

Harnessing Biomineralization Potential of Metal-Sequestering Bacteria for Rare Earth Elements (REE) Material Synthesis

April 2022

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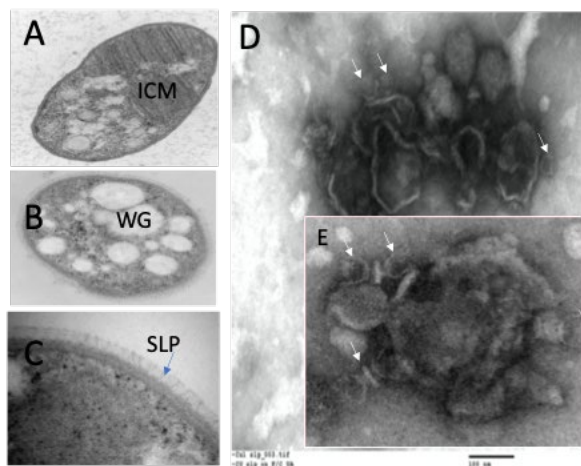
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Introduction

Extremophilic methylotrophs, such as *Methyloviummicrobium alcaliphilum* 20ZR are known for their natural ability to carry out REE capture and uptake. It has been predicted that proteins associated with surface layers facilitate transport and homeostasis of essential metals. Indeed, electron microscopic analysis coupled with antibody staining revealed that cell envelop of metal-grown *M. alcaliphilum* 20ZR contains metal-binding proteins in the base of the cup-shaped structures of S-layers. While less explored, the proteins associated with S-layers are also predicted to contribute to scavenging of other minerals essential for core metabolism. Here, we employed bottom-up proteomic study to compare the protein content of S-layer fractions of *M. alcaliphilum* 20ZR cultures grown in the presence and absence trace metals to identify putative components REE-scavenging machinery.



Summary of Results

To characterize the proteome of the *M. alcaliphilum* 20ZR, S-layers were extracted from cells grown with or without trace elements. After fractionation, a subset of samples was sent to our collaborators at SDSU (Profs. Kalyuzhnaya and Collins) for electron microscopy imaging to validate the purity of the S-layer fractions.

Upon confirmation of purity, the separated fractions were analyzed by bottom up proteomics. The overview of the proteomic analyses and normalization are shown in 2. The peptide distribution across samples reasonable and required minor normalization A-B). The averages of normalized data for fractions collected from cells grown at normal metal-limited conditions were compared. 97 proteins were more abundant or only present layer preparation, obtained from cells exposed metal limitation (Table 1). These included proteins associated with iron, molybdenum, and zinc uptake addition, as well as numerous predicted TonB-dependent receptors. The obtained list of S-layer proteins enriched in the presence of trace metals provides a unique insight into the putative molecular machinery of methanotrophic bacteria involved in the sequestration of metal including rare earth elements.

Figure 1. EM-images of *M.alcaliphilum* 20ZR cells and cell fractions. (A) Typical TEM image of cells grown with copper; (B) Typical TEM image of cell grown without copper; (C) TEM of cell surface depicting S-layer; (D-E), S-layer (SLP) fraction. Images courtesy of D. Collins D.& M. Kalyuzhnaya

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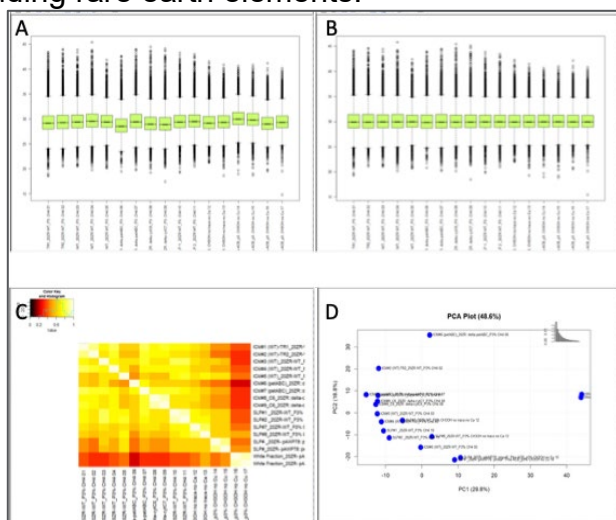


Figure 2. Overview of proteomics data. A. Log(2) peptide data before normalization; B. Log(2) peptide data after mean central tendency normalization; C. Correlation plot of log(2) peptide data; D. 2-D PCA plot of log(2) normalized peptide abundances.

Table 1. Fold change and spectral counts for top 24 proteins, which are only present or more abundant in cells grown under metal limitation.

