacterial group capable of anaerobic ammonium oxidation—the "anammox" process [3]. Originally, these organisms were found in a pilot wastewater treatment plant, and further application of molecular techniques showed wide distribution of anammox bacteria in marine and estuarine environments [4–6]. The anammox planctomycetes cannot be grown in pure culture yet, but their physiological and metabolic traits have been studied in detail [6].

Recently, we have shown that members of the phylum *Planctomycetes* represent a numerically significant bacterial group in boreal *Sphagnum* peat bogs [7]. Analysis of the depth distribution of planctomycete abundance in the peat bog Bakchar, Tomsk oblast, revealed the highest population size of these bacteria in the uppermost, oxic layer of the bog profile [8]. Interestingly, a sharp decline of planctomycete abundance with depth was followed by a second population maximum at a depth of 30 cm below the water table level. Since this analysis was performed using a single set of samples taken from a singe site within the peat bog Bakchar, the goal of the present study was to verify these intriguing data by taking into account a larger number of individual profiles within this particular wetland.

MATERIALS AND METHODS

The peat samples used in this study were collected in July 2005 from six different sites of oligomesotrophic *Sphagnum* peat bog Bakchar (56°51' N, 82°50' E, Tomsk oblast, South Vasyugan, Plotnikovo

[1] Corresponding author; e-mail: dedysh@mail.ru.

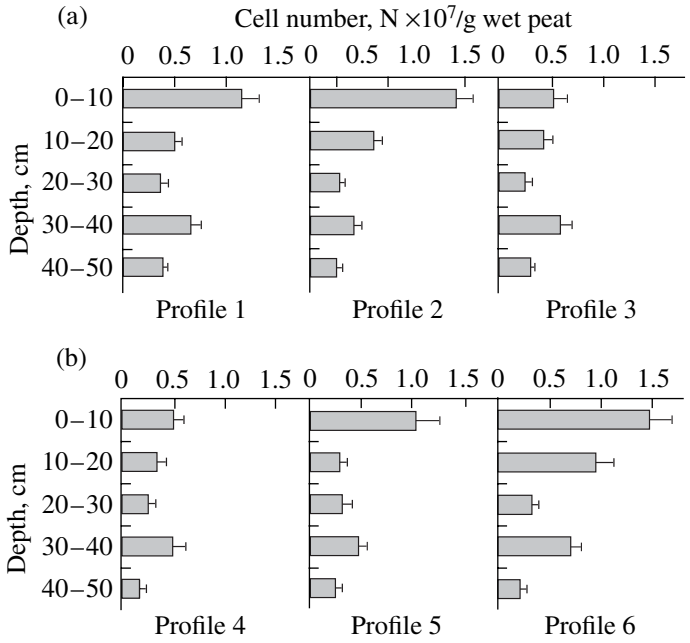

Fig. 1. Depth distribution of cells detected by means of in situ hybridization with the probes PLA46 and PLA886, reported to be group-specific for the phylum *Planctomycetes*, in six different sites of the *Sphagnum* peat bog Bakchar: (a) sites under a *Sphagnum*-dominated plant community; (b) sites under a *Carex*-dominated plant community.

field station of the Institute of Soil Science and Agrochemistry, Siberian Division, Russian Academy of Sciences). The plant communities in three sampling sites were dominated by *Sphagnum* mosses (*Sphagnum angustifolium* and *Sphagnum magellanicum*), whereas the three other sites were in a *Carex*-dominated part of the wetland. The sampling was performed along the peat bog profile to a depth of 50 cm (the water table was approximately at a depth of 0–5 cm). The sampled peat was fixed immediately. To do this, the samples were subjected to homogenization treatment in BagFilter® disposable sterile plastic bags as described previously [7], and the resulting peat suspension was mixed with 100% ethanol (1 : 1, vol/vol) and stored at –20°C until use.

Sphagnum peat sampled at a depth of 30 cm was used for the laboratory experiment on stimulation of planctomycete growth in anoxic conditions. 10 g of peat was placed in 100-ml serum vials and amended with N-acetylglucosamine (0.5 mg per g of wet peat). After this, the vials were closed, thoroughly flushed with sterile dinitrogen, and incubated at 15°C (the temperature characteristic of this peat depth in summer time). The incubations were performed in triplicate. The absence of oxygen in the headspace of vials was checked chromatographically. After four weeks of incubation, samples were taken and fixed for analysis.

