Targeted metaproteomic analysis suggests the use of oxidative stress to survive zinc exposure
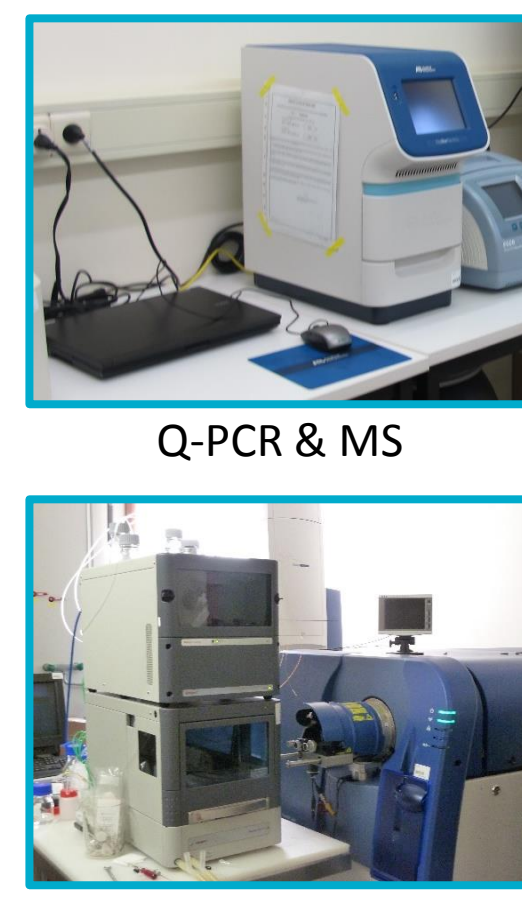
Mélanie Beraud, David C. Gillan, Ruddy Wattiez

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

Materials and Methods

Community Controls — Community + Zn 0.5mM — 5 & 28 days — Q-PCR & MS

Taxonomy: Quantitative-PCR
Functionality: Metaproteomics

(16 °C, 90 rpm, marine medium)

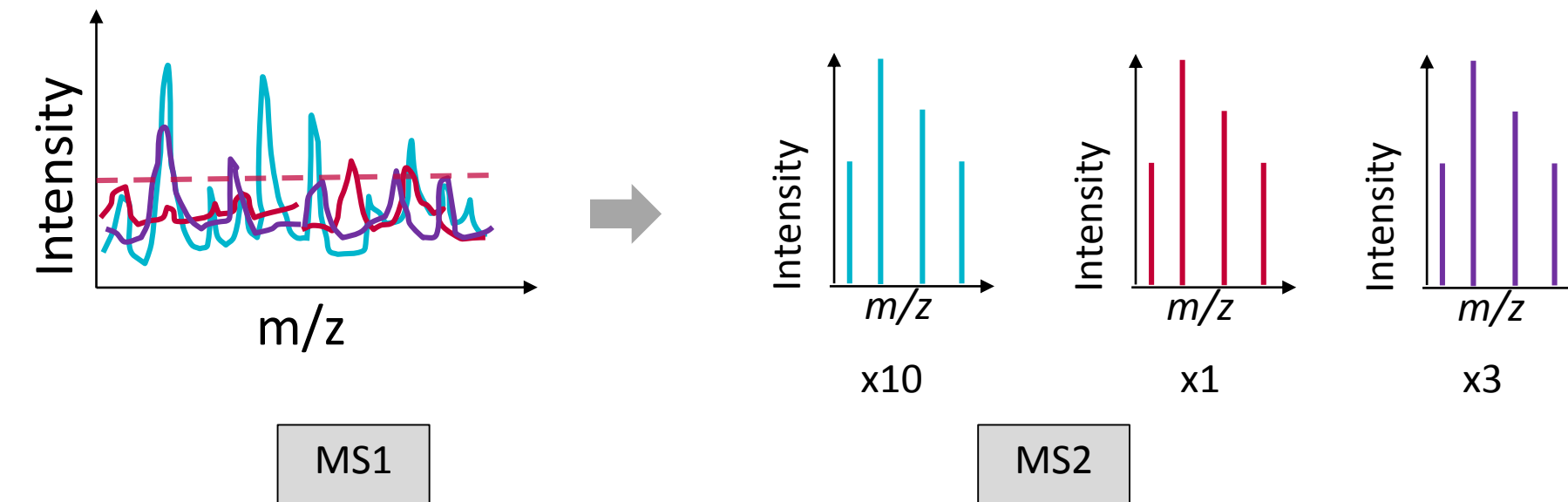| STRAIN		SPECTRAL LIBRARY
1. *Pseudomonas putida*	PSEPK	Yes
2. *Cupriavidus metallidurans*	CUPMC	Yes
3. *Escherichia coli*	ECOBD	Yes
4. *Shewanella baltica*	SHEB5	Yes
5. *Shewanella frigidimarina*	SHEFN	No
6. *Burkholderia xenovorans*	BURXL	No
7. *Burkholderia glumae*	BURGL	No
8. *Mycobacterium vanbaalenii*	MYCVP	No

