First record of the endophytic bacteria of *Deschampsia antarctica* E. Desv. from two distant localities of the maritime Antarctica

O. Podolich[1], I. Parnikoza[1,2], T. Voznyuk[1], G. Zubova[1], I. Zaets[1], N. Miryuta[2], G. Myryuta[1], O. Poronnik[1], I. Kozeretska[2,3], V. Kunakh[1], A.M. Pirttila[4], N. Kozyrovska[1]

[1]*Institute of Molecular Biology and Genetics NAS of Ukraine, 150 Zabolotnogo Str., Kyiv, 03143, Ukraine*

[2]*State Institution National Antarctic Scientific Center, Ministry of Education and Science of Ukraine, 16 Shevchenko Ave, Kyiv, 01601, Ukraine*

[3]*Taras Shevchenko National University of Kyiv, 60 Volodymyrska Str., Kyiv, 01601, Ukraine*

[4]*Department of Ecology and Genetics, University of Oulu, FIN-90014 Oulu, Finland*

email address of the corresponding author: ivan.parnikoza@gmail.com

Abstract

The vascular plant *Deschampsia antarctica* samples were collected for endophytic bacteria study from two regions in the maritime Antarctic 400 km distant from one another: Point Thomas oasis (King George Island) and Argentine Islands (Galindez Island). The endophytes were isolated from roots and leaves of *D. antarctica*, cultivated and identified by using a partial sequencing of the 16S rRNA gene served as a phylomarker. Endophyte isolates from two sites of Galindez Island were represented mainly by *Pseudomonas* species and by *Gammaproteobacteria*, *Firmicutes* and *Actinobacteria*. The vast majority of the isolates had specific for endophytes cellulase and pectinase activities, however, *Bacillus* spp. did not express both activities. A group-specific PCR screening at the four sites of Galindez Island and two sites of King George Island, indicated *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Firmicutes*, *Cytophaga-Flavobacteria* and *Actinobacteria*. Notably, the number of endophytic bacteria taxa was significantly larger in leaves than in roots of plants.

Keywords: Antarctica, endophytic bacteria, *Deschampsia antarctica*

Introduction

Endophytic microorganisms live inside the asymptomatic host plants as commensals (van Overbeek and Saikkonen 2016) and are known to have beneficial effect on plants such as promoting the plant growth and the plant protection from different biotic and abiotic stresses. Endophytic bacteria are ubiquitous inside of all plant species and create together with the host plant a superorganism – a

multispecies community that functions as an organizational unit (Podolich et al. 2015). The major source of the endophytes in plants is rhizo- and phyllosphere (Edwards et al. 2015).

The maritime Antarctica presents the global southern limit for a spread of the vascular plants, with only two vascular plants species found, and one of these species is Antarctic hairgrass *Deschampsia antarctica* E. Desv. (*Poaceae*) (Parnikoza et al. 2009; Parnikoza et al. 2017). The adaptation of the Antarctic hairgrass to cold environment could be supported by individual and unique cross-species interactions, *e.g., D. antarctica* has no specific adaptive mechanisms, therefore its survival and colonization of the Antarctica may depend on the interactions with another organisms, like birds (Parnikoza et al. 2018) or bacteria. Moreover, plants, living in extreme environments, can be a rich source of associated bacterial species, beneficial for biotechnological purposes, *e.g.,* as producers of enzymes active under low temperatures (Kuddus 2018). Antarctic plant-associated bacteria have been recovered from mosses (Park et al. 2013), as well as endophytic fungi were found in *Deschampsia* (Santiago et al. 2016). However, there is no information about the prokaryotic endophytes of *D. antarctica* from the different maritime Antarctica.

