

Isolation and Identification of Anaerobic Bacteria from Arctic Tundra Soils



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Abstract

The Korea Polar Research Institute (KOPRI) has operated the Polar and Alpine Microbial Collection (PAMC) since 2012. Although, PAMC holds approximately 7,000 strains of bacteria, not many of the strains were isolated from anaerobic conditions. The soil samples were collected from a total of 36 sampling sites in tussock tundra of Council, Alaska. The soil samples were stocked in 15% glycerol, and transported to KOPRI under frozen and anaerobic conditions. The inoculation of bacteria was performed in the anaerobic chamber, using TSA, R2A and ISP4 as the isolation media. The culturing temperature was 20°C. After sub-culturing, the isolated strains were stocked in 40% liquid glycerol and stored at -80°C. The revival test was performed on the anaerobic bacteria that had been stocked for 2~3 months. The strains that had passed the revival test were stored in PAMC. Total of 209 strains have been isolated, among them, 127 strains have been identified on the basis of 16S rRNA genes. They belonged to 14 genus and 27 species, with more than half being *Paenibacillus*. Most of the strains were facultative anaerobic strains except an obligate anaerobe *Clostridium algidixylanoyticum*. The revival test and identification of the remaining strains are still under process, and taxonomic studies for candidate strains for new species.

Procedure

Isolation of bacteria under anaerobic condition



Preservation of Isolated stock (-80 °C)



Revival test and identification confirmation

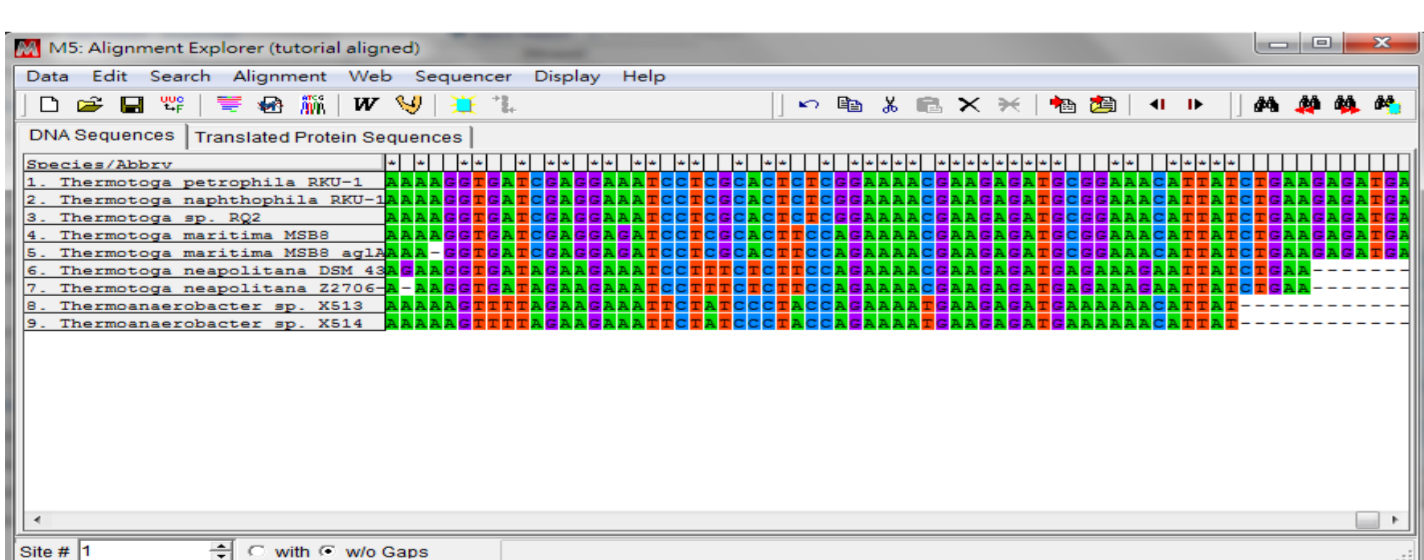


Registration for PAMC



Anaerobic Culture System

- Culture procedure was carried out in anaerobic chamber.
- Used media: TSA, R2A, ISP4, etc. with concentration modification
- The culture plates are place in an anaerobic jar.
- Incubation temperature: 4, 10, 15 and 20°C



