

ABSTRACT

Microbial activity is considered by many to have potentially adverse effects on nuclear waste repositories. Due to insufficient knowledge about the microbial communities indigenous to the Waste Isolation Pilot Plant (WIPP) at the time of its inception, those same effects were attributed to these organisms as well. However, significant differences exist between the communities present in low- and high-ionic strength matrices.

Studies have been underway to gain a better understanding of the microbial ecology in the WIPP and the potential effect of microbial activity on the waste therein. These studies have found organisms from all three domains of life—*Archaea*, *Bacteria*, and *Eukarya*—with different tolerances to salinity and constrained metabolic capability [1-3].

Results suggest that the halophilic microorganisms detected in the WIPP will play a much smaller role than that predicted by performance assessments, thereby supporting the level of conservatism built into the WIPP model.

METHODS

Collection

Samples were retrieved from two different panels within the WIPP horizon. A long-handled pick was used to pry away the outer layer of halite, then to remove halite from the underlying stratum. Samples were handled aseptically, segregated by halite appearance (clear versus argillaceous), and placed into sterile plastic bags.

Cultivation

Samples were not sterilized prior to processing in the lab. Approximately 50 grams of halite were dissolved in generic growth media at two salt concentrations. Media contained (in g/L): NaCl 100 or 175; yeast extract, 0.5; casamino acids, 0.5; soluble starch, 0.2; MgCl₂·6H₂O, 20; KCl, 2.0; CaCl₂·2H₂O, 0.2; sodium pyruvate, 0.02, ATCC trace elements, 1 ml; pH adjusted with Tris buffer to ~7.5. Flasks were incubated at room temperature in the dark, while stirring. Periodic subcultures were made onto solid media.

