17. Both sand particles and organic substances enhance activity of free-living, nitrogen-fixing bacteria inhabiting soil of permafrost terrain in East Siberia

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Abstract

Despite boreal forests produce large biomass, available nitrogen in the soil is poor because of low mineralizing activities of the soil microorganisms. Nitrogen imbalance in boreal forest due to low temperature has, thus, long time been a mystery for soil scientists and forest ecologists. In 2002, a research group of Swedish University of Agricultural Science has reported that cyanobacteria symbiotically attaching on the surface of a ground-covering feather moss play an important role in nitrogen supply to the boreal forest in Sweden.[1)] In Siberian boreal forest throughout East Siberian plain where Siberian larch (*Larix gmelinii*) is a predominant tree species on the permafrost area, the nitrogen balance suggests the presence of unknown nitrogen source of the forest ecosystem. The East Siberian forest is heathland forest, mainly composed of *L. gmelinii* and heather (*Vaccinium vitis-idaea*) as ground-covering vegetation, so the forest bed is poor in moss carpets, unlike the Swedish boreal forest. Hence, it is important to search and characterize main nitrogen-providing source in the heathery boreal forest in order to maintain and rehabilitate the permafrost forest ecosystem.

Using soil microflora obtained from sandy soils sampled at Spasskaya Pad and Viluy in 2003 and 2004, their nitrogen-fixing activity was analyzed by means of C_2H_2 reduction assay as a preliminary investigation. When soil suspension was inoculated to nitrogen-free, soft gel medium (Winogradsky's mineral mixture, 1.5% glucose, and 0.3% gellan gum at pH 6.2), many bacteria emerged in the medium. Their C_2H_2 reduction activity was, however, trace and beside did not show any reproducibility. We, hence, added some materials (such as autoclaved sandy soil and/or extracted chemicals from the litters of the plants there) to imitate natural soil conditions. When 1 mg of MeOH extract prepared from *Vaccinium vitis-idaea* root mat was added to 10 ml of the nitrogen-free soft gel medium, 2 samples out of three replications showed a burst activity of ethylene (C_2H_4) production. The C_2H_2 reducing activity of the test microorganisms was dependent to both organic chemicals of plant species and the sample soils prepared from different depth, but these effects were generally unstable. On the other hand, when autoclaved sand particles were added to the soil bacteria-inoculated soft gel medium, some test samples showed more stable, and reproducible ethylene (C_2H_4) production. When the sand particles from the sample soil were replaced with sea sand particles commercially available, clear nitrogen-fixing abilities of the soil microorganisms were also apparent. Light microscopic observation revealed that bacteria in the active vials actively colonized on the surface of sand particles. Taken together, we hypothesized that oligotrophic, nitrogen-fixing soil bacteria require both organic substances (for chemical stimulants or carbon source) and scaffold (for biofilm formation) to provide mineral nitrogen to boreal forest ecosystem on permafrost terrain. Partial 16S rRNA gene sequence determination for the culturable, oligotrophic bacteria suggested that major nitrogen-fixer is morphologically unique-shaped b-proteobacteria close to a group of genus *Burkholderia*.

1) Deluca TH, Zackrisson O, Nilsson MC, Sellstedt A. Nature, 419: 917-919 (2002).