Evaluation of the SWATH-MS potential for comparative metaproteomics of complex ecosystems

Mélanie Beraud, Giuseppe Giambarresi, David C. Gillan, Ruddy Wattiez

Laboratory of Proteomic and Microbiology

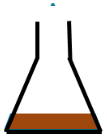
Experimental Setup

Synthetic Community

STRAINS	UNIPROT
1. *Pseudomonas putida* KT2440	PSEPK
2. *Cupriavidus metallidurans* CH34	RALME
3. *Escherichia coli* BL21-DE3	ECOBD
4. *Shewanella baltica* OS155	SHEB5
5. *Shewanella frigidimarina* ACAM591	SHEFN
6. *Burkholderia xenovorans* LB400	BURXL
7. Burkholderia glumae P1-22-1	BURGL
8. Methylibium petroleiphilum PM1	METPP
9. *Mycobacterium vanbaalenii* PYR-1	MYCVP

- Zn 0.5 mM

NoZ



+ Zn 0.5 mM

Z

28- days

16°C, 900 rpm

Analysis

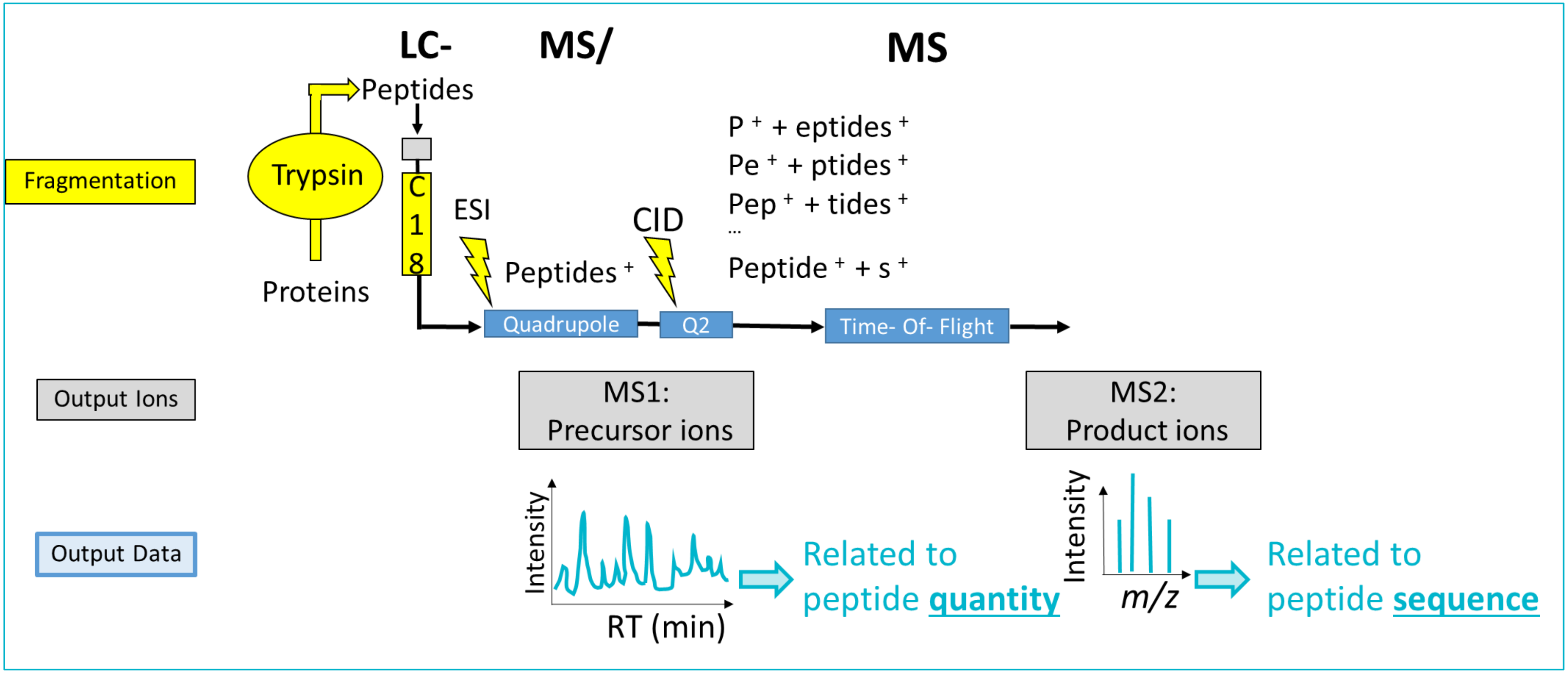
Taxonomy

Q-PCR

Functionality

Metaproteomics

DDA-MS: Label free



LC- MS/ MS

Fragmentation

Output Ions

Output Data

Trypsin

Proteins

Peptides

C 1 8

ESI

Peptides $^{+}$

CID

Quadrupole

Q2

Time- Of- Flight

P $^{+}$ + eptides $^{+}$
Pe $^{+}$ + ptides $^{+}$
Pep $^{+}$ + tides $^{+}$
…
Peptide $^{+}$ + s $^{+}$

MS1: Precursor ions

MS2: Product ions

Related to peptide **quantity**

Related to peptide **sequence**

LC-MS/MS Triple TOF 5600 (ABSciex)
Background proteome : Home-made with specific genomes (9 species)

In DDA-MS, protein detection is uneven

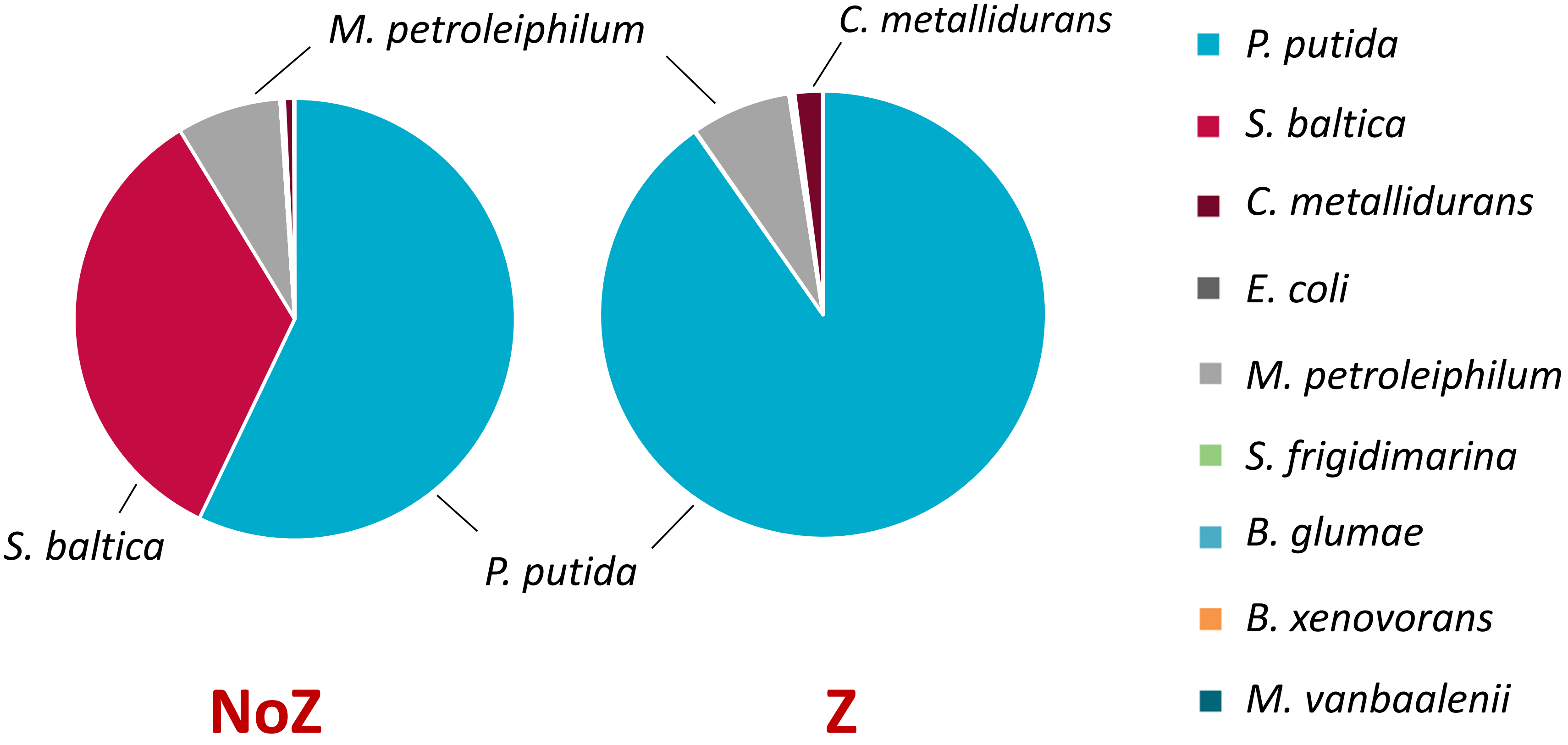
Background proteome :
Home-made with specific genomes (9 species)

