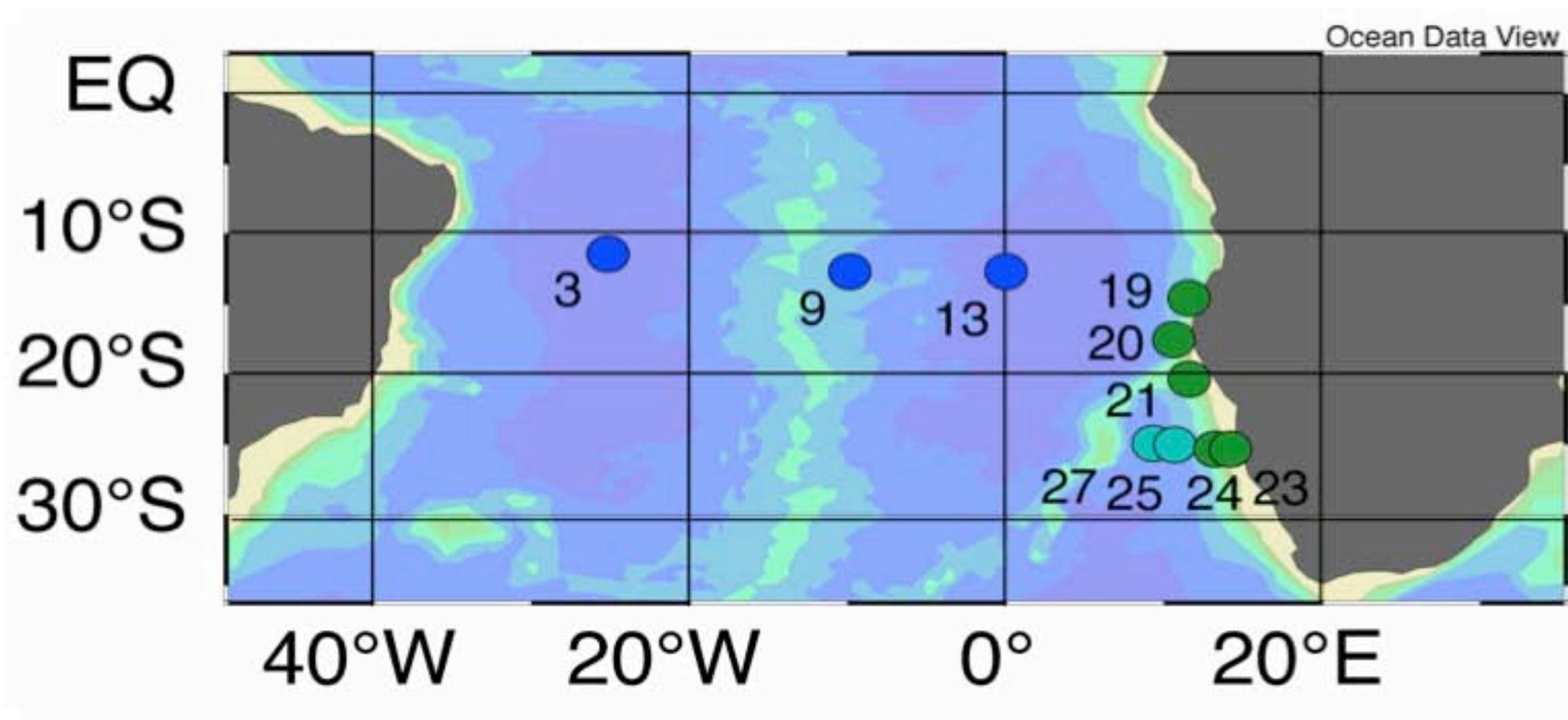


Comparative Metaproteomics of Marine Bacteria and Archaea

Robert M. Morris (Bob)

University of Washington, School of Oceanography



Morris et al., in press (ISME)

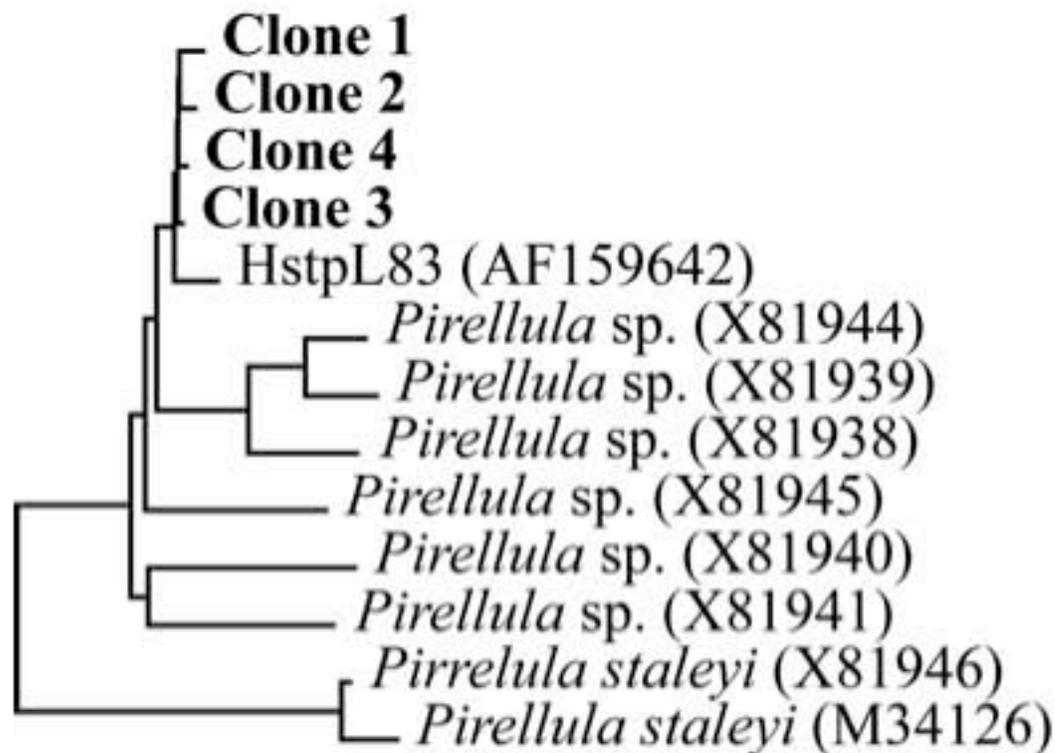
Meta-data to describe environmental conditions

