Fishing up rare bacterial proteomes using SWATH-MS in synthetic microbial communities

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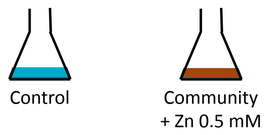
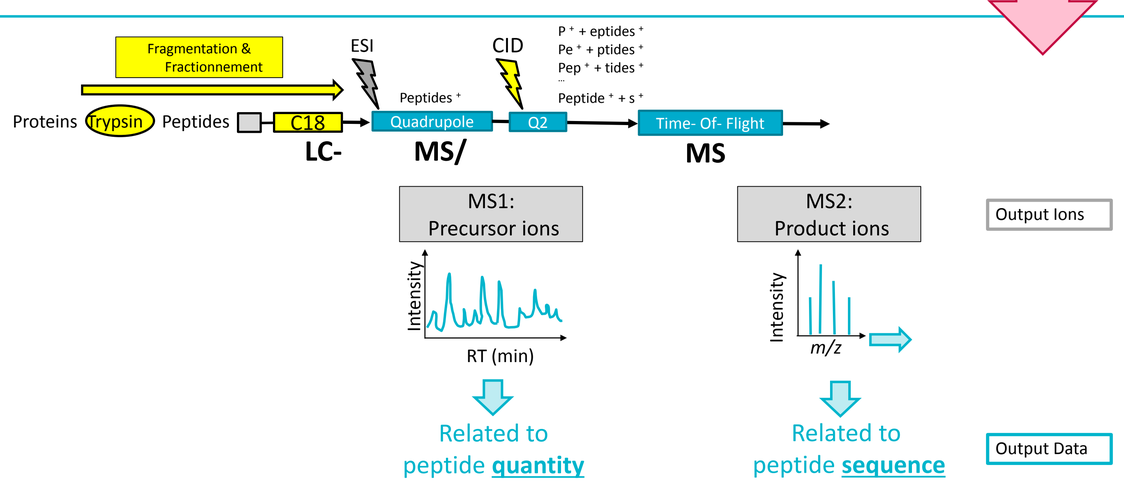


Fig. 1| Communities were grown at 16 °C, 90 rpm in marine medium.

We created a **synthetic community** composed of **9 bacteria** in order to analyse the effect of metals. This community was cultivated in marine conditions and stressed for 28 days using zinc 0.5 mM (**Fig. 1**).



Metaproteomic (LC-MS/MS) pipeline using TripleTOF™ 5600 (AB Sciex, ESI ionization source).

SWATH-MS

To increase the number of Precursor Ions, MS1 Spectra were decomposed in **Sequential Windows** (Fig. 4A), each of these being fully used for MS2. The resulting MS2 spectra are then composed of **every peptides whatever their abundance**. This approach is called "Data-Independent Acquisition" (DIA) using **SWATH** (Sequential Windows Acquisition of all THeoretical fragment ion; Gillet *et al.*, 2012).

A reference spectral library composed of known peptides is then used as a map of the different Precursor Ions that can theoretically be obtained with the species of the community. This library is prepared using DDA-MS analyses of **each bacterial species** individually cultivated in the same conditions (Fig. 4B).