 # PROTEINS WITH >1 PEPTIDE

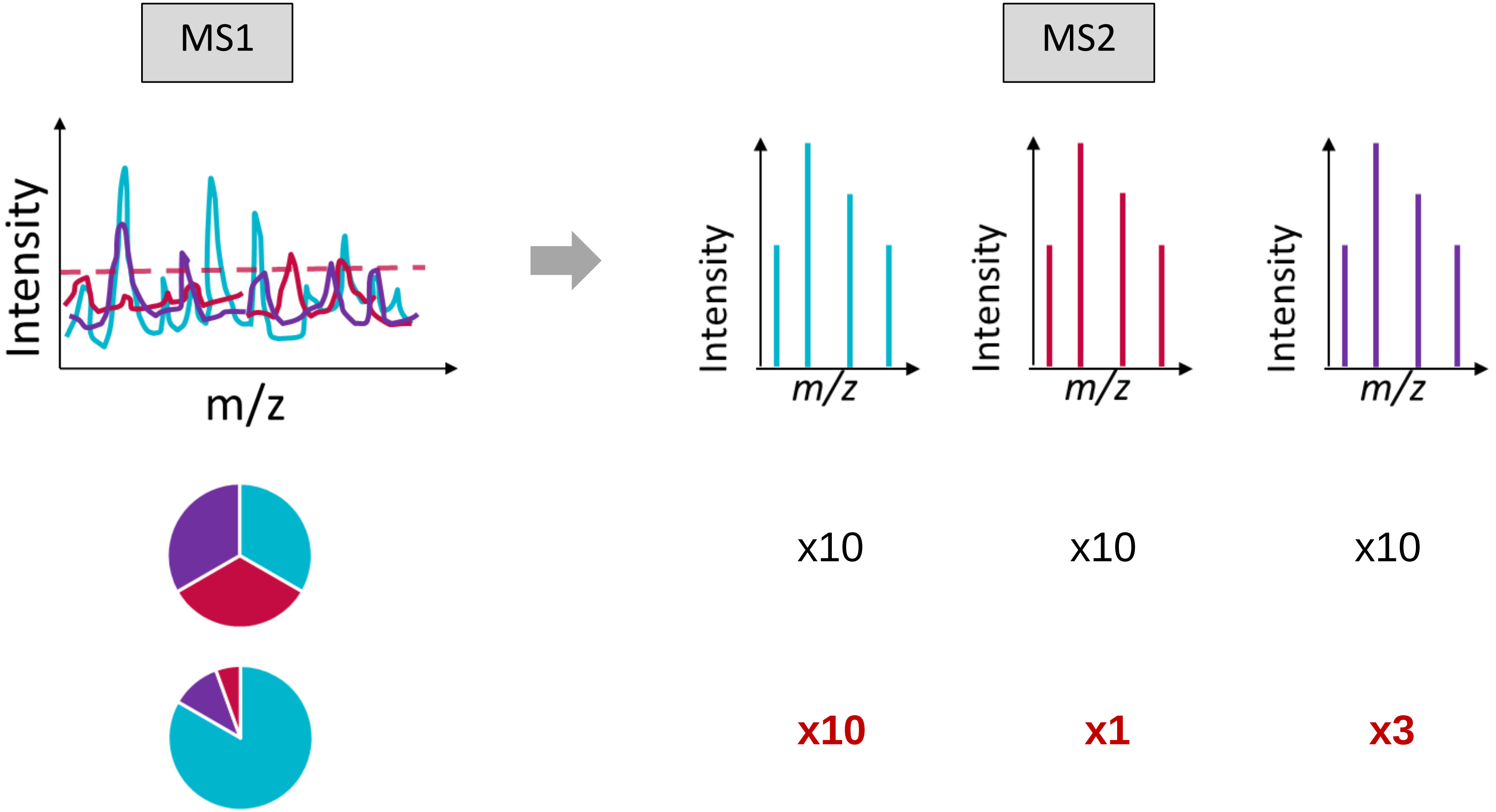
Strain	**DDA-MS**	**%**
P. putida	521	**86.7 %**
S. baltica	50	8.3 %
C. metallidurans	23	3.8 %
E. coli	1	0.2 %
Others	6	1.0

