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Proteomic characterization of the psychrophile *Pedobacter cryoconitis* based on both ^{15}N metabolic labeling and *de novo* sequencing

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Aim

To characterise the proteome of an unsequenced psychrophile grown with different carbon sources and under temperature extremes employing ^{15}N metabolic labeling, 2DGE, tandem mass spectrometry, N-constrained ortholog searching, and *de novo* sequencing.

Introduction

Figure 1 *Pedobacter cryoconitis*[1] is an unsequenced psychrophile able to co-metabolically degrade petroleum hydrocarbons. Initial experiments showed that its maximum growth temperature is influenced by medium composition. Therefore growth and protein characterizations were made at 1 °C and 20 °C (the maximum growth temperature in mineral medium), and with different carbon sources, maltose and glucose.

^{15}N metabolic labeling is a non-invasive technique used for protein identification and quantification[2], and recently shown its potential as a proteomic tool for protein identification via *de novo* sequencing[3]. Here, the first example of a proteome characterization of this unsequenced psychrophile is reported employing 2 dimensional gel electrophoresis (2DGE), tandem mass spectrometry, ^{15}N metabolic labeling, nitrogen-constrained ortholog searching, and *de novo* sequencing.

Methods

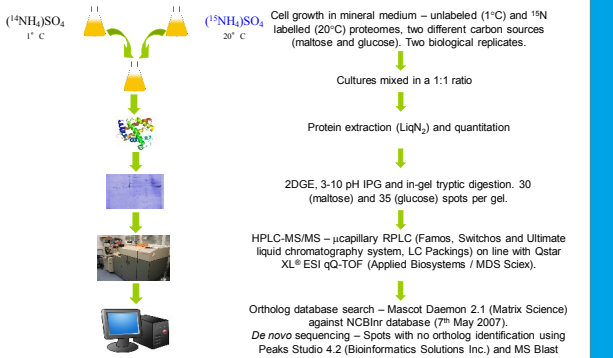

Figure 2 Experimental and data flow.

Analysis of Results

I. N-constrained ortholog database search and *de novo* sequencing

→ Identified proteins in both replicates and with two or more peptides.

→ Validated by light and heavy peptide through Mascot search and Peaks protein identification algorithms.

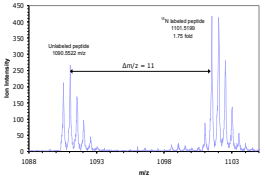

Figure 3 Spectra of light/unlabeled (M: 2181.1044 amu and N_2 composition: 22; $(M+2H)^{2+}$: 1090.5522 m/z and N_2 composition: 11) and heavy/labeled (M: 2203.0398 amu; $(M+2H)^{2+}$: 1090.5522 + 11 N_2 = 1101.5199 m/z, **validating** the peptide) peptide LTDQPFLMPVEDVFSITGR, a peptide from Elongation factor Tu (EF-Tu) protein, up-regulated at 20ºC by 1.75 fold.

Table 1 List of proteins identified and validated per spot for each phenotype. (a) Maltose; (b) Glucose. NI: No confident identification. NA: No Answer

a)

