Hub E2S MeSMic
Metals in Environmental Systems Microbiology

An integrated approach to unravel metal ion interactions with microbial ecosystems at the molecular, cellular and community levels

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Hub MeSMIC: 6 scientific leaders and ca. 20 scientific & technical staff members from IPREM (CNRS/UPPA)

Applicants (Leaders)	Applicants (Partners)	Engineering support
D. Amouroux, DR (biogeochemistry) **R. Duran, PR (microbial ecology)** **M. Goñi, MCF HDR (microbiology)** **R. Grimaud, PR (molecular biology)** **B. Lauga, PR (microbial ecology)** **R. Lobinski, DR (analytical chemistry)**	C. Cagnon (molecular biology), C. Cravo-Laureau (microbial physiology) O. Donard (analytical chemistry) B. K. Hassani (genetics) R. Guyoneaud (microbial physiology) L. Ouerdane (analytical chemistry) F. Rigal (biostatistics) Z. Pedrero (analytical chemistry) L. Ronga (organic chemistry) M. Sebilo (biogeochemistry) P. Sivadon (molecular biology)	MARSS/IPREM O. Donard S. Berail K. Bierla J. Szpunar PREMICE/IPREM C. Gassie F. Hakil L. Urios A. Carbon

Hub MeSMic: Research workplan based on key scientific questions and achievements

Environmental relevance of metal-involving biological interactions:

-trace metals affect essential biological functions of microorganisms, health of ecosystems and global cycling of carbon and nitrogen.

-metal-biomolecule interactions from cellular to community level allow to better predict ecosystem responses and environmental risk.

WP 0	**Management, coordination, dissemination (scientific leaders)**
WP 1	**Mercury, thiols and transformation processes in bacterial strains and communities (WPL: M. Goñi, D. Amouroux)**
WP 2	**Molecular mechanisms of the acquisition of Iron in heterotrophic bacteria degrading particulate organic matter (WPL: R. Grimaud)**
WP 3	**Metal mediated-metabolic interactions in complex multi-species microbial assemblages (WPL: B. Lauga, R. Duran)**
WP 4	**Large scale high-throughput methods for metal binding ligands and metal-ligand complexes analysis and bioinformatics (WPL: R. Lobinski)**

Hub MeSMic: A significant additional human potential - 6 Postdoc fellows and 6 Doctoral students during 5 years (2018-2023)