Taxonomy: Uneven community

Q-PCR analysis: 16S DNA => % of DNA



DDA-MS is not relevant for uneven community analysis



MS1

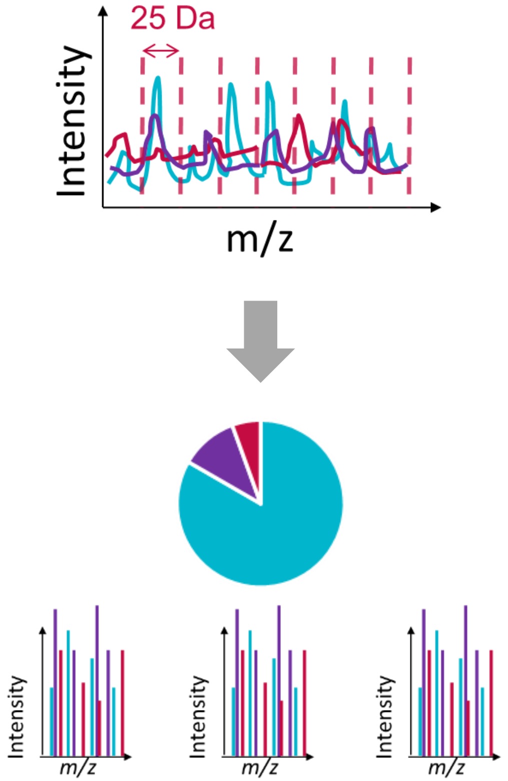
MS2

x10 x10 x10

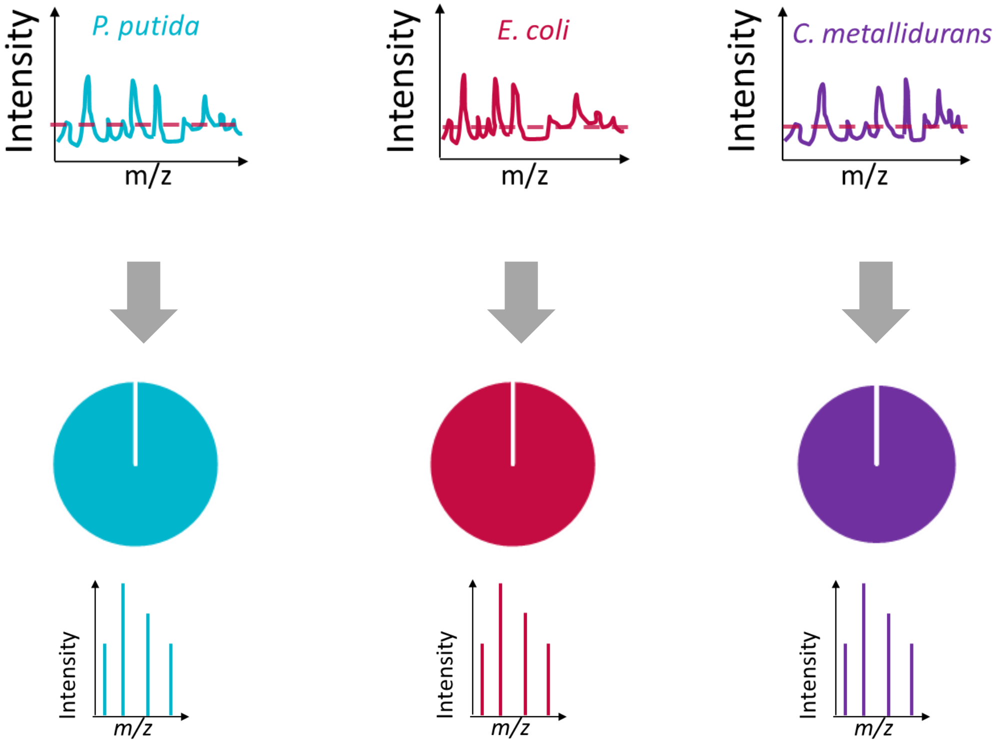
x10 **x1** **x3**

SWATH-MS gives similar potentiality to each bacterial species

SWATH-MS community



DDA-MS species alone Spectral Library



SWATH-MS gives similar potentiality to each bacterial species

Spectral Library:
- 4 species
- 3 biological replicates
- 2 conditions +/- Zn 0.5mM

