

Alive After 250 Million Years: Isolation of Live Permian Microorganisms

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In the past, the scientific worlds of geo-chemists and microbiologists were separate entities with little actual interaction. However, recognition of the involvement of microbes in mineral precipitation and biogeochemical cycling has moved these disciplines ever closer. Recent interdisciplinary experiments, conducted on salt crystals taken from the Permian Salado Formation in Southeastern New Mexico, have shown that some ancient crystals still contain viable micro-organisms trapped within fluid inclusions. The careful use of stringent geological and microbiological selection criteria support the hypothesis that the bacteria are at least 250 million years old. The salt crystal that contained the organisms was taken from an ancient dissolution pipe located within primary sedimentary beds 564 meters below ground surface. The entire sedimentary layer was examined before removing the crystal, to be sure that the sample was taken from a primary bed. Individual crystals were examined for any evidence of penetration or perturbation. Only crystals that showed primary features without evidence of secondary dissolution, extensive pressure changes or fractures were selected for microbiological sampling. These selection

criteria resulted in the final selection of 56 individual crystals (<300 grams) from 45 Kg of starting material. One of the selected crystals contained three brine filled inclusions large enough (>0.5 mm³) for sampling. Before microbiological sampling, the crystal was surface sterilized, using concentrated (10 M) NaOH and HCl. All sampling occurred inside a Biological Level three isolation room using a dedicated laminar flow hood and sterile equipment. The techniques used ensured a probability of contamination as low as one chance in one billion (1 X 10⁻⁹). Each of the inclusions were sampled using sterilized wire drills. Two of these inclusions held trapped, micro-organisms that were still viable. The isolated microbes are salt tolerant *Bacillus* sp. that grow best in media containing 8% NaCl, and respond to concentrated brines by forming spores. One of the organisms is, phylogenetically, related to several modern day bacilli but does have several unique characteristics. This talk will trace the interdisciplinary data and procedures supporting this discovery. It will describe the various isolated bacteria and will present the future direction of this project.