ABSTRACT

Microflora trapped in the salt deposits of Pakistan's Khewra salt mine has evolved to harsh temperatures and nutrient deprivation in order to survive. Bacteria have frequently gone undiscovered since it was assumed that halophilic archaea dominated hypersaline habitats. There have been few reports on extremely halophilic bacteria endemic to the Khewra mine, leaving the salt mine's biodiversity mostly unexplored. Halophilic bacteria isolated and cultivated from rock salt and brine samples were selected for morphological and phylogenetic analysis. Here in this research, isolated nineteen halophilic bacteria, out of which four were sequenced using 16S rRNA. This study investigated the culturable bacterial biodiversity and phylogenetic analysis of isolates native to the Khewra salt mine in Pakistan. PCR amplification of isolates' 16S rRNA was performed using universal primers 27F and 1492R, and the results were sequenced. These genetic sequences are compared to other gene sequences in the GenBank databases to identify closely similar sequences. A phylogenetic tree for these bacteria was constructed by aligning these sequences with sequences available in the GenBank database. The majority of the isolates belonged to various *Bacillus* species, with 90-100% 16S rRNA similarity to their respective type strains. Other isolates had close similarities with *Priesta megasterium*, *Bacillus sonorensis*, *Bacillus tequilensis* and *Bacillus safensis* respectively. Conclusively, isolation and molecular characterization of the extremely halophilic bacteria from Khewra mine indicated their important ecological role and contribution towards the microbial diversity. The ability to thrive in extreme conditions also highlights the potential of the isolates as valuable resources for significant biotechnological applications.