<table>
<tr><th rowspan="2">Work packages</th><th rowspan="2">Tasks</th><th colspan="10">Months</th></tr>
<tr><th>1 - 6</th><th>7–12</th><th>13 –18</th><th>19 –24</th><th>25 –30</th><th>31 -36</th><th>37-42</th><th>43-48</th><th>49-54</th><th>55-60</th></tr>
<tr><td rowspan="2">WP0</td><td rowspan="2">Coordination and management</td><td colspan="10">Admin</td></tr>
<tr><td>D0.0</td><td>D0.1</td><td>D0.1, D02</td><td>D0.1, D0.2, D0.3</td><td>D0.1</td><td>D0.1, D0.2</td><td>D0.1</td><td>D0.1, D0.2, D0.3</td><td>D0.1</td><td>D0.1, D0.2</td></tr>
<tr><td rowspan="2">WP1</td><td rowspan="2">Task 1.1 Metal (Hg) binding thiols involved in transformation by bacteria</td><td colspan="6">PhD1</td><td></td><td></td><td></td><td></td></tr>
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<tr><td rowspan="2">WP1</td><td rowspan="2">Task 1.2 Competition and exchanges of thiols in microbial consortia</td><td></td><td></td><td colspan="6">PD2</td><td></td><td></td></tr>
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<tr><td></td><td>D2.1</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td rowspan="2">WP2</td><td rowspan="2">Task 2.2 Genes involved in the production of metallophores</td><td></td><td></td><td colspan="2">PhD3</td><td colspan="2">PhD3, PD3</td><td colspan="2">PD3</td><td></td><td></td></tr>
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D2.4</td><td></td><td></td><td></td><td></td></tr>
<tr><td rowspan="2">WP2</td><td rowspan="2">Task 2.3 Interactions in biofilms degrading POM under iron limitation</td><td></td><td></td><td></td><td></td><td colspan="2">PD1</td><td colspan="4">PD3</td></tr>
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<tr><td rowspan="2">WP3</td><td rowspan="2">Task 3.1 Inventory of metallophores and identification of producers</td><td></td><td colspan="3">PhD4, PD5</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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<tr><td rowspan="2">WP3</td><td rowspan="2">Task 3.2 Metabolite production and metal species transformation in microcosms</td><td></td><td></td><td></td><td></td><td colspan="3">PhD4, PD5, PD4</td><td colspan="2">PD4</td><td></td></tr>
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D3.3</td><td>D3.6</td><td></td><td></td></tr>
<tr><td rowspan="2">WP3</td><td rowspan="2">Task 3.3 In silico analysis of metagenomics and genomic data</td><td></td><td></td><td></td><td></td><td></td><td colspan="4">PD4</td><td></td></tr>
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<tr><td rowspan="2">WP4</td><td rowspan="2">Task 4.1 High throughput multielemental analysis of microsamples</td><td colspan="4">PhD5</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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<tr><td rowspan="2">WP4</td><td rowspan="2">Task 4.2 Large scale analysis for metal-ligand and metal-binding ligands</td><td colspan="4">PhD5, PD6</td><td>(PhD6)</td><td colspan="5">(PhD5) PhD6, PD7</td></tr>
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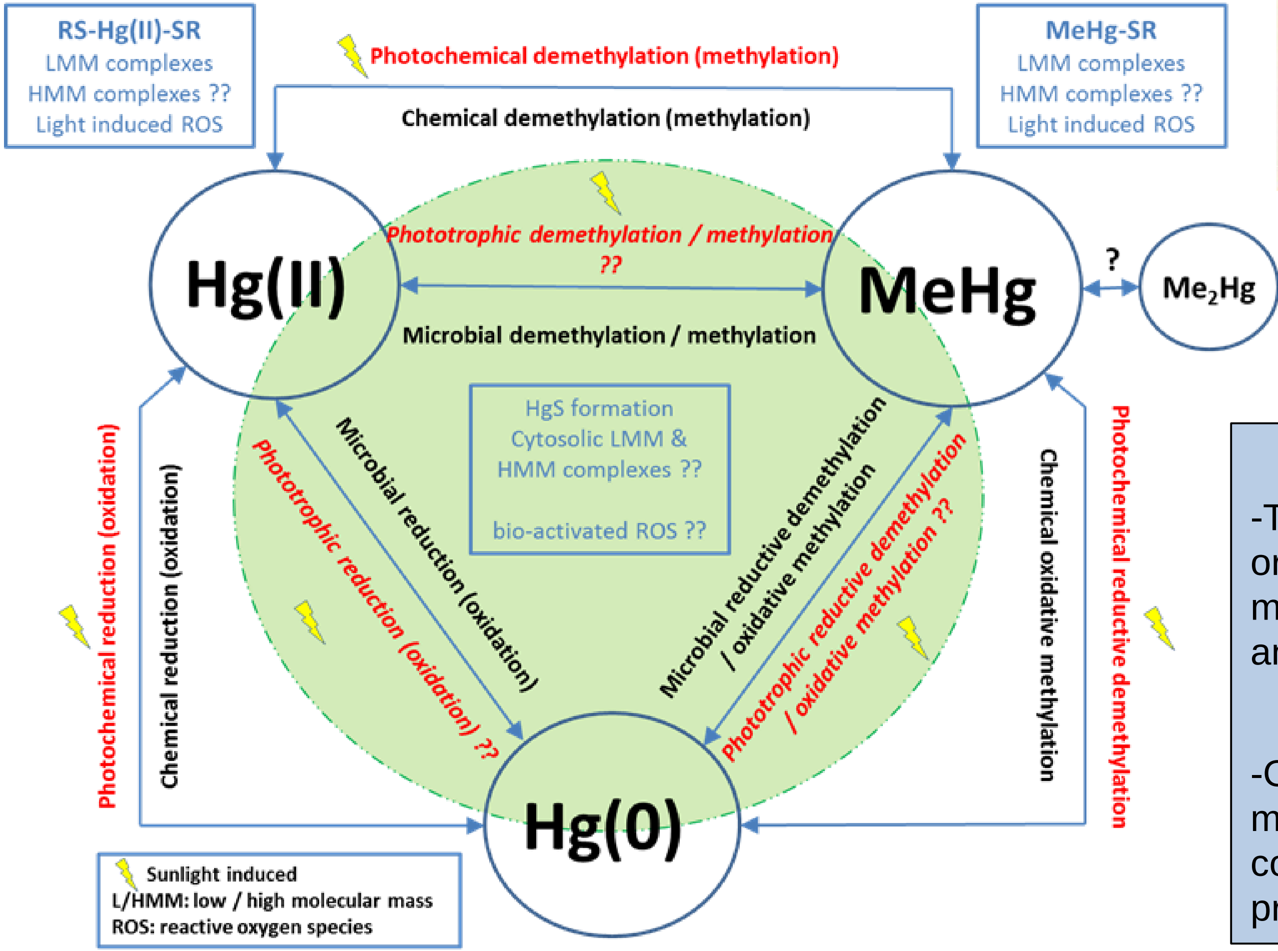
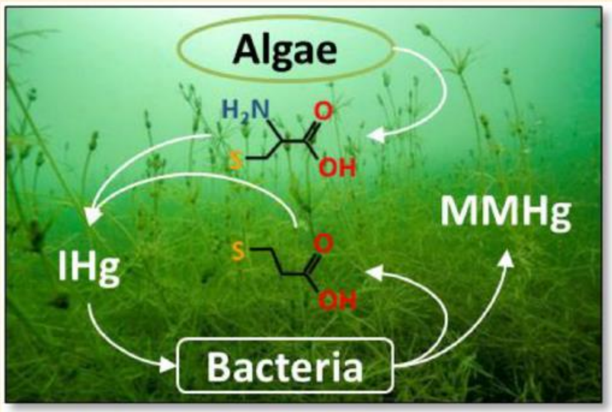
PhD: Doctorate student, PD: Post-doc fellow, Admin: Administrative technician, D: Deliverables

