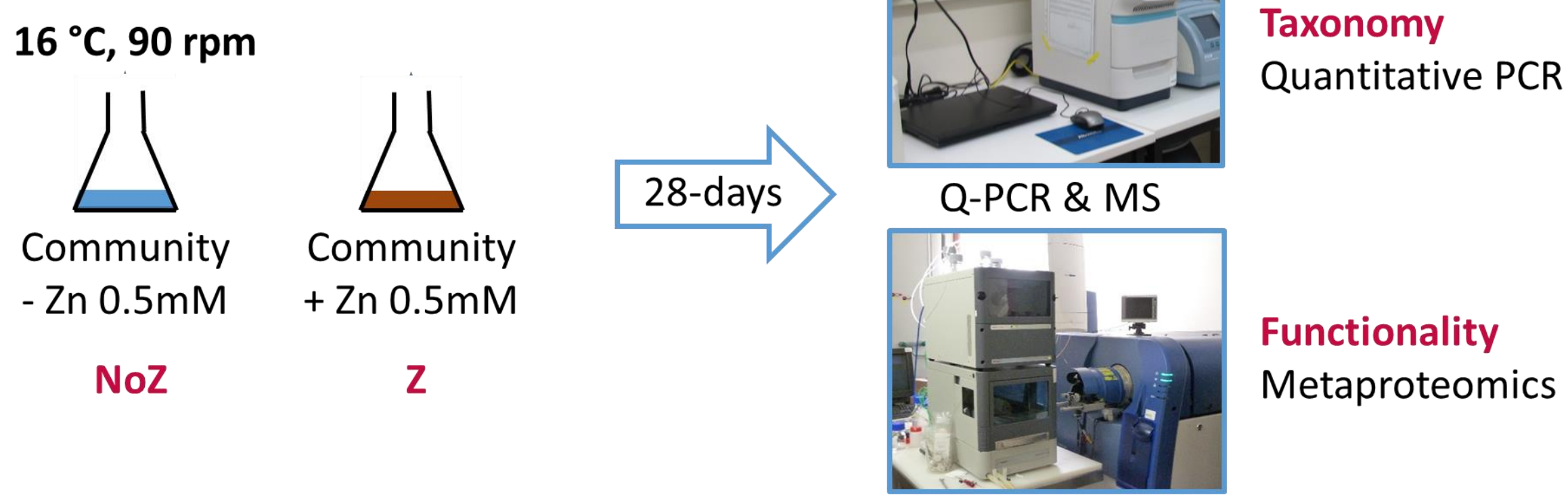# SWATH-MS increases coverage in metaproteomics. Application to a Zn-stressed marine synthetic community

