Quantitative proteomic analysis of Ibuprofen-degrading Patulibacter sp. strain I11

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Introduction

The increase in diversity and quantity of Pharmaceutically Active Compounds (PhACs) detected in the effluents of wastewater treatment plants is an issue of great concern due to health and environmental associated risks of the PhACs [1].

Ibuprofen, a non-steroidal anti-inflammatory drug, is considered one of the most frequently occurring PhACs in the influent wastewater, typically being found in the range of 10-400 µg/L. Typical Ibuprofen removal efficiencies range from 80-100%, depending on operational conditions and wastewater treatment plant configuration[2,3]. The elimination of ibuprofen is being ascribed primarily to biodegradation. However, in order to investigate the conditions for better removal of compounds like ibuprofen, we need to know the identity of the organisms involved and how their ibuprofen degradation activity depend on the controlling parameters. For this purpose we wanted to identify the genes involved and develop quantitative molecular tools for determining the activity of these genes.

Objective

The main objective of this study was to investigate the biochemical pathway of ibuprofen degradation in the ibuprofen degrading strain *Patulibacter sp.* Strain I11 using quantitative tandem mass spectrometry.

Methods

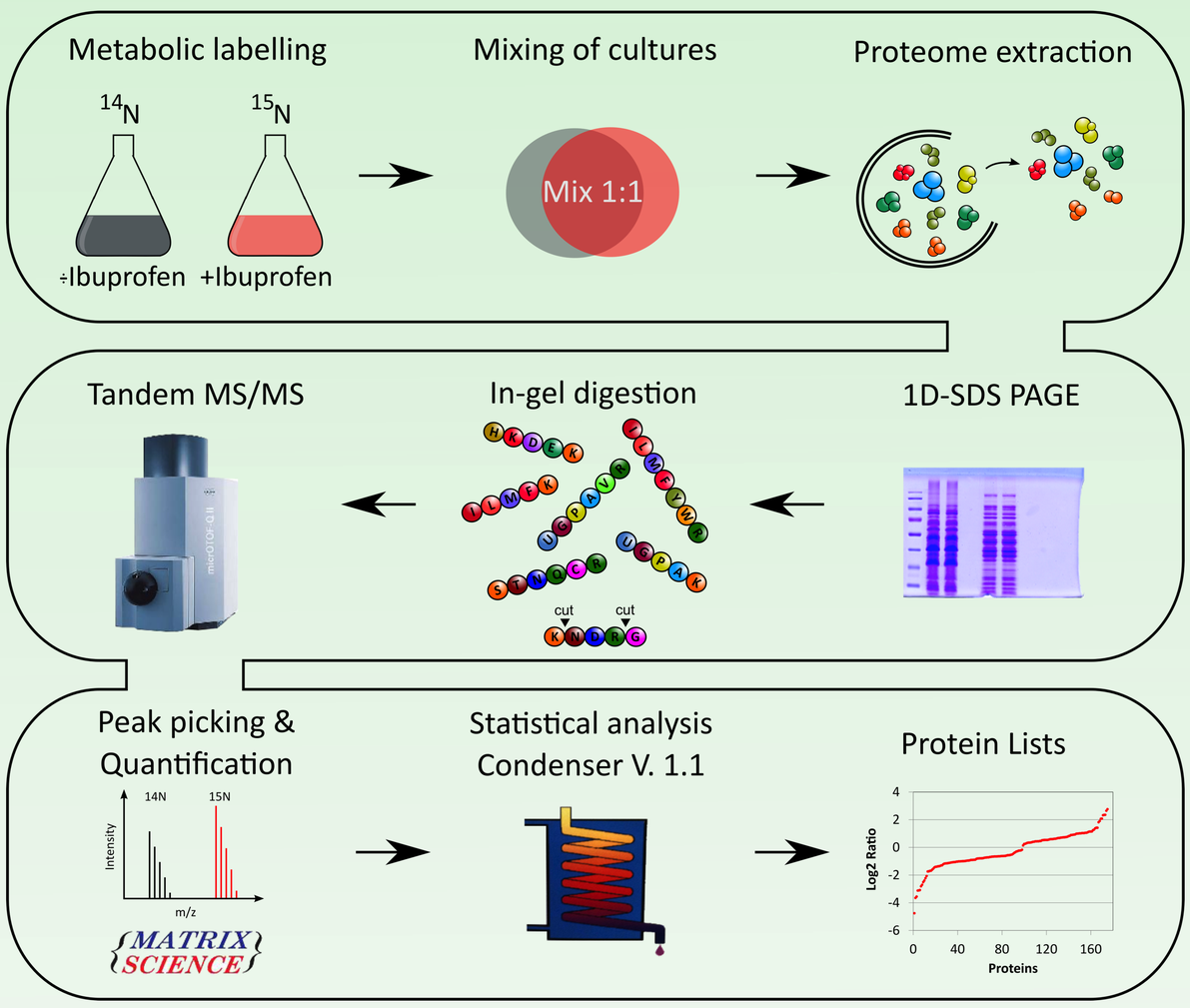


Fig. 1 The differential changes in the proteome of *Patulibacter sp.* strain I11, grown in the presence and absence of ibuprofen, were characterised by the combination of stable isotope metabolic labelling and 1-D gelbased shotgun Proteomics. The genome of *Patulibacter sp.* Strain I11 was sequenced and annotated and used as the reference database for the subsequent MS-based protein identification (the sequencing and annotation part of the genome of *Patulibacter sp.