## Environmental diversity, distribution, and evolution of microbial metal ion transport systems in global hydrothermal systems

FLAVIA MIGLIACCIO<sup>1</sup>, DAVIDE CORSO<sup>1</sup>, MARTINA CASCONE<sup>1</sup>, DEBORAH BASTONI<sup>1</sup>, MATTEO SELCI<sup>1,2</sup>, GABRIELLA GALLO<sup>1</sup>, ALESSIA BASTIANONI<sup>1</sup>, FELICIANA OLIVA<sup>1</sup>, BERNARDO BAROSA<sup>1</sup>, FRANCESCO MONTEMAGNO<sup>1</sup>, ANNARITA RICCIARDELLI<sup>1</sup>, LUCA TONIETTI<sup>1,3,4</sup>, MONICA CORREGGIA<sup>1</sup>, LUCIANO DI IORIO<sup>1</sup>, COSTANTINO VETRIANI<sup>2,5</sup>, PETER H BARRY<sup>5</sup>, REBECCA TYNE<sup>5</sup>, KAREN LLOYD<sup>6</sup>, GERDHARD JESSEN<sup>7</sup>, AGOSTINA LAURA CHIODI<sup>8</sup>, MAARTEN DE MOOR<sup>9,10</sup>, CARLOS RAMÍREZ-UMAÑA<sup>11</sup>, ANGELINA CORDONE<sup>1</sup> AND DONATO GIOVANNELLI<sup>1,2,5,12,13</sup>

Transition metals are crucial for microbial metabolism, serving as catalytic cofactors in enzymes or playing structural roles. The ubiquity of trace metals in physiological processes requires their uptake from the extracellular space. However, their bioavailability as a response to different redox states and their potential toxicity due to high reactivity selected for tight homeostasis regulation. Microorganisms evolved a plethora of metal transport systems to cope with variable environmental trace metals availability, which has in turn fluctuated during the history of our planet. These changes might have played a key role in the emergence and evolution of metal transporters.

The present study aims at describing the diversity and distribution of microbial metal transport systems across several geothermal environments, with a specific focus on shallow water hydrothermal vents and terrestrial deeply sourced seeps. In these ecosystems, microbial diversity and metabolism are tightly linked to the elements supplied by the water-rock interactions, providing an excellent model to investigate the diversity of microbial metal transport systems.

To this purpose, we performed shotgun metagenomics on

geofluids from around 200 thermal features across the world, and we carried out functional annotation of sequencing reads with a manually curated database of metal transport genes sequences. Metagenomics data were coupled to high resolution geochemical analysis, such as Ion Chromatography and Inductively-Coupled Plasma Mass Spectrometry.

Our results suggest the geochemical regime and trace metal abundance influence the diversity and abundance of microbial metal transporters. Specifically, we find an inverse correlation between metal transporters presence and trace metals environmental availability. Active metal transport systems tend to prevail over passive ones, thus indicating that metal homeostasis in geothermal environments requires energy consumption. Furthermore, metal uptake systems negatively correlate with metal abundance, while metal efflux systems increase with metal availability.

These relationships point to a dynamic regulatory mechanism, where microorganisms may adapt their metal uptake strategies in response to fluctuating metal concentrations, potentially offering new insights into microbial evolution of metal transport systems. Such findings could have broader implications for understanding microbial evolution in extreme environments, providing more insights into the fundamental role of metal availability in the regulation of microbial diversity.

<sup>&</sup>lt;sup>1</sup>University of Naples Federico II

<sup>&</sup>lt;sup>2</sup>Rutgers University

<sup>&</sup>lt;sup>3</sup>Parthenope University of Naples

<sup>&</sup>lt;sup>4</sup>Osservatorio Astronomico di Capodimonte

<sup>&</sup>lt;sup>5</sup>Woods Hole Oceanographic Institution

<sup>&</sup>lt;sup>6</sup>University of Southern California

<sup>&</sup>lt;sup>7</sup>Austral University of Chile

<sup>&</sup>lt;sup>8</sup>Instituto de Bio y Geociencias del Noroeste Argentino (IBIGEO, CONICET-UNSa), Salta, Argentina

<sup>&</sup>lt;sup>9</sup>National University

<sup>&</sup>lt;sup>10</sup>University of New Mexico

<sup>&</sup>lt;sup>11</sup>Servicio Geológico Ambiental de Costa Rica (SeGeoAm), San José, Costa Rica

<sup>&</sup>lt;sup>12</sup>National Research Council

<sup>&</sup>lt;sup>13</sup>Tokyo Institute for Technology