Nutrients, temperature, salinity
(environmental data matrix, 9 variables)

	<i>Temp (°C)</i>	<i>PO4</i>	<i>SiO4</i>	<i>NO2</i>	<i>NO3</i>	<i>NH4</i>
ST3	25.0	0.136	0.81	0.048	0.212	0.034
ST9	22.7	0.167	0.88	0.014	0.066	0.005
ST13	21.0	0.242	0.69	0	0.162	0
ST19	21.1	1.022	3.58	0.24	11.96	0.203
ST20	16.1	1.431	3.12	0.786	21.114	0.175
ST21	16.3	1.124	1.84	0.405	12.905	0.189
ST23	13.1	1.792	31.48	0.31	25.61	0.4
ST24	16.6	0.851	1.44	0.371	7.809	1.268
ST25	18.4	0.431	1.09	0.093	2.087	0.054
ST27	19.2	0.231	1.59	0.002	0.288	0.081

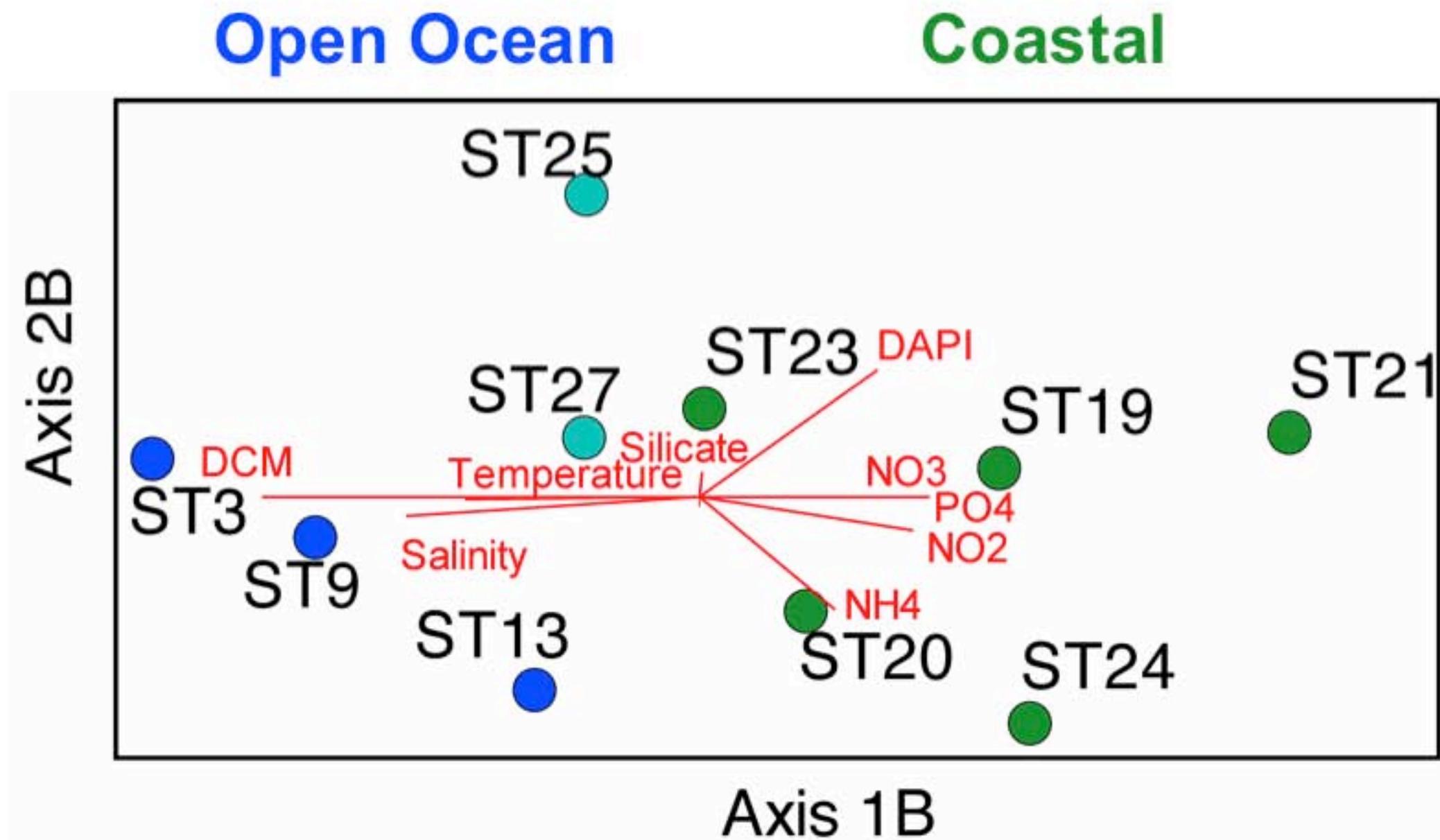
Molecular data to describe microbial communities

Ten 16S rRNA gene clone libraries
(community data matrix, 32 variables)



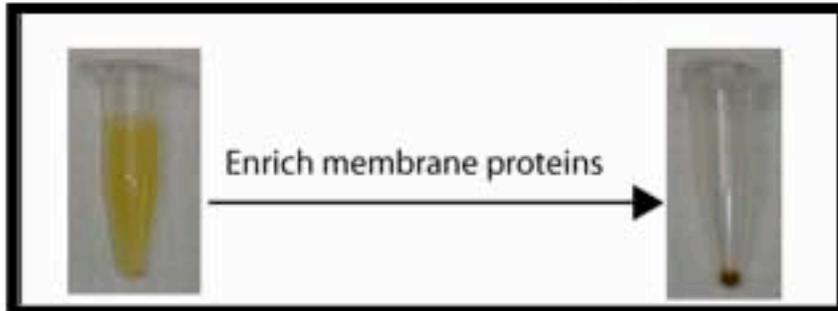
	<i>Actino</i>	<i>Proch</i>	<i>Roseo</i>	<i>SAR11</i>
ST3	13	48	5	18
ST9	15	28	4	22
ST13	18	12	7	9
ST19	19	0	7	8
ST20	30	0	9	16
ST21	5	0	19	4
ST23	23	0	13	29
ST24	29	0	6	3
ST25	12	2	11	43
ST27	20	0	7	25