POTENTIALITY OF DETECTION ⇨

Strain	**Spectral Library > 1 peptide**
P. putida	**1268**
S. baltica	**1294**
C. metallidurans	**1106**
E. coli	**1403**

Better sensitivity

DETECTION OF RARER SPECIES IS INCREASED

Strain	**# proteins with > 1 peptide**	
	DDA-MS	**SWATH-MS**
P. putida	521	468
S. baltica	50	46
C. metallidurans	23	**40**
E. coli	1	**5**
	601	559

Better accuracy

QUANTIFICATION ROBUSTNESS IS INCREASED

Strain	**% of proteins with 2-fold difference +/- Zn 0.5 mM**		**% of proteins significatively different (pval < 0.05)**	
	DDA-MS	**SWATH-MS**	**DDA-MS**	**SWATH-MS**
P. putida	44 %	47 %	**22 %**	49 %
S. baltica	52 %	65 %	**20 %**	37 %
C. metallidurans	61 %	50 %	**4 %**	45 %
E. coli	0 %	60 %	**0 %**	40 %

+ and -

-

Proteins need to be present in the spectral library => Poster 5

Spectral Library can be large and time consuming

+

Better sensitivity

Better accuracy

High-throughput: 1 Spectral Library = numerous samples

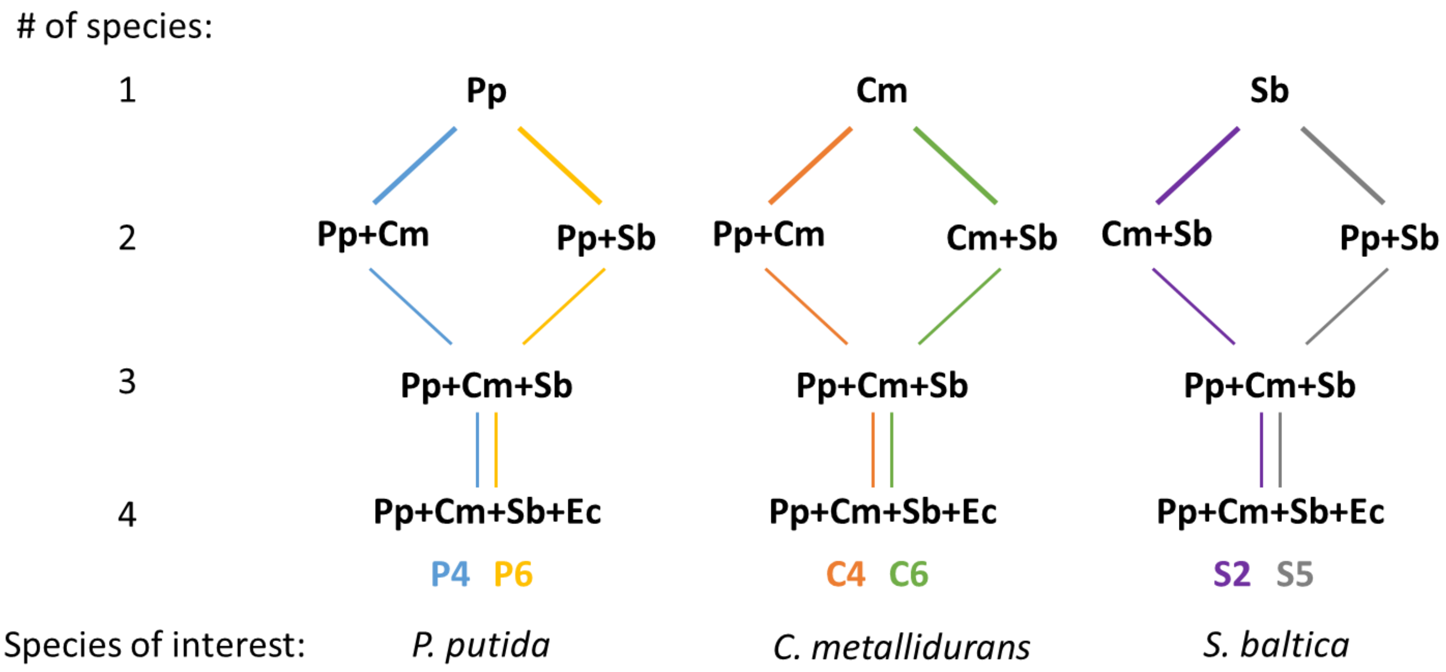
Dying versus active low abundance bacteria => Poster 5