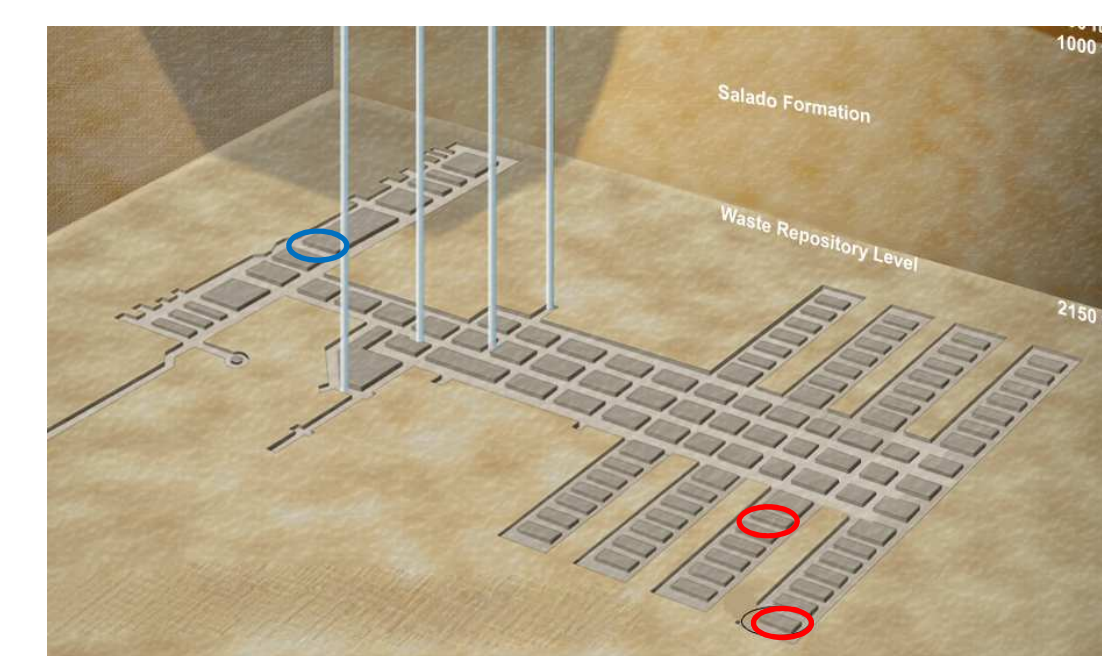


Figure 1: Sampling site locations in WIPP

Cultivation-independent analyses

Halite was dissolved in sterile 20% NaCl, and passed through a 0.22 micron nylon filter. The filter was transferred to the MoBio Ultra Clean Water DNA Purification kit bead tube, and DNA was extracted according to the manufacturer's directions. Small-subunit ribosomal RNA-encoding genes (or fungal internal transcribed spacer genes) were amplified using polymerase chain reactions. Amplicons were ligated into the pCR 4.1 vector using Invitrogen's TOPO TA cloning kit, transformed into DH5α *E. coli* cells, and sequenced by SeqWright, Inc. (Houston, TX). Sequences were processed in Sequencher 4, screened for chimeras using Bellerophon (greengenes.lbl.gov), and aligned in Clustal X. Operational taxonomic groupings were made in Sequencher at 97% sequence similarity. DNA was extracted from isolates using MoBio's Ultra Clean Soil DNA Purification Kit, amplified by PCR, and shipped for sequencing.