Material and Methods

Sampling was conducted in two regions of the maritime Antarctica, separated by a distance of 400 km from one another: Point Thomas oasis, King George Island, South Shetland Islands, and the Argentine Islands during the austral summer season 2014, and 2017/18. We collected green plants with a near root substrata (leptosols) – three specimens of *D. antarctica* plants from each locality (Supplement Table 1), packed them in sterile plastic boxes and transported to the laboratory. Endophytic bacteria were isolated from two locations of Galindez Island, namely, Karpaty Ridge and Metheo Point. For isolation of endophytic bacteria, *D. antarctica* plants were surface-sterilized in 70% ethanol for 1 min and in 6% calcium hypochlorite for 20 min and rinsed three times for 5 min in sterile distilled water. The last washing was controlled on dissemination by inoculation of DW on nutrient agar and no microbial growth was observed. The plant material was crushed in mortar with a pestle, serially diluted and cultivated on KB, LB and M9 agar media. Bacterial DNA isolation was performed with innuSPEED bacteria/fungi DNA isolation kit (Analytik Jena AG). Endophytic bacterial isolates were identified by a PCR amplification, using standard primers 27F and 1492R (Fredriksson et al. 2013). The PCR products were sequenced by the Sanger method using Big Dye Terminator Sequencing Standard Kit v3.1 (Applied Biosystems, USA) and apparatus 3130 Genetic Analyser (Applied Biosystems). The 16S rDNA sequences were binned by BLASTN programs search through

the NCBI (USA). GenBank accession numbers: MG916945-MG916956. Neighbour-joining phylogenetic tree were conducted in MEGA 7. (Supplement, Fig.1).

The endophytic isolates were examined primarily for cellulolytic (Wood 1981) and pectinolytic (Starr et al. 1977) activities by plate assays.

For group-specific bacterial amplification, total DNA was isolated from the surface-sterilized roots and leaves of *D. antarctica* plants from all localities, using Power Plant DNA isolation kit (MoBio). The isolated DNA (100 ng) was amplified with group-specific bacterial primers: Alf28f/Alf684r for *Alphaproteobacteria*, Beta359f/Beta682r for *Betaproteobacteria*, Gamma395f/Gamma871r for *Gammaproteobacteria*, Firm350f/Firm814r for *Firmicutes* (Mühling et al. 2008), ACT235f/ACT878r for *Actinobacteria*, CF315f/ CF967r (Stach et al. 2003), *Cytophaga – Flavobacterium* (Xihan et al. 2008), and the PCR products were separated in a 2.0% agarose gel.

Results and Discussion

Identification and phylogenetic analysis (Supplement, Fig.1) of the endophytic bacterial isolates from the roots of *D. antarctica* showed that representatives of the *Pseudomonas* genus were the most common in the plant interiors of both localities (Supplement, Table 2). Interestingly, all gram-negative endophytic isolates were represented exclusively by *Gammaproteobacteria*. The vast majority of them had cellulase or pectinase activities; *P. migulae, P. rhodesiae, P. orientalis* and *P. antarctica* had both activities, but *P. graminis* and *P. fluorescens* did not exhibit such traits. It is known that bacterial genus *Pseudomonas* has appeared frequently in different Antarctic environments (Vásquez-Ponce et al. 2018). Also, the genus *Pseudomonas* is tightly associated as endophytes with circumpolar grass *Deschampsia flexuosa* (L.) Trin., growing in subarctic Aeolian sand dune area (Poosakkannu et al. 2014). Thus, the results indicate that bacteria of the genus *Pseudomonas* frequently inhabit extremely cold environment.

Gram-positive bacteria were present only in plant samples from Metheo Point, and they belonged to the phyla *Firmicutes* and *Actinobacteria*. Remarkably, isolated *B. subtilis* and *B. pumilis* did not express both activities, although it is known that cellulose activity is common for *Bacillus* spp., occupying different mainland econiches (Gupta et al. 2015), including endophytic *Bacillus* and *Paenibacillus* strains exhibited both cellulase and pectinase activities (Zhao et al. 2015).