WP1 – Context and Hypothesis

Hg transformation pathways drive Hg exposure, toxicity and remediation solutions





Bouchet et al., ES&T 2018

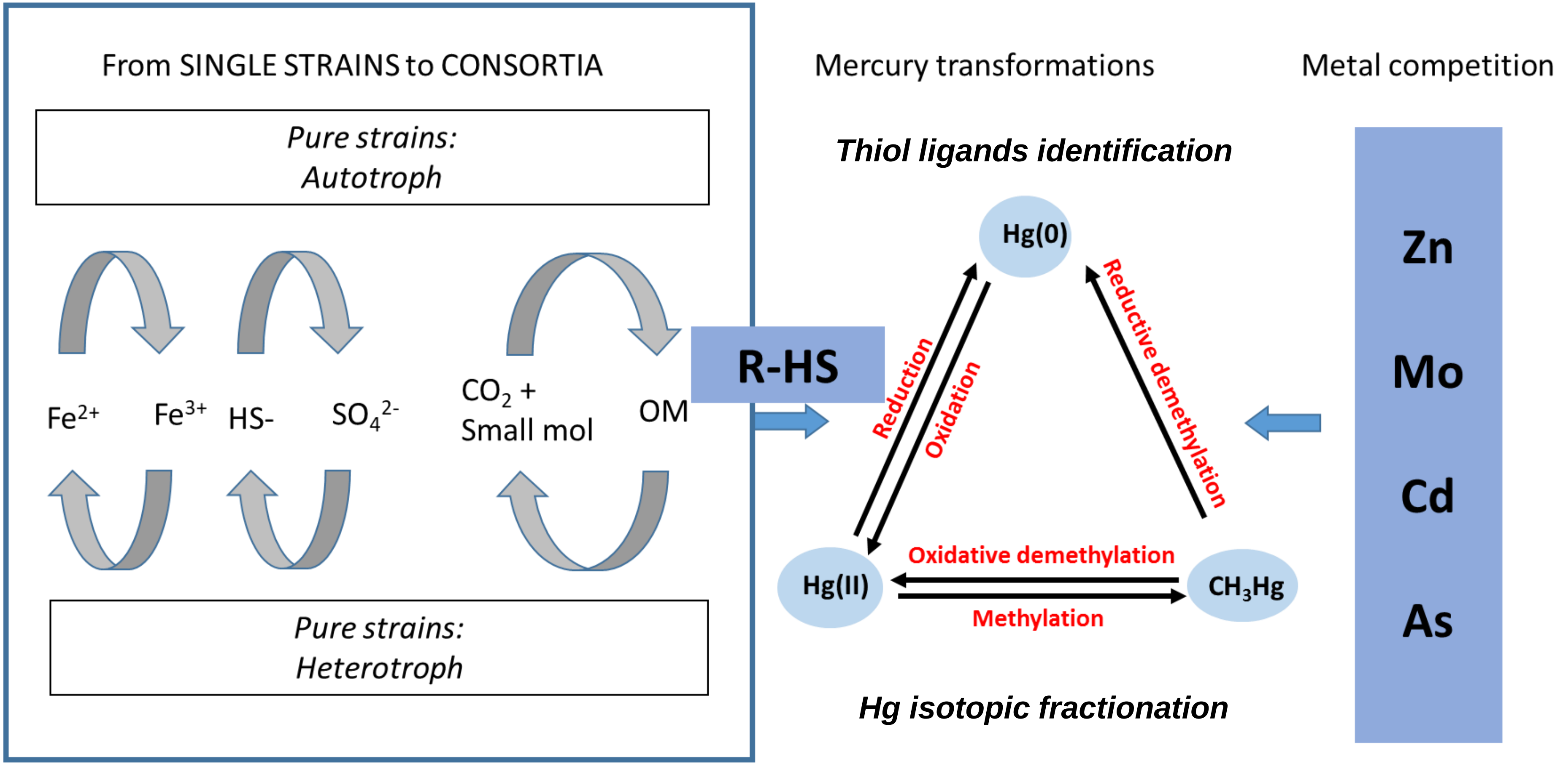
Hypothesis:

