Although ubiquitous organisms in water and soil, only recently mycobacteria have been found in thermal environments. Norris Geyser Basin in Yellowstone National Park is one of the biggest hot springs field in the word. *Mycobacterium parascrofulaceum* was found in a system, in Norris Geyser Basin, composed by two acidic (pH 3.0) springs with temperatures between 56°C at the source and 40°C at the confluence of both springs. *Mycobacterium parascrofulaceum* was isolated in all the temperature gradient by culture and molecular methods and identified by 16s rRNA sequencing.

Growth and survival assays at 56°C for 90 days were performed to confirm the origin of the isolates. Auramine-Rhodamine and Live/Dead bacterial viability fluorescent microscopy stains were performed to confirm the origin of the isolated strains. Both staining methods gave positive results during the entire assay confirming the origin of the *Mycobacterium parascrofulaceum* strains. Further assays are needed to determine possible temperature-related resistance mechanisms.