Mélanie BERAUD, Giuseppe GIAMBARRESI, Ruddy WATTIEZ and David C. GILLAN

Laboratory of Proteomic and Microbiology, Mons University, Avenue du Champ de Mars, 6, 7000 Mons, Belgium
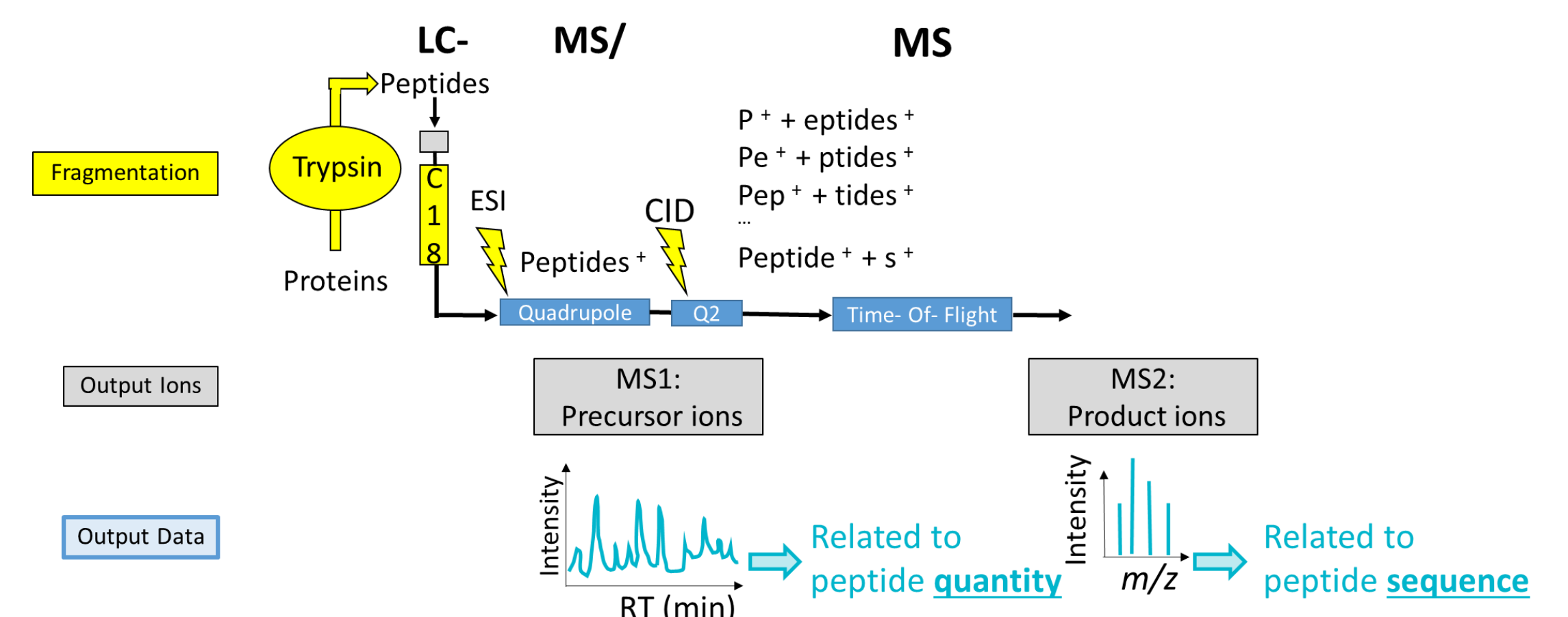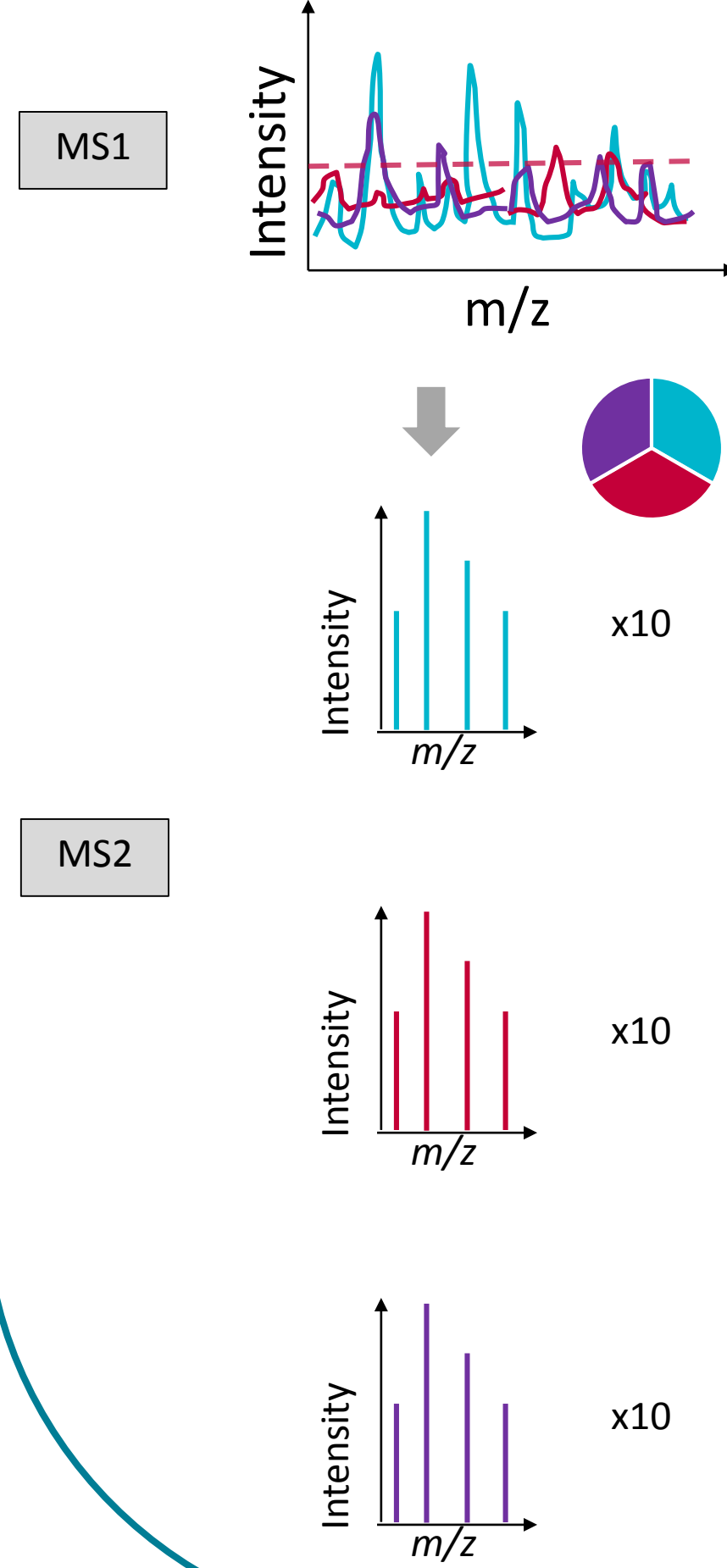
Material and Methods

