Metal Ion Uptake in *Lactobacillus plantarum* as a Model Organism for Studying the Human Gut Microbiota

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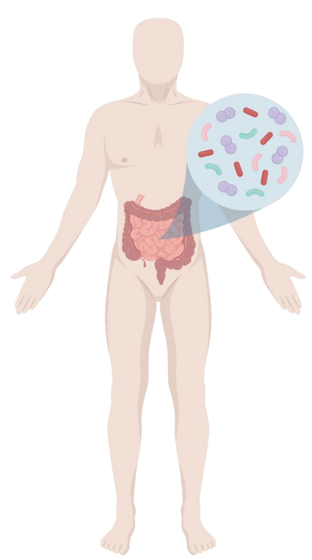
Introduction



The human body maintains a commensal relationship with millions of microbes, known as the **microbiota**. Approximately 98% of the microbiota resides in the gut, where its resident microbes digest food, take up nutrients, and protect the body from pathogens, in part by limiting **essential trace metal** ions, such as **zinc** and **iron**.

The concentrations of essential trace metal levels are tightly regulated in cells and, in commensal microbes, are affected by the host diet. Changes in essential trace metal levels are correlated to **increased likelihood of infection and gut diseases** such as obesity and diabetes.

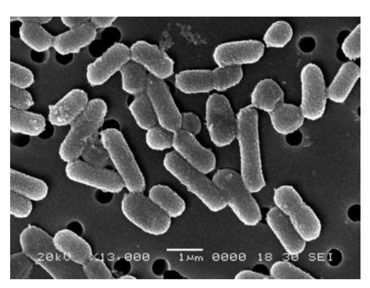


Image of Lactobacillus plantarum.[2]

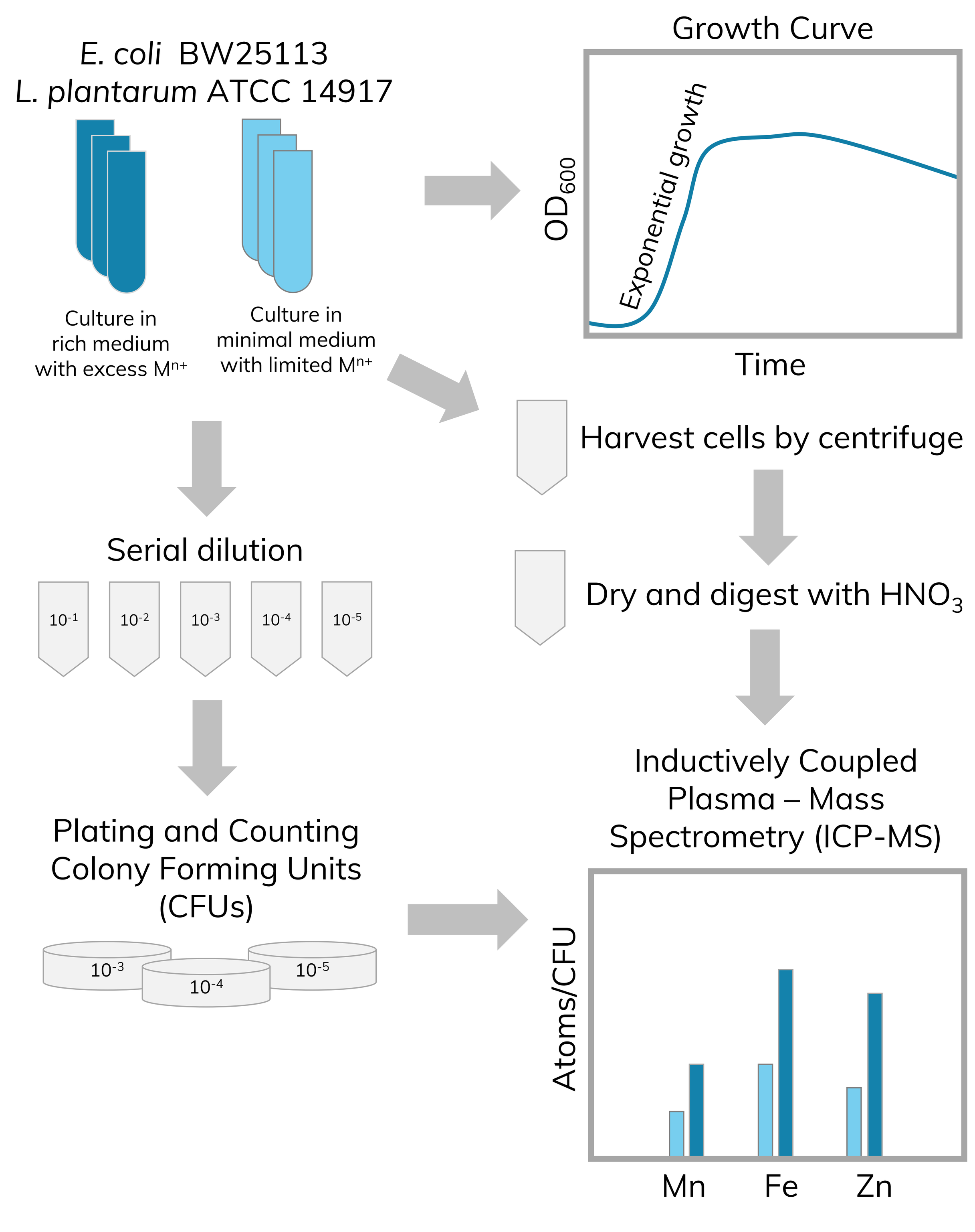
Lactobacillus plantarum is a species of lactic acid bacteria and is commonly used in probiotic formulations. The strain *L. plantarum* ATCC 14917 was sourced from the human microbiota and is a reference strain for the NIH Human Microbiome Project.