PROTEOMIC
Spectral Library:
DDA-MS acquisition with LC-MS/MS Triple TOF 5600 (ABSciex)
Samples: *S. baltica, E. coli, C. metallidurans & P. putida* alone, 3 replicates +/- Zn 0.5mM
Analysis Software: ProteinPilot v 4.5 (ABSciex)
Database: Home made extracted from UNIPROT, 8 species

Community:
SWATH-MS with LC-MS/MS Triple TOF 5600 (ABSciex)
SWATH acquisition : 32 windows of 25 Da each
Analysis against Spectral library using PeakView 2.1 (ABSciex)

Analysis:
Servers: UNIPROT, NCBI, PATRIC 3.3.10, RAST, SEED Viewer, Blast 2.3.0+ (Stand alone)
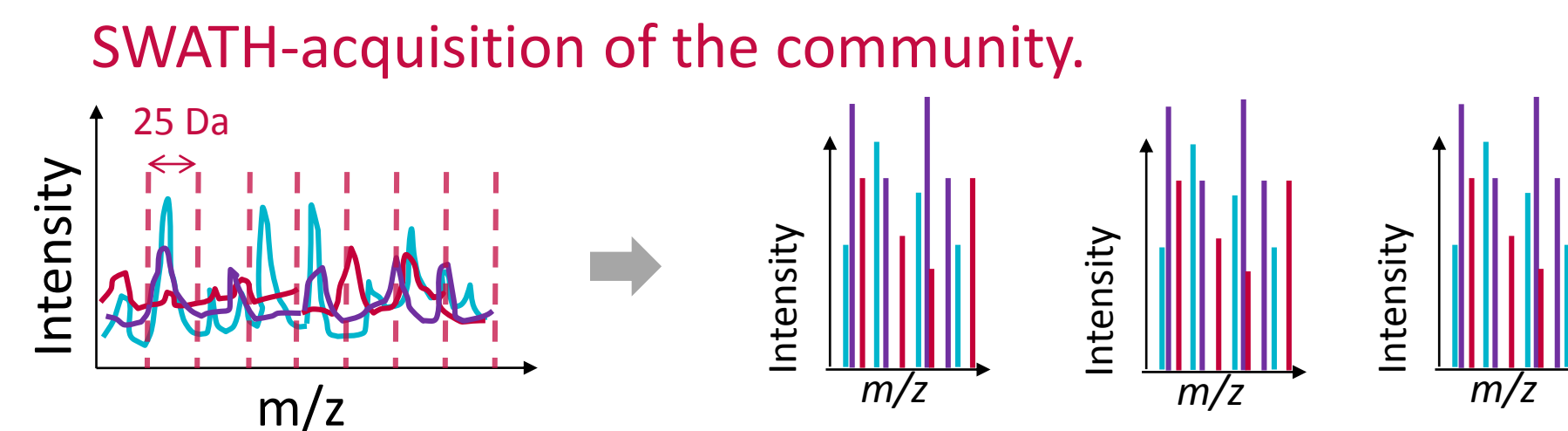Softwares: Excel (Microsoft), R, Rstudio

