# Proteomic analysis of *Lactobacillus* species

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## Introduction and Summary

- **Gut microbiota**
  - complex population of microorganisms in the gastrointestinal tract
  - important to health, but many molecular mechanisms remain unclear
  - composition impacted by changes in diet, antibiotics, diseases, infection, or dietary metal intake.[2]
- **Probiotics**
  - live microorganisms that positively affect the host when administered in sufficient amounts.
  - *Lactobacillus* species are common probiotics and are gut bacteria primarily found in the small intestine.[3]
- **Proteomic analysis**: systemic identification and quantification of complete complement of a biological system's proteome (e.g. cells, tissues, or organs).
- **Proteome**:
  - set of proteins produced in an organism, system, or biological context
  - play many critical roles in the organism's functions
  - genes are made up of DNA and are the instructions to make proteins in organisms.
  - Gene ontology provides information regarding specific roles of genes and their products, such as proteins, in living cells at molecular, cellular, and biological levels.[4]

- **Metalloproteomics:** is a study of metalloproteins expressed in an organism
- **Metalloproteins**
  - have metal ions in their structure or bind to metals and utilize them for biological purposes
  - essential for all domains of life
  - mediate biological processes such as enzyme activity, cell signaling, metal binding, metal uptake and storage, and intracellular transport of metals in bacteria.[5]
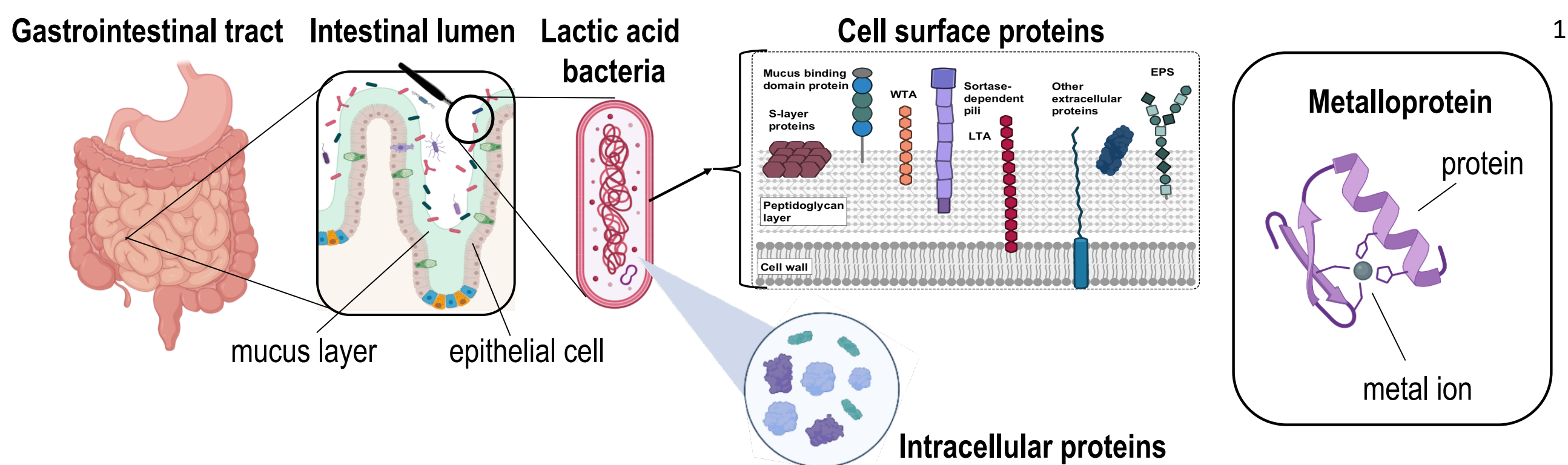


