## Deep terrestrial saline fluids harbor an indigenous microbial community dominated by *Candidatus* Frackibacter

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Characterization of microbial communities living within Earth's deep subsurface is important for understanding Earth's biogeochemistry and providing a baseline for investigating habitability and potential subsurface life beyond the Earth. Over the past decade, the Kidd Creek Observatory at 2.39 km depth has served as an astrobiology analog site where saline fracture fluids have been shown to be stored in the subsurface with mean residence times of hundreds of millions to greater than one billion years. In this study, long term sampling of two boreholes was undertaken to characterize microbial communities. Analysis of 16S rRNA gene amplicons from biosamplers that were isolated from the external mine environment revealed the dominance of a putatively anaerobic and halophilic bacterial species from the Halobacteroidaceae family, classified as Candidatus Frackibacter. In contrast, microbial communities from other boreholes where the biosamplers were exposed to the mine environment, as well as borehole-adjacent biofilms sampled on the rock wall near a borehole outlet, revealed Candidatus Frackibacter mixed with putatively aerobic Sphingomonas taxa. Phospholipid fatty acid (PLFA) analysis of biosamplers indicated that microbial growth was of the same magnitude for all biosampler units. Stable carbon isotope analysis of PLFA, and putative functional predictions derived from 16S rRNA gene profiles, showed the importance of anaerobic chemoheterotrophy by Candidatus Frackibacter, particularly for the packered system. These results demonstrate the presence of a Candidatus Frackibacter population that is adapted to highly saline and anoxic fracture fluids, where their carbon source may be derived from ancient carbon-rich layers common in these systems, sharing some important characteristics with shale bed environments where Candidatus Frackibacter was previously found in a much younger geologic setting. Further, results here suggest this organism may be indigenous to the subsurface and that Candidatus Frackibacter and other members of the Halobacteroidaceae can survive in a wider range of geologic settings than previously recognized, including deep subsurface halophilic groundwaters hosted in carbon-rich rocks. Such deep subsurface environments may be a refuge for life to

persist over long periods in the Earth's subsurface, with implications for saline waters and brines that might be present in the subsurface of other planets and moons.