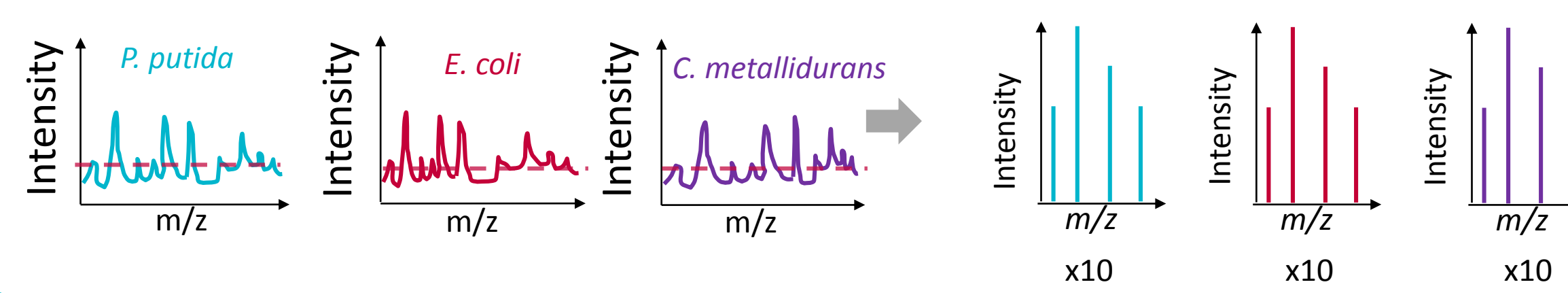## DDA-MS is not relevant for uneven communities
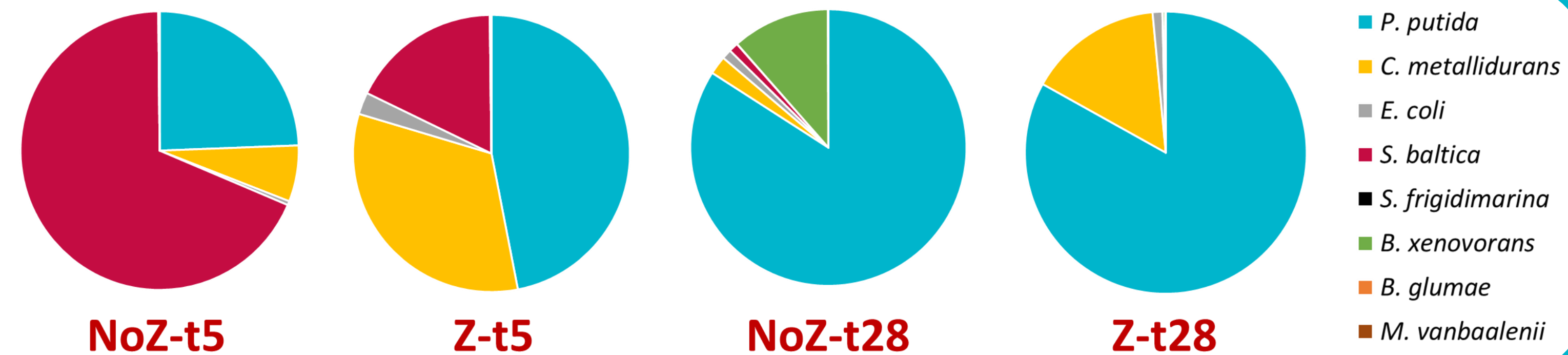
A. DDA-MS
HORIZONTAL DIVISION
MS1 — MS2 — x10, x1, x3

B. SWATH-MS[1] (DIA-MS)
SWATH-acquisition of the community. 25 Da
VERTICAL DIVISION

Spectral Library (using DDA- MS of the different strains alone)
P. putida, *E. coli*, *C. metallidurans* — x10, x10, x10

% OF CELLS (Q-PCR)

NoZ-t5 — Z-t5 — NoZ-t28 — Z-t28

- *P. putida*
- *C. metallidurans*
- *E. coli*
- *S. baltica*
- *S. frigidimarina*
- *B. xenovorans*
- *B. glumae*
- *M. vanbaalenii*

COMMUNITY RESPONSE — Normalisation by Total Area

Community Response: ALL SPECIES

SEED Category vs Total Area — NoZ-t5, Z-t5, NoZ-t28, Z-t28

Amino Acids and Derivatives; Cell Division and Cell Cycle; Cell Wall and Capsule |* 0.011; Membrane Transport |* 0.001; Metabolism of Aromatic...; Miscellaneous; Motility and Chemotaxis |* 2.6 e⁻⁴; Nitrogen Metabolism; Nucleosides and Nucleotides |* 3.8 e⁻⁵ |* 0.043; Phosphorus Metabolism; Potassium metabolism |* 0.008; Respiration |* 0.002 |* 0.005 |* 0.028 |* 0.023; RNA Metabolism; Secondary Metabolism |* 0.042; Stress Response; Sulfur Metabolism; Virulence, Disease and Defense |* 0.028

Pseudomonas putida

Amino Acids and Derivatives |* 0.007; Cell Division and Cell Cycle; Cell Wall and Capsule; Membrane Transport; Metabolism of Aromatic Compounds; Miscellaneous; Motility and Chemotaxis |* 0.093; Nitrogen Metabolism; Nucleosides and Nucleotides |* 0.025; Phosphorus Metabolism |* 0.015 |* 3.7e⁻⁴; Potassium metabolism |* 0.026; Respiration |* 0.002 |* 0.002 |* 0.043 |* 0.040; RNA Metabolism; Secondary Metabolism; Stress Response; Sulfur Metabolism; Virulence, Disease and Defense |* 0.019

	COMM	PSEPK	CUPMC	ECOBD	SHEB5
COMM	1	0.99	0.85	0.73	0.88
PSEPK	0.99	1	0.81	0.69	0.83
CUPMC	0.85	0.81	1	0.77	0.83
ECOBD	0.73	0.69	0.77	1	0.67
SHEB5	0.88	0.83	0.83	0.67	1

