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Enrichment and isolation of carbonate-precipitating bacteria from a lava tube cave in South Korea

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Cave microorganisms associated with calcareous speleothems have been reported to help facilitate calcium carbonate precipitation via crystal nucleation and mineral growth [1]. Here, we investigate drip water and stalagmite biofilm samples collected from Yongcheon cave, a lava tube located on Jeju Island in South Korea, for the growth of carbonate-precipitating microorganisms. Microbial enrichments were performed at 15 °C and 25 °C under ureolytic, non-ureolytic, and oxalotrophic conditions relevant to carbonate biomineralization via urea hydrolysis [2], biofilm adsorption [3], and oxalate degradation [4], respectively. Isolates from successful enrichments were generated after three consecutive rounds of streak-plate technique. To date, ten isolates (five under ureolytic conditions and five under non-ureolytic conditions) have been established, and two additional isolations are currently ongoing for enrichments under oxalotrophic conditions. Five of the generated isolates have been confirmed as pure cultures both microscopically and through 16S rRNA gene sequence analysis. The phylogenetic identities of the three non-ureolytic isolates analyzed thus far all fell within the genus *Pseudomonas*, with 99 % to 100 % gene sequence similarity with *Pseudomonas brenneri* (previously isolated from mineral water; [5]), *Pseudomonas gessardii* (previously isolated from mineral water and from Antarctica; [6-7]), and *Pseudomonas fluorescens* (previously isolated from biofilm on cave wall; [8]), among others. The two ureolytic isolates analyzed thus far fell within the genera *Pseudomonas* (similar to the non-ureolytic isolates) and *Bacillus*, with 99 % to 100 % gene sequence similarity with *Bacillus toyonensis* (previously isolated from deep-sea and from caves; [9-10]) and *Bacillus cereus* (previously isolated from air in a cow shed; [11]), among others. Current efforts will result in the isolation and phylogenetic identification for a total of twelve isolates established through this study. Generated ureolytic, non-ureolytic, and oxalotrophic isolates will be used to further investigate carbonate precipitation mechanisms associated with cave environments.

[1] Sanchez-Moral et al. (2012); [2] Phillips et al. (2013); [3] Hemayati et al. (2023); [4] Sun et al. (2019); [5] Baida et al. (2001); [6] Verhille et al. (1999); [7] Maharana and Singh (2020); [8] Turrini et al. (2020); [9] Luo et al. (2021); [10] Jan et al. (2022); [11] Frankland and Frankland (1887)