16 °C, 90 rpm

Community - Zn 0.5mM — **NoZ**
Community + Zn 0.5mM — **Z**

28-days

Q-PCR & MS

Taxonomy Quantitative PCR

Functionality Metaproteomics

STRAINS	Provider	Class
1. *Pseudomonas putida* KT2440	Gift	γ-Proteobacteria
2. *Cupriavidus metallidurans* CH34	Lab strain	β-Proteobacteria
3. *Escherichia coli* BL21-DE3	Lab strain	γ-Proteobacteria
4. *Shewanella baltica* OS155	DSM	γ-Proteobacteria
5. *Shewanella frigidimarina* ACAM591	DSM	γ-Proteobacteria
6. *Burkholderia xenovorans* LB400	BCCM	β-Proteobacteria
7. *Burkholderia glumae* P1-22-1	BCCM	β-Proteobacteria
8. *Methylibium petroleiphilum* PM1	BCCM	β-Proteobacteria
9. *Mycobacterium vanbaalenii* PYR-1	DSM	Actinobacteria

Q-PCR:
16S DNA of each species transformed into cell #
Metaproteomics:
LC-MS/MS Triple TOF 5600 (ABSciex)
DDA-MS & SWATH-MS
Spectral Library: *S. baltica, E. coli, C. metallidurans.* & *P. putida*
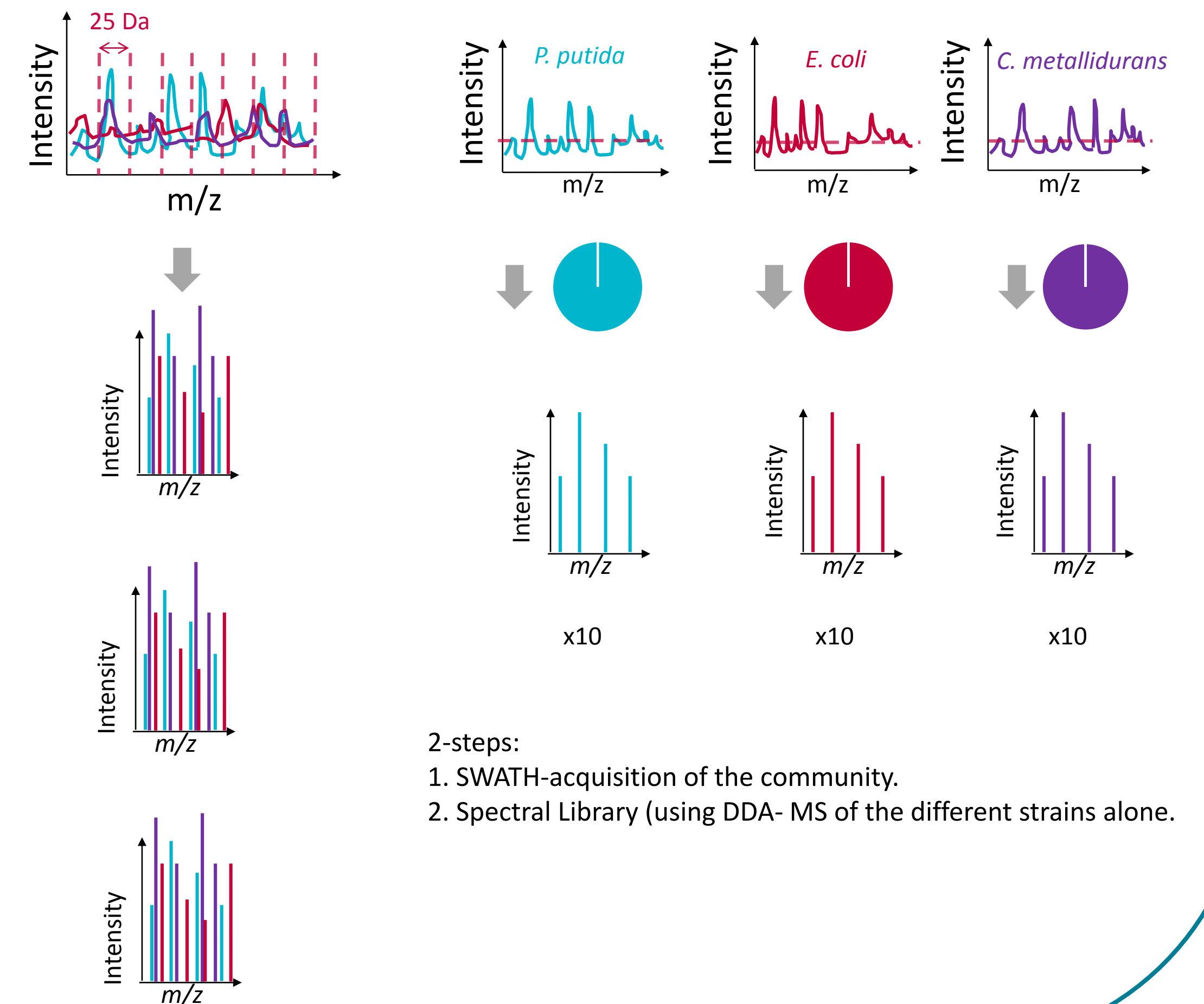**Analysis:** UNIPROT, NCBI, Excel, R (R Studio), SEED Viewer, RAST, PATRIC, Blast (Stand alone), Venny…
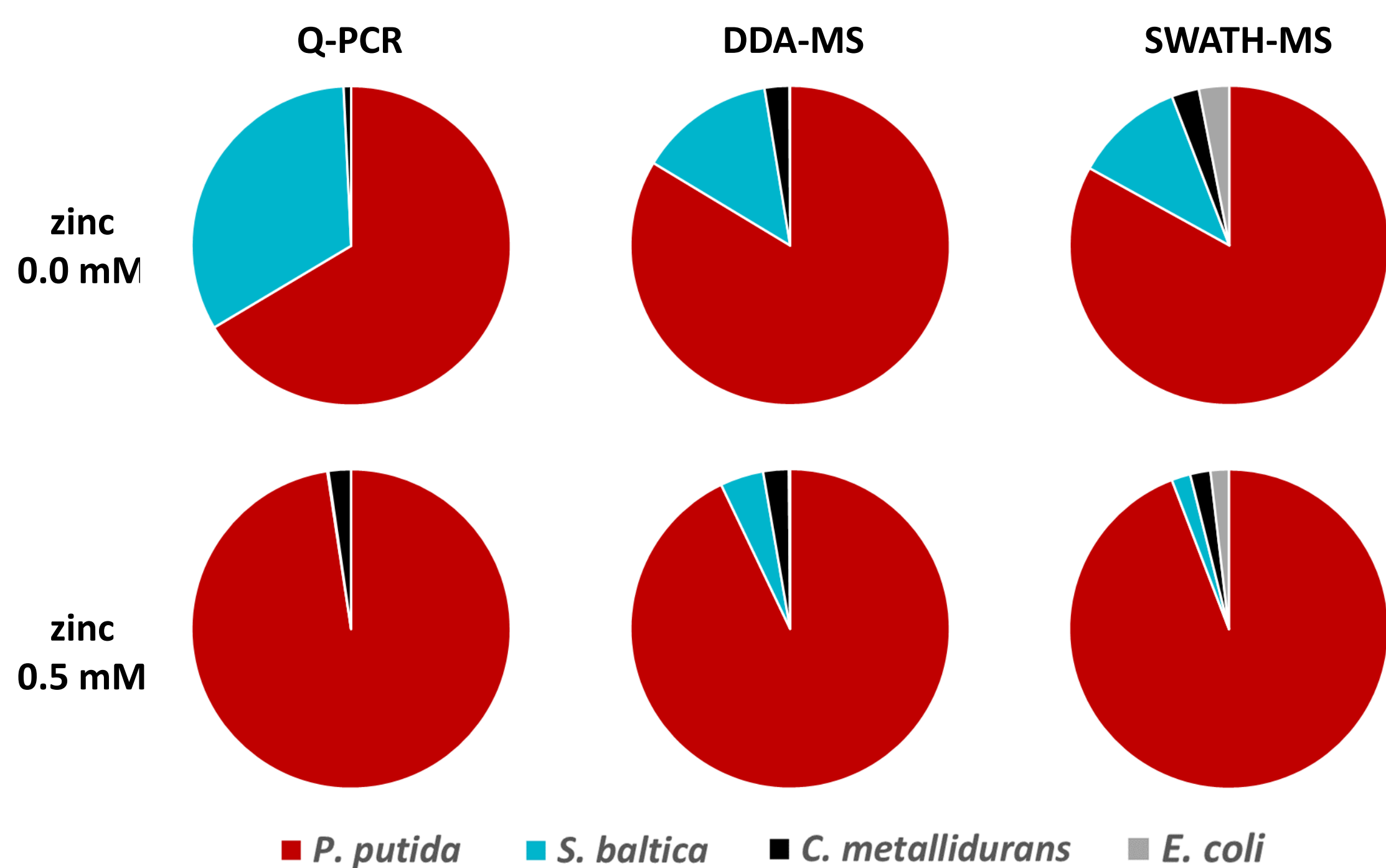
LC-MS/MS