Species normalization may be required => Poster 5

Biological data => Poster 5

Minimum number of species required in the Spectral Library

1. SET OF SPECTRAL LIBRARY OF DIFFERENT COMPOSITIONS:



# of species:

1 **Pp** **Cm** **Sb**

2 **Pp+Cm** **Pp+Sb** **Pp+Cm** **Cm+Sb** **Cm+Sb** **Pp+Sb**

3 **Pp+Cm+Sb** **Pp+Cm+Sb** **Pp+Cm+Sb**

4 **Pp+Cm+Sb+Ec** **Pp+Cm+Sb+Ec** **Pp+Cm+Sb+Ec**

P4 **P6** **C4** **C6** **S2** **S5**

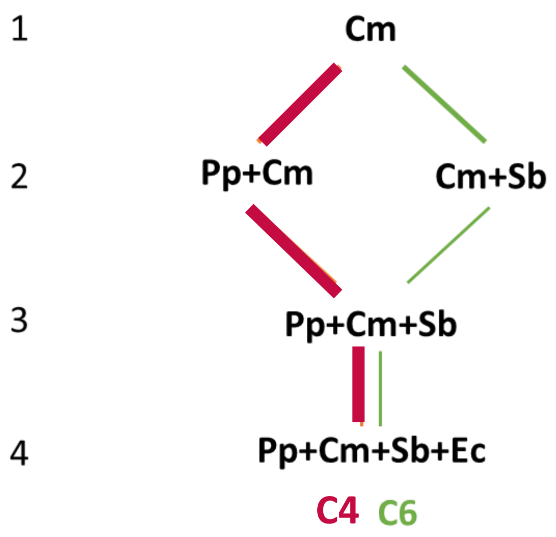
Species of interest: *P. putida* *C. metallidurans* *S. baltica*

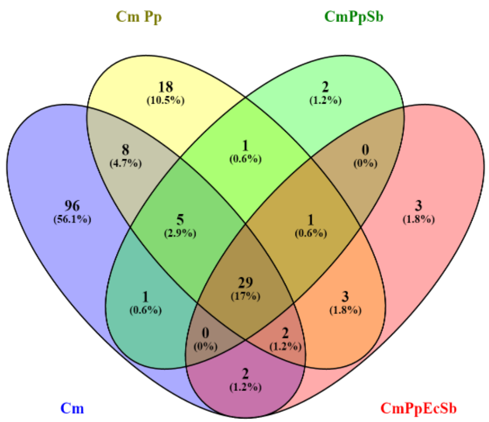
2. ANALYSIS OF THE COMMUNITY SWATH- ACQUIRED

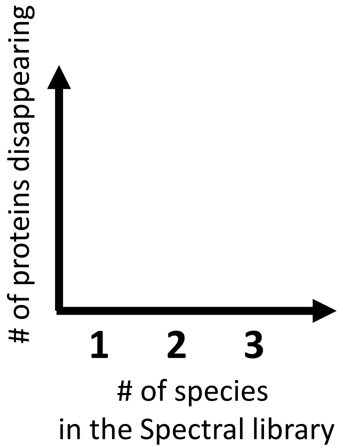
Minimum number of species required in the Spectral Library