Identification

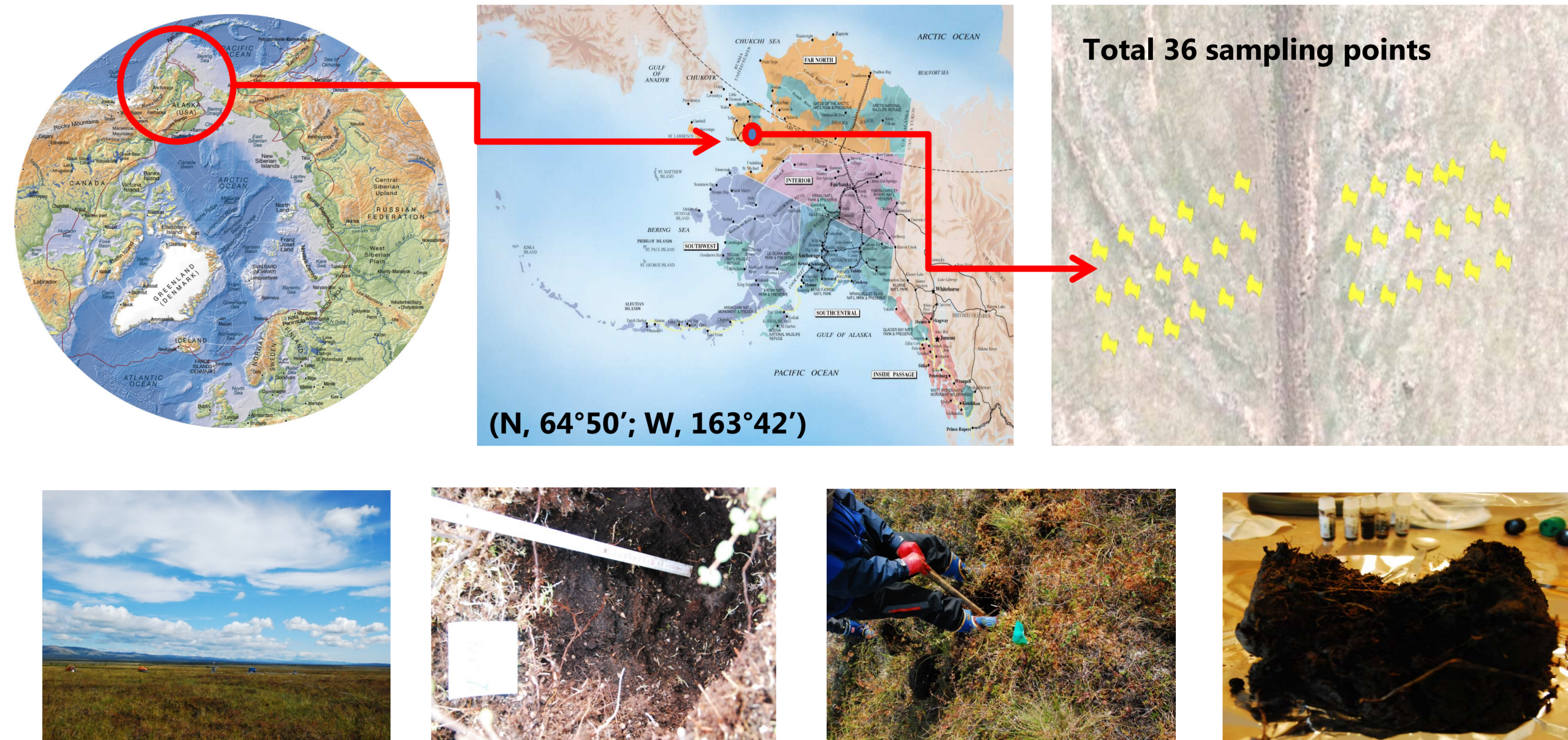
- 16S rRNA gene sequencing (27F & 1492R)
- Using EZ-taxon and Mega