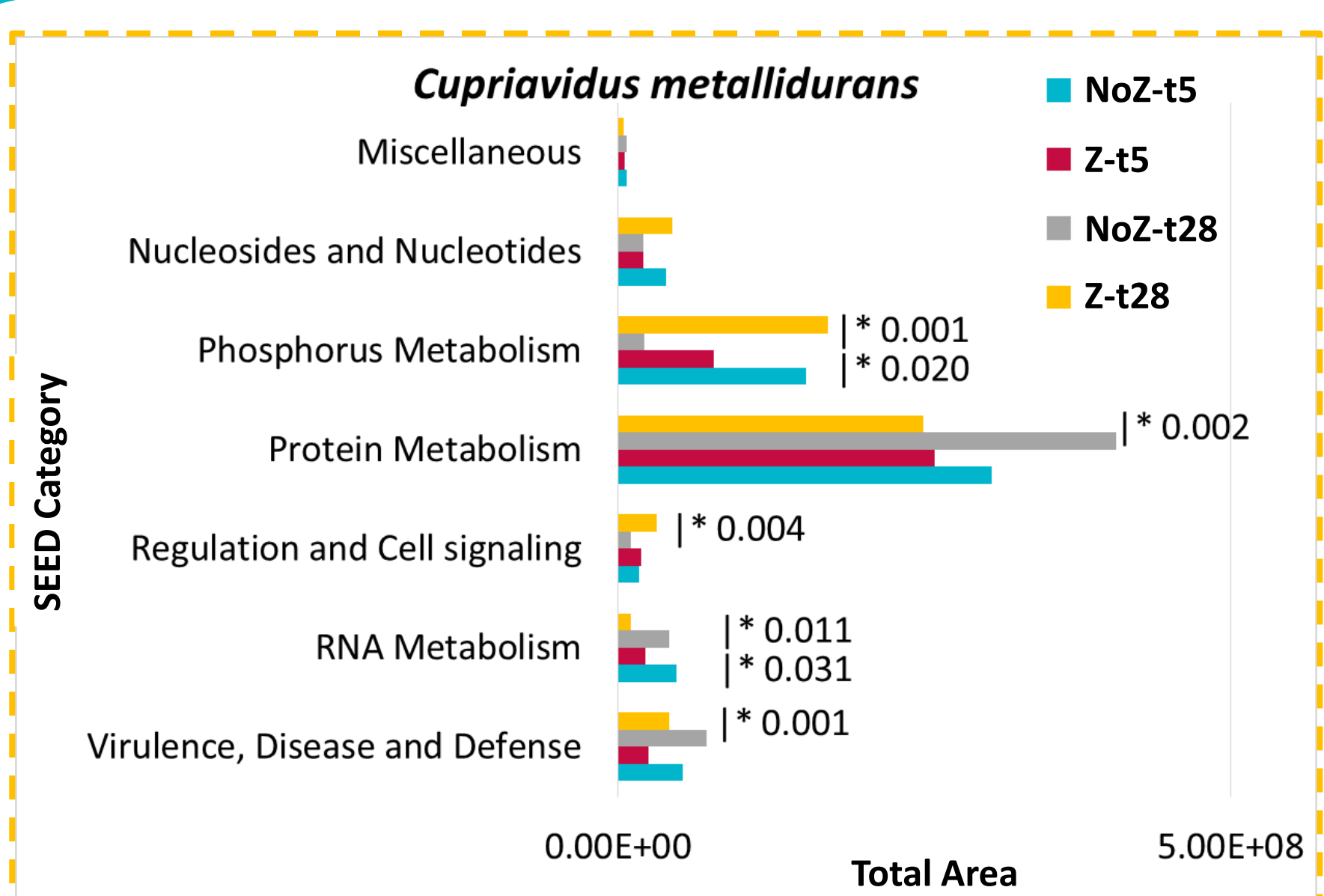**Community response follows the dominant species response**

