

## Isolation of gram-positive spore-forming piezophilic bacteria from 1.5 and 2.4 km-deep subseafloor sediment core samples

JIASONG FANG<sup>1,2</sup>, CHIAKI KATO<sup>3</sup>, GABRIELLA RUNKO<sup>1</sup>, YUICHI NOGI<sup>3</sup>, TONOYUKI HORI<sup>4</sup>, YUKI MORONO<sup>5</sup>, AND FUMIO INAGAKI<sup>5</sup>

<sup>1</sup>Department of Natural Sciences, Hawaii Pacific University, Honolulu, HI 96813, USA

<sup>2</sup>The Hadal Science and Technology Research Centre, Shanghai Ocean University, Shanghai 201306, China

<sup>3</sup>Department of Marine Biodiversity Research, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka 237-0061, Japan

<sup>4</sup>National Institute of Advanced Industrial Science and Technology, Tsukuba 305-8569, Japan

<sup>5</sup>Kochi Institute for Core Sample Research, JAMSTEC, Kochi 780-0862, Japan

Microorganisms of the three domains of life persist in the deep subseafloor environments. However, the depth (pressure) and habitability limits of the deep subseafloor life remain poorly constrained because of their high resistance to the cultivation in the laboratory. In this study, we successfully isolated several gram-positive piezophilic bacterial strains and characterized two pure-isolates, 19R1-5 and 29R7-12, from very deep subseafloor sediment samples (coaly shale) obtained at 1,498 m and 2,406 m below seafloor, respectively, during IODP Expedition 337 in the north western Pacific off the Shimokita Peninsula, Japan (1,180 m in water depth). The isolates were low-GC gram-positive, spore-forming, facultative anaerobic piezophilic bacteria. Phylogenetic analysis based on the 16S rRNA gene indicated that strains 19R1-5 and 29R7-12 were closely related to *Virgibacillus pantothenicus* and *Bacillus subtilis* in the phylum *Firmicutes*, respectively. The growth behaviour and lipid chemistry of the isolates were found to notably differ from the reference strains. The optimal pressure and temperature conditions for growth were 20 MPa and 45°C for 19R1-5, and 10 MPa and 55°C for 29R7-12, which are close to the conditions *in situ*. This is the first report of high-temperature and high-pressure adapted piezophilic *Firmicutes* isolated from the deep subseafloor sedimentary biosphere.