Stock preservation & PAMC registration

- Storage solution: 5% DMSO or 40% Glycerol
- Storage temperature: -80°C
- PAMC supported liquid nitrogen (LN₂) tank for backup
- Databases (information of sample & species identity) uploading to PAMC
- More information: pamc.kopri.re.kr

Sampling area



Results and Discussion

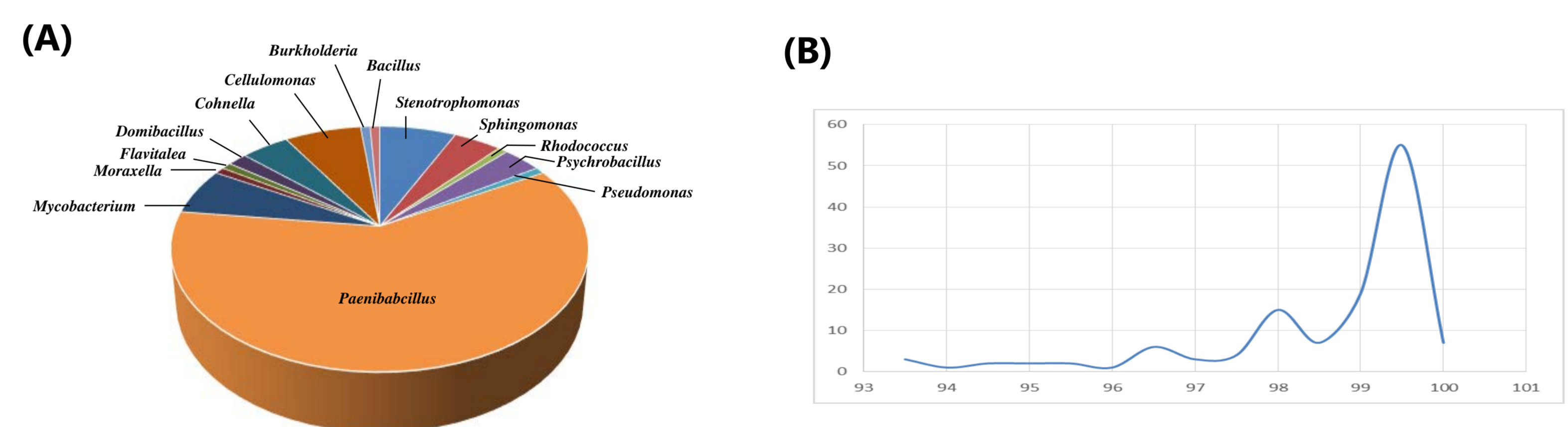


Fig. 1. The community structure of the isolates at genus level (A) and sequence similarity of the isolates analyzed in this study (B).