-Thiol ligands produced by bacteria or microbial consortia affect mercury transport, transformation and impact.

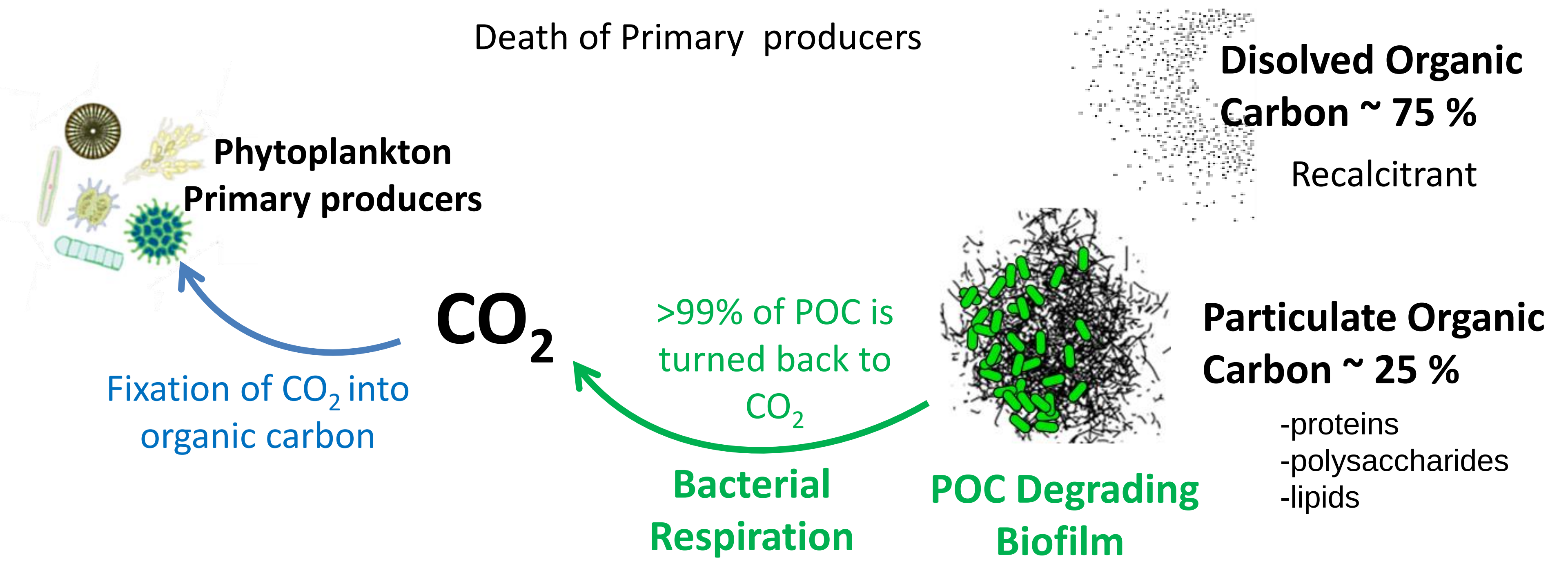
-Growth conditions and (co-) metabolism of bacteria or microbial consortia affect thiol ligands production and the fate of mercury.

WP1 - Objectives and Methods

Objectives: Decipher mercury species transformation pathways and interactions with biotic ligands (thiols) produced by microorganisms of different ecological role.