Shift in community structure along gradient in nutrient concentrations

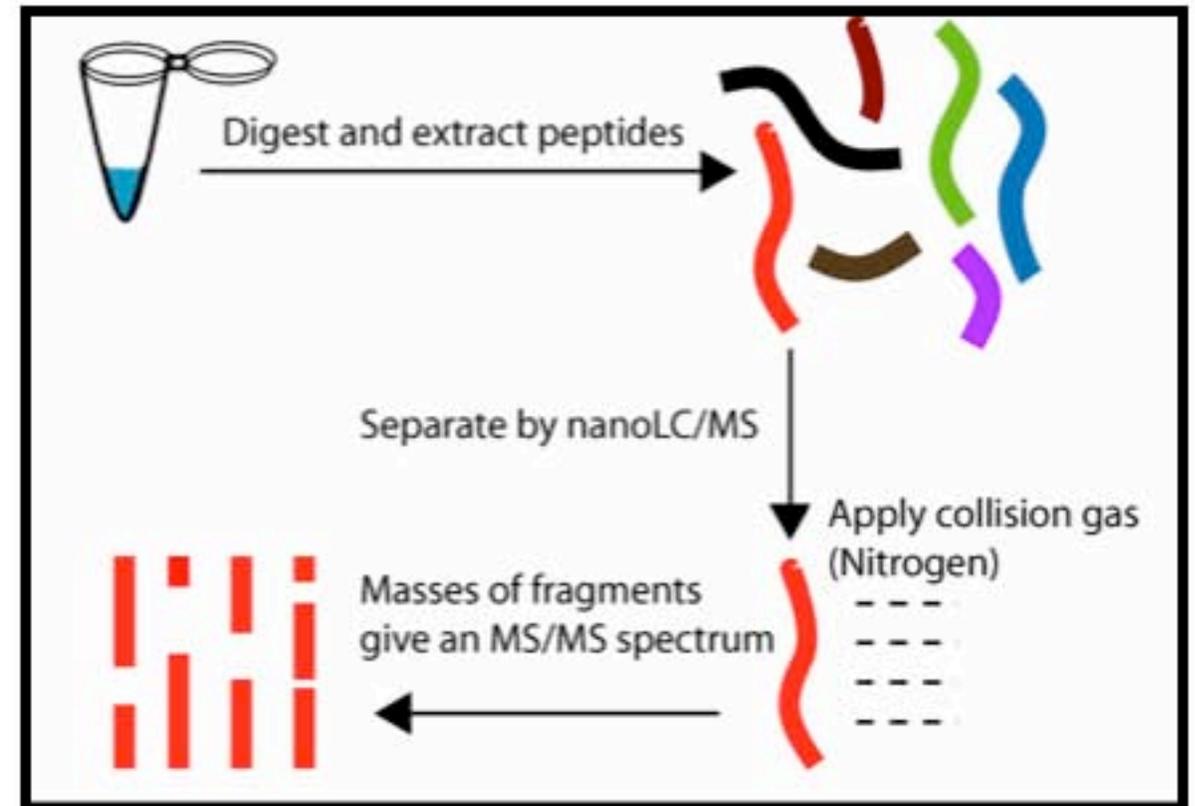


Metaproteomics (MS/MS)

