Table S1. Identified soil bacteria obtained as culturable on MWA' containing 0.25% D-glucose and 0.25% DL-malic acid

Soil / Isolate	V5	V10	V20	V30
G'1	*Stenotrophomonas* sp.	NI	*Arthrobacter* sp.*	*Paenibacillus* sp.
G'2	*Pseudomonas* sp.	*Luteibactor* sp.	*Leifsonia* sp.*	*Leifsonia* sp.
G'3	*Stenotrophomonas* sp.	NI	*Leifsonia* sp.*	*Burkholderia* sp.
G'4	*Pseudomonas* sp.	*Burkholderia* sp.	*Paenibacillus borealis**	*Burkholderia* sp.
G'5	*Leifsonia* sp.	NI	*Leifsonia* sp.	*Leifsonia* sp.
G'6	*Pseudomonas* sp.	*Burkholderia* sp.	Microbacteriaceae	Micrococcineae
G'7	*Janthinobacterium* sp.	NI	*Paenibacillus* sp.	*Burkholderia* sp.
G'8	*Pseudomonas* sp.	*Leifsonia* sp.	*Arthrobacter* sp.	*Paenibacillus* sp.
G'9		*Burkholderia* sp.	*Microbacterium* sp.	NI
G'10		*Pseudomonas* sp.	*Arthrobacter* sp.	NI
G'11		*Pseudomonas* sp.	*Arthrobacter* sp.	
G'12		*Burkholderia* sp.		

Table footnote

Conditions and methods for isolation of bacteria on MWA' were the same as for Table 4, except 1.5% agar for gel matrix. Distinguishable colony-forming bacteria in the plate culture were isolated as many as possible. Isolates were preliminary identified with their 16S rRNA gene regions partially sequenced (approximately 150 pb containing the V6 region of their 16S rRNA gene). Note that MWG'-culturable bacteria of genera *Janthinobacterium, Paenibacillus, Arthrobacter* and *Luteibacter* obtained were all minor isolates.

Table S2. Effect of gellan gum as matrix to increase acetylene reduction of microfloral communities in Shizunai farmland Andisol.

Source	Medium (0.05% D-mannitol-containing Winogradsky's mineral mix.)		
	Liquid	0.2% agar soft gel	0.3% gellan gum soft gel
	Activity as nmol d^{-1} of C_2H_4		
Shizunai 5	0 ± 0	0.7 ± 0.6	42.7 ± 20.1
Shizunai 10	0.6 ± 1.3	0.5 ± 1.1	26.0 ± 3.4
Shizunai 15	2.4 ± 1.4	3.2 ± 0.2	308.0 ± 412.9

After preculturing for 14 days, acetylene gas was injected to the headspace of each culture vial to a 10% concentration (v/v), and the culture was further incubated for 7 days at 15°C. Three different conditions (liquid, 0.2% agar, and 0.3% gellan gum) for Winogradsky's medium (0.05% D-mannitol as carbon source) were tested and incubation was as previously described for acetylene reduction. Values are means and standard deviations for five replicates.