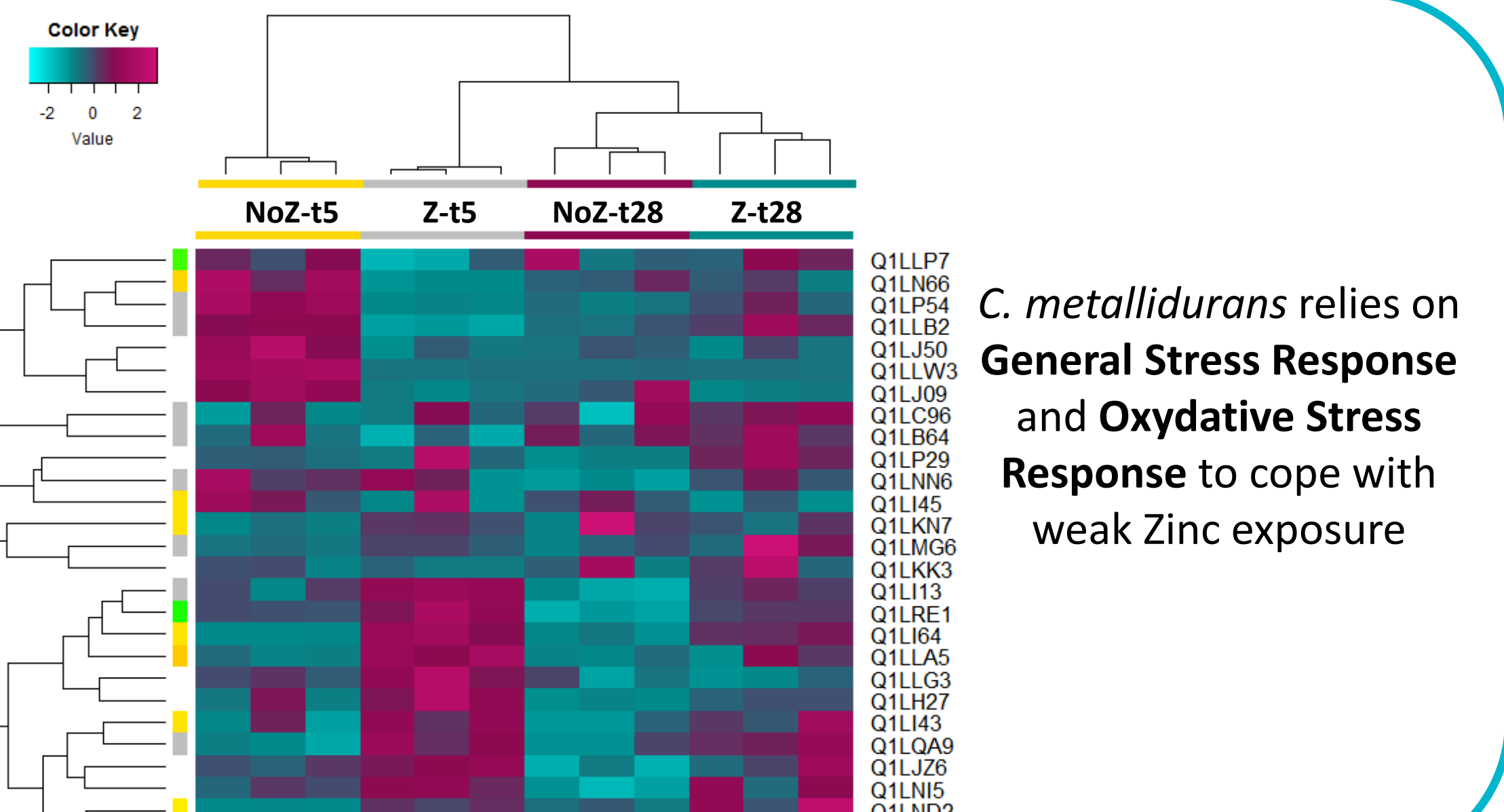**NORMALISATION PER SPECIES is required to catch species responses**