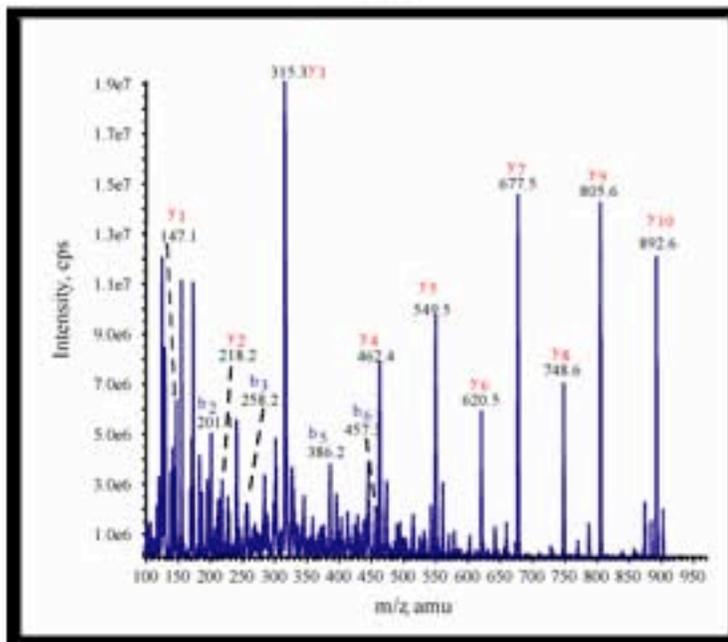
1. Concentrate proteins



2. Digest and fragment peptides



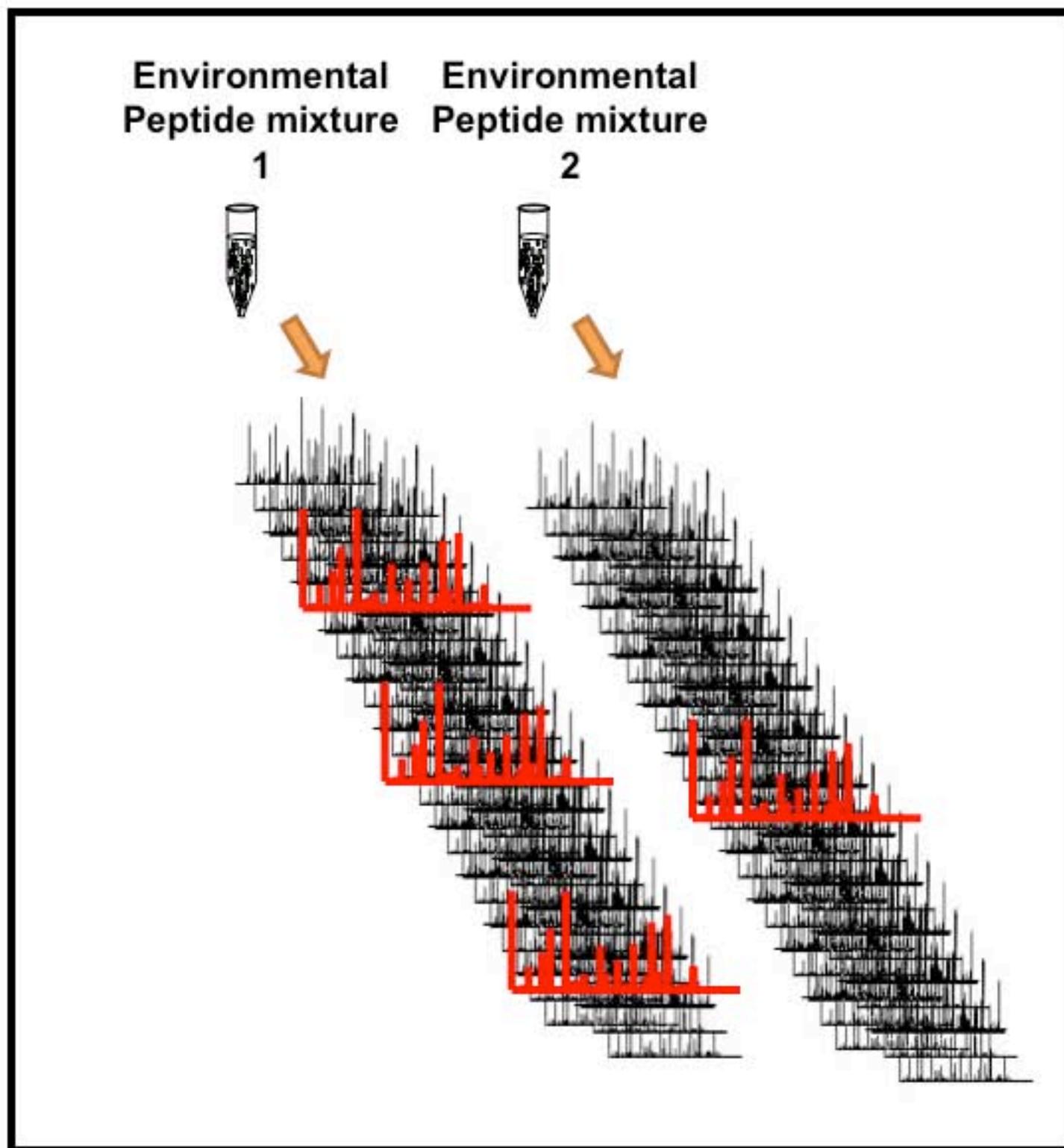
3. Interrogate MS/MS spectra



3. Inventory peptides

ATLVSAGTR
TVAGSTYLK

Cluster and count MS/MS spectra



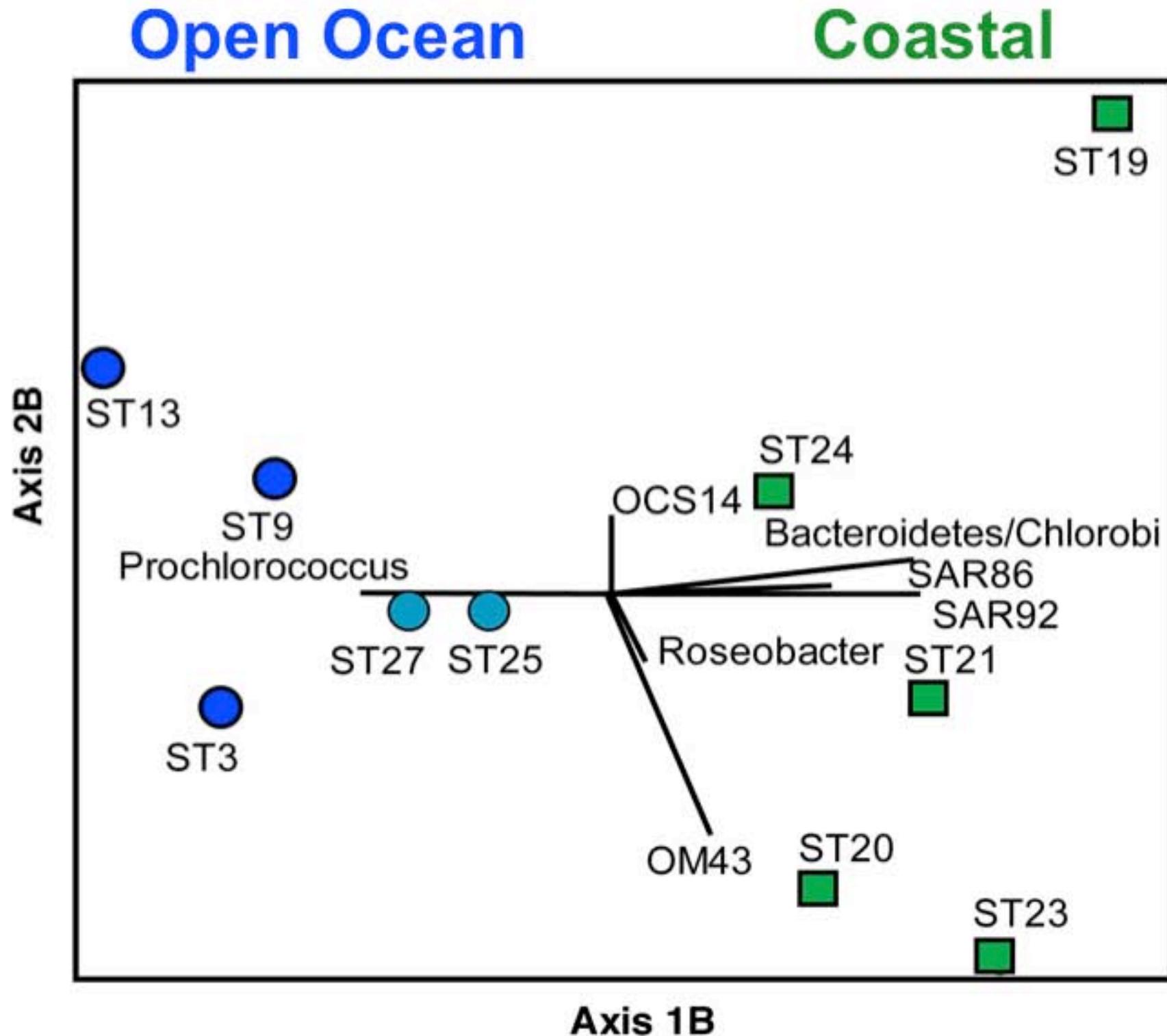
MS/MS spectra matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
ST3	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	1
ST9	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	2
ST13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
ST19	0	0	0	0	0	0	0	0	1	3	0	2	0	0	0	0	0
ST20	0	0	0	0	0	1	0	0	0	0	7	1	2	0	2	0	0
ST21	14	0	0	1	1	0	0	1	0	0	4	1	1	0	1	1	0
ST23	1	0	0	0	0	0	0	0	0	0	7	0	4	0	1	0	0
ST24	10	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0
ST25	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0
ST27	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0