The abundance of planctomycete cells in peat was determined by means of in situ hybridization with an equimolar mixture of the 16S rRNA-targeted oligonucleotide probes PLA46 and PLA886 that had been designed for specific detection of members of the *Planctomycetes* [9]. To differentiate the cells of the known aerobic planctomycetes of the genera *Gemmata, Planctomyces, Pirellula*, and *Isosphaera* from other planctomycetes, the probe PLA886 alone was used for hybridization. The probes were synthesized and labeled with Cy3 by Syntol (Moscow, Russia). Hybridization of peat samples with oligonucleotide probes and further staining with the universal DNA stain 4',6'-diamidino-2-phenylindole (DAPI, 1 μM) was performed as described before [10].

The specimens were analyzed using a Zeiss Axioplan 2 microscope (Jena, Germany). The counting of cells hybridized with the probes was performed in 100 randomly chosen fields of view for each sample. This value was further used to calculate the number of planctomycete cells per gram of wet peat. The diameter of cells detected with the probes was measured using the corresponding tool of the Axiovision software package (Jena, Germany). Statistic tests were performed using MS Excel 2000.

RESULTS AND DISCUSSION

Distribution of planctomycetes over the bog profile. Figure 1 shows the depth distribution of planctomycete cells in six different sites of the peat bog Bakchar. Profiles nos. 1–3 correspond to sites under *Sphagnum*-dominated plant community, whereas profile nos. 4–6 correspond to sites within the *Carex*-dominated part of the wetland. The results obtained for these two sets of samples were quite similar. Significant spatial variation of planctomycete abundance was

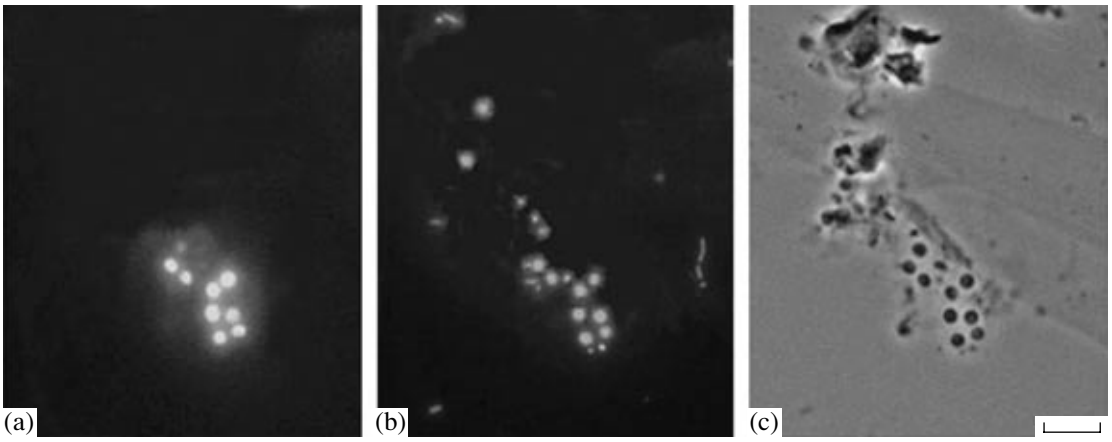

Fig. 2. In situ hybridization of *Sphagnum* peat sampled at a depth of 30–40 cm with fluorescently labelled oligonucleotide probes PLA46 and PLA886, reported to be group-specific for the phylum *Planctomycetes*: (a) epifluorescent micrograph of in situ hybridization with Cy3-labelled probes; (b) DAPI staining; (c) phase-contrast image. The scale bar, 5 μm.

observed for both *Sphagnum*- and *Carex*-dominated sites. The presence of two distinct population maxima of planctomycete development was typical of all six profiles examined. The first population maximum was detected in the uppermost (0–10 cm), oxic layer of the bog profile. The number of cells detected in this layer with the probes PLA46 and PLA886 was in the range of $0.5–1.5 \times 10^7$ cells per g^{-1} of wet peat. A sharp decline of planctomycete abundance in underlying anoxic peat layers was followed by a second population maximum at a depth of 30–40 cm. It is particularly remarkable that the population sizes of planctomycetes at a depth of 30–40 cm were of the same order of magnitude as those in the uppermost layer and comprised $0.4–0.7 \times 10^7$ cells per g^{-1} of wet peat (Fig. 2). In profiles no. 3 and 4, the abundances of planctomycete cells in the uppermost layers were equal to those at a depth of 30–40 cm.