Correlation Matrix, Ward method:
COMM = All species normalised per sample.
Others = Normalised per species.
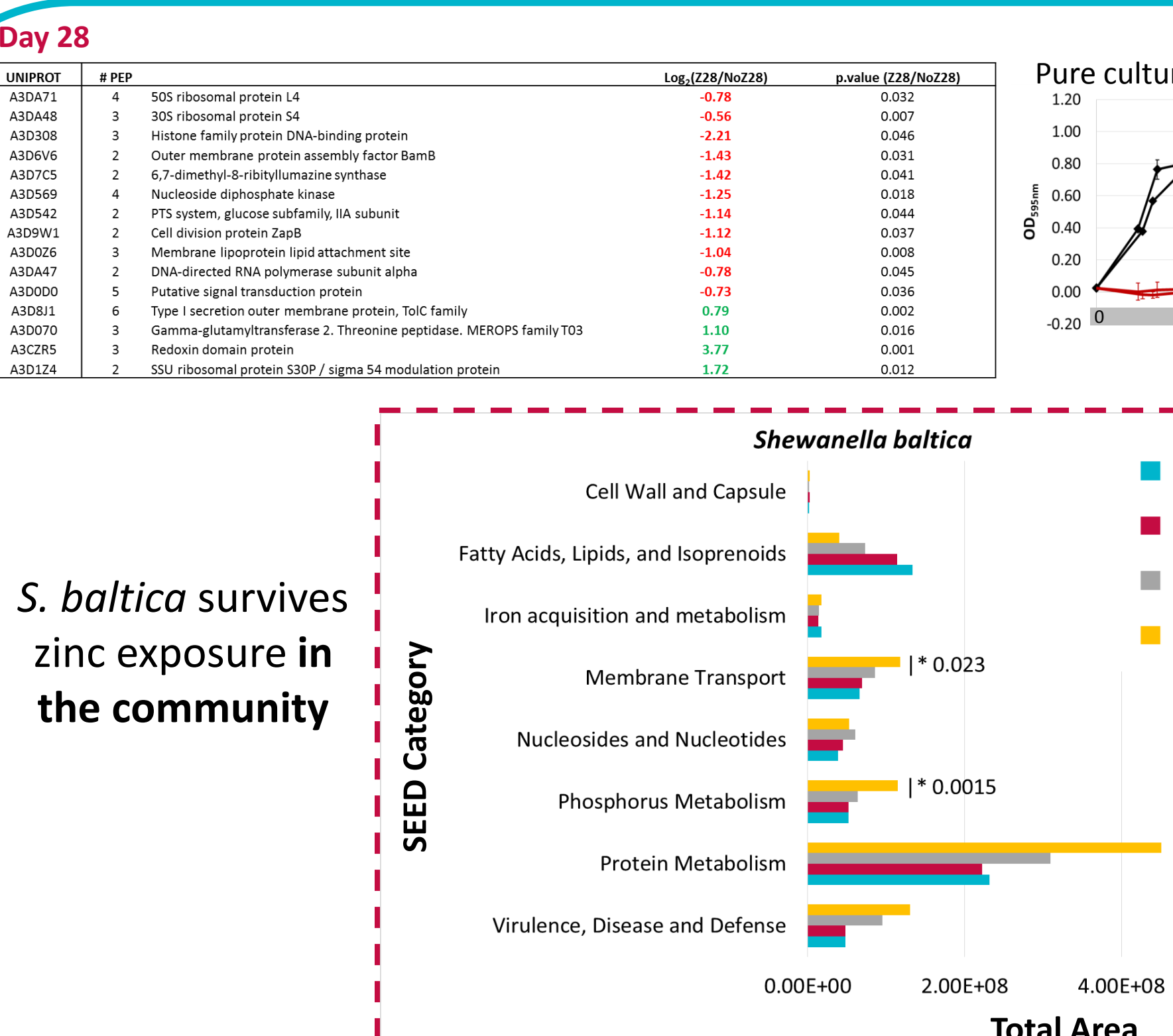
RARER SPECIES

Cupriavidus metallidurans

SEED Category — NoZ-t5, Z-t5, NoZ-t28, Z-t28

Miscellaneous; Nucleosides and Nucleotides; Phosphorus Metabolism |* 0.001 |* 0.020; Protein Metabolism |* 0.002; Regulation and Cell signaling |* 0.004; RNA Metabolism |* 0.011 |* 0.031; Virulence, Disease and Defense |* 0.001

Day 5

UNIPROT	# PEP	Group	Log₂(Z5/NoZ5)	p.value (Z5/NoZ5)
Q1LLW3	2	L-Aspartate decarboxylase	-4.18	0.002
Q1LLQ9	2	Phosphate transport system-binding protein pstB	-3.35	0.018
Q1LLP54	4	Putative hydroxydechloroatrazine ethylaminohydrolase, AtzB (Hydroxyatrazine hydrolase)	-3.24	0.011
Q1LLB2	5	Phosphate-binding protein PstS	-2.98	0.001
Q1LL50	2	Polyprenyl-pyrophosphate binding protein	-2.11	0.017
Q1LN66	2	Cyclic nucleotide-binding domain (CNMP-BD) protein	-1.56	0.045
Q1LLP7	2	2-oxoglutarate decarboxylase, thiamin-requiring (E1 component)	-0.94	0.034
Q1LB64	2	Ferredoxin	0.24	0.044
Q1LC96	2	Catalase-peroxidase	0.35	0.010
Q1LLG3	5	Thioredoxin	0.61	0.029
Q1LLD6	2	Uncharacterized protein	0.65	0.029
Q1LHZ7	2	Histone-like nucleoid-structuring protein HNS-like: transcriptional regulator	0.88	0.007
Q1LMG6	2	Catechol 1,2-dioxygenase	1.00	0.041
Q1LND2	5	Inosine-5'-monophosphate dehydrogenase	1.30	0.013
Q1LKN7	2	Elongation factor P	1.49	0.025
Q1LLA5	2	Transcription elongation factor GreA	2.11	0.012
Q1LQA9	2	Ribosomal silencing factor RsfS	2.18	0.044
Q1LI38	2	50S ribosomal protein L4	0.80	0.027
Q1LI64	2	50S ribosomal protein L17	3.61	0.009