A. DDA-MS

B. SWATH-MS[1] (DIA-MS)

2-steps:
1. SWATH-acquisition of the community.
2. Spectral Library (using DDA- MS of the different strains alone.

Results

A. Taxonomy

Q-PCR | DDA-MS | SWATH-MS

zinc 0.0 mM

zinc 0.5 mM

■ *P. putida* ■ *S. baltica* ■ *C. metallidurans* ■ *E. coli*

B. Number of proteins

Strain	# proteins with > 1 peptide		Spectral Library > 1 peptide
	DDA-MS	SWATH-MS	
P. putida	521	468	1268
S. baltica	50	46	1294
C. metallidurans	23	**40**	1106
E. coli	1	**5**	1403
Others	6	-	28

Potentiality of detection is increased for rarer species.
Protein detection depends on abundance.

C. Accuracy of quantification

MS / Strain	% of proteins with at least 2-fold difference +/- Zn 0.5 mM	
	DDA-MS	SWATH-MS
P. putida	44 %	47 %
S. baltica	52 %	65 %
C. metallidurans	61 %	50 %
E. coli	0 %	60 %

MS / Strain	% of proteins significatively different +/- Zn 0.5 mM (p-value < 0.05)	
	DDA-MS	SWATH-MS
P. putida	22 %	**49 %**
S. baltica	20 %	**37 %**
C. metallidurans	4 %	**45 %**
E. coli	0 %	**40 %**

Cupriavidus metallidurans

Number of proteins

DDA-MS 31 | Spectral Library for SWATH-MS 1106 | SWATH-MS 1106

Proteins involved in Stress resistance

Systems	DDA	Spectral Library	SWATH-MS
Czc	0	3	0
Cop	0	7	0
Ars	0	1	0
Cad	0	0	0
PstS	1	1	1
PtxB	1	1	1
Thioredoxin	1	1	1
Peroxiredoxin	1	1	1
ABC-receptors	1	5	5

