Immunoanalytical approach for detecting and identifying ancestral peptide biomarkers in early Earth analogue environments

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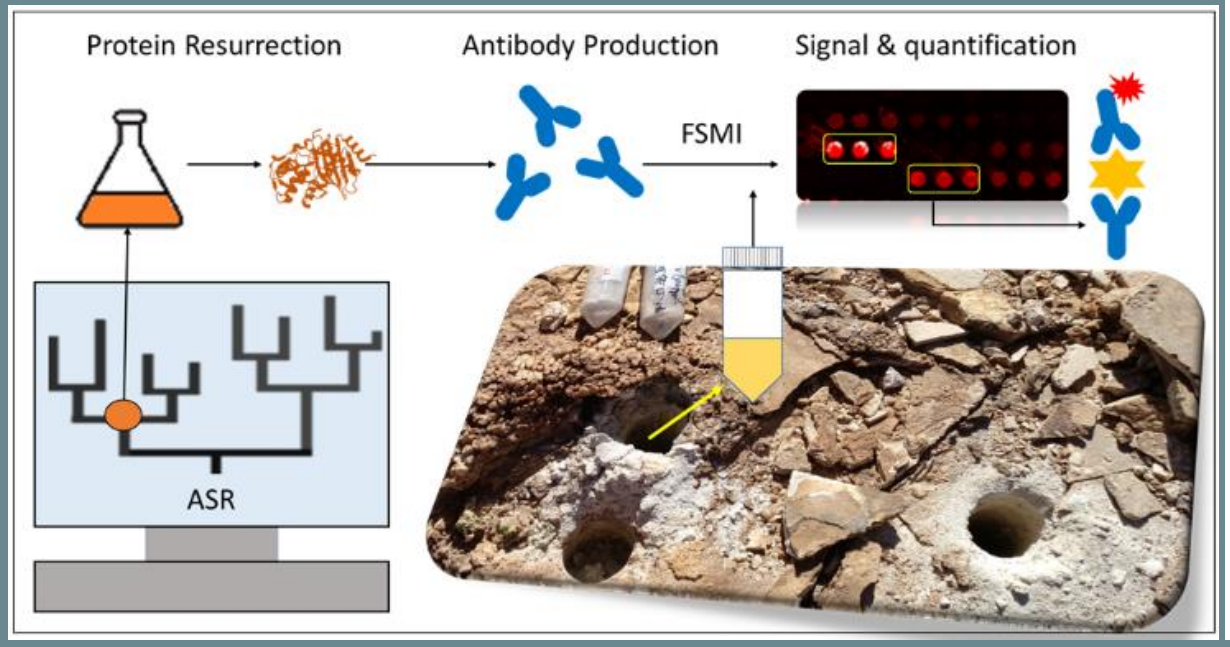
Ancestral Sequence Reconstruction (ASR) and Protein Resurrection to search for biomarkers in extreme environments analogous to other planetary environments using Fluorescence Sandwich Microarray Immunoassay (FSMI). Microorganisms from El Tatio (Chile), an analogue of hydrothermalism on early Mars, produce β-lactamase and thioredoxin proteins that still retain ancestral features in their structure. Photo credit: Laura Sanchez-García



Several mass spectrometry and spectroscopic techniques have been used in the search for molecular biomarkers on Mars. A major constraint is their capability to detect and identify large and complex compounds such as peptides or other biopolymers. Multiplex immunoassays can detect these com- pounds, but antibodies must be produced for a large number of sequence-dependent molecular targets. Ancestral Sequence Reconstruction (ASR) followed by protein "resurrection" in the lab can help to narrow the selection of targets.

We propose an immunoanalytical method to identify ancient and universally conserved protein/peptide sequences as targets for identifying ancestral biomarkers in nature. We have developed, tested, and validated this approach by producing antibodies to eight previously described ancestral resurrected proteins (three β-lactamases, three thioredoxins, one Elongation Factor Tu, and one RuBisCO, all of them theoretically dated as Precambrian), and used them as a proxy to search for any potential feature of them that could be present in current natural environments. By fluorescent sandwich microarray immunoassays (FSMI), we have detected positive immunoreactions with antibodies to the oldest β-lactamase and thioredoxin proteins (ca. 4 Ga) in samples from a hydrothermal environment. Fine epitope mapping and inhibitory immunoassays allowed the identification of well-conserved epitope peptide sequences that resulted from ASR and were present in the sample. We corroborated these results by metagenomic sequencing and found several genes encoding analogue proteins with significant matches to the peptide epitopes identified with the antibodies.

The results demonstrated that peptides inferred from ASR studies have true counterpart analogues in Nature, which validates and strengthens the well-known ASR/protein resurrection technique and our immunoanalytical approach for investigating ancient environments and metabolisms on Earth and elsewhere.

Severino, R.; Moreno-Paz, M.; Puente-s, F.; Laura, S.; Risso, V. A.; Sanchez-Ruiz, J. M.; Cabrol, N.; Parro, V. *Analytical Chemistry.* **2023**. https://doi.org/10.1021/acs.analchem.2c05386.