Rows = stations (10)

Columns = spectra (2,273)

Shifts in membrane proteins along gradient in nutrient concentrations



Identified range of taxonomic origins and functions by consensus annotation



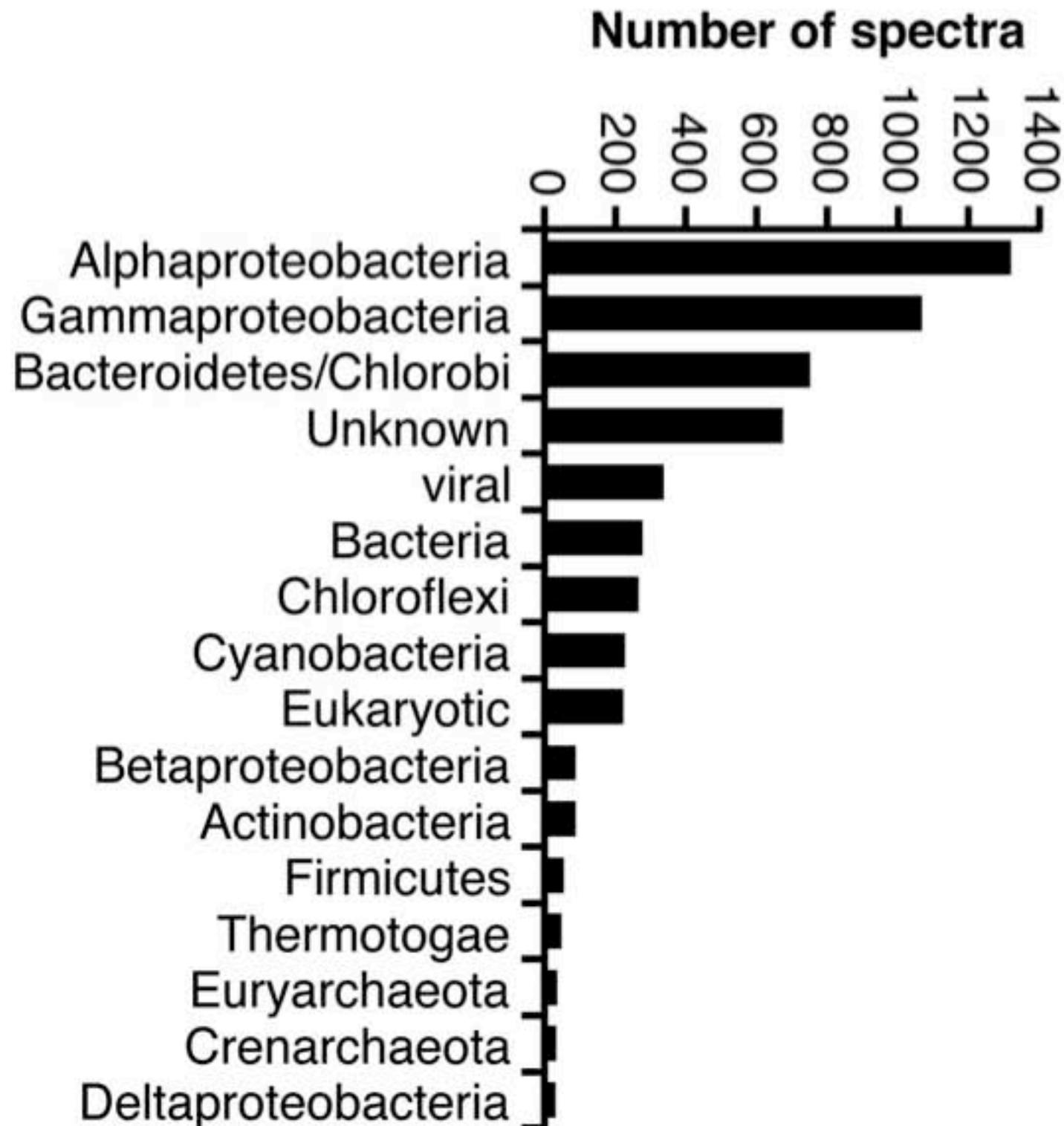
Representative JCVI proteins	JCVI proteins (total)	Top BLAS T score (taxonomic ID)	Top BLAS T score (annotation)
JCVI_PEP_1096700264879	2	Streptomyces coelicolor A3(2)	chaperonin GroEL
JCVI_PEP_1096686443737	15	Streptomyces avermitilis MA-4680	putative class I heat-shock
JCVI_PEP_1096697004939	1	Mycobacterium sp. 'graecum DL049'	heat shock protein 65
JCVI_PEP_1096685962599	7	Nocardioides sp. JS614	chaperonin GroEL
JCVI_PEP_1096686312649	2	Mycobacterium avium subsp. paratuberculosis	heat shock protein 65
JCVI_PEP_1096682475363	1	Mycobacterium smegmatis st r. MC2 15 5	chaperonin GroL
JCVI_PEP_1096699563923	4	Frankia sp. EAN1pec	chaperonin Cpn60/TCP-1
JCVI_PEP_1096700383965	1	Nocardia farcinica IFM 10152	chaperonin GroEL
JCVI_PEP_1096684894731	1	Frankia sp. Ccl3	chaperonin GroEL
JCVI_PEP_1096696263705	1	Janibacter sp. HTCC2649	chaperonin GroEL
JCVI_PEP_1096675798027	21	Kineococcus radiotolerans SRS30216	chaperonin Cpn60/TCP-1
JCVI_PEP_1096674473623	1	Thermobifida fusca YX	chaperonin GroEL
JCVI_PEP_1096668872213	3	Thermobifida fusca YX	chaperonin Cpn60/TCP-1
JCVI_PEP_10966889850	11	Streptomyces albus G	60 kDa chaperonin
JCVI_PEP_1096670437	115	Caldicellulosiruptor saccharolyticus DSM 8903	chaperonin GroEL
JCVI_PEP_1096697905649	3	Propionibacterium acnes	60 kDa chaperonin
JCVI_PEP_10966787	1183	Brevibacterium linens BL2	chaperonin GroEL
JCVI_PEP_1096682501903	6	Arthrobacter aureescens TC1	chaperonin GroEL
JCVI_PEP_1096677709109	2	Clostridium phytofermentans ISDg	chaperonin GroEL

