

Investigation of nitrogen-fixing potential in soil bacterial microbiota from Lapland boreal forest limit

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Abstract

In forest ecosystem of high latitude or high elevation, the nitrogen cycle has not been elucidated, although nitrogen-fixing bacteria may play an important role in forest bed soil for nitrogen supply. In this study, bacterial nitrogen fixation in soil of Northern high latitude area in Pallas, Lapland (68°N, forest limit) was investigated, and effective of incubation conditions for nitrogen fixation such as concentration of carbon source in medium and temperature, were examined. Although these bacterial microflora did not show any nitrogen fixation in a medium containing 1.0% carbon source, a concentration that is widely used for rhizobia and other diazotrophs, the Lapland soil showed nitrogen-fixing abilities at relatively low (0.3–0.02%) concentration of carbon source. DNA was extracted from the soil and from cultured bacteria which were incubated in nitrogen-poor medium under alternative conditions. Using DGGE (denaturing gradient gel electrophoresis) technique, analyses for bacteria community of soil of high latitude forest limit area is undertaken in order to reveal specific features of the nitrogen-fixing bacterial community.

Key Words

Nitrogen fixation, boreal forest limit, soil bacteria, gellan gum soft gel.

Introduction

Although the ecology and distribution of boreal forest limit have been studied for a long time, the factors that prevent tree growth and forest establishment at the boreal forest limit are not fully understood. Nitrogen is often considered as one of the most important factors for primary production in terrestrial plants (Vitousek and Howarth 1991), especially in high latitudes, where soils are exposed to cold temperature and are subject to leaching of organic matter during summer season. Previous studies have emphasized that annual nitrogen mineralization failed to account for the annual nitrogen demand of plants in boreal forest ecosystems because of low temperature (Kielland 1994; Schimel and Chapin 1996). Recently, Kielland (1994) suggested that organic nitrogen, particularly dissolved amino acids, constitute a large portion of the nitrogen budget for plants in boreal forest limit ecosystems, but the source of the organic nitrogen itself has not been clarified yet. In boreal Scandinavian spruce forests, rate of decomposition is also low but cyanobacterium inhabiting the ubiquitous feather moss covering the forest bed is thought to be a nitrogen supplier (Delca *et al.* 2002).

In contrast, in studies on nitrogen fixation in high latitude or permafrost area where moss carpet does not develop, the nitrogen cycle has not completely been clarified. In the case of East Siberian forest, soil microbiota and free-living nitrogen-fixing bacteria in the active layer showed relatively high acetylene reduction in soilless medium solidified with gellan gum (Hara *et al.* 2009). Furthermore, the soil microbiota of East Siberian forest bed also showed higher acetylene reduction under conditions similar to soil environments compared to some conventional methods for rhizobia or tropical diazotrophs (Hara *et al.* 2010).

In this study, we measured nitrogen-fixing capability of soil bacteria sampled from a boreal forest limit ecosystem in Pallas, Lapland and investigated effects of culture conditions, including molecular species and concentration of carbon sources for nitrogen fixation.

Methods

Soil sampling and site description

Spruce forest bed soils were collected in mid-September 2009 from the Pallas, Lapland, northern part of Finland. In the Pallas National Park (68°02'N, 24°04'E, alt 710 m), high altitude boreal forest limit area, organic layer (O-horizon), podzol soil (E-horizon) and mineral soil (B-horizon) were sampled in three sites. As a reference, organic layer (O-horizon) and mineral soil (A- and B-horizon) of artificial larch forest established on volcanic sand in Tomakomai, Japan (42°39'N, 141°47'E, alt 25 m) were sampled. These soils were kept at 4°C until use.

Culture medium and bacterial inoculation

Winogradsky's nitrogen-poor mineral medium was prepared as described in previous study (Hara *et al.* 2009). In the current study, D-mannitol or a mixture of D-fructose, D-glucose, sucrose, D-mannitol, succinic acid, DL-malic acid, molar ratio of 2: 2: 2: 2: 1: 1, were used as carbon source as mimicking root exudates (Bürgrman *et al.* 2006). pH of the solution was adjusted to 5.0 with 2M H₂SO₄ approximate soil pH, and the solution was solidified with 3 g/L gellan gum.

Incubation and acetylene reduction assay

In the acetylene reduction assay, 300 mg of soils from each horizon were incubated in 10 mL medium in 30-mL gas chromatographic vial. After 7-day pre-incubation, a 10% volume of acetylene gas was injected in headspace and the culture medium was further incubated for more 3 days. Headspace gas containing ethylene gas converted from acetylene was analysed by gas chromatography.

WSOC (water-soluble organic carbon) and pH

For WSOC, 2 g of each soil was mixed with 20 mL of MilliQ water and shaken for 1 h at 130 rpm. To measure the organic carbon, extracts were filtered by 0.45 µm filter and content of organic carbon of them were measured. For pH measuring, 3 g of each soil was mixed with 15 mL of deionized water and shaken for 30 min by hand, and pH of suspensions were measured.