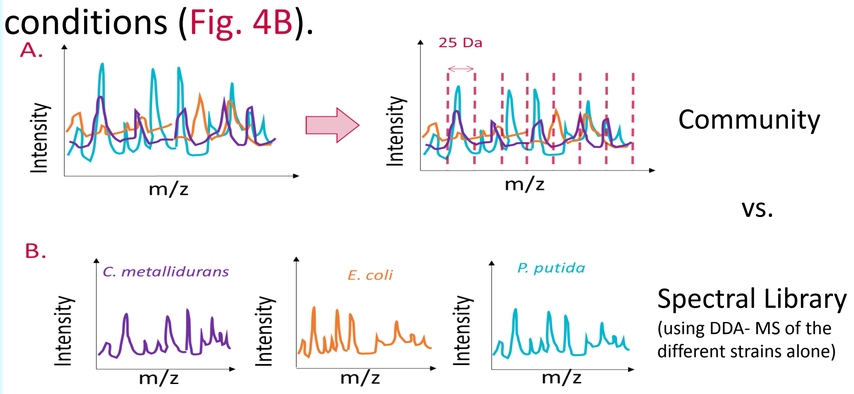
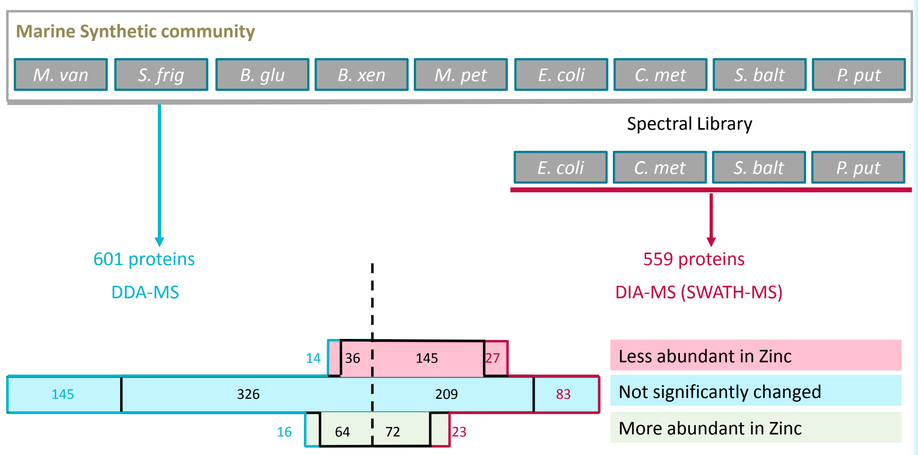


Fig. 4| SWATH-MS first divides the acquisition process in 32 Sequential overlapping Windows of 25 Da (A) and compares the resulting spectra to a spectral library composed of known peptides for identification (B).

Fig. 5| Protein amount for each experiments.
Most of the proteins are found in both analyses (in black).

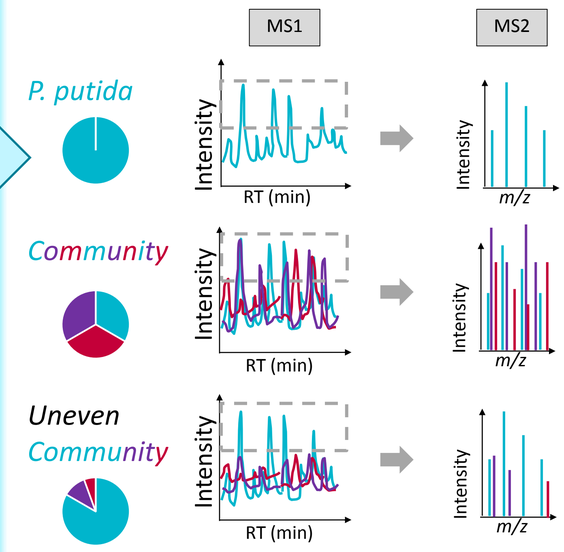


When comparing the 2 conditions(+/- zinc), a subsequent part of the proteins (*i.e.* 117 proteins) that were not significantly found upregulated or downregulated by metal during DDA-MS analysis became significantly upregulated or downregulated (Fig. 5).

As quantification is based on the Fragment ions (MS2 step), the SWATH-MS approach generally increases the accuracy of the quantification for all peptides including low abundance peptides.

DDA-MS

The community composition was studied using **Q-PCR** (targeting 16S DNA of each bacteria). In parallel, **metaproteomics** was used in order to detect proteins involved in metal stress resistance.



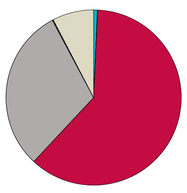
The most abundant Precursor Ions were selected for MS2 (**Fig. 2**). Subsequently only a subset of proteins were identified and quantified. This approach is called "Data- Dependent Acquisition"-MS (**DDA-MS**).

If a bacterium dominates in the community, mostly peptides from this bacterium are identified. No conclusions can be drawn for the other species.

Fig. 2| DDA-MS selects the most abundant peptides.

As an example, within the Marine synthetic community, mainly *P. putida* proteins were identified (**Fig. 3**).