## Methods
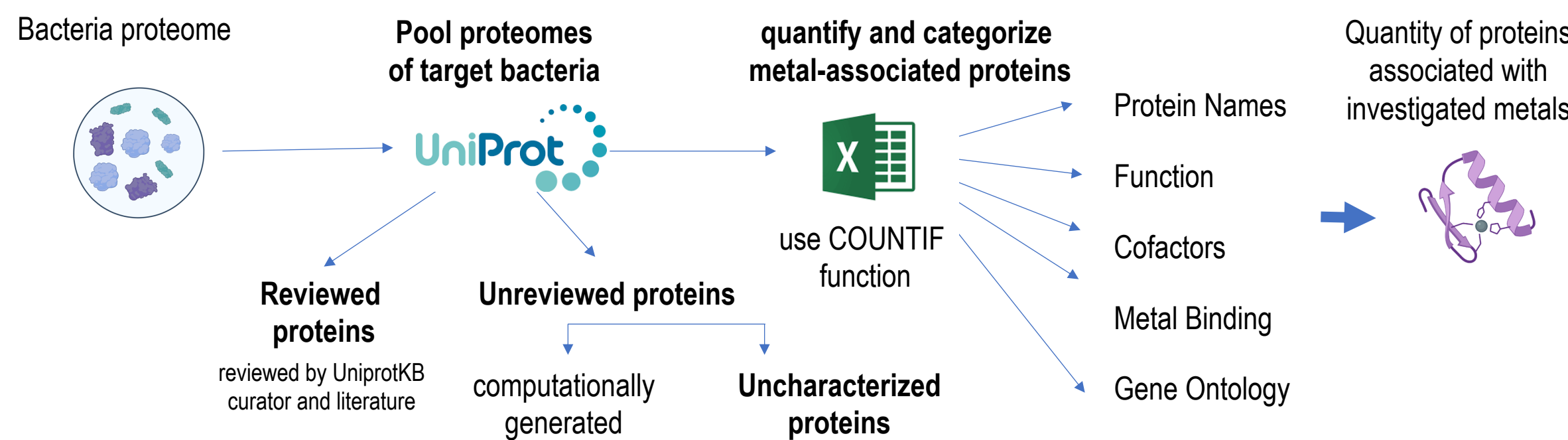


- The main goal of this study is to analyze the proteomes of target organisms to quantify and categorize metal-associated (metal-binding) proteins based on their names, functions, and gene ontology.
- **Bacterial strains:** *E. coli* K12, and 5 prominent *Lactobacillus* species.
- **Method:**
  - Bacterial proteome was acquired from UniProtKB database.
  - Employed Excel functions, such as COUNTIF formula, to categorize and quantify the the number of proteins associated with metals, and specifically zinc, copper, manganese, iron, and calcium and categorized them based on protein names, function, cofactors, metal binding, and gene ontology. These metals are known to be essential to bacteria, and might play important roles to *Lactobacillus* species.[7]
- **UniprotKB** [6]
  - a central database of protein sequences with accurate, consistent and functional annotations
  - comprises of two sections
    - **reviewed**: contains manually annotated records, extracted from literature and evaluated by UniprotKB curators
    - **unreviewed:** contains computationally generated records, awaiting full manual annotation.
    - **uncharacterized:** among unreviewed proteins and weren't characterized, thus remains unknown
- **Protein names:** provide information regarding the biological activity of the proteins.
- **Function**
  - describes the biological function(s) of a protein
  - include specific information such as **cofactor** and **metal-binding**. A cofactor is any foreign substance required in a protein's structure to function properly, in this study, we are looking for metals act as cofactor.
- **Gene ontology**[4]
  - provide information if a protein requires metals to function at molecular and biological levels such as being enzyme, transporter, ligands, etc.
- After that, we combined all information extracted from the above categories to quantify in **"total"** the percentage each kind of metal-associated proteins take up in the entire proteomes. Our goal is to explore if and which metals might play important roles in particular *Lactobacillus* species.

## Results



**E. coli K12**

| | Total | Protein names | Function | Cofactor | Metal binding | Gene ontology |
|---|---|---|---|---|---|---|
| non metal-associated proteins | 55.00% | 85.01% | 83.75% | 82.50% | 67.66% | 66.67% |
| other metals | 19.84% | 1.48% | 1.59% | 2.61% | 13.94% | 12.50% |
| calcium | 0.38% | 0.01% | 0.04% | 0.05% | 0.22% | 0.19% |
| iron | 6.24% | 0.75% | 1.99% | 0.34% | 2.66% | 4.38% |
| manganese | 1.67% | 0.08% | 0.06% | 0.68% | 0.82% | 0.88% |
| copper | 0.65% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| zinc | 4.13% | 0.40% | 0.29% | 1.65% | 2.59% | 2.89% |

**L. reuteri DSM 20016**

| | Total | Protein names | Function | Cofactor | Metal binding | Gene ontology |
|---|---|---|---|---|---|---|
| zinc | 2.99% | 0.21% | 0.1% | 1.07% | 1.65% | 2.45% |
| copper | 0.05% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| manganese | 0.69% | 0.00% | 0.00% | 0.32% | 0.32% | 0.27% |
| iron | 1.39% | 0.48% | 0.11% | 0.11% | 0.23% | 0.80% |
| calcium | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| unidentified metals | 8.80% | 0.69% | 0.11% | 0.43% | 4.64% | 5.55% |
| non metal-associated proteins | 63.34% | 78.98% | 80.26% | 78.23% | 71.61% | 69.26% |