Results

Although all the soils from every B-horizon showed acetylene reduction, those from podzol (E-horizon) showed trace activity. In appropriate carbon source selection, each soil bacterium showed higher activity in 0.05% carbon source mixture than in 0.05% D-mannitol (max of site 2 B2-horizon soil, 0.39 µmol C₂H₄/d/vial for carbon source mixture cf. 0.058 µmol C₂H₄/d/vial). Soils from B-horizon of site 2 were tested for acetylene reduction assay at different concentrations (0.01, 0.03, 0.1, 0.32, 1.0%) of the carbon source mixture. Soils from the B-horizon and B2-horizon showed activity at 0.03, 0.1, 0.32% of the carbon source mixture, but did not at 1.0% (Figure 1A). On the other hand, soil of A-horizon from Tomakomai with richer supply of organic matter from litter fall showed the highest activity at 1.0% carbon source in the medium (Figure 1B). In further investigation, soils of B1-horizon and B2-horizon showed relatively high activity in 0.02% carbon source containing medium (B1-horizon, 0.63 µmol C₂H₄/d/vial; B2-horizon, 0.46 µmol C₂H₄/d/vial).

Table 1. Characteristics of the soils.

	Horizon	Depth (cm)	WSOC (ppm)	pH
Pallas site 1	O	-20 ~ -10	-	3.86
	H	-10 ~ 0	-	4.62
	E	0 ~	47.17	4.91
Pallas site 2	O	-2 ~ 0	-	4.13
	E	0 ~ 5	32.29	4.93
	B1	5 ~ 10	10.31	5.12
	B2	10 ~	10.57	5.20
Pallas site 3	O	-3 ~ 0	-	4.04
	E	0 ~ 8	45.68	4.73
	B	8 ~	43.67	5.01
Tomakomai	O	-5 ~ 0	-	5.53
	A	0 ~ 20	43.31	5.64
	B	20 ~	16.85	6.58

Conclusion

As revealed by the incubation method used in this study, soil bacteria from B-horizon in boreal forest limit area showed relative high nitrogen fixation. In this study, the highest performance for the nitrogen fixation was brought at low concentration (0.02-0.3%) of carbon source mixture, which is imitated root exudation. In the Tomakomai larch forest soil, soil bacteria from the A-horizon, which is considered to be relatively nutrient rich due to rich litter production, showed the highest activity in the medium containing 1.0% carbon source mixture, while samples obtained from the B-horizon showed activity only at low concentration of carbon source, similar to those from boreal forest limit. These results indicate the importance of low concentration of carbon source, imitating the local soil conditions. Not only carbon source including its components, but also incubation temperature, medium pH and other physical and chemical conditions are

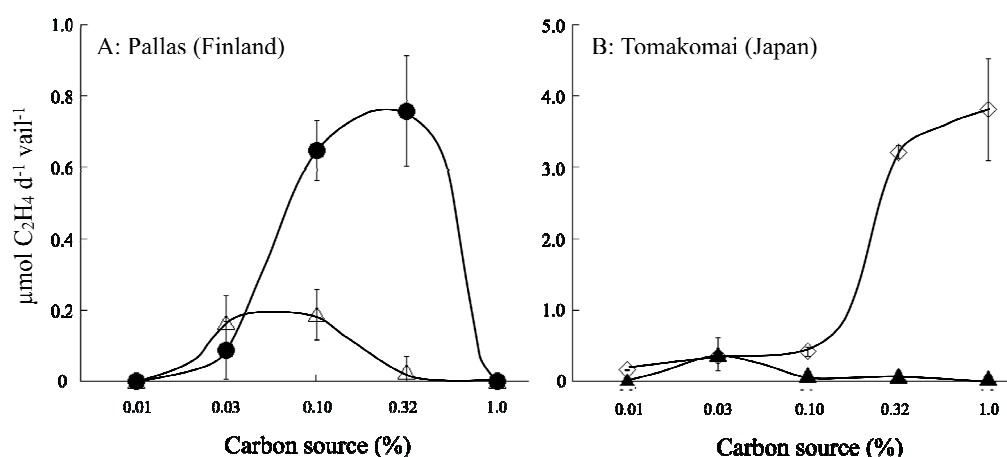


Figure 1. Acetylene reduction under altered carbon source concentration. Soil bacterial microbiota from B-horizon of Pallas (Finland) and those from A- and B-horizon of Tomakomai (Japan) were incubated in medium containing 0.01, 0.03, 0.10, 0.32 or 1.0% concentration of carbon source mixture (n=3, ±SD). (A): site 2 forest limit in Pallas (Finland), filled circle (●) and open triangle (Δ) are B1- and B2-horizon soils respectively. (B): larch forest in Tomakomai (Japan), open rhombus (◊) and filled triangle (▲) are A- and B-horizon respectively.

possible environmental factors characteristic of the location and highly affective on nitrogen fixation. Therefore, effect of these conditions on nitrogen fixation should be tested. To clarify the relationship between microbial succession and culture/or soil conditions, bacterial composition should be examined by means of metagenomic analysis using DGGE techniques targeted at bacterial 16S rRNA gene. Sampling should include soil microbiota extracted directly from the soil and from the soil microbiota culture medium for acetylene reduction assay.

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