Consensus annotation: Two spectra (peptides)

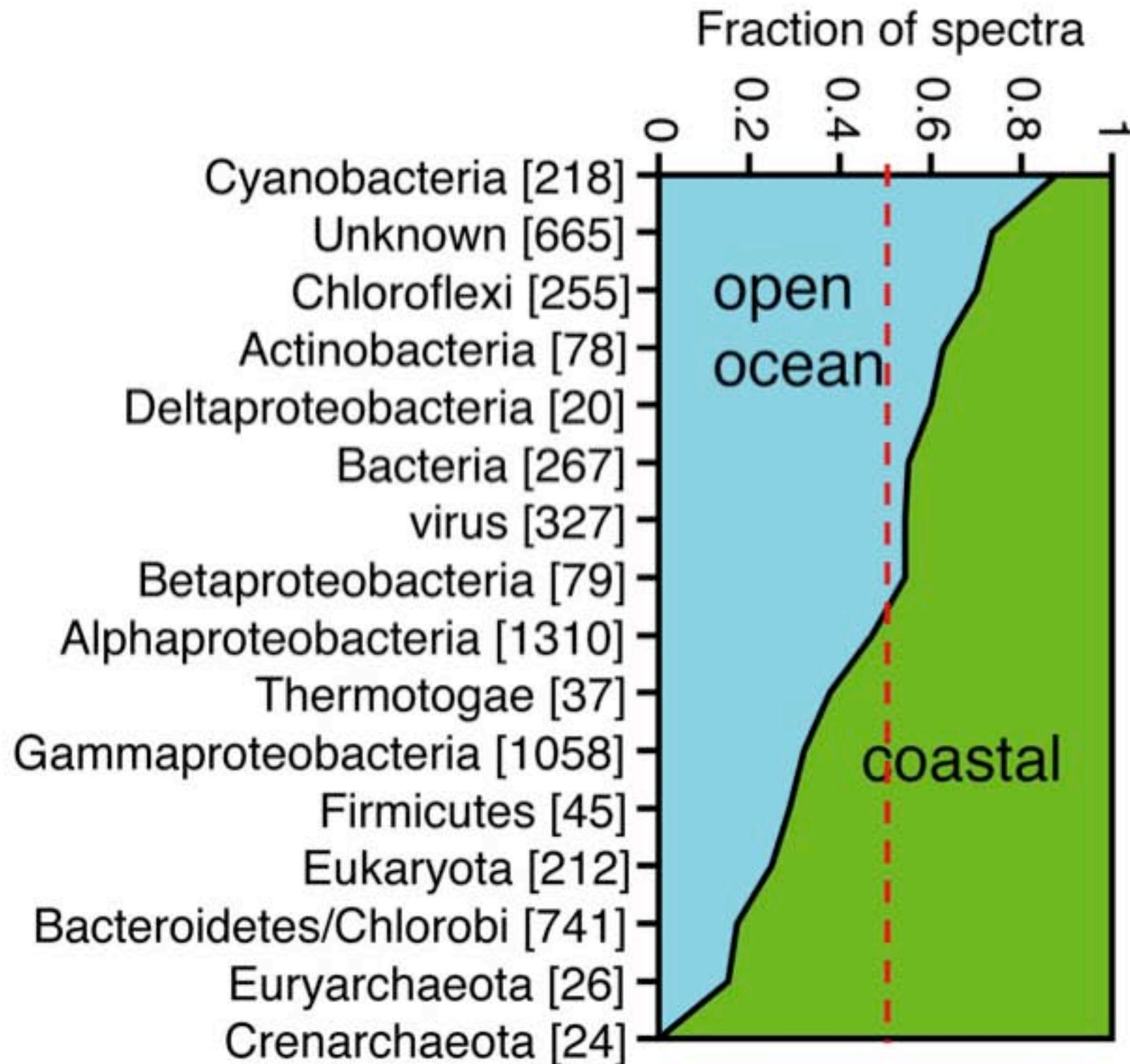
Total JCVI: 82

Bacteria (chaperonin GroEL)