RESULTS

Archaea and Bacteria

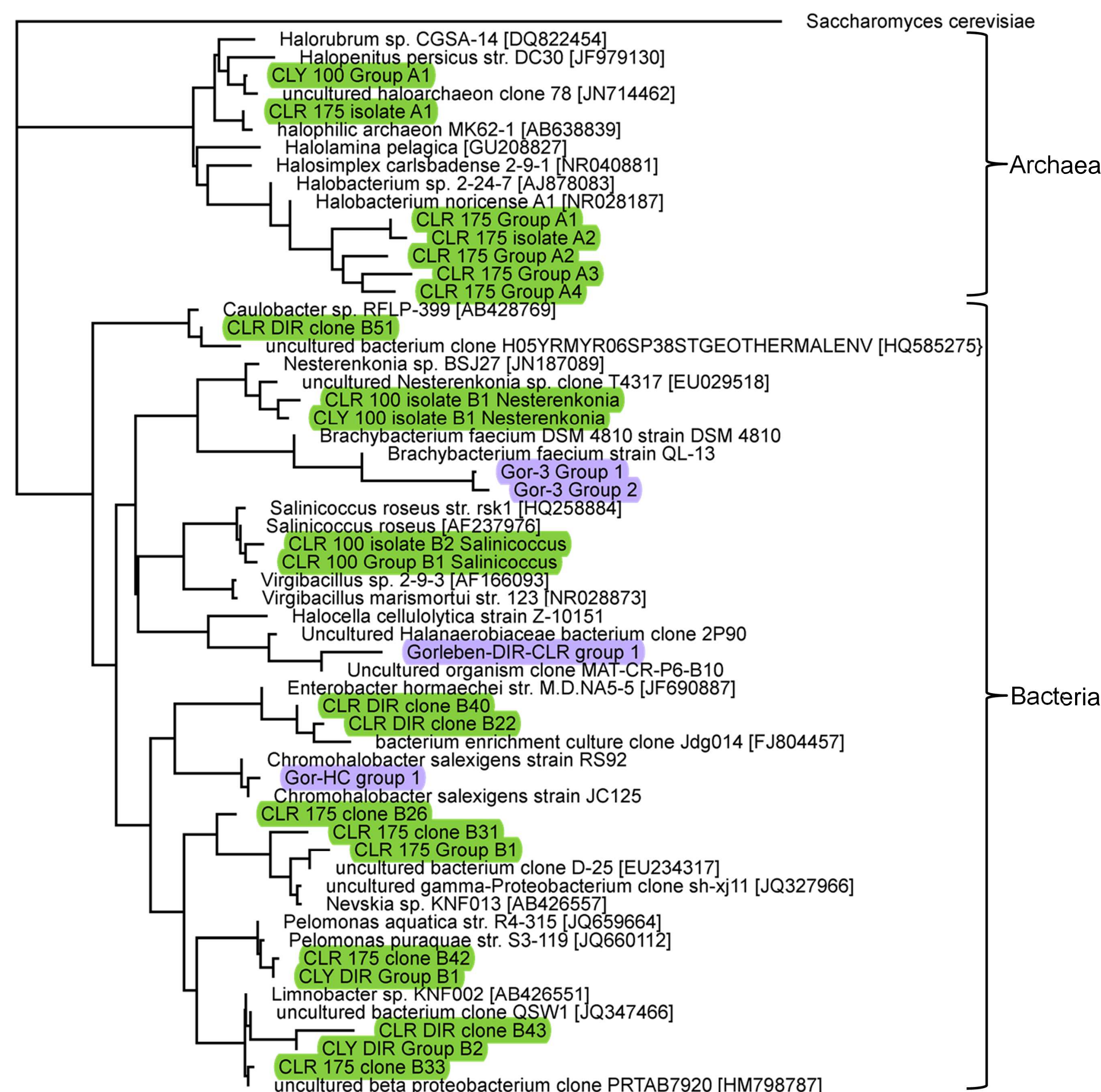


Figure 2: Phylogenetic tree based on 16S bacterial and archaeal rRNA. Contiguous groupings are based on a 97% minimum match. Tree is rooted to *Saccharomyces cerevisiae* as an outgroup.