Like many lactic acid bacteria, *L. plantarum* has minimal iron requirements and may grow under host conditions that limit essential metal ions from pathogens.[1] The associated proteins have not been identified and little is known about the requirements of *L. plantarum* for other transition metal ions, such as zinc.

Our approach to elucidating the mechanisms of how lactic acid bacteria respond to changes in metal nutrients is to study the effects of metal concentration on bacterial growth and identify and characterize the associated metalloproteins, including those involved in uptake, efflux, transport, and storage.

Computational metalloproteomics may be used to exclude known, characterized metalloproteins and determine which unknown metalloproteins may play key roles in metal-response mechanisms.

Experimental Method

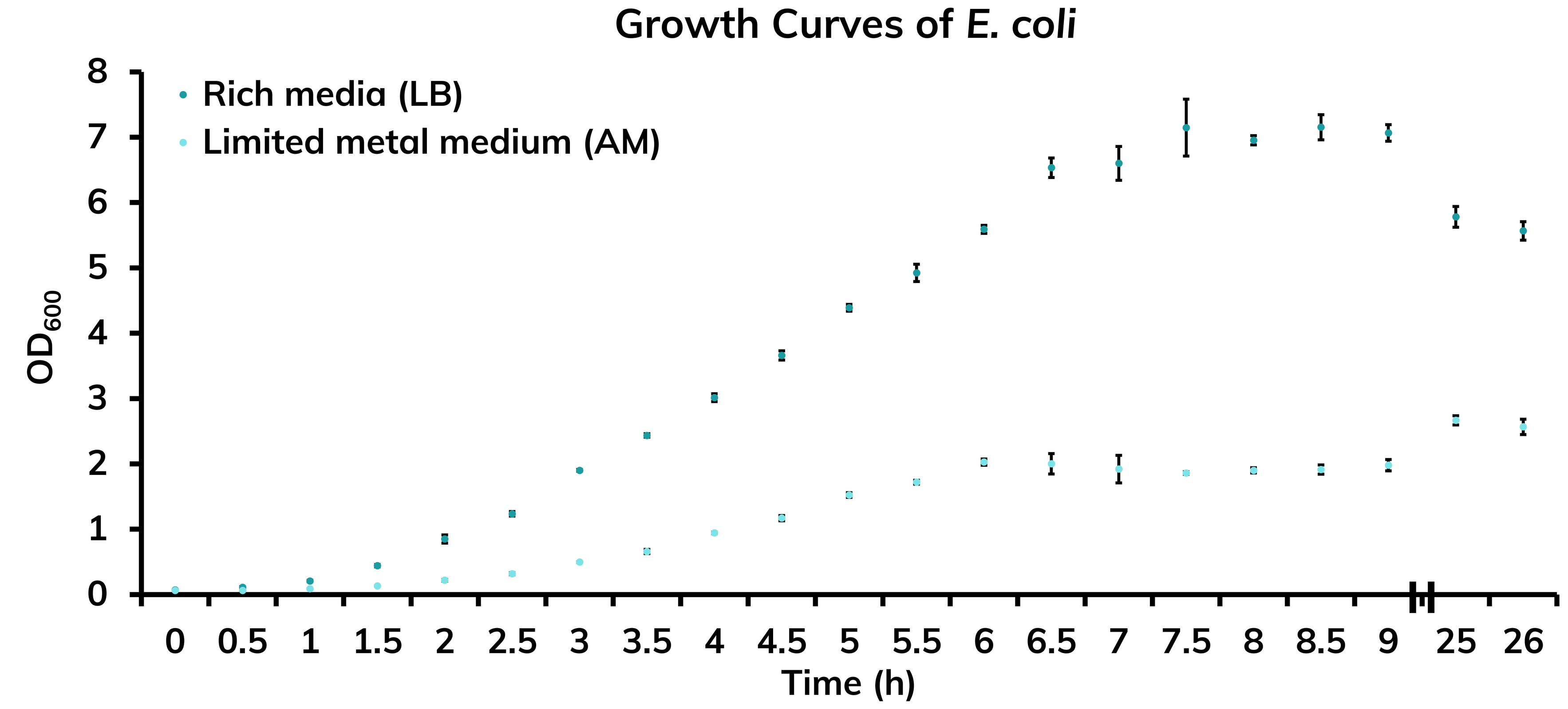


Experimental Results

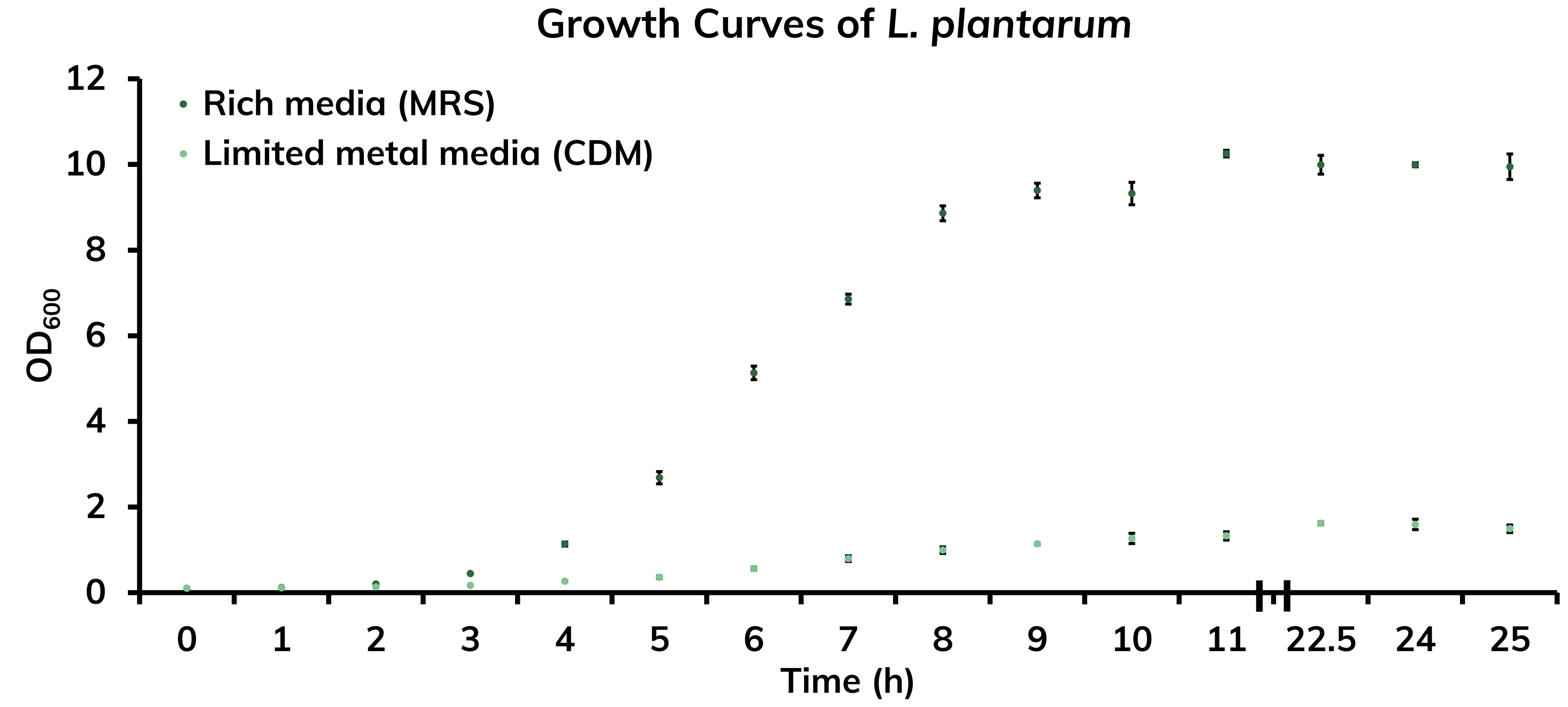
Table 1. Growth conditions of *E. coli* and *L. plantarum*