Day 28

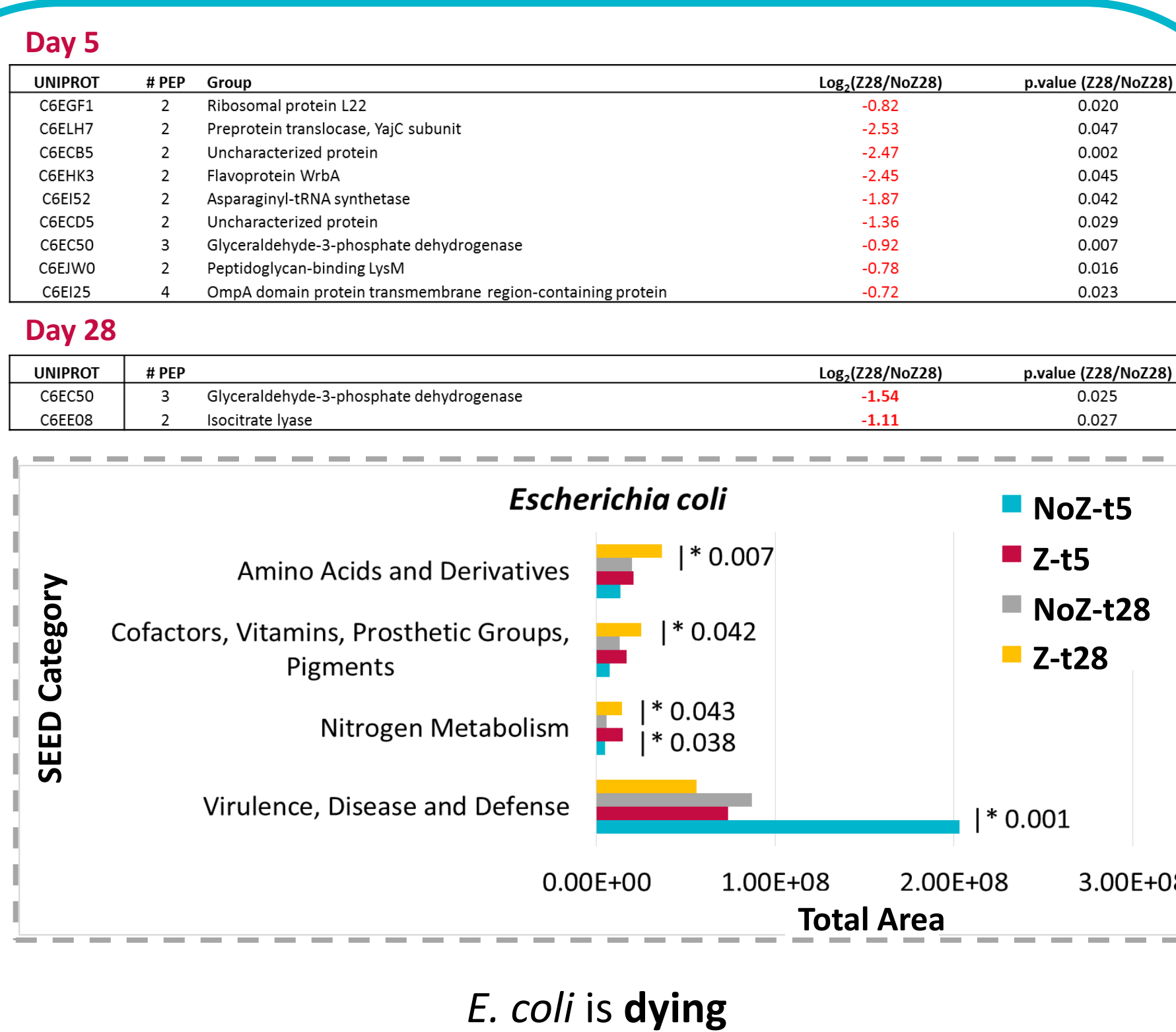| UNIPROT	# PEP	Group	Log₂(Z28/NoZ28)	p.value (Z28/NoZ28)
Q1LLI45	2	50S ribosomal protein L29	-1.57	0.010
Q1LLK3	2	2-keto-D-gluconate reductase (Glyoxylate reductase) (2-ketoaldonate reductase)	0.41	0.023
Q1LHZ7	2	Histone-like nucleoid-structuring protein HNS-like: transcriptional regulator	0.88	0.007
Q1LI13	5	Elongation factor Tu	1.19	0.027
Q1LRE1	4	Glutamate and aspartate transporter subunit periplasmic-binding component of ABC superfamily	1.34	0.001
Q1LN45	3	Putative peptide transporter subunit; periplasmic-binding component of ABC superfamily	1.80	0.009
Q1LP29	3	Putative ABC transporter, periplasmic substrate-binding protein	1.99	0.025
Q1LVN6	4	Acetyl-CoA acetyltransferase (Acetoacetyl-CoA thiolase)	1.99	0.022
Q1LQA9	2	Ribosomal silencing factor RsfS	1.40	0.010
Q1LI43	2	30S ribosomal protein S3	1.51	0.039
Q1LI64	2	50S ribosomal protein L17	2.55	0.023