Results of a group-specific PCR assay, which was used for characterization of both culturable and unculturable endophytes of the hairgrass, represented in Supplement, Fig. 2. For all studied regions, cultivation-independent approach showed more taxons in endophyte community structures than a culture-based one: *Proteobacteria (Alpha-, Beta-, Gammaproteobacteria), Firmicutes, Cytophaga-Flavobacteria* and *Actinobacteria*. There was some difference in the studied groups of

endophytic bacteria between the roots and leaves. The number of taxa of studied endophytic bacteria was wider in leaves than in roots of the plants from the two sites of Galindez Island (Karpaty Ridge and Magnit Cape) and one site of King George Island (Point Thomas, Puchalski grave). Such difference was especially noticeable in plants from the site of Magnit Cape (Galindez Island). It could be explained by specific conditions in substrates (Zaets et al. 2012), *e.g*., the high content of ions of trace elements in soils in some localities of the maritime Antarctic. Homogeneity of endophytic community structure was observed for the developed *D. antarctica* cenoses within distant locations, *e.g*., the plants from King George Island, Point Thomas, Puchalski grave and Galindez Island, Cemetry Ridge had similar endophytic communities in both roots and leaves.

Conclusion

Summarizing, we may conclude that in isolates from *D. antarctica* most abundant genus was *Pseudomonas.* Some of isolated cultures had pectinase and cellulase activities. Remarkably, the representatives of *Firmicutes* did not possess cellulase and pectinase activities, as compared to homologous species in another parts of the world. This may mean that horizontal gene transfer events could take place rarer in the maritime Antarctic than on the mainland. In some regions, the culture independent approach indicated a higher number of bacterial taxons in leaves than in roots, which may be explained by extreme Antarctic environmental conditions.

Acknowledgments

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Compliance with Ethical Standards

This study was not accompanied by the emergence of potential conflicts of interest and did not include Human Participants or Animals.

