The first record of genus *Neocystis* from Kamchatka volcano soils, confirmed by genetic data

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The first record of genus *Neocystis* from Kamchatka volcano soils, confirmed by genetic data

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Abstract. Two strains of green algae having kidney-like cells in mucilage envelope without pyrenoids were isolated during the floristic study on algae in volcanic soil samples collected in the vicinity of Mutnovsky volcano (Kamchatka peninsula, Russia). Based on morphology, the strains were assigned to the genus *Neocystis*. 18S rRNA gene sequence comparisons confirmed, that the new isolates are members of the Neocystis clade. Thus, the genus *Neocystis* was reported from Kamchatka for the first time. The newly isolated *Neocystis* strains have good potential for use in biotechnology as sours of extracellular polymeric substances.

1. Introduction

The genus *Neocystis* F. Hindák originally was described from the freshwater plankton [1], but later it was reported from different terrestrial habitats in several continents including the Antarctic as well [2-4]. Morphologically its members are hardly distinguishable from representatives of many other green coccoid algae and molecular data are necessary for precise identification.

Kamchatka peninsula is one of the most volcanically and seismically active regions of the world that is rich in extreme habitats including volcanic soils. Only ecologically resistant organisms with unique physiology adaptations and the ability to produce biologically active substances can survive there. However, there is very little information on the diversity of soil microorganisms and terrestrial algae from this region.

Here we present data on two strains of green algae from volcanic soils of Kamchatka peninsula with Neocystis-like morphology obtained with a polyphasic approach.

2. Methods

Two strains of green algae were isolated from volcanic soil samples collected at the Mutnovsky volcano slopes (Kamchatka peninsula, Russia). The cultures were established by the enrichment method on the agar medium [5,6]. The strains were cultivated on 1.5% agar-solidified BBM media [7] at 4°C in a refrigerator with a transparent door et natural daylight regime. Observations of algae were conducted by the Zeiss Axio Imager A2 microscope with DIC optics and Axio Vision 4.9 visualization system.

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Microphotographs were taken with an Axio Cam MRc camera on magnification ×1000. For identification of species, relevant key books and publications were used [8,9,3].

For the DNA analysis, the culture was harvested during the exponential growth phase and concentrated by centrifugation. Total genomic DNA was extracted as described before [10] with some modifications [11]. 18S rRNA gene and ITS region were amplified with primers EAF3 and ITS4R as described in detail in previous publications [12] using the Encyclo Plus PCR kit (Evrogen, Moscow, Russia) with a T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, California, USA). The PCR products were purified by ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix, Santa Clara, California, USA) and sequenced in both directions at the Instrumental Centre of Biotechnology and Gene Engineering of Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS using an ABI 3500 genetic analyzer (Applied Biosystems, Foster City. California, USA) with a BigDye terminator v3.1 sequencing kit (Life Technologies Corporation, Austin, Texas, USA). Sequences were assembled with the Staden Package v1.4 [13], aligned manually in the SeaView program [14]. Phylogenetic analysis was performed using RAxML web server version 7.7.1 (http://embnet.vital-it.ch/raxml-bb/) [15].

3. Results and discussion

Among the algae strains, isolated from the Kamchatka volcanic soils near Mutnovsky Volcano, two strains K1 and K2, similar to *Neocystis*, were found. These algae formed groups of kidney-like or elongated cells with length 3,5- 7,2 µm and width 2,5-3,9 µm without pyrenoids in the mucilage envelope (figure 1). However, the same morphology is characteristic for several other genera of algae, for example, representatives of *Nephrodiella* Pascher and *Ellipsoidion* Pascher (Xanthophyceae) [3].

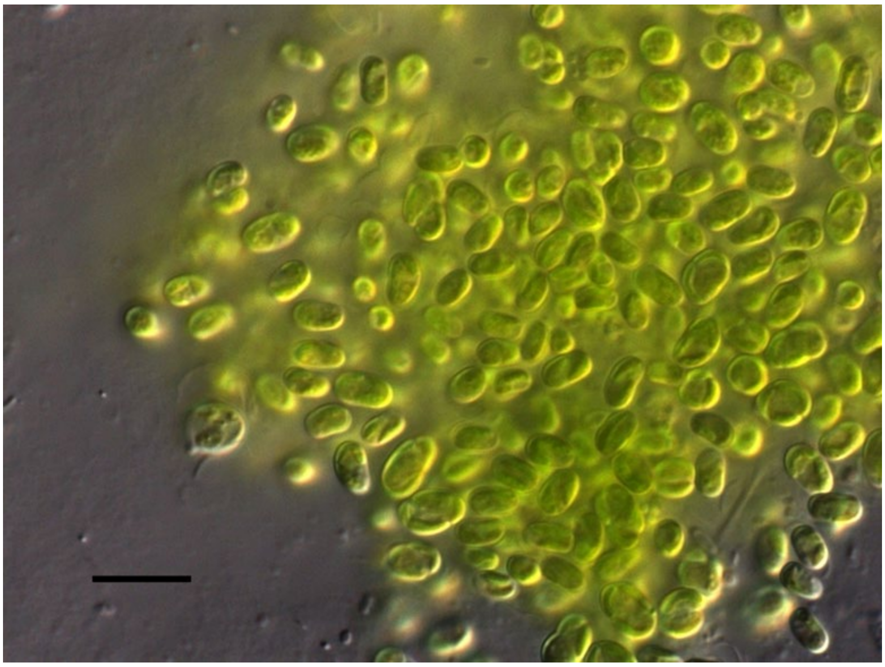

Figure 1. Morphology of strain K1 *Neocystis sp*. Scale bar – 10 µm

Phylogenetic analysis of the 18S rRNA gene reveals, that Kamchatka's isolates are members of the *Neocystis* clade, composed of *N. brevis* and *N. mucosa* (figure 2). As in most phylogenetic analyzes, Neocystis was a sister to the *Prasiola* clade [16-19]. It was suggested, that *Neocystis* could be the basal branch of this clade in fact [20].