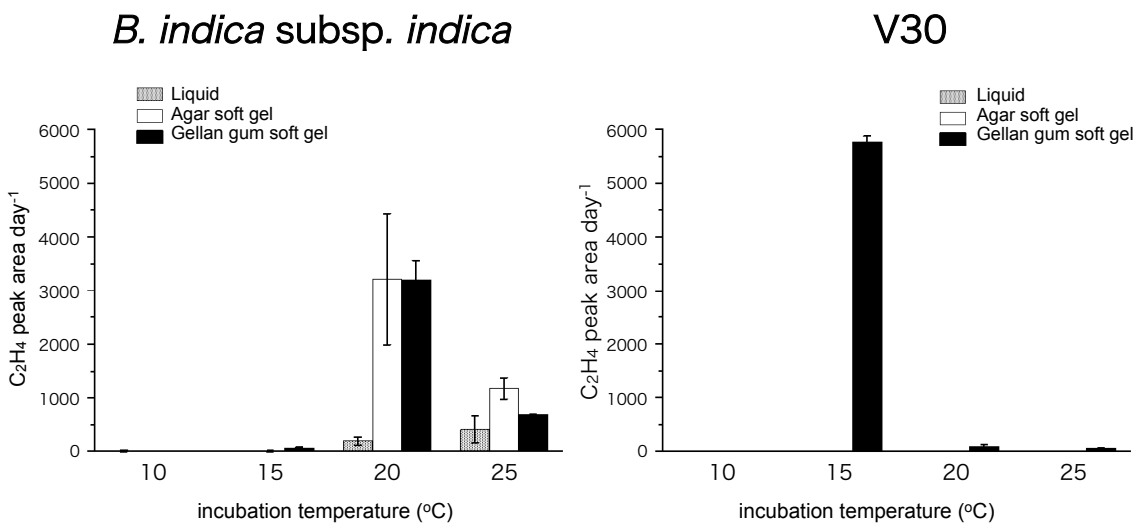
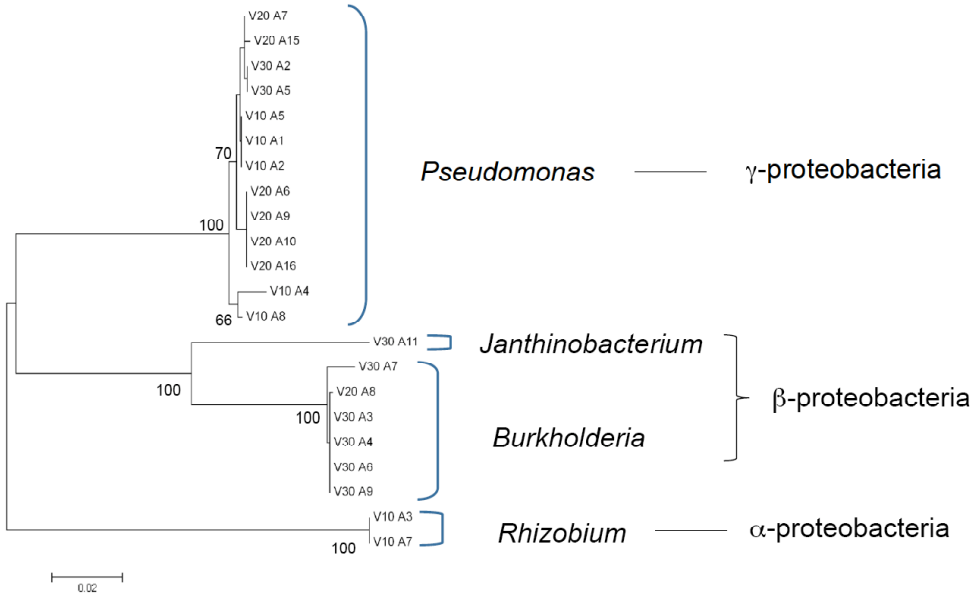


Fig. S1. Appropriate temperature for N_2-fixation by cryophilic bacterial microbiota

Responses of *Beijerinckia indica* subsp. *indica* IFO 3744 and V30 microfloral community to incubation temperature. The most active bacterial microbiota sample from East Siberian Taiga forest bed soil, V30, showed high acetylene reduction at 15°C when cultured in a gellan gum soft gel. This representative response of V30 as a cryophilic microbiota was also shown at higher temperature. Even in gellan gum medium, V30 failed to exhibit acetylene reduction at either 20°C or 25°C. Thus, the profitable temperature range for acetylene reduction of cryophilic bacterial microbiota was narrow, near 15°C. In contrast, *B. indica* subsp. *indica* IFO 3744, used as a reference bacterium for free-living N-fixers, showed higher acetylene reduction at 20°C and 25°C, but low at 15°C. In addition, this bacterium showed almost the same level of acetylene reduction in agar and gellan gum.