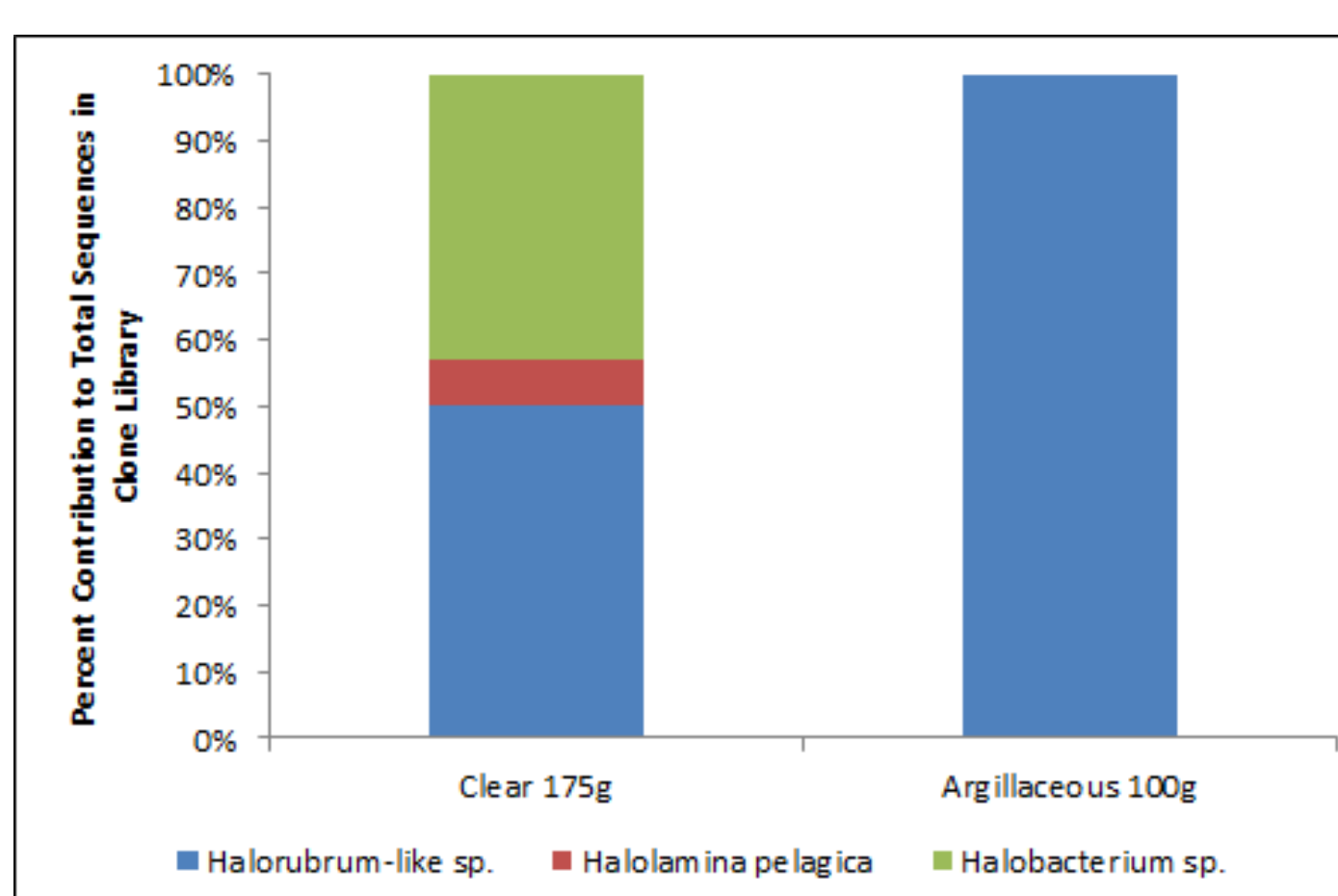


Figure 3: Comparison of archaeal species contribution to clone libraries in clear and argillaceous halite samples