Our finding provides new information important for understanding the ecology and biogeography of *Neocystis*. This genus was found in Signy Island in Antarctica (for example, *Neocystis mucosa* JQ920367, *Neocystis mucosa* JQ920366) and in different soils mostly in Europe (*Neocystis brevis*

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JQ920360, *Neocystis brevis* JQ920362, *Neocystis brevis* JQ920364) [3]. Likely, *Neocystis* is widely distributed in terrestrial ecosystems around the world. Resistance to extreme habitat conditions may be due to mucilage production, and the ability to produce biologically active substances. It is known, that strain *Neocystis mucosa* SX synthesize extracellular polymeric substances (EPS) [21]. EPS of green algae possess a wide spectrum of bioactivities, including antifungal and antibacterial effects [22, 23], and may be used for creating the drugs with anti-phytopathogenic activity. We think, that further investigation of physiology and biochemistry of newly isolated from Kamchatka *Neocystis* strains is necessary.

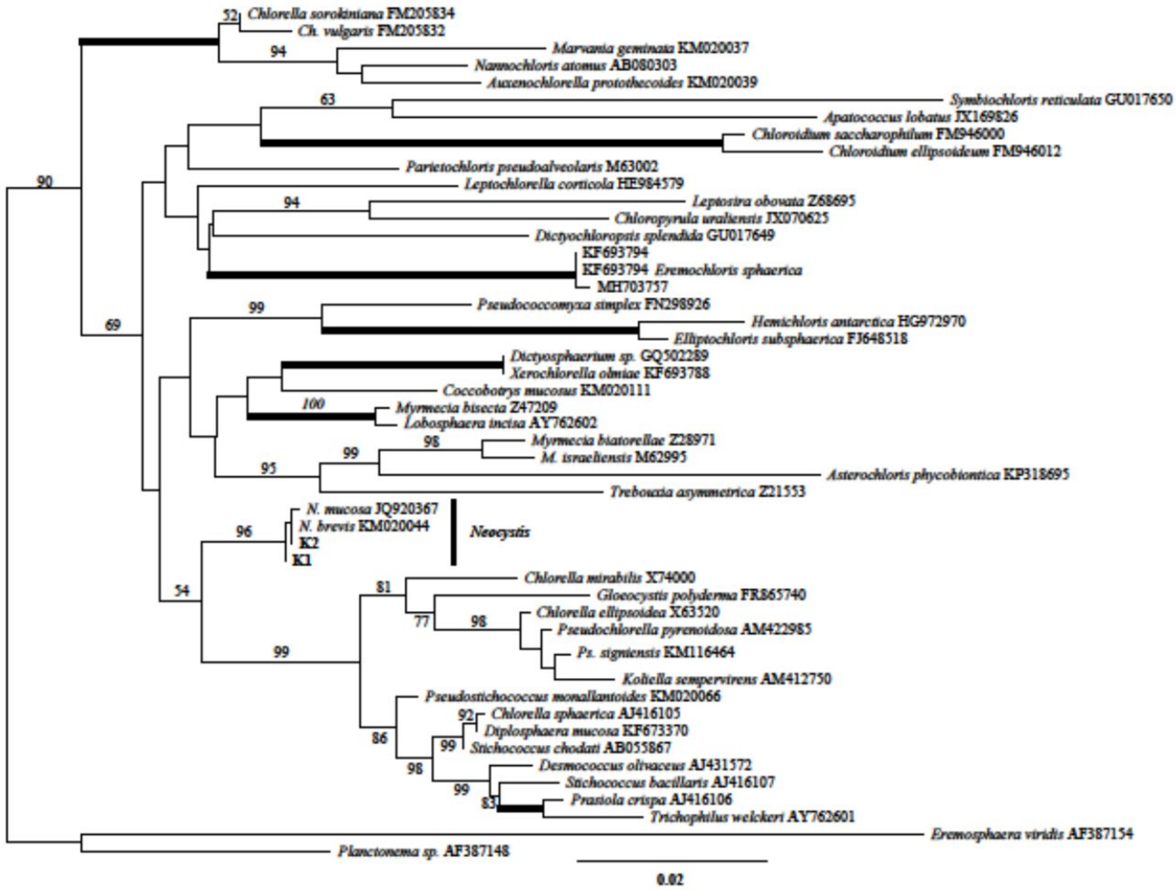

Figure 2. Placement of the genus *Neocystis* in the phylogenetic tree of Trebouxiophyceae, based on a comparison of 50 sequences of 18S rRNA gene (1689 nt) by Maximum Likelihood method in RaxML program (Stamatakis, 2014). Representatives of the fam. Oocystaceae were used as outgroup. Bootstrap values above 50% are shown above or below the branches. Branches with 100% support are shown bold.

4. Conclusion

Our results indicate that a polyphasic approach is important for correct identification of many green algae inhabiting soils. For estimation of real biodiversity of terrestrial algae broad study of many strains, including representatives of genus *Neocystis*, is necessary. Analysis of molecular data is an essential tool for assessing the biodiversity of terrestrial algae.

The polyphasic approach allows us to record the representatives of genus Neocystis in volcanic soils of Kamchatka peninsula. The newly isolated *Neocystis* strains may be used in biotechnology as a source of biologically active substances, for example, extracellular polymeric substances.

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