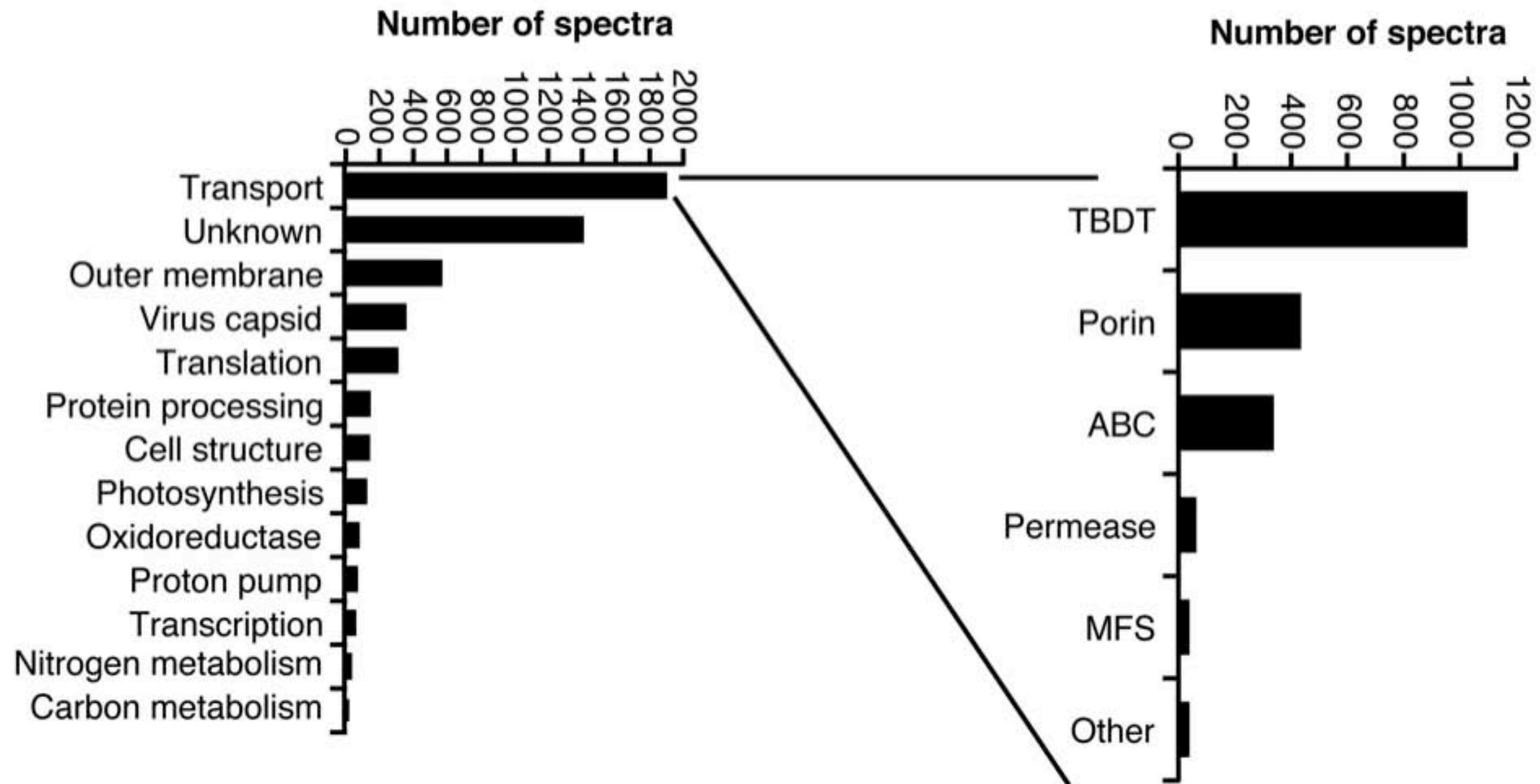
Community structure at the level of the expressed protein



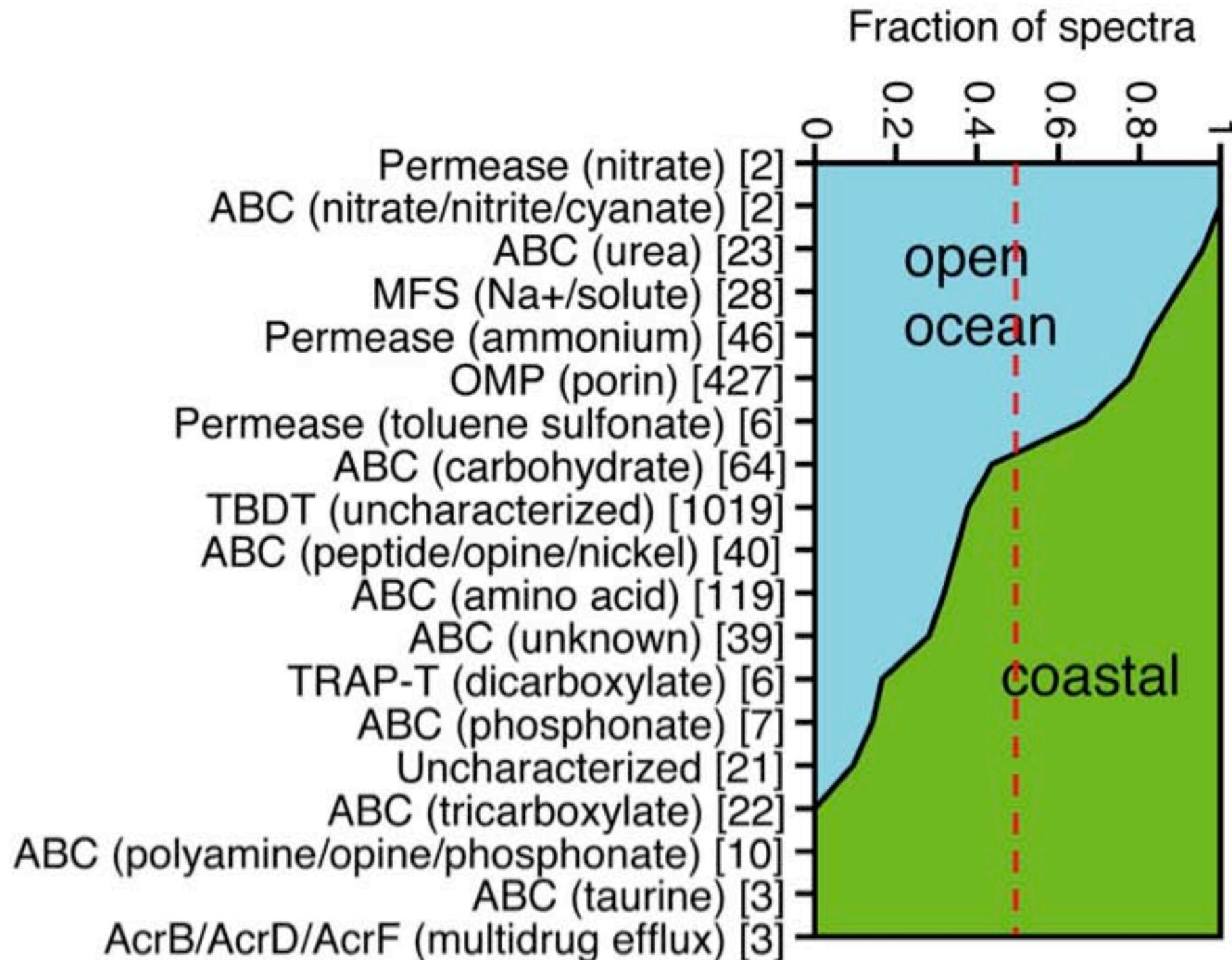
Comparative metaproteomics (structure)