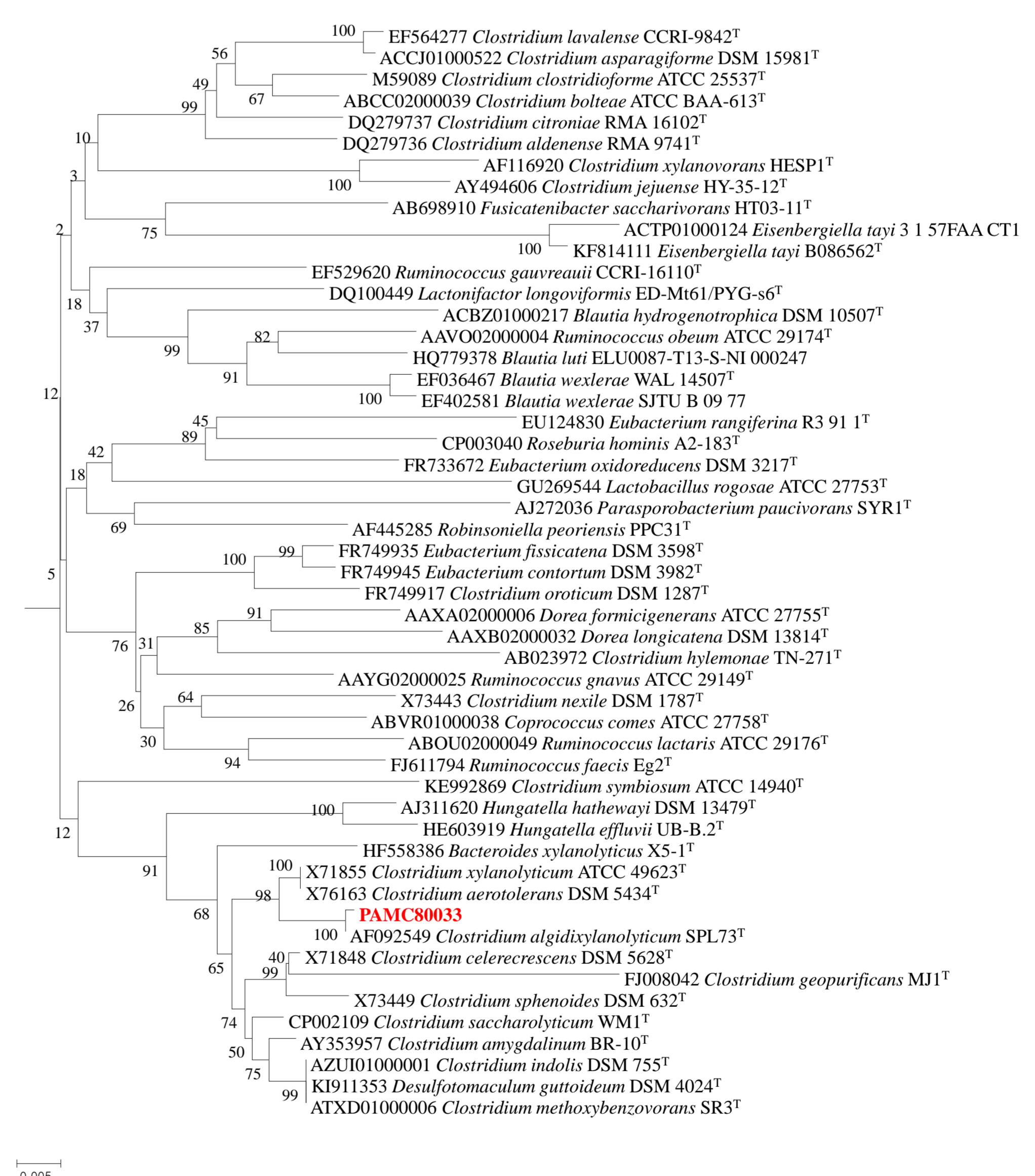


Fig. 2. Phylogenetic tree of the isolate PAMC80033. The isolate was obligate anaerobe.

- Total of 209 strains have been isolated and 127 strains have been identified 14 genus and 27 species.
- More than 50% of the identified strains belonged to *Paenibacillus*.
- Most of the strains were facultative anaerobic strains except an obligate anaerobe *Clostridium algidixylanoyticum* (PAMC80033).
- More than 20% of the identified strains are candidates for new species.
- To isolate diverse isolates, we are going to culture the anaerobic bacteria using various samples & media.

ACKNOWLEDGEMENTS

This study was supported by the National Research Foundation of Korea Grant funded by the Korean Government (MSIP) (NRF-2011-0021067) (PN14082, KOPRI) and by Korea Polar Research Institute (PE15080).

Thank you for your attention!