**L. paracasei ATCC 334**

| | Total | Protein names | Function | Cofactor | Metal binding | Gene ontology |
|---|---|---|---|---|---|---|
| zinc | 2.32% | 0.18% | 0.07% | 0.85% | 1.18% | 1.64% |
| copper | 0.15% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| manganese | 0.74% | 0.00% | 0.00% | 0.41% | 0.37% | 0.15% |
| iron | 0.96% | 0.22% | 0.11% | 0.04% | 0.15% | 0.59% |
| calcium | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| unidentified metals | 7.68% | 1.22% | 0.11% | 0.52% | 3.84% | 4.87% |
| non metal-associated proteins | 61.77% | 74.17% | 76.05% | 74.02% | 68.97% | 67.16% |

**L. gasseri ATCC 33323**

| | Total | Protein names | Function | Cofactor | Metal binding | Gene ontology |
|---|---|---|---|---|---|---|
| zinc | 3.17% | 0.26% | 0.16% | 1.25% | 1.92% | 2.50% |
| copper | 0.21% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| manganese | 0.62% | 0.10% | 0.00% | 0.42% | 0.36% | 0.26% |
| iron | 0.78% | 0.21% | 0.19% | 0.05% | 0.31% | 0.36% |
| calcium | 0.05% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| unidentified metals | 9.00% | 1.25% | 0.16% | 0.42% | 5.46% | 5.04% |
| non metal-associated proteins | 73.27% | 88.51% | 90.22% | 87.94% | 79.98% | 79.88% |

**L. plantarum ATCC 14917**

| | Total | Protein names | Function | Cofactor | Metal binding | Gene ontology |
|---|---|---|---|---|---|---|
| zinc | 2.57% | 0.35% | 0.06% | 0.89% | 1.27% | 1.93% |
| copper | 0.16% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| manganese | 0.57% | 0.03% | 0.00% | 0.29% | 0.22% | 0.25% |
| iron | 0.82% | 0.22% | 0.06% | 0.03% | 0.19% | 0.44% |
| calcium | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| unidentified metals | 7.16% | 0.35% | 0.13% | 0.32% | 3.55% | 4.60% |
| non metal-associated proteins | 60.79% | 73.15% | 74.10% | 72.49% | 67.64% | 65.26% |

**L. acidophilus ATCC 4356**

| | Total | Protein names | Function | Cofactor | Metal binding | Gene ontology |
|---|---|---|---|---|---|---|
| zinc | 2.57% | 0.35% | 0.06% | 0.89% | 1.27% | 1.93% |
| copper | 0.16% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| manganese | 0.57% | 0.03% | 0.00% | 0.29% | 0.22% | 0.25% |
| iron | 0.82% | 0.22% | 0.06% | 0.03% | 0.19% | 0.44% |
| calcium | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% | 0.00% |
| unidentified metals | 7.16% | 0.35% | 0.13% | 0.32% | 3.55% | 4.60% |
| non metal-associated proteins | 60.79% | 73.15% | 74.10% | 72.49% | 67.64% | 65.26% |

## Discussion

- Overall, based on the current reported proteomes on UniprotKB, ~15-30% of the proteomes of *Lactobacillus* species are metal-associated. Metal-associated proteins might have metal ions in their structure or bind to metals and utilize them for biological purposes, such as enzyme activity, cell signaling, metal binding, metal uptake and storage, and intracellular transport of metals in bacteria.
- Our proteomic analysis revealed that there are significantly less proteomic data available on UniprotKB database for *Lactobacillus* species compared to the well-studied *E. coli* K-12 strain.
- Among investigated metals, zinc associated-proteins are the most abundant for all of 5 *Lactobacillus* species, followed by manganese, then iron. Manganese promotes the growth of *Lactobacilli* and protects them from oxygen toxicity.[8] Zinc deficiency is linked to gastrointestinal diseases and impaired immune systems,[9] while zinc overload associated with increased infection susceptibility.[10]
- On the other hand, *E. coli* has the most iron-associated proteins, followed by zinc, and manganese. It is consistent with the literature which revealed *E. coli* cells took up more iron and zinc than other essential transition metals.[11] Besides, iron can promote *E. coli* growth and its iron uptake system is essential for its successful colonization in the intestines.[12]
- The analysis confirms that metal-associated properties of proteins are reported in gene ontology annotation and metal-binding section.
- In the future, we aim to expand our analysis on more *Lactobacillus* and probiotic species to reveal the roles of metal in *their* proteomes. In addition, we will carry out full proteomic analysis to uncover additional effects on unexpected or previously uncharacterized surface proteins and intracellular proteins

## Conclusion

Our study revealed the importance of metals, especially zinc and manganese, in *Lactobacillus* species, as well as the importance of more attention on bacterial metal-associated proteomic analysis will provide more insight into the treatment of diseases caused by metal-associated pathogen and probiotic therapy.