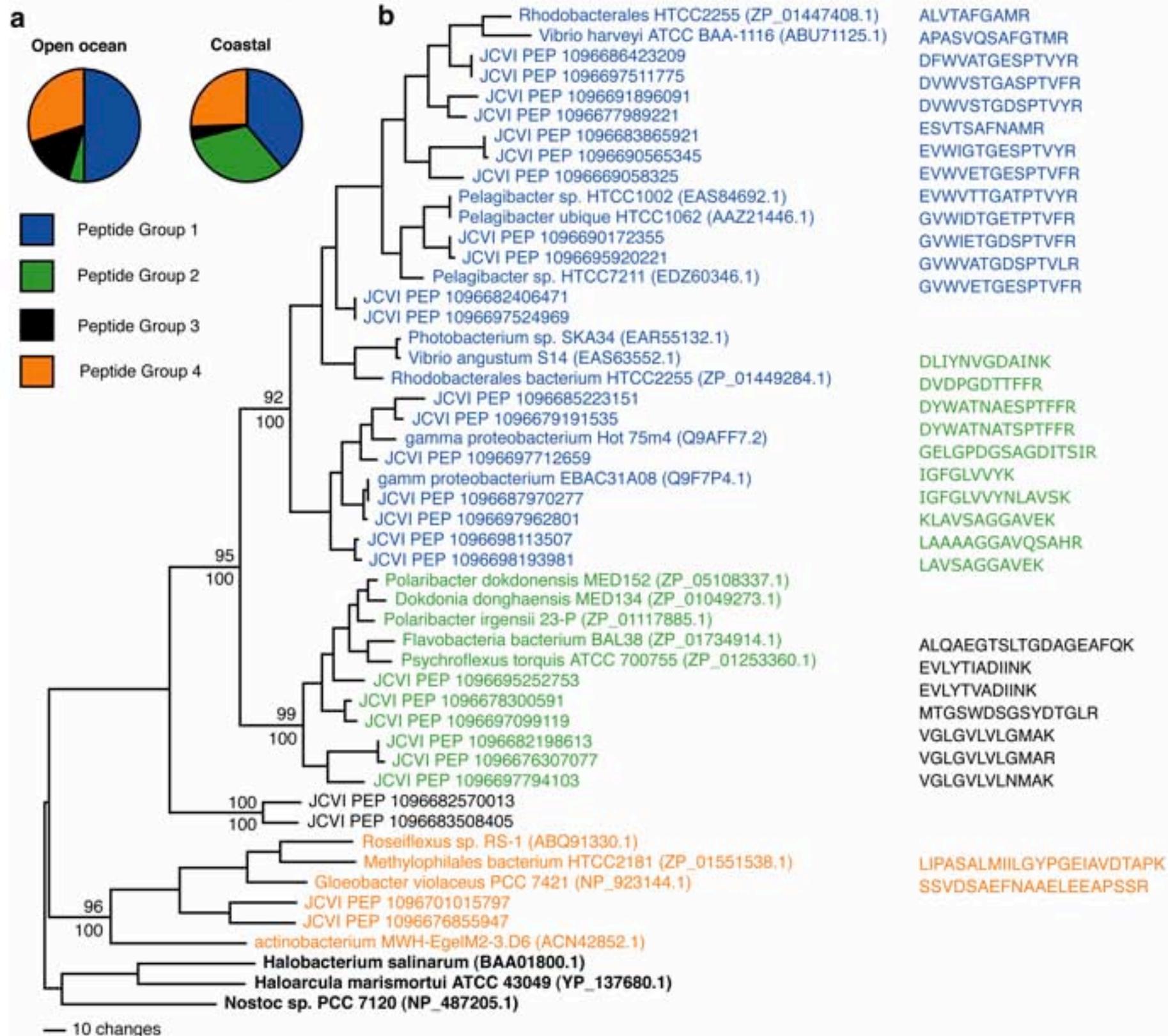
Community function at the level of the expressed protein



Comparative metaproteomics (function)



Light-harvesting rhodopsins identified throughout the South Atlantic



Major findings

Proteins for nutrient transport and energy transduction shifted from the low nutrient oligotrophic gyre (open ocean) to the highly productive Benguela upwelling region (coastal).

Uncharacterized TonB-dependent transporters accounted for 17% of all proteins identified throughout the South Atlantic Ocean.

Diverse light-harvesting rhodopsins were identified in open ocean and coastal samples.

Archaeal nitrification was identified in the Benguela upwelling region.

Puget Sound Metaproteome

LTQ-Orbitrap



GOS proteins 9,507
Protein groups 3,804
>90% identified by 1 peptide

SOLiD



Puget Sound sample 1	Number of sequences	Average number of nucleotides or AA
High quality trimmed and filtered reads	8,573,296	40
Six frame translation (>5AA)	39,084,285	12
Tryptic peptides (>5AA)	59,495,128	9

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