Only proteins represented in the Spectral Library can be detected in SWATH-MS
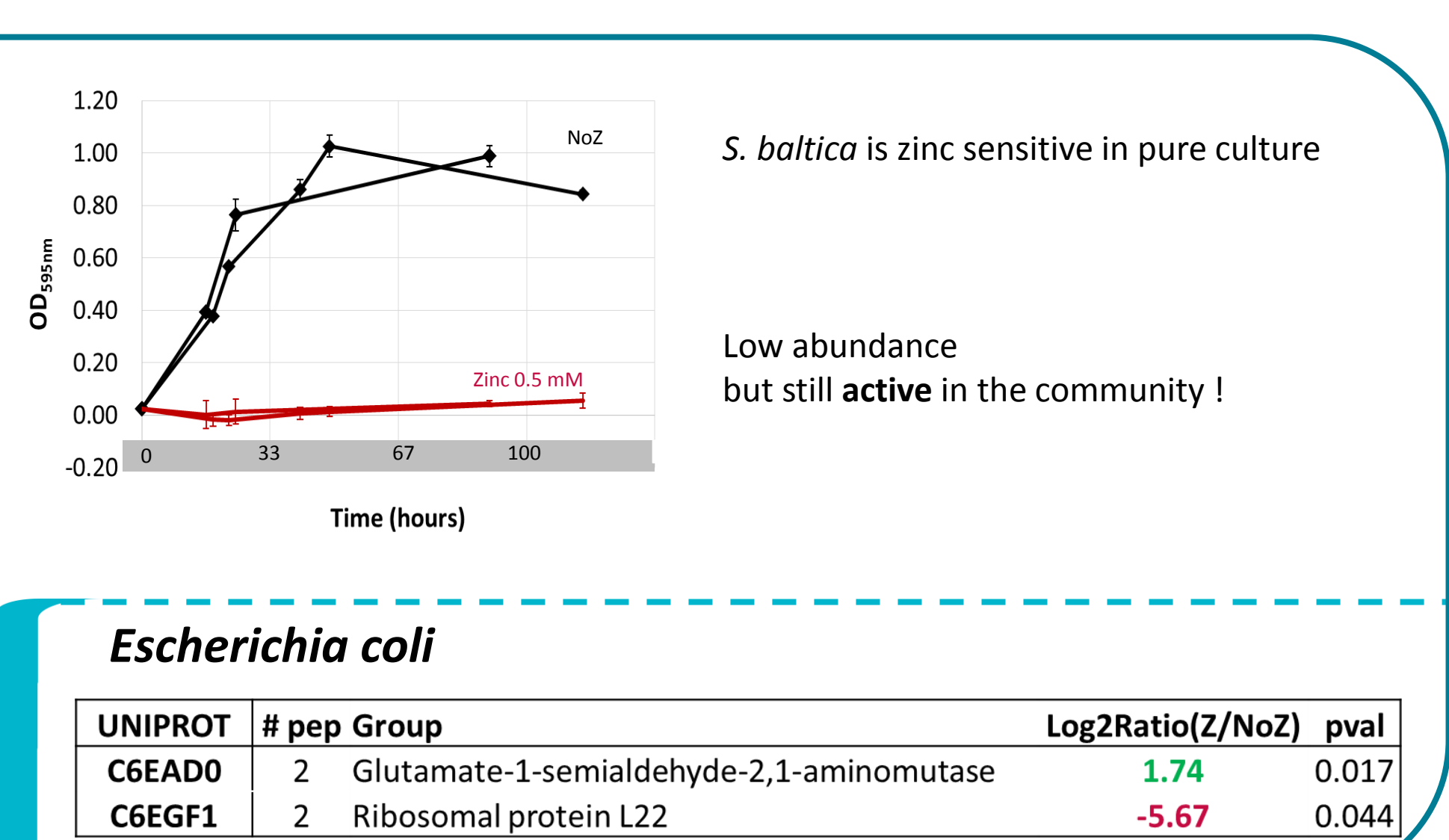
Significativity and Ratios

