

# Identification of Novel Key Microorganisms for Alkanes Degradation in the Deepest Biosphere, Sediment of the Mariana Trench

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The Mariana Trench is the deepest biosphere in the Earth, with ~11,000 m at the Challenger Deep. Recent studies report that highly abundant alkane content existed in the sediment of the Mariana Trench, and a few alkane-degrading bacteria were identified in the Mariana Trench. However, comparative studies about short-chain and long-chain alkane degradation and key degraders in the sediment of the Mariana Trench under low- and high-pressures conditions are lack. Here, based on culture-dependent and culture-independent methods, enrichment of the 11,000 m sediment with short-chain (C<sub>7</sub>-C<sub>17</sub>) or long-chain (C<sub>18</sub>-C<sub>36</sub>) alkanes were performed under 100 MPa, 4 °C or 0.1 MPa, 4 °C for 7-139 days. Surprisingly, several novel alkane-degrading microbes such as *Ralstonia*, *Halomonas*, and *Thalassolituus* were observed. The alkane-degrading microbial communities under 100 MPa were significantly different from 0.1 MPa ( $P < 0.05$ ). *Ralstonia* and *Halomonas* were responsible for n-alkanes utilization under in-situ pressure (100 MPa), whereas *Thalassolituus* dominated the degradation of n-alkanes at 0.1 MPa. On the other hand, the key microbes for degradation of the short- and long-chain alkanes were also different. For instance, *Halomonas* were mainly long-chain alkane-degrading taxa, however, the short-chain alkane-degrading bacteria were included *Pseudomonas* and *Ralstonia*. Additionally, the temporal variation of community succession were obviously observed in the processes of n-alkanes degradation. An abrupt increase in the relative abundance of *Thalassolituus* at day 18-30 at the expense of *Halomonas* during long-chain alkane degradation at 0.1 MPa. Similarly, after incubation for 139 days, the relative abundance of *Ralstonia* for short-chain degradation sharply increased to 28% at 100 MPa. On the contrary, the relative abundance of *Marinobacter* at 0.1 MPa decreased dramatically, changed from 23% to 1%. Our results, for the first time, revealed that novel alkane-degrading microorganisms were present in the deepest sediment of Mariana Trench, and demonstrated the extreme hydrostatic pressure (100 MPa) affected significantly the processes of microbial-mediated alkane degradation.