Best Protein	Predicted Function	Fold change	Fold change	SLP+ trace	SLP-no trace
gi 802898031 ref WP_046061248.1	Putative heme degradation protein		Only in SLP WT CHEOH, passes LOQ	0	40.5
gi 351716508 emb CCE2168.1	Type II SS (PuIG, metallophores (Pseudopaline) secretion pathways, chelate multiple transition metal ions; Duplicate proteins: gi 503912983 ref WP_014146977.1		Only in SLP WT CHEOH, passes LOQ	0	26
gi 351719746 emb CCE25422.1	ABC-type hemein transport system, periplasmic component [Methylobacterium alcaliphilum 20Z]; Duplicate proteins: gi 503916181 ref WP_014150175.1		Only in SLP WT CHEOH, passes LOQ	0	13
gi 503916188 ref WP_014150182.1	TonB-dependent receptor [Methylobacterium alcaliphilum]; Duplicate proteins: gi 351719753 emb CCE25429.1		Only in SLP WT CHEOH, passes LOQ	0	27
gi 351716168 emb CCE21819.1	protein of unknown function in methanotrophs [Methylobacterium alcaliphilum 20Z]; Duplicate proteins: gi 503912643 ref WP_014146637.1	673	higher in SLP WT CHEOH, both pass LOQ (673.87 fold)	1.5	36
gi 351716784 emb CCE22446.1	conserved protein of unknown function [Methylobacterium alcaliphilum 20Z]	112	higher in SLP WT CHEOH, both pass LOQ (112.22 fold)	26	11
gi 351716584 emb CCE22244.1	putative TonB-dependent receptor [Methylobacterium alcaliphilum 20Z]; Duplicate proteins: gi 503913058 ref WP_014147052.1	100	higher in SLP WT CHEOH, both pass LOQ (100.24 fold)	18.5	145.5
gi 503914949 ref WP_014148943.1	ferric iron transporter B [Methylobacterium alcaliphilum]; Duplicate proteins: gi 351718496 emb CCE24167.1	98	higher in SLP WT CHEOH, both pass LOQ (98.18 fold)	0.5	19
gi 351719893 emb CCE25569.1	import inner membrane translocase subunit Tim44 [Methylobacterium alcaliphilum 20Z]; Duplicate proteins: gi 503916324 ref WP_014150318.1	48	higher in SLP WT CHEOH, both pass LOQ (47.92 fold)	1.5	11.5
gi 351719751 emb CCE25427.1	conserved exported protein of unknown function [Methylobacterium alcaliphilum 20Z]; Duplicate proteins: gi 503916186 ref WP_014150180.1	36	higher in SLP WT CHEOH, both pass LOQ (35.98 fold)	5	56
gi 351719556 emb CCE25232.1	protein of unknown function, specific for methanotrophic bacteria [Methylobacterium alcaliphilum 20Z]; Duplicate proteins: gi 503915993 ref WP_014149987.1	25	higher in SLP WT CHEOH, both pass LOQ (24.73 fold)	67	23.5
gi 503914784 ref WP_014148778.1	MULTISPECIES: hemerythrin [Methylobacterium]; Duplicate proteins: gi 351718328 emb CCE23997.1	19	higher in SLP WT CHEOH, both pass LOQ (19.25 fold)	2	17.5
gi 503915114 ref WP_014149108.1	branched chain amino acid aminotransferase [Methylobacterium alcaliphilum]; Duplicate proteins: gi 351718666 emb CCE24340.1	19	higher in SLP WT CHEOH, both pass LOQ (19.24 fold)	5.5	16.5
gi 503915100 ref WP_014149094.1	MULTISPECIES: serine hydrolase [Methylobacterium]; Duplicate proteins: gi 351718652 emb CCE24326.1	19	higher in SLP WT CHEOH, both pass LOQ (19.01 fold)	2	12
gi 503913445 ref WP_014147439.1	TonB-dependent receptor [Methylobacterium alcaliphilum]; Duplicate proteins: gi 351716973 emb CCE22638.1	16	higher in SLP WT CHEOH, both pass LOQ (15.98 fold)	7	35
gi 503916385 ref WP_014150379.1	biopolymer transporter MotA/roQ/EshB proton channel-related [Methylobacterium alcaliphilum]; Duplicate proteins: gi 351719954 emb CCE25630.1	16	higher in SLP WT CHEOH, both pass LOQ (15.73 fold)	8.5	29
gi 802899102 ref WP_046061498.1	cysteine sulfinatedesulfase [Methylobacterium alcaliphilum]	15	higher in SLP WT CHEOH, both pass LOQ (14.71 fold)	4	17.5
gi 351716552 emb CCE22212.1	particulate methane monooxygenase C subunit [Methylobacterium alcaliphilum 20Z]; Duplicate proteins: gi 503913026 ref WP_014147020.1	14	higher in SLP WT CHEOH, both pass LOQ (14.09 fold)	78	38.5
gi 802898746 ref WP_046061274.1	hypothetical protein [Methylobacterium alcaliphilum]	13	higher in SLP WT CHEOH, both pass LOQ (13.45 fold)	1.5	12
gi 802899045 ref WP_046061441.1	TonB-dependent receptor [Methylobacterium alcaliphilum]	11	higher in SLP WT CHEOH, both pass LOQ (10.98 fold)	25	88.5
gi 351716669 emb CCE22331.1	Histidinol dehydrogenase (HDH) [Methylobacterium alcaliphilum 20Z]; Duplicate proteins: gi 503913143 ref WP_014147137.1	11	higher in SLP WT CHEOH, both pass LOQ (10.91 fold)	4	15
gi 802899000 ref WP_046061397.1	glutathione disulfide reductase [Methylobacterium alcaliphilum]	11	higher in SLP WT CHEOH, both pass LOQ (11.17 fold)	2	11
gi 351716650 emb CCE22312.1	putative amylase, alpha amylase [Methylobacterium alcaliphilum 20Z]; Duplicate proteins: gi 503913124 ref WP_014147118.1	10	higher in SLP WT CHEOH, both pass LOQ (10.43 fold)	2	11
gi 351717539 emb CCE23204.1	Procorrin-6γ C5,15-methyltransferase (Deacetylating), ChIE subunit [Methylobacterium alcaliphilum 20Z]; Duplicate proteins: gi 503914004 ref WP_014147998.1	10	higher in SLP WT CHEOH, both pass LOQ (10.44 fold)	2.5	10.5

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