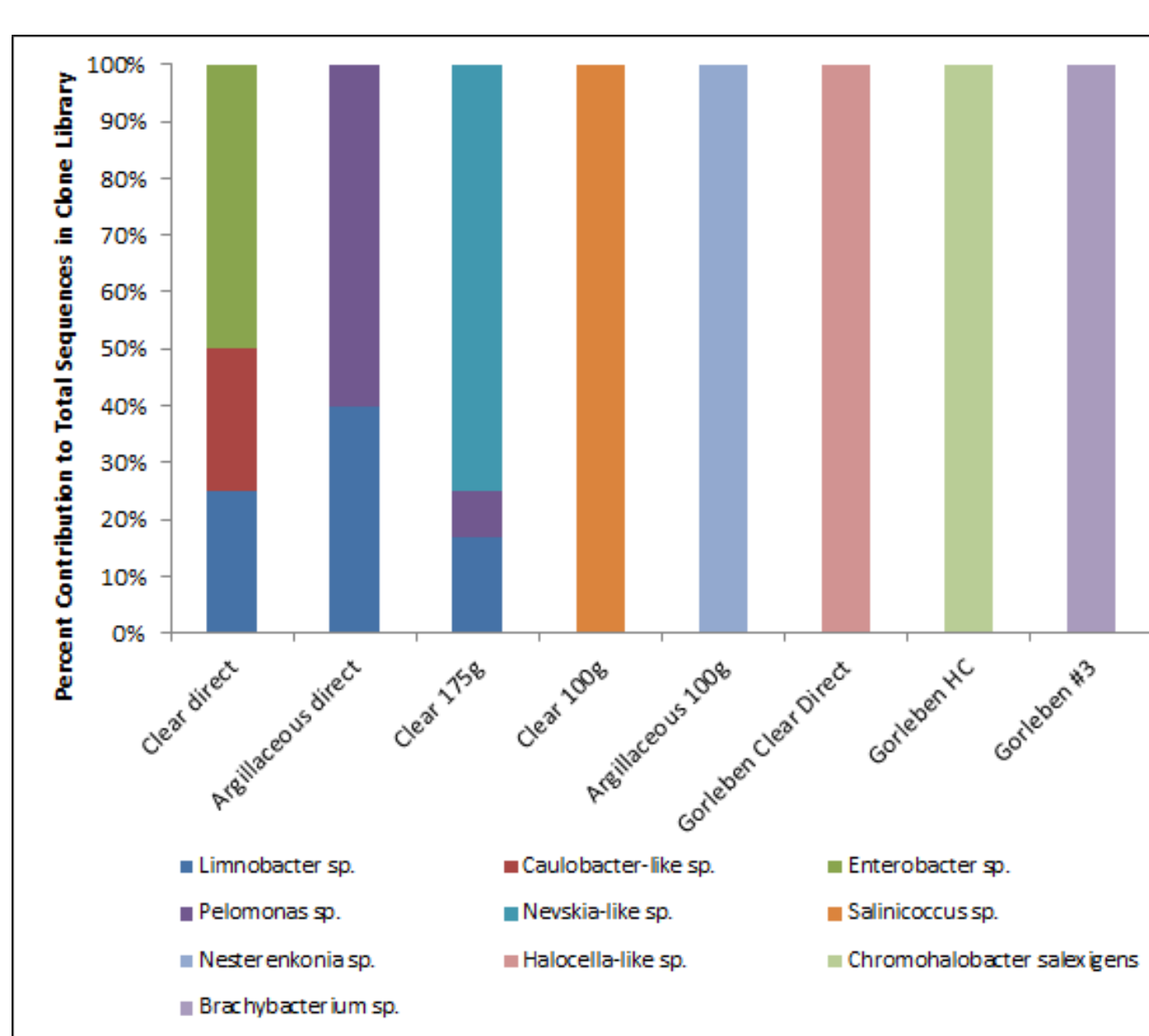


Figure 4: Comparison of bacterial species contribution to clone libraries in clear, argillaceous, and Gorleben halite samples.