◇ DDA-MS ◆ SWATH-MS ● p.value < 0.05

Cupriavidus response

UNIPROT	# pep	Group	Log2(Z/NoZ)	pvalue	Function
Q1LKG0	3	Malate dehydrogenase	2.65	0.001	
Q1LIV8	3	Fused malic enzyme oxidoreductase phosphotransacetylase	0.78	0.015	
Q1LLB2	5	Phosphate-binding protein PstS	1.94	0.001	(ABC) transporter Phosphate
Q1LJO9	2	Phosphite transport system-binding protein ptxB	1.75	0.004	(ABC) transporter Phosphonate
Q1LRE1	3	Glu and asp transporter, PBP (ABC superfamily)	1.88	0.001	(ABC) transporter
Q1LKG9	2	Leu/Ileu/val transporter subunit, PBP (ABC superfamily)	1.68	0.003	(ABC) transporter
Q1LPZ9	3	Putative ABC transporter, periplasmic substrate-binding protein	1.07	0.007	(ABC) transporter
Q1LIR19	2	Extra-cytoplasmic Solute Receptor protein (Bug, PBP)	1.07	0.001	
Q1LLZ7	3	Alkyl hydroperoxide reductase, peroxiredoxin (Thioredoxin-dependent peroxide reductase)	2.29	0.001	Peroxide reductase
Q1LLG3	2	Thioredoxin	1.24	0.005	Thioredoxin
Q1LRG2	2	Aspartate–tRNA(Asp/Asn) ligase	1.52	0.005	Aspartyl-trna synthetase
Q1LAZ9	2	Cold-shock DNA-binding protein family	0.97	0.018	
Q1LQS5	3	10 kDa chaperonin	2.42	0.002	
Q1LKM2	3	Acyl carrier protein	2.43	0.001	Lipid Transport and Metabolism
Q1LH35	6	Acetyl-CoA acetyltransferase	1.78	0.000	Lipid Transport and Metabolism
Q1LU52	2	50S ribosomal protein L6	2.52	0.001	Ribosomal protein
Q1LU55	3	50S ribosomal protein L30	1.09	0.029	Ribosomal protein
Q1LU99	3	30S ribosomal protein S20	-3.83	0.029	Ribosomal protein

Shewanella baltica

UNIPROT	# pep	Group	Log2Ratio(Z/NoZ)	pval
A3DA60	2	50S ribosomal protein L5	2.54	0.000
A3DAK3	3	Pirin domain protein domain protein	2.52	0.001
A3D554	2	Iron-containing alcohol dehydrogenase	2.42	0.012
A3DAR4	2	ATP synthase subunit beta	2.13	0.001
A3D5Y4	2	Cell division protein ZipA homolog	1.83	0.013
A3D018	2	Uncharacterized protein	1.21	0.030
A3D4Q1	2	Glyceraldehyde-3-phosphate dehydrogenase	0.96	0.034
A3D1L6	4	Putrescine-binding periplasmic protein	-0.81	0.028
A3DBA4	3	50S ribosomal protein L1	-0.94	0.045
A3D1P1	2	N-acetylglucosamine kinase	-1.27	0.038
A3D5S0	2	Peptidyl-prolyl cis-trans isomerase	-1.40	0.013
A3D3B6	2	Acyl carrier protein	-1.57	0.025
A3DBA0	4	Elongation factor Tu 2	-1.91	0.009
A3D2H5	3	Uncharacterized protein	-1.92	0.013
A3D4Q4	3	Transketolase, central region	-2.44	0.045
A3DA67	2	50S ribosomal protein L22	-3.45	0.021
A3D2D5	5	Phosphate binding protein	-5.10	0.009

S. baltica is zinc sensitive in pure culture

Low abundance but still **active** in the community !

Escherichia coli

UNIPROT	# pep	Group	Log2Ratio(Z/NoZ)	pval
C6EAD0	2	Glutamate-1-semialdehyde-2,1-aminomutase	1.74	0.017
C6EGF1	2	Ribosomal protein L22	-5.67	0.044

CONCLUSIONS :

1. **Rare species become visible** by the SWATH approach

2. Proteins quantification is more **accurate**

3. Representation in **Spectral Library** is required

4. **Dying versus rare but active** bacteria can be differenciated

Université de Mons