The cell size of planctomycetes in oxic and anoxic zones. The cells of planctomycetes detected in oxic and anoxic zones of the peat bog profile exhibited similar morphology. In both cases, most cells that hybridized with the probes had spherical shape, although the cells in the anoxic zone were slightly smaller. The mean diameter of the cells in the oxic zone was 1.5 ± 0.2 μm, whereas at the depth of 30–40 cm, it was 1.3 ± 0.2 μm.

Differential enumeration of planctomycete cells in the layer of "anoxic maximum." The difference in the target specificity of probes PLA46 and PLA886 allowed us to retrieve additional information concerning the phylogenetic affiliation of the planctomycetes that were detected in the anoxic layer of the peat bog profile. The probe PLA46 possesses a very wide scope and targets all known representatives of the *Planctomycetes*, including anaerobic Anammox planctomycetes and phylogenetically related subgroups, represented by uncultured organisms only. By contrast, the scope of PLA886 is more limited and includes the taxonomically characterized aerobic chemoorganotrophic planctomycetes of the genera *Gemmata, Planctomyces, Pirellula*, and *Isosphaera*. Enumeration of cells detected with the probe PLA886 alone was performed for peat sampled at a depth of 30–40 cm in profiles 1 and 6, which presented the most vivid examples of high planctomycete abundance in anoxic layers. This cell count revealed that only 25–30% of the cells detected by the combination of probes PLA46 and PLA886 in these peat samples were targeted with the probe PLA886 alone. By contrast, the proportion of planctomycete cells detected by PLA886 in the uppermost, oxic layers of these bog profiles comprised 88–95% of the planctomycete cell counts determined by simultaneously use of both probes. Thus, most planctomycete cells in the anoxic peat layer do not belong to the above-listed taxonomically characterized genera within the *Planctomycetes*.

Laboratory experiment on the stimulation of planctomycete growth in anoxic conditions. We attempted to promote growth of planctomycetes in anoxic conditions by means of peat amendment with organic substrate. As the substrate, we used N-acetylglucosamine, since it represents the most universal carbon and nitrogen source for all currently characterized aerobic planctomycetes. The analysis of substrate-amended peat samples after four weeks of incubation showed not only the absence of increase, but even a certain decrease in the population size of planctomycetes. Specifically, the original sample contained $0.7 \pm 0.1 \times 10^7$ planctomycete cells per g^{-1} of wet peat, while after anoxic incubation, their number decreased to $0.4–0.6 \pm 0.1 \times 10^7$ cells per g^{-1} of wet peat.

In summary, this study convincingly confirmed the existence of a second maximum of planctomycete abundance in the anoxic part of the peat bog profile. The nature of the planctomycetes that inhabit this layer of anoxic maximum remains unclear. Those few cells

that hybridized with PLA886 might belong to well-known planctomycete genera such as *Gemmata, Planctomyces, Pirellula*, or *Isosphaera*, since we cannot exclude the possibility that some representatives of these genera are capable of growth in anoxic conditions. Detection of *Pirellula*-like planctomycetes among the actively metabolizing community members in a freshwater sediment is indirect evidence in favor of this possibility [11]. In addition, nucleotide sequences of the 16S rRNA genes of representatives of the genera *Pirellula* and *Planctomyces* were recently detected in the suboxic zone of the Black Sea [12]. Nonetheless, in the case of a *Sphagnum* peat bog, most planctomycete cells in the anoxic layer did not hybridize with the probe PLA886 but were detected only with PLA46, which possesses a much wider detection scope. This suggests the affiliation of these peat-inhabiting anaerobic planctomycetes to one of the yet undescribed and uncultured phylogenetic subgroups within the *Planctomycetes*. Due to lack of ammonium, the major growth substrate of Anammox planctomycetes, the occurrence of these organisms in *Sphagnum* peat bogs is highly unlikely. At the same time, in spite of the negative results of our incubation experiment with peat amended with N-acetylglucosamine, the possibility of chemoorganotrophic growth of peat-inhabiting planctomycetes in anoxic conditions is not excluded. Further studies are needed to clarify the question as to the type of metabolism of the planctomycetes that were detected in the anoxic zone of the peat bog profile.

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