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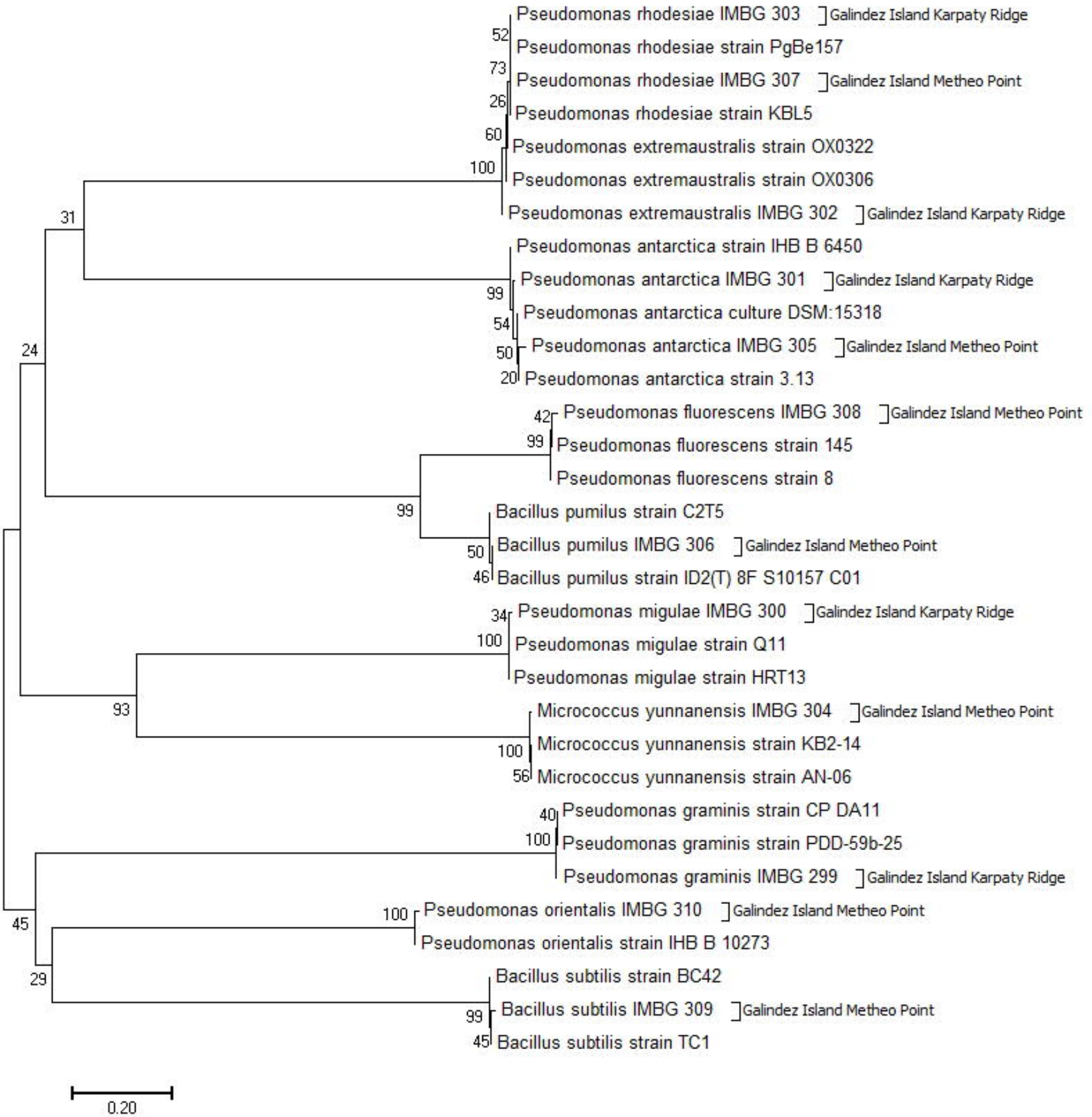
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Galindez Island Karpaty Ridge
Pseudomonas rhodesiae strain PgBe157
Pseudomonas rhodesiae IMBG 307
Galindez Island Metheo Point
Pseudomonas rhodesiae strain KBL5
Pseudomonas extremaustralis strain OX0322
Pseudomonas extremaustralis strain OX0306
Pseudomonas extremaustralis IMBG 302
Galindez Island Karpaty Ridge
Pseudomonas antarctica strain IHB B 6450
Pseudomonas antarctica IMBG 301
Galindez Island Karpaty Ridge
Pseudomonas antarctica culture DSM:15318
Pseudomonas antarctica IMBG 305
Galindez Island Metheo Point
Pseudomonas antarctica strain 3.13
Pseudomonas fluorescens IMBG 308
Galindez Island Metheo Point
Pseudomonas fluorescens strain 145
Pseudomonas fluorescens strain 8