A: MWA-grown bacteria



B: MWG'-culturable bacteria

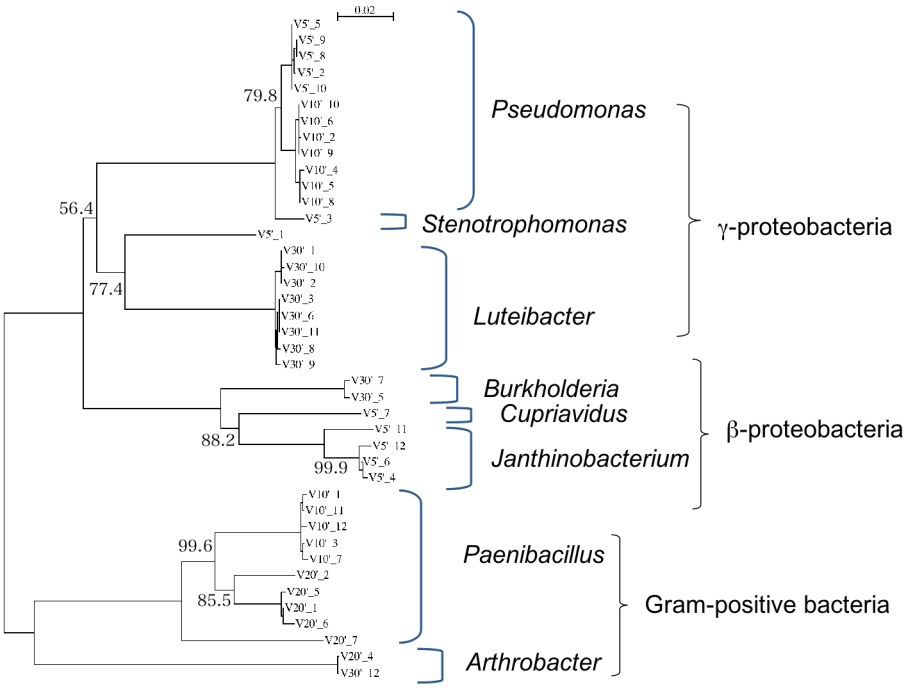


Fig. S2 Phylogenetic alignment for 16S rRNA gene sequences of the soil bacteria from East Siberian forest bed soil

Phylogenetic trees were constructed for the bacterial isolates obtained from 1.0% sucrose-containing MWA plates (**A**) and 0.25% D-glucose plus 0.25% DL-malic acid-containing MWG (MWG') plates (**B**). For the phylogenetic analysis, Clustal W was used. In this program, 1.36 pkb (positioned from 86 to 1443 of *Escherichia coli*) of the 16S rRNA gene sequences among the collections of MWA-grown bacteria was compared. Genera *Pseudomonas* and *Burkholderia* formed two large groups among the isolates on MWA plate culture. These two genera are diverse in bacterial species based on their clustering in tree of **A**. A 1.24 kbp region (positioned from 105 to 1345 of *E. coli*) of the 16S rRNA gene sequences was used in the clustering for the MWG'-culturable bacteria as shown in tree **B**. A cluster of *Pseudomonas* spp. shows that the soil bacterial microbiota of samples is diverse at the species level of genus *Pseudomonas*. Genera *Luteibacter* and *Paenibacillus* show a similar tendency.

