

Insights into the population history of free-living bacteria as counted by their CRISPR inventory

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The CRISPR-cas is an adaptive and heritable immune system encoded by prokaryotes, which provides insights into genetic diversity within bacterial populations. The CRISPR array contains short repeats interspersed with unique spacers. The spacers are small pieces of DNA derived from foreign nucleic acid and represent chronological records of viruses infecting the cell. We investigated the population history of *Hydrotalea* sp. based on their CRISPR repertoire. Single-cells from an early-stage acid mine drainage were sorted and amplified following the Bigelow SCGC pipeline. Six single-amplified genomes (SAGs) of Chitinophagaceae were partially reconstructed. The phylogeny and average nucleotide identity analysis suggest that all SAGs represent a same species within the genus *Hydrotalea*, at the species boundary of *H. flava*. They form two clades representing different strains and cells inside the same clade are clones. CRISPR-cas were recovered across SAGs. Cells L11, B16, P17 and J04 encode two co-existing CRISPR loci, type I-B and II-C. Newly described repeat sequences are shared by SAGs, and all spacer sequences are divergent among the cells from two clades. Considering type I-B, there is evidence of loss of older spacers in SAG B16. Analyzing the genome content of other Chitinophagaceae species we observe only type II CRISPR loci, our hypothesis is that the SAGs or their ancestor cells had a CRISPR-Cas type II-C that is conserved in the Chitinophagaceae family, and also a CRISPR-Cas type I-B system that was likely acquired through HGT. It is important to mention that the SAGs harbor transposases and features related to conjugative transposons (CTns). The CRISPR based heterogeneity of population documented here, as well as the loss of spacers, suggest that organisms are adjusting to a quickly changing selective pressure in a microhabitat scale. A very likely form of such selective pressure is phage predation.

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