Spot	gi Number	Protein Name	Organism	Mascot score (matched peptides)	Peaks ID score (matched peptides)	MS Blast score (matched peptides)
1	NI					
2	gi|23502010	enolase	*Brucella suis 1330*	73(3)	96(4)	NA
3	gi|24374410	superoxide dismutase, Fe	*Shewanella oneidensis MR-1*	55 (3)	96(3)	NA
4	NI					
5	NI					
6	NI					
7	gi|110639474	chaperone protein (heat shock protein 70)	*Cytophaga hutchinsonii ATCC 33406*	241(4)	37(2)	NA
8	gi|110638228	60 kDa chaperonin	*Cytophaga hutchinsonii ATCC 33406*	452(8)	99(5)	NA
9	gi|110639548	translation elongation factor EF-Tu	*Cytophaga hutchinsonii ATCC 33406*	286 (7)	99(5)	NA
10	NI	3 (2',5'-bisphosphate nucleotidase	*Photobacterium profundum SS9*	NA	NA	69(1)
11	NI					
12	gi|110639548	translation elongation factor EF-Tu	*Cytophaga hutchinsonii ATCC 33406*	137(3)	99(4)	NA
13	gi|110639548	translation elongation factor EF-Tu	*Cytophaga hutchinsonii ATCC 33406*	159(4)	87(2)	NA
14	NI					
15	NI					
16	gi|91215257	malate dehydrogenase	*Psychroflexus torquis ATCC 700755*	79(1)	63(1)	NA
17	gi|21231962	triosephosphate isomerase	*Xanthomonas campestris pv. c ampestris str. ATCC 33913*	85(4)	77(4)	NA
18		Superoxide dismutase	*Symbiobacterium thermophilum*	NA	NA	196(4)
19		unknown	*Environmental sequence BEA_C7G_3ZA TQ367F*	NA	NA	146(3)
20	NI					
21	NI					
22	NI					
23		Malate dehydrogenase	*Bacteroides thetaiotaomicron VPI-5482*	NA	NA	65(1)
24	gi|89890610	transaldolase	*Cellulophaga sp. MED134*	168(3)	86(2)	NA
25	NI					
26		DNA-directed RNA polymerase alpha chain	*Bacteroides thetaiotaomicron VPI-5482*	NA	NA	79(1)
27	NI					
28	gi|34540241	ATP-dependent Clp protease, proteolytic subunit	*Porphyromonas gingivalis W83*	66(1)	63(1)	NA
29	gi|56539539	S-adenosyl-L-homocysteine hydrolase	*Gluconobacter oxydans 621H*	234(3)	99(3)	NA
30	gi|73764583	60 kDa chaperonin	*uncultured bacterium*	459(6)	459(6)	NA

b)

Spot	gi Number	Protein Name	Organism	Mascot score (matched peptides)	Peaks ID score (matched peptides)	MS Blast score (matched peptides)
1	gi|155581	enolase	*Zymomonas mobilis*	85(2)	86(2)	NA
2	NI					
3	gi|155581	enolase	*Zymomonas mobilis*	85(3)	99(5)	NA
4	NI					
5	NI					
6	NI					
7	gi|1169497	Elongation factor Tu (EF-Tu)	*Taxeobacter ocellatus*	169(4)	85(2)	NA
8	gi|110639543	50S ribosomal protein L7/L12	*Cytophaga hutchinsonii ATCC 33406*	132(3)	93(4)	NA
9	NI					
10	gi|73764583	60 kDa chape ronin	*uncultured bacterium*	488(9)	96(4)	NA
11	NI					
12	NI					
13	NI					
14	NI					
15	NI					
16	gi|110639474	chaperone protein (heat shock protein 70)	*Cytophaga hutchinsonii ATCC 33406*	90(1)	63(1)	NA
17	gi|94496879	Chaperone DnaK	*Sphingomonas sp. SKA58*	182(2)	39(2)	NA
18	gi|110637634	isocitrate dehydrogenase	*Cytophaga hutchinsonii ATCC 33406*	63(1)	97(3)	NA
19		DNA-directed RNA polymerase alpha chain	*Porphyromonas gingivalis W83*	NA	NA	106(2)
20	gi|110638338	deoxyhypusine synthase	*Cytophaga hutchinsonii ATCC 33406*	77(2)	76(3)	NA
21	NI					
22		Phosphoglycerate kinase	*Listeria innocua*	NA	NA	223(5)
23	gi|82184760	glyceraldehyde-3-phosphate dehydrogenase	*Chlamydophila abortus S26/3*	125(5)	99(5)	NA
24	gi|86131770	3-hydroxybutyryl-CoA dehydrogenase	*Cellulophaga sp. MED134*	91(1)	63(1)	NA
25	NI					
26	NI					
27	gi|110637557	ketol-acid reductoisomerase	*Cytophaga hutchinsonii ATCC 33406*	254(5)	99(8)	NA
28		Elongation factor Tu (EF-Tu)	*Taxeobacter ocellatus*	NA	NA	164(3)
29	gi|53715581	hypothetical protein BF4299	*Bacteroides fragilis YCH46*	72(1)	63(1)	NA
30	NI					
31	gi|110639649	triosephosphate isomerase	*Cytophaga hutchinsonii ATCC 33406*	68(1)	68(2)	NA
32	gi|88802653	Transaldolase	*Polaribacter irgensii 23-P*	131(2)	63(2)	NA
33	gi|89130096	transal dolase	*Cellulophaga sp. MED134*	153(3)	86(2)	NA
34	gi|82184760	glyceraldehyde-3-phosphate dehydrogenase	*Chlamydophila abortus S26/3*	154(3)	97(6)	NA
35	NI					

Figure 3 Summary of validated identified proteins per carbon sources and mode of identification. Results show the proteome characterization differs among the cells grown with different carbon sources, and only ~1/3 of the proteins were identified in both phenotypes.

