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Comparative Genomic Analysis Reveals Habitat-Specific Genes and Regulatory Hubs within the Genus *Novosphingobium*

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ABSTRACT Species belonging to the genus *Novosphingobium* are found in many different habitats and have been identified as metabolically versatile. Through comparative genomic analysis, we identified habitat-specific genes and regulatory hubs that could determine habitat selection for *Novosphingobium* spp. Genomes from 27 *Novosphingobium* strains isolated from diverse habitats such as rhizosphere soil, plant surfaces, heavily contaminated soils, and marine and freshwater environments were analyzed. Genome size and coding potential were widely variable, differing significantly between habitats. Phylogenetic relationships between strains were less likely to describe functional genotype similarity than the habitat from which they were isolated. In this study, strains (19 out of 27) with a recorded habitat of isolation, and at least 3 representative strains per habitat, comprised four ecological groups—rhizosphere, contaminated soil, marine, and freshwater. Sulfur acquisition and metabolism were the only core genomic traits to differ significantly in proportion between these ecological groups; for example, alkane sulfonate (*ssuABCD*) assimilation was found exclusively in all of the rhizospheric isolates. When we examined osmolytic regulation in *Novosphingobium* spp. through ectoine biosynthesis, which was assumed to be marine habitat specific, we found that it was also present in isolates from contaminated soil, suggesting its relevance beyond the marine system. *Novosphingobium* strains were also found to harbor a wide variety of mono- and dioxygenases, responsible for the metabolism of several aromatic compounds, suggesting their potential to act as degraders of a variety of xenobiotic compounds. Protein-protein interaction analysis revealed β-barrel outer membrane proteins as habitat-specific hubs in each of the four habitats—freshwater (Saro_1868), marine water (PP1Y_AT17644), rhizosphere (PMI02_00367), and soil (V474_17210). These outer membrane proteins could play a key role in habitat demarcation and extend our understanding of the metabolic versatility of the *Novosphingobium* species.

IMPORTANCE This study highlights the significant role of a microorganism's genetic repertoire in structuring the similarity between *Novosphingobium* strains. The results suggest that the phylogenetic relationships were mostly influenced by metabolic trait enrichment, which is possibly governed by the microenvironment of each microbe's respective niche. Using core genome analysis, the enrichment of a certain set of genes specific to a particular habitat was determined, which provided insights on the influence of habitat on the distribution of metabolic traits in *Novosphingo-*

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