Fungi

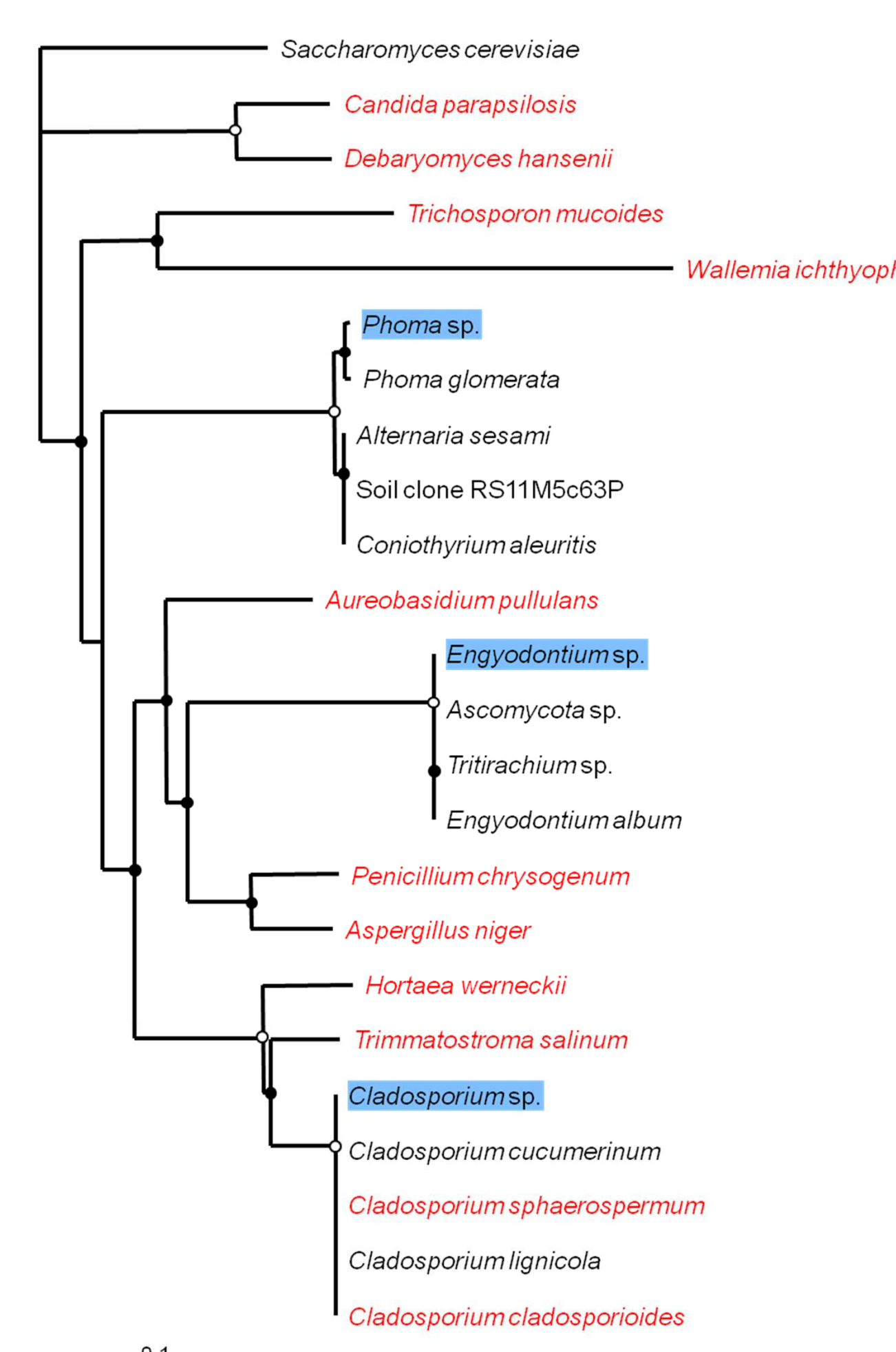


Figure 5: Phylogenetic tree based on fungal ITS region. Contiguous groupings are based on a 97% minimum match. Tree is rooted to *Saccharomyces cerevisiae* as an outgroup.

DISCUSSION

The microbial ecology within the WIPP repository is limited to halotolerant and halophilic organisms. Haloarchaea (*Halobacterium*, *Halorubrum*, *Natronomonas*, *Halolamina*, unclassified) dominated cultures enriched under high-salt conditions and were found in both pure and argillaceous halite samples. These cultures have been shown to survive in generic growth media, as well as more stringent WIPP brines. Halophilic and halotolerant bacteria and fungi (*Chromohalobacter*, *Nesterenkonia*, *Salinicoccus*; *Cladosporium*, *Engyodontium*, *Phoma*) dominated cultures enriched at lower salt concentrations, although some species survived higher concentrations as well. Additional bacterial DNA sequences were detected, but those organisms were not isolated (*Enterobacter*, *Nevskia*, *Caulobacter*, *Pelomonas*, *Limnobacter*). It is probable that their DNA was preserved although no viable cells were present or that culture conditions were inadequate for their growth. Fungal species were likely introduced from the mine surface on equipment and personnel or via the air-intake shaft.

Although the haloarchaea are the most adapted to survival at the ionic strength expected in the repository, they are the least capable of utilizing the more complex organic substrates present in the waste (e.g. cellulose). While some haloarchaea ferment small organics (e.g. amino acids) or reduce nitrate, they are essentially limited to aerobic metabolism [1, 2]. These organisms may function during the repository's oxic phase and degrade organic complexing agents in the waste. Still, the longevity of these organisms in halite crystals is well established; therefore, they are likely to be present throughout repository history [4].

Bacteria detected within the WIPP range from moderately (*Nesterenkonia*, *Salinicoccus*) to extremely halophilic (*Chromohalobacter*). *Nesterenkonia* and *Salinicoccus* spp. exhibit cellulase activity, and *Salinicoccus* and *Chromohalobacter* spp. are capable of citrate utilization [5-7]. However, none have been shown to survive long in WIPP brines and all are aerobic, suggesting their roles will also be limited to the early oxic phase. A *Chromohalobacter* sp. was also detected in enrichment cultures of hydrocarbon-bearing Gorleben halite and has been previously shown to degrade some HCs, suggesting it may play a role in HC transformation during this repository's oxic phase [8].

Two of the fungal isolates, *Cladosporium* and *Phoma*, were found to be cellulolytic and, should they come into contact with waste, may be able to hydrolyze cellulose to smaller compounds for resident *Bacteria*. Still, they are also obligate aerobes whose growth will be limited.

In conclusion, the impact of microbial activity on the WIPP is expected to be far less than that predicted by the performance model, based on limited microbial metabolic capability and survival in WIPP brines, thus affirming the model's conservatism. The extension of this implication to other salt-based repositories is contingent upon their waste contents and expected repository conditions and should be an area of ongoing research. Continued efforts are focused on anaerobic enrichments of halite and the degradation of cellulose by indigenous organisms.

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