WP2 – Context and Hypothesis



Respiratory chain contains 94 % of cellular iron

Working hypothesis

- Because of their high respiration rate, bacteria in POM-degrading biofilms should have a high iron demand
- They may have specific iron acquisition systems

WP2 - Objectives and Methods

Objectives

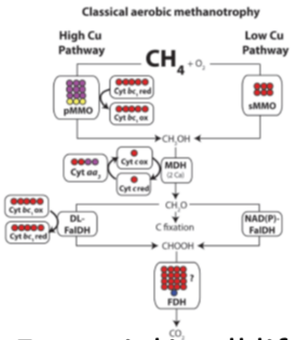
- Identification of metallophore produced in POM degrading biofilms
- Identification of the genes/proteins involved in metallophores production
- Regulation of expression of metallophore genes in POM degrading biofilms.
- Role of the amphiphilic and photoreactive characters of siderophore
- Exploration of isotopic fractionation of iron in POM degrading bacteria

Methods

- Model bacteria forming biofilms on proteins lipids hydrocarbons
- Genes identification: Tn mutagenesis, Tnseq, transcriptomic
- Biofilm characterization: flurescence microscopy, biochemistry
- Siderophore identification: HPLC- high resolution mass accuracy MS

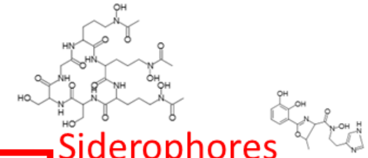
WP3 – Context and Hypothesis

Hypothesis: Metallophores contribute to microbial community structuration



Essential in all life forms

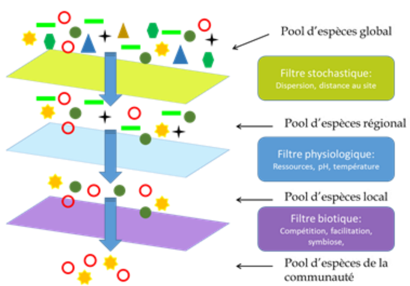
Essentiality



Siderophores

Metals

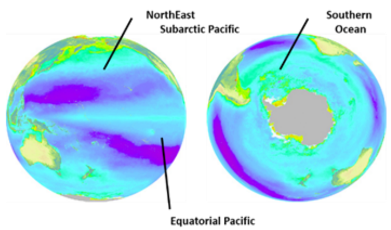
- toxicité/essentialité
- biodisponibilté



Community structure

- Assembly
- Functioning

Competition, cheating, commensalism, etc..

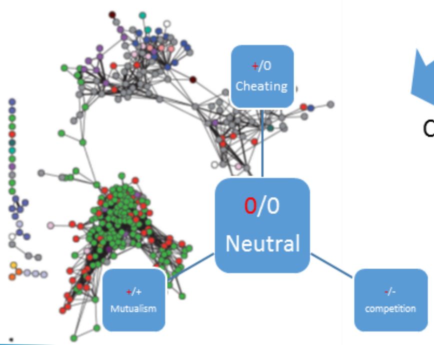


Regions of iron limitation (HNLC areas)

HNLC = High-macronutrient, low-chlorophyll (biomass)

Ecosystem functioning

→ major impact at global level



WP3 - Objectives and Methods

Objectives

- Inventory of metallophores (Met) produced in natural environments
- Identification of potential Met producers in natural environments
- Identification of genes involved in the synthesis of Met
- Linking Met production to community structure and functioning
- In silico mining to retrieve eco-evolutionary significance of Met production

Methods

- Prospecting natural environment considering gradients in a marine context
- Taxonomic and functional characterization of communities (omics + isotopic)
- Natural environments /// Microcosms experiments
- Mining genomes and metagenomes for genes involved in Met biosynthesis

WP4 – Context and Hypothesis

<u>Metal ion paradox (?)</u>

Environments with increasing competition/depletion for metal ions

Weakly- and randomly-bound metal ions

High proportion — Slow dissemination / Low stability — Low specificity (strong competition) — *Low biological significance*

« In vitro »

Immobilized surface-bound metal ions

Competing organisms and metal ions

« In situ »

Low proportion — Fast dissemination / High stability — High specificity (weak competition) — **High biological significance**

Metallophore-bound metal ions

WP4 - Objectives and Methods

- The **identification** and **quantification** of the **metal-binding species produced by microbiota**

Targeted approach	**Pseudo-targeted approach**	**Non-targeted approach**
-Search for **known** metallophores -**Available standards**	-Search for **known** metallophores -**No standards**	-Search for **unknown** metallophores -**No standards**

- Improve **metallophore-dedicated methods** for:

- sample preparation → preconcentration and selective extraction

- analytical development → effective LC MS couplings

- data treatment → high throughput and big data

- microbiota culture conditions → trigger metallophore production

Merci de votre attention

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