	E. coli	*L. plantarum*
Environment	Aerobic	Aerobic with 5% CO_2
Temperature	37°C	37°C
Rich media	Luria-Bertani (LB)	De Man, Rogosa, Sharpe (MRS)
Minimal media	A minimal (AM) • Ammonium sulfate • Monopotassium phosphate • Dipotassium phosphate • Magnesium sulfate • Sodium citrate dihydrate • Glucose • All 20 L-amino acids	Chemically defined media (CDM)[3-4] • Ammonium sulfate • Dipotassium phosphate • Magnesium sulfate heptahydrate • Manganese chloride • para-aminobenzoic acid • Glucose • All amino acids EXCEPT glutamine and asparagine • Vitamins B1, B2, B3, B5, B7, B11, & B12 • Nucleobases: adenine, guanine, xanthine, uracil

Growth Curves of *E. coli*

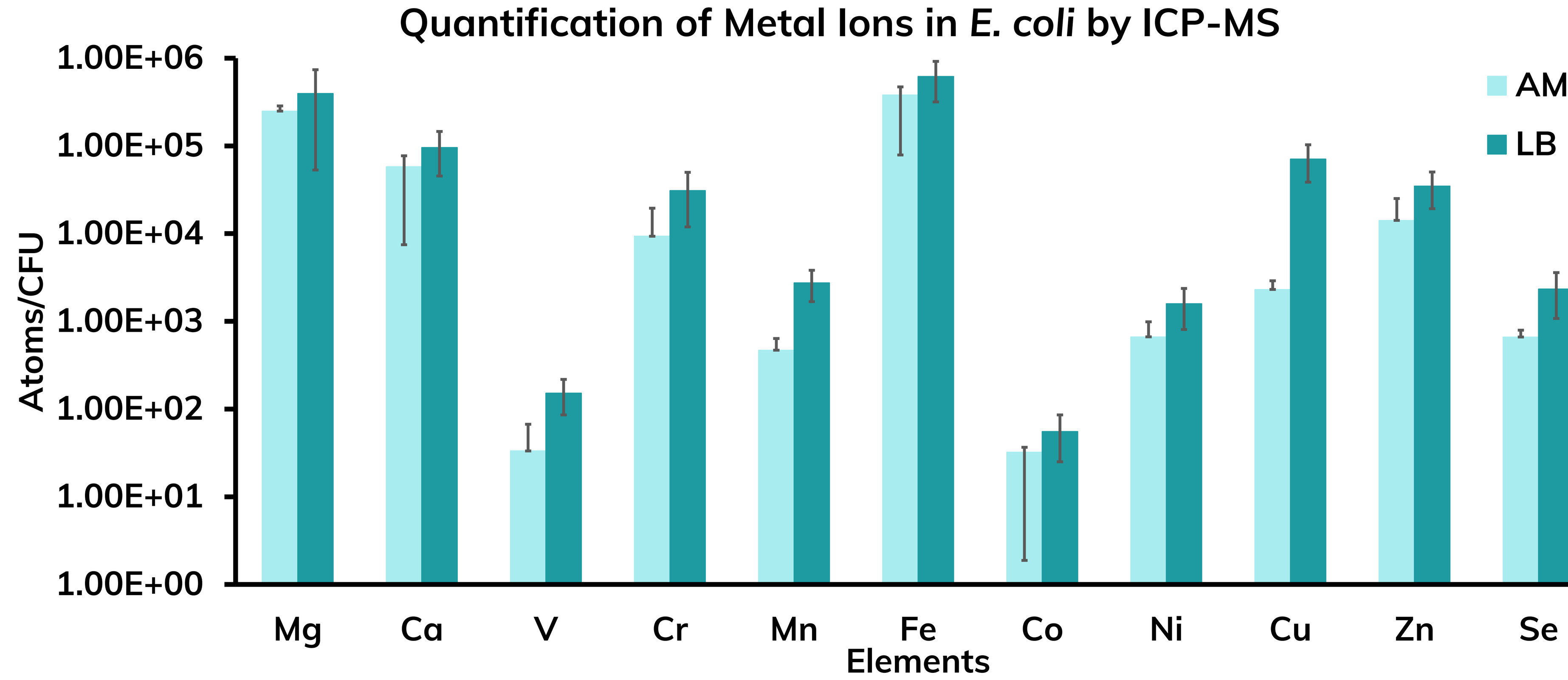


Growth Curves of *L. plantarum*



- The reported values are the mean of three independent measurements; error bars are SDs.
- When critical OD_{600} (≥0.5) was reached, samples were diluted before measurement by a defined dilution factor, *d*. The measured value was divided by *d* to obtain the corrected OD.