Bacillus pumilus strain C2T5
Bacillus pumilus IMBG 306
Galindez Island Metheo Point
Bacillus pumilus strain ID2(T) 8F S10157 C01
Pseudomonas migulae IMBG 300
Galindez Island Karpaty Ridge
Pseudomonas migulae strain Q11
Pseudomonas migulae strain HRT13
Micrococcus yunnanensis IMBG 304
Galindez Island Metheo Point
Micrococcus yunnanensis strain KB2-14
Micrococcus yunnanensis strain AN-06
Pseudomonas graminis strain CP DA11
Pseudomonas graminis strain PDD-59b-25
Pseudomonas graminis IMBG 299
Galindez Island Karpaty Ridge
Pseudomonas orientalis IMBG 310
Galindez Island Metheo Point
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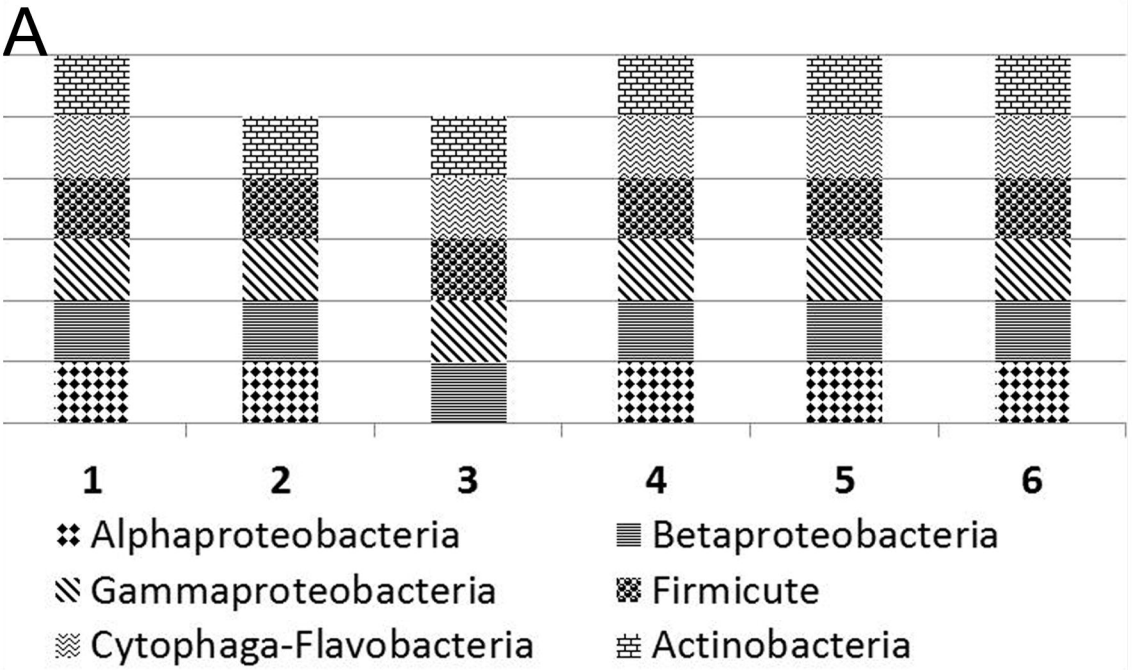
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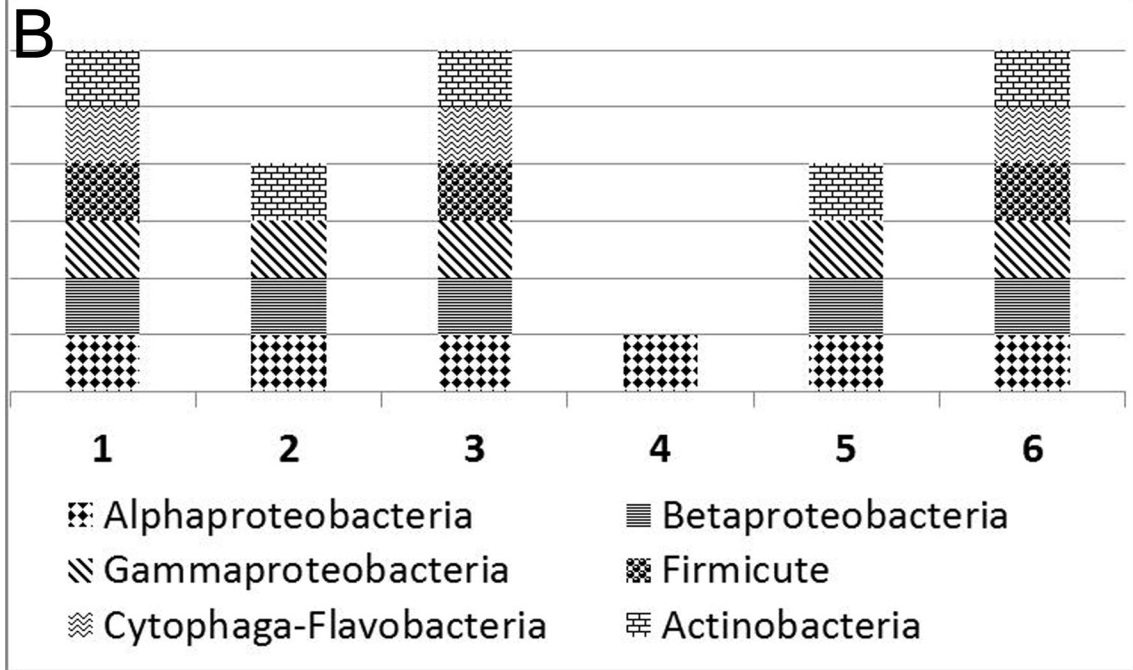
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