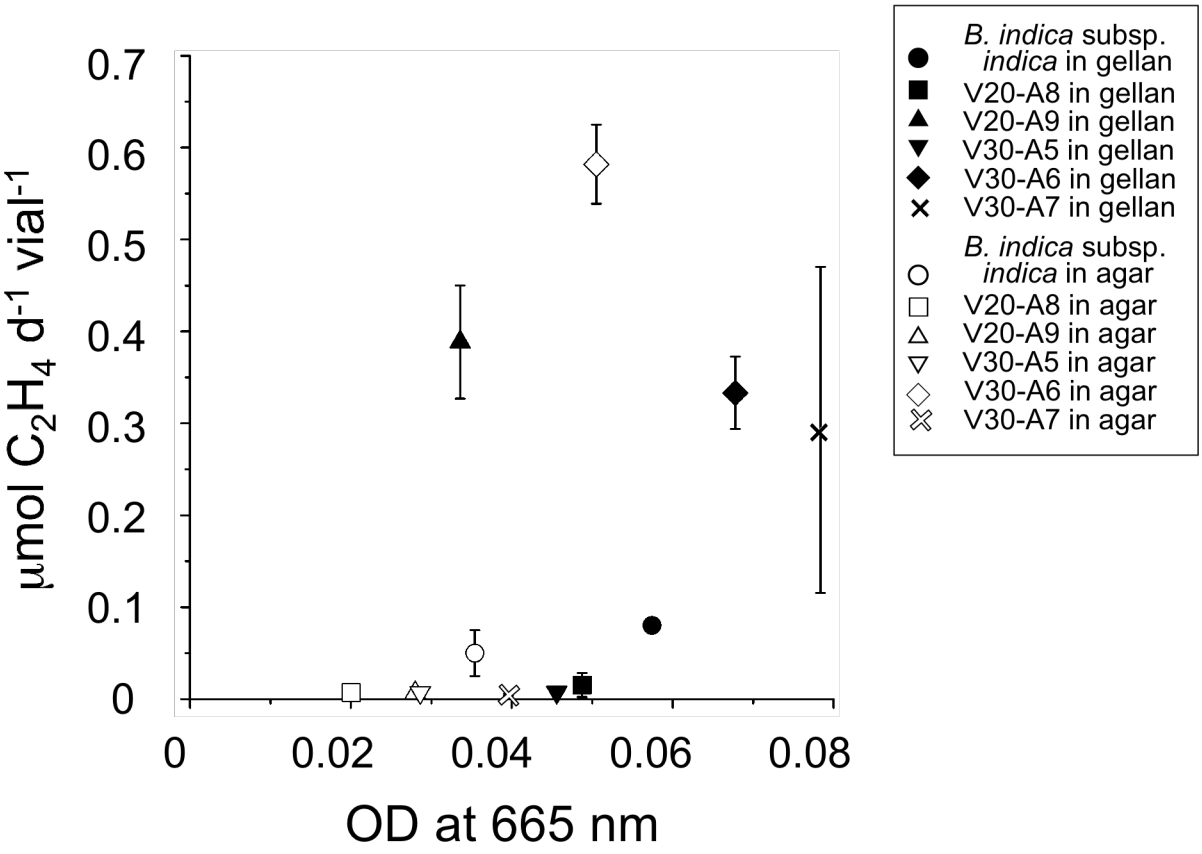

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
µmol C_2H_4 d^{-1} $vial^{-1}$
0
0.02
0.04
0.06
0.08
OD at 665 nm
B. indica subsp. *indica* in gellan
V20-A8 in gellan
V20-A9 in gellan
V30-A5 in gellan
V30-A6 in gellan
V30-A7 in gellan
B. indica subsp. *indica* in agar
V20-A8 in agar
V20-A9 in agar
V30-A5 in agar
V30-A6 in agar
V30-A7 in agar

Fig. S3. Validation of correlations between acetylene reduction and bacterial cell growth (OD at 665 nm)

In this preliminary experiment, we used pure isolates to show acetylene reduction-positive responses. Acetylene reduction of the tested bacteria in gellan gum medium is shown with filled symbols, while open symbols indicate those cultured in agar medium. The assays for each bacterial isolate were replicated five times, but the OD was not very consistent because of the presence of the gel in the medium. Nevertheless, this figure shows that some bacteria, particularly those cultured in gellan gum, showed slight positive correlation between OD and acetylene reduction. This is reasonable because bacteria that are able to grow in nitrogen-deficient medium beyond an OD of 0.05 at 665 nm are most likely diazotrophic bacteria. However, a few exceptions are also observed. For example, V20-A9 (*Pseudomonas* sp.) showed a relatively low OD, but it showed relatively high acetylene reduction in gellan gum medium. Another remarkable bacterium was V30-A6 (*Burkholderia xenovorans*), which uniquely both grew in agar and gellan gum media and had the highest acetylene reduction among tested bacteria. OD at 665 nm was measured in 96-well plates, in which 200 μl of agitated gelling medium had been poured (depth of the medium, 5.2 mm), using a CENios microplate reader (Tecan, Männedorf, Switzarland). The value was used without any correction for the light path length. Because there was less-thick medium in the well, OD values were relatively low, but the standard curve gave reliable values.