C. metallidurans relies on **General Stress Response** and **Oxydative Stress Response** to cope with weak Zinc exposure

Day 28 (Shewanella baltica)

UNIPROT	# PEP	Group	Log₂(Z28/NoZ28)	p.value (Z28/NoZ28)
A3D4T1	4	50S ribosomal protein L4	-0.78	0.032
A3DA48	3	30S ribosomal protein S4	-0.96	0.003
A3D308	3	Histone family protein DNA-binding protein	-1.43	0.011
A3D6V6	2	Outer membrane protein assembly factor BamB	-1.53	0.045
A3D7C5	2	6,7-dimethyl-8-ribityllumazine synthase	-1.42	0.041
A3D569	4	Nucleoside diphosphate kinase	-1.16	0.018
A3D542	2	PTS system, glucose subfamily, IIA subunit	-1.14	0.044
A3D9W1	2	Cell division protein ZapB	-1.08	0.022
A3D0D6	3	Membrane lipoprotein lipid attachment site	-1.04	0.008
A3DA47	2	DNA-directed RNA polymerase subunit alpha	-0.85	0.043
A3D0D0	5	Putative signal transduction protein	0.39	0.036
A3DB1L	6	Type I secretion outer membrane protein, TolC family	0.79	0.002
A3DD70	2	Gamma-glutamyltransferase 2. Threonine peptidase. MEROPS family T03	1.54	0.007
A3D000	5	Redoxin domain protein	3.77	0.001
A3D1Z4	2	SSU ribosomal protein S9OP / sigma 54 modulation protein	1.72	0.012

Pure culture: OD₆₀₀ vs Time (hours) — NoZ, Zinc 0.5 mM

S. baltica survives zinc exposure **in the community**

Shewanella baltica

SEED Category — NoZ-t5, Z-t5, NoZ-t28, Z-t28

Cell Wall and Capsule; Fatty Acids, Lipids, and Isoprenoids; Iron acquisition and metabolism; Membrane Transport |* 0.023; Nucleosides and Nucleotides; Phosphorus Metabolism |* 0.0015; Protein Metabolism; Virulence, Disease and Defense

Day 5 (Escherichia coli)

UNIPROT	# PEP	Group	Log₂(Z28/NoZ28)	p.value (Z28/NoZ28)
C6E5F1	2	Ribosomal protein L22	-0.82	0.020
C6ELH7	2	Preprotein translocase, YajC subunit	-2.53	0.047
C6ECB5	2	Uncharacterized protein	-2.47	0.002
C6EHK3	2	Flavoprotein WrbA	-2.45	0.045
C6EIS2	2	Asparaginyl-tRNA synthetase	-1.87	0.042
C6ECD5	2	Uncharacterized protein	-1.36	0.029
C6ECS0	3	Glyceraldehyde-3-phosphate dehydrogenase	-0.92	0.007
C6EW0	2	Peptidoglycan-binding LysM	-0.78	0.016
C6E25	4	OmpA domain protein transmembrane region-containing protein	-0.72	0.023

Day 28

UNIPROT	# PEP	Group	Log₂(Z28/NoZ28)	p.value (Z28/NoZ28)
C6ECS0	2	Glyceraldehyde-3-phosphate dehydrogenase	-1.54	0.025
C6EE08	2	Isocitrate lyase	-1.11	0.027

Escherichia coli

SEED Category — NoZ-t5, Z-t5, NoZ-t28, Z-t28

Amino Acids and Derivatives |* 0.007; Cofactors, Vitamins, Prosthetic Groups, Pigments |* 0.042; Nitrogen Metabolism |* 0.043 |* 0.038; Virulence, Disease and Defense |* 0.001

E. coli is **dying**

CONCLUSIONS :

Rare species become visible by the SWATH approach

Species normalization is required to describe **uneven communities.**

Dying versus rare but active bacteria can be differenciated

Université de Mons