II. Relative quantitation and biological analysis

Table 2 List of some up-regulated proteins based on unlabeled and ^{15}N labelled peptides, as described in Figure 3.

Accesion No.	Protein description	Organism	1ºC:20ºC fold	Carbon source
gi|2437440 10	superoxide dismutase, Fe	*Shewanella oneidensis MR-1*	1.29 ± 0.09	Maltose
gi|110638 228	60 kDa chaperonin	*Cytophaga hutchinsonii ATCC 33406*	1.56 ± 0.54	Maltose
gi|110639 548	translation elongation factor EF-Tu	*Cytophaga hutchinsonii ATCC 33406*	1.50 ± 0.04	Maltose
gi|21231962	triosephosphate isomerase	*Xanthomonas campestris pv. campestris str. ATCC 33913*	2.15 ± 0.20	Maltose
gi|94496879	Chaperone DnaK	*Sphingomonas sp. SKA58*	1.41 ± 0.02	Glucose
gi|169497	Elongation factor Tu (EF-Tu)	*Taxeoba cter ocellatus*	1.75 ± 0.45	Glucose
gi|86131770	S-adenosyl-L-homocysteine hydrolase	*Cellulophaga sp. MED134*	2.22 ± 0.08	Glucose
gi|110637557	ketol-acid reductoisomerase	*Cytophaga hutchinsonii ATCC 33406*	1.75 ± 0.39	Glucose
gi|86130096	transaldolase	*Cellulophaga sp. MED134*	1.75 ± 0.04	Glucose

Analysis on some typical representative proteins

→ translation elongation factor

Its increase at 20°C compared to 1°C (1.50 to 1.75 fold) may be explained by the fact that the growth rate (and accordingly the rate of protein biosynthesis) was slower at 1°C than at 20°C, independent of carbon source.

→ chaperone proteins (heat shock proteins) / chaperonins

Its apparent increase may correspond to a response to sudden temperature increase.

Proteins involved in stress response (some up-regulated at 20°C - temperature close to the maximum growth in complex medium (25°C) represents stress) and carbohydrate metabolism were identified.

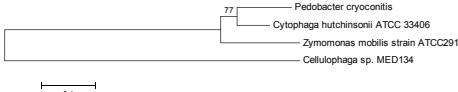

Figure 4 Phylogenetic tree phylogram comparing three organisms that reported ortholog protein identification. *Cytophaga hutchinsonii ATCC 33406* reported more similar proteins and is seen to be closer to *P. cryoconitis* than the other organisms. The genus *Cytophaga* belongs to the phylum Bacteroidetes, as does *Pedobacter.*

Conclusions

→ Protein identification via N-constrained ortholog searching and *de novo* sequencing of ^{15}N metabolic labelled cells of an unsequenced psychrophilic bacterium has been achieved for the first time.

→ *De novo* sequencing was time consuming, but allowed for additional proteins to be identified.

→ Additionally, relative quantitation of labelled and unlabelled proteins corresponding to different phenotypes (1 and 20ºC and glucose vs maltose) has been carried out.

→ The proposed protocol provides a practical alternative to identify proteins from unsequenced organisms and to relatively quantify proteins of two different phenotypes.

→ A more thorough manual spectral study will be carried out to further analyse the 27 spots with no valid identification.

→ Further work will improve the throughput of protein identification by improving the sample preparation, MS analysis, and data analysis throughput (especially for *de novo* identification) for the study of a larger amount of proteins.

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[2] Snijders, A.P., de Vos, M.G., de Koning, B., and Wright, P.C. (2005). A fast method for quantitative proteomics based on a combination between two-dimensional electrophoresis and 15N-metabolic labelling. Electrophoresis, 26(16):3191-9.

[3] Snijders, A.P.L., deVos, M.G.J., and Wright, P.C. (2005). Novel Approach for Peptide Quantitation and Sequencing Based on ^{15}N and ^{13}C Metabolic Labeling. J. Proteome Res., 4(2):578-585.

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