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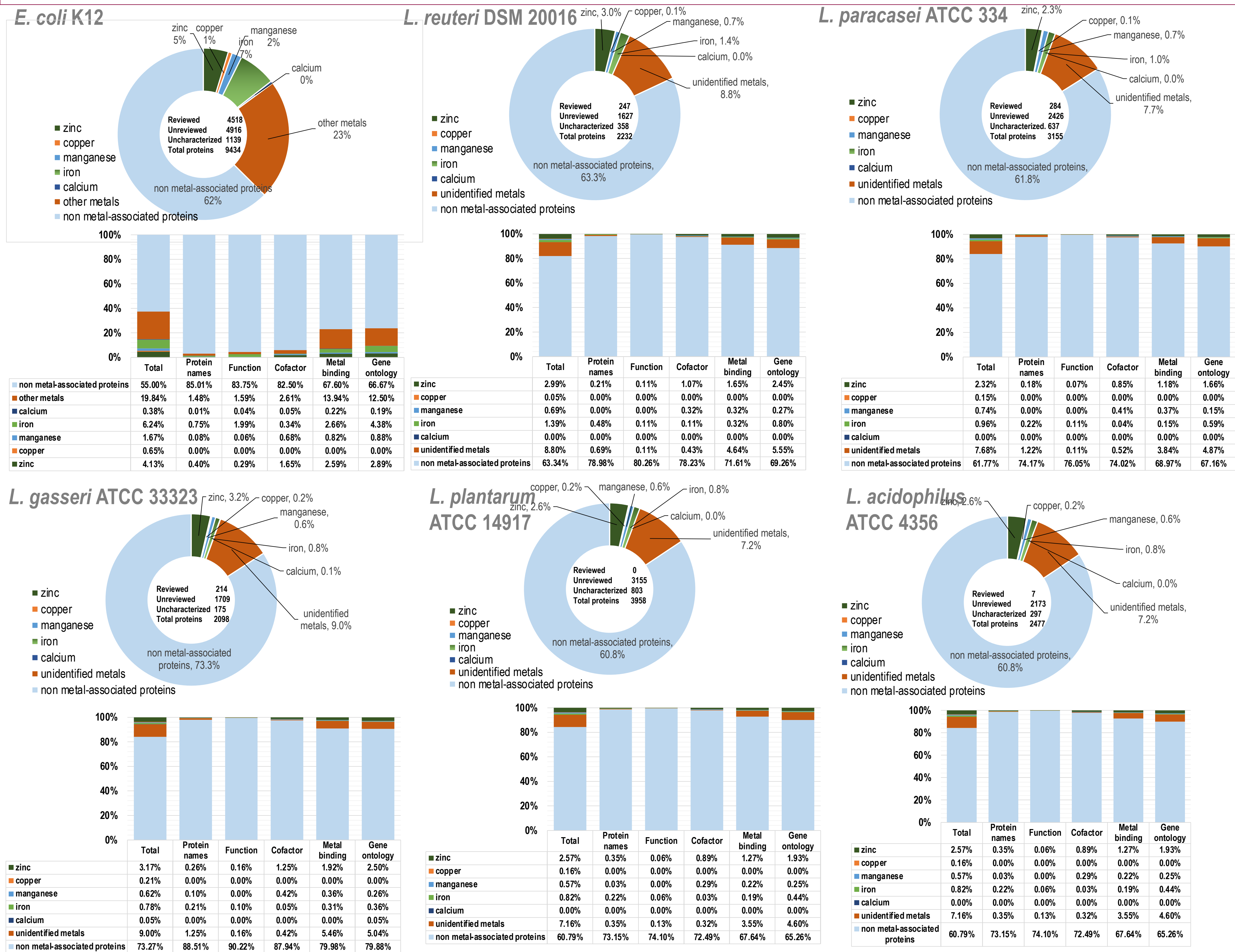
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