A. Q-PCR



B. DDA-MS

	No Zinc	0.5 mM Zinc	Strain	# proteins	Identified with… peptides 1 2 3 4 5 6
	0.610	0.900	***P. putida***	521	121 83 60 52 35 291
	0.301	0.001	***S. baltica***	50	14 13 13 6 7 11
	0.078	0.074	***M. petroleiphilum***	-	1
	0.007	0.021	***C. metallidurans***	23	11 5 8 2 2 6
	0.002	0.002	***B. xenovorans***	-	1
	0.001	0.002	***M. vanbaalenii***	-	
	0.000	0.001	***B. glumae***	-	1
	0.000	0.000	***E. coli***	1	2 1
	0.000	0.000	***S. frigidimarina***	6	1 1 3 2

Fig. 3| Q-PCR and metaproteomics of the synthetic community: (A) Ratio of cells based on 16S DNA Q-PCR and (B) Number of proteins identified using DDA-MS according to species. « # proteins » indicates the total number of relevant proteins (*i.e.* with more than 1 peptide).

SWATH-MS specifically increased the number of proteins identified for rare species. 40 proteins of *C. metallidurans* were identified in total *i.e.* twice more than for DDA analysis.

UNIPROT	# pep	Group	Log2Ratio(NoZ/Z)	pval	EggNOGG
Q1LLB2	5	Phosphate-binding protein PstS	-1.94	0.001	Inorganic ion transport and metabolism
Q1LJ09	2	Phosphite transport system-binding protein ptxB	-1.75	0.004	Inorganic ion transport and metabolism
Q1LRE1	3	Glu and asp transporter, PBP (ABC superfamily)	-1.88	0.001	Amino acid transport and metabolism
Q1LKG9	3	Leu/ileu/val transporter subunit, PBP (ABC superfamily)	-1.68	0.003	Amino acid transport and metabolism
Q1LP29	3	Putative ABC transporter, periplasmic substrate-binding protein	-1.07	0.007	Function unknown
Q1LR19	4	Extra-cytoplasmic Solute Receptor protein (Bug, PBP)	-2.06	0.001	Function unknown
Q1LKG0	3	Malate dehydrogenase	-2.65	0.001	Energy production and conversion
Q1LIV8	3	Fused malic enzyme oxidoreductase phosphotransacetylase	-0.78	0.015	Energy production and conversion
Q1LH35	6	Acetyl-CoA acetyltransferase	-1.78	0.000	
Q1LKM2	3	Acyl carrier protein	-2.43	0.000	Lipid transport and metabolism
Q1LLG3	2	Thioredoxin	-1.24	0.005	Post-translational modification, protein turnover, and chaperones
Q1LLZ7	3	Alkyl hydroperoxide reductase, peroxiredoxin (Thioredoxin-dependent p	-2.29	0.001	Post-translational modification, protein turnover, and chaperones
Q1LQS5	3	10 kDa chaperonin	-2.42	0.002	Post-translational modification, protein turnover, and chaperones
Q1LAZ9	2	Cold-shock DNA-binding protein family	-0.97	0.018	
Q1LRG2	2	Aspartate--tRNA(Asp/Asn) ligase	-1.52	0.005	Translation, ribosomal structure and biogenesis
Q1LJ99	2	30S ribosomal protein S20	3.83	0.029	Translation, ribosomal structure and biogenesis
Q1LI52	2	50S ribosomal protein L6	-2.52	0.001	Translation, ribosomal structure and biogenesis
Q1LI55	2	50S ribosomal protein L30	-1.09	0.029	Translation, ribosomal structure and biogenesis

Fig. 6| Protein found differentially expressed with Zn 0.5 mM. UNIPROT code, # of peptides used for identification (« # pep »), Log 2 Ratios NoZ/Z, pvalue of Student t-test and EggNOG first level of annotation are given.

Most of the *Cupriavidus* proteins more abundant in the presence of Zn were involved either in various sensing and transport systems (6 out of 18) or in stress response (Fig. 6).

SWATH-MS is a major breakthrough in the field of functional microbiology. It increases the **quantity of proteins detected for rare bacteria** as well as the **overall accuracy of the quantification**. Thanks to this approach, we were able to analyse the influence of metals on *C. metallidurans* in a synthetic community composed of 9 strains. We are currently increasing the Spectral Library to expand the identification of the other rare species in the community.

- SWATH-MS increases the accuracy of quantification.

- SWATH-MS specifically increases the number of proteins identified for rare species.

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Gillet LC, Navarro P, Tate S, Rost H, Selevsek N, Reiter L, *et al.* (2012). Targeted Data Extraction of the MS/MS Spectra Generated by Data-independent Acquisition: A New Concept for Consistent and Accurate Proteome Analysis. *Molecular & Cellular Proteomics* **11**: O111.016717