Species of interest: *C. metallidurans*

# of species:

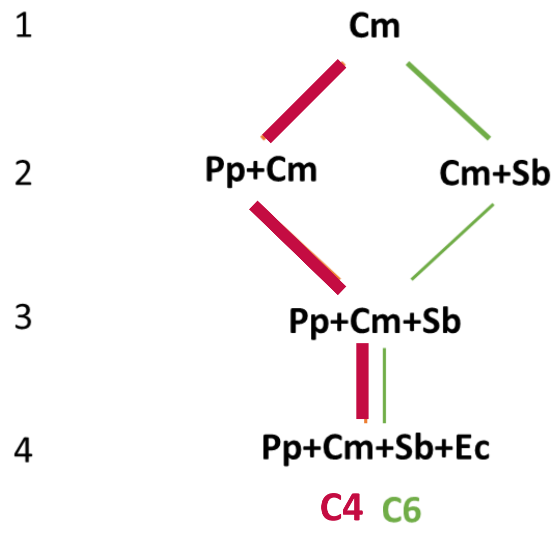






Minimum number of species required in the Spectral Library

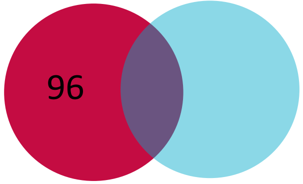
Species of interest: *C. metallidurans*



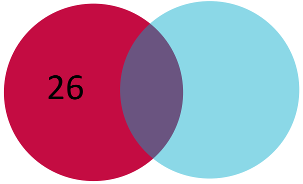
# of species:		
1	**Cm**	
2	**Pp+Cm**	**Cm+Sb**
3	**Pp+Cm+Sb**	
4	**Pp+Cm+Sb+Ec**	

C4 **C6**

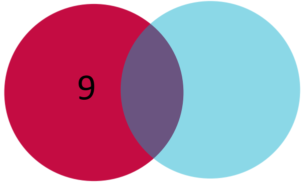
1 species: Cm only — **2 species:** Cm + Pp