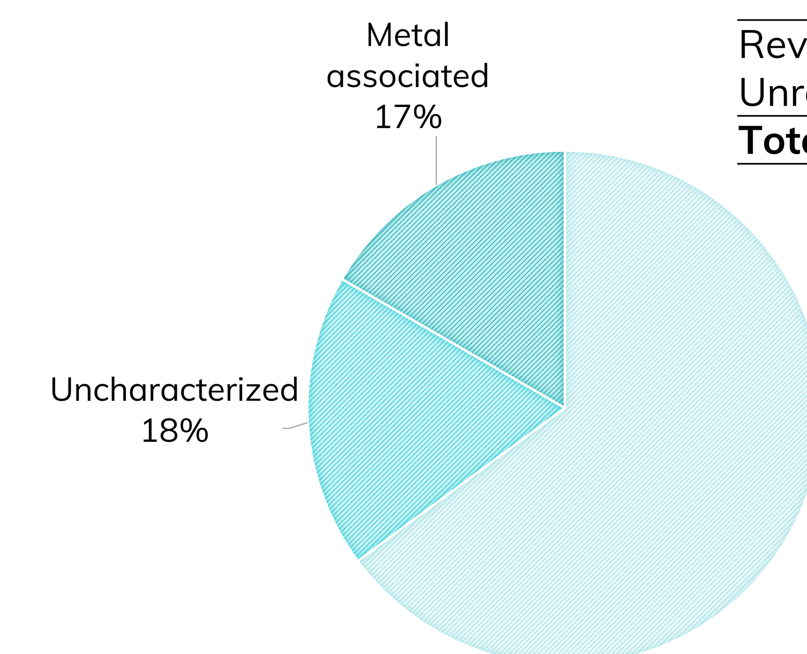
Quantification of Metal Ions in *E. coli* by ICP-MS



Metalloproteomic Analysis

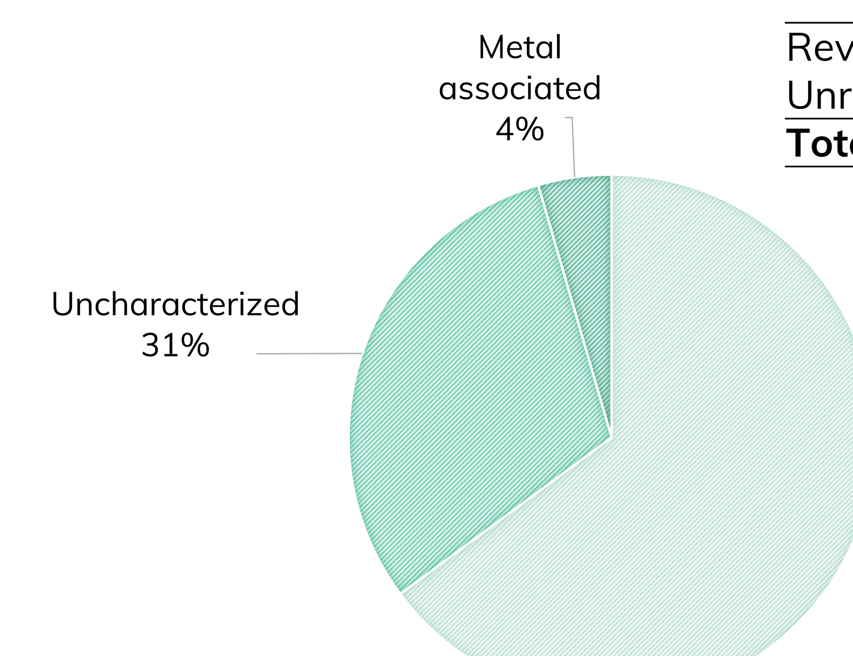
PROTEOME OF E. COLI BW25113

Reviewed	4443
Unreviewed	54
Total proteins	**4497**



PROTEOME OF L. PLANTARUM ATCC 14917

Reviewed	0
Unreviewed	3155
Total proteins	**3155**



Conclusion

- Observed more growth in rich media than minimal media for both *E. coli* and *L. plantarum*
- Metal uptake followed similar trend to published work[8] in *E. coli*
- Established foundation for future work

Future Work

- Quantify metal ions in rich and limited metal media for *L. plantarum* with ICP-MS
- Examine the effects of metals on the growth of L. plantarum under aerobic and anaerobic conditions to build foundation for future experiments with obligate anaerobes

Acknowledgements

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