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Note

Spatially-correlated mass spectrometric analysis of microbe–mineral interactions

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Abstract

A new methodology for examining the interactions of microbes with heterogeneous minerals is presented. Imaging laser desorption Fourier transform mass spectrometry was used to examine the colonization patterns of *Burkholderia vietnamiensis* G4 (previously *Burkholderia cepacia* G4) on a heterogeneous basalt sample. Depth-profile imaging found that the bacterium preferentially colonized the plagioclase mineral phase within the basalt.

Keywords: Minerals; Bacteria; Laser desorption; Chemical imaging; Mass spectrometry; *Burkholderia vietnamiensis*

The interactions of microorganisms with minerals is important for interpreting the origins of life on Earth and on other planets, understanding the formation and transformation of mineral deposits, and developing industrial processes for the recovery of metals from mineral ores. Examining the impact of microorganisms on mineral chemistry is complex due to the heterogeneity of natural geomatrices. Our approach to examining the interaction of microorganisms with heterogeneous minerals uses spatially-correlated, depth-profiling chemical imaging by a laser desorption Fourier transform mass spectrometer (LD-FTMS).

The Laser-based Optical and Chemical Imager (LOCI) combines a LD-FTMS, a unique laser-scanning system, custom optics for fluorescence detection, and software for automated data acquisition and analysis (McJunkin et al., 2002; McJunkin and Scott, submitted for publication; Scott and Tremblay, 2002; Scott et al., 2003). The laser-rastering system was designed to enable the layer-by-layer spatially-correlated analysis of surface associated cells and materials (e.g., cell exudates, sorbed ions) and underlying mineral phases (Scott and Tremblay, 2002). The custom software, Spectral IDentification Inference Engine© (SIDIE), is used to interpret spectra and develop micro-scale chemical maps of materials being examined (McJunkin et al., 2002; McJunkin and Scott, submitted for publication; Scott et al., 2003). In this study, the spectral library included the minerals associated with the basalts of the Snake River Plain such as olivine, plagioclase, ilmenite, and augite (Scott et al., 2003; Yan et al., in press) and the bacterium *Burkholderia vietnamiensis* G4. For each laser desorption spot, the raw data was converted into a peak-list by the Odyssey Analyzer software (ThermoFinnigan, Bremen, Germany). Based on comparison with the spectral library, the data interpretation

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software assigned a classification to each spectrum associated with each *x*,*y*-coordinate. Compiled information was graphically displayed using different colors to represent the different mineral phases and biomass. The data for each spot on the maps was derived from a spectrum obtained from a single laser shot. Spectral peaks have a mass error of $\pm 4 \times 10^{-4}$ amu with an average resolution of 30,000.

LOCI was used to characterize the micro-scale distribution of the mineral phases in a basalt sample collected in the Snake River Plain at the Idaho National Laboratory (INL) site. A basic square-grid laser desorption scan over the surface using a 184 × 184 array of 6 μm diameter contiguous laser desorption spots was performed using a laser wavelength of 355 nm and a laser fluence of 2×10^5 W. The mineral phases were identified and compiled as a chemical image of the basalt sample (Fig. 1). The surface of this basalt sample was predominantly ilmenite with a moderate amount of olivine and relatively small amounts of plagioclase and augite. These findings are in contrast to those found for other samples obtained at the INL in which the predominant mineral phases were olivine and plagioclase (Ingram et al., 1999; Yan et al., in press).

A basalt thin section was incubated in the presence of *B. vietnamiensis* G4 for ~2 d, then rinsed and analyzed by depth-profiling using a 150 × 150 μm array of 6 μm spots on 7.5 μm centers. The chemical image depicted in Fig. 2A was derived from the first square-grid laser desorption scan of the surface of basalt thin section colonized by the bacterium. This scan depicts the presence of microbial biomass on the surface of the basalt. The chemical image obtained for the second, deeper laser scan indicated that plagioclase was the predominant mineral underlying the microbial biomass (Fig. 2B). The detection of microbial signatures on the second pass may have been due to the presence of microbial biomass located within the pores in the basalt sample or the incomplete laser ablation of a thicker layer of biofilm. The preferential binding of microbial biomass to the plagioclase mineral phase as determined by imaging LD-FTMS was consistent with another sample in which the location of the bacteria was determined using epi-fluorescent microscopy and the identification of mineral phases of the basalt accomplished using optical mineralogy and scanning electron microscopy/energy dispersive X-ray analysis prior to the incubation of the basalt in the presence of the bacterium (Kauffman et al., 2000).

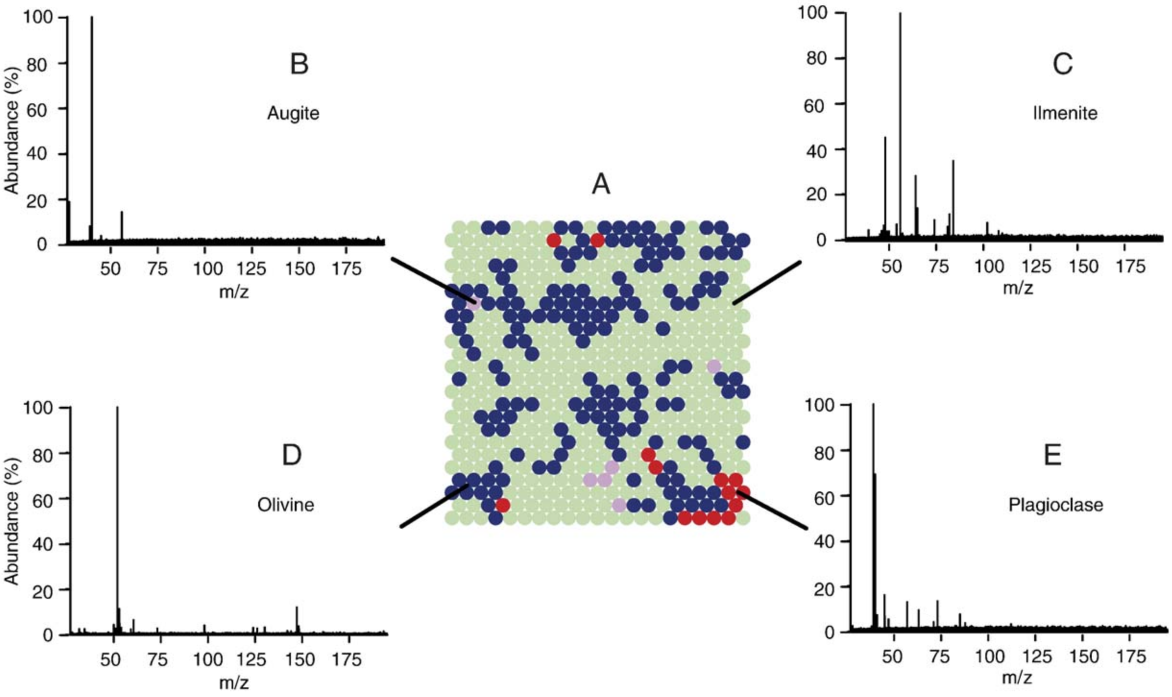

Fig. 1. The chemical image of a basalt sample (A) was compiled from spectra obtained for B) augite; C) ilmenite; D) olivine; and E) plagioclase. Key: , augite; , ilmenite; , olivine; , plagioclase.