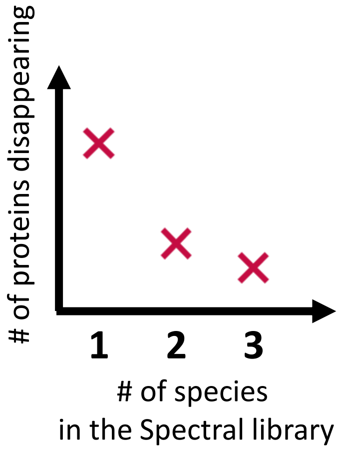


2 species: Cm + Pp — **3 species:** Cm + Pp + Sb

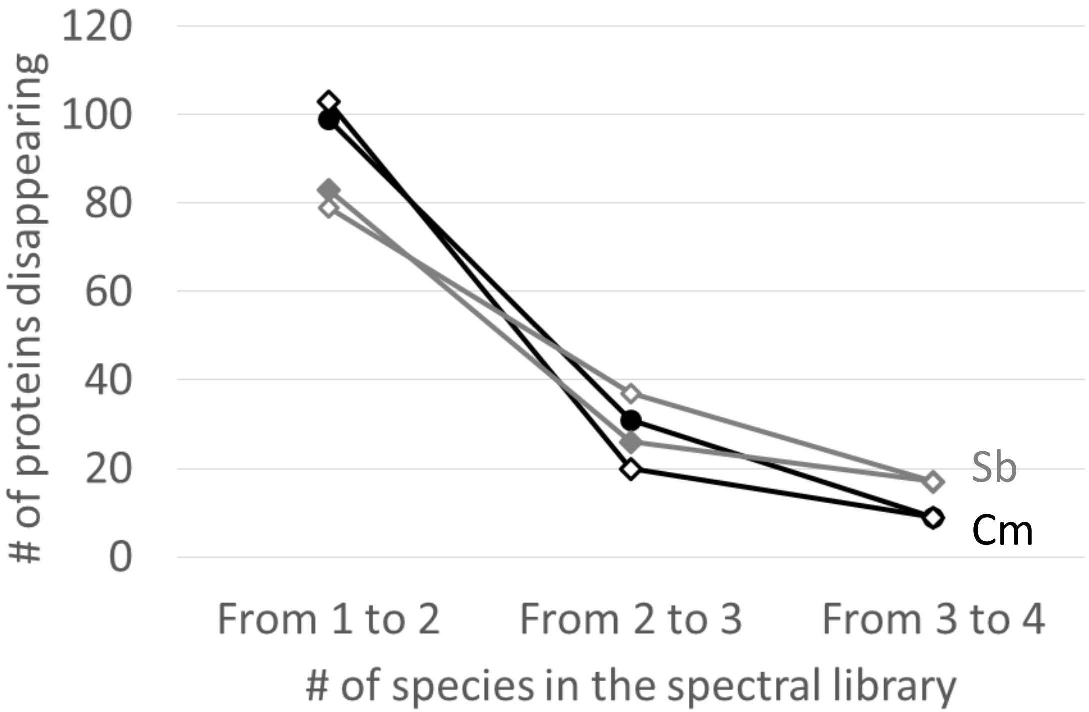


3 species: Cm + Pp + Sb — **4 species:** Cm + Pp + Sb + Ec





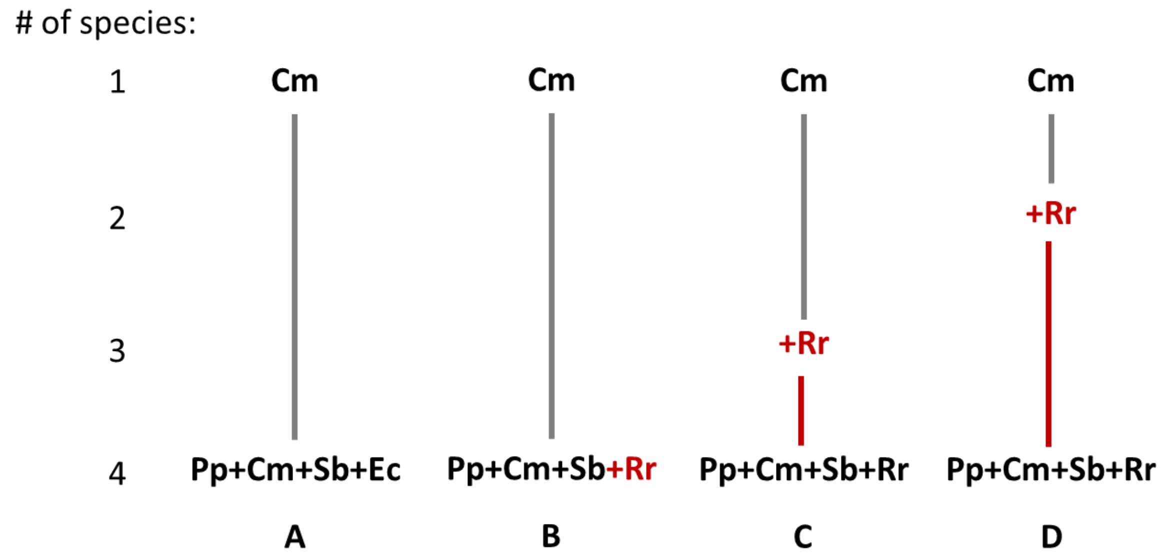
Minimum number of species required in the Spectral Library



2 SPECIES ARE SUFFICIENT IN THE SPECTRAL LIBRARY FOR GOOD SPECIFICITY

- Matching parameters
- "Dirtyness" of the Spectral library
- Good community representation required

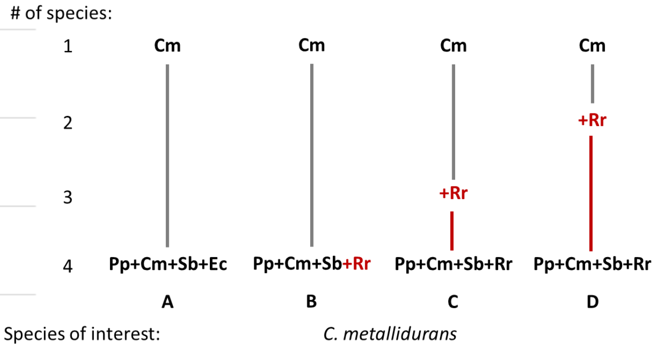
« Dirtyness » of the Spectral library



Species of interest: *C. metallidurans*

Rr (*Rhodospiriullum rubrum*)
Is **NOT** in the community

« Dirtyness » of the Spectral library



DIRTYNESS DOES NOT CHANGE MUCH WHEN SUFFICIENT NUMBER OF SPECIES

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Ruddy Wattiez
David Gillan
Mélanie Beraud
Giuseppe Giambarresi

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