

Pitch Lake in Trinidad & Tobago is a natural asphalt reservoir nourished by pitch seepage, a form of petroleum consisting of mostly asphaltines, from the surrounding oil rich region. During upward seepage, pitch mixes with mud and gases under high pressure and the lighter portion evaporates or is volatilized producing a liquid asphalt residue characterized by low water activity, recalcitrant carbon substrates and noxious chemical compounds. An active microbial community of archaea and bacteria, many of them novel strains (particularly from the new Tar Arc groups) and reaching a biomass of up to  $10^7$  cells per gram, was found to inhabit the liquid hydrocarbon matrix of Pitch Lake. Geochemical and molecular taxonomic approaches revealed diverse, novel, and deeply branching microbial lineages with the potential to mediate anaerobic hydrocarbon degradation processes in different parts of the asphalt column. In addition, we found markers for archaeal methane metabolism and specific gene sequences affiliated with facultative and obligate anaerobic sulfur and nitrite oxidizing bacteria. The microbial diversity encountered at Pitch Lake was unique when compared to microbial communities analyzed at other hydrocarbon-rich environments, which included Rancho Le Brea, a natural asphalt environment in California, USA, and an oil well and a mud volcano in Trinidad & Tobago, among other sites. These results open a window into the microbial ecology and biogeochemistry of recalcitrant hydrocarbon matrices, and establish the site as a terrestrial analog for modeling the biotic potential of hydrocarbon lakes such as those found on Saturn's largest moon Titan.