* strain I11 have been omitted in the above flowchart). The setup was carried out in biological duplicates using a forward and reverse labelling strategy (only the forward labelled duplicate is depicted above). For the reverse labelled duplicate the metabolic labels were reversed, i.e. yielding (^{14}N + Ibuprofen) and (^{15}N ÷ Ibuprofen). The forward and reverse labelling strategy served the purpose of evaluating potential bias of the ^{14}N- and ^{15}N- medium on protein expression levels.

Results

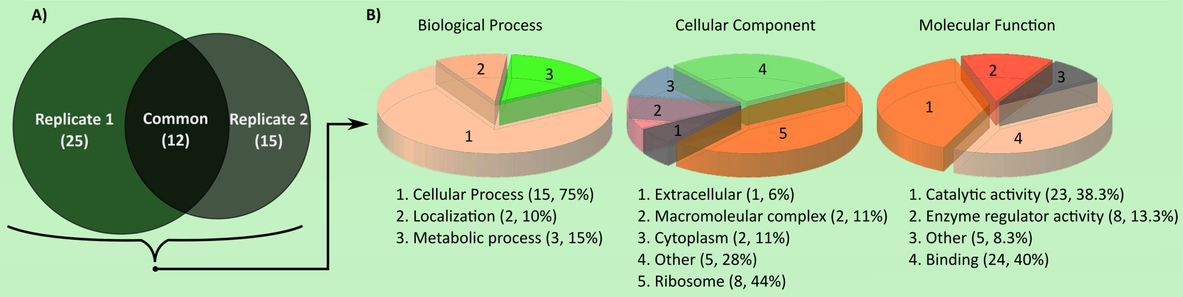
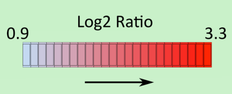


Fig. 2 A) Metabolic labelling was carried out in biological duplicate and proteins considered up-regulated (proteins with log2 ratio ≥ 0.9) were **B)** pooled and Gene Ontology-annotated at three different levels: Biological Process, Cellular Component and Molecular Function. Each pie slice is labelled with the GO subcategory name, number of GO annotations within the category as well as the percentage fraction of annotations. Replicate 1 corresponds to the forward labelled replicate (14N ÷ Ibuprofen, 15N + Ibuprofen) whereas Replicate 2 corresponds to the reverse labelled replicate (14N + Ibuprofen, 15N ÷ Ibuprofen).



	Accession[A]	Description[B]	Log2 Ratio[C] Replicate 1	Log2 Ratio[C] Replicate 2	Protein Score[D] Replicate 1	Protein Score[D] Replicate 2	#[E] Replicate 1	#[E] Replicate 2
●	D3F114	Enoyl-CoA hydratase/isomerase		-	145		2	
	D3F2P4	Nuclear export factor GLE1			335	(388)	17	(25)
●	D3F178	Putative uncharacterized protein		-	482		24	
	E6SEB9	Aminotransferase class-III		-	96		8	
●	E1VKI1	AMP-dependent synthetase and ligase		-	314		6	
	D3FA23	Putative uncharacterized protein			174	(382)	6	(10)
	D3F6B1	Putative uncharacterized protein			311	(292)	7	(14)
	Q47N65	Putative uncharacterized protein			235	(219)	4	(4)
	D3F293	Putative uncharacterized protein			747	(723)	40	(46)
●	D3F3V3	Rieske (2Fe-2S) iron-sulphur domain protein			313	(366)	13	(13)
	D3FFG0	Putative uncharacterized protein			2195	(1785)	45	(97)
	B0CFJ3	Putative uncharacterized protein			113	(110)	4	(6)
	F1YMM4	Glycosidase		-	749		16	
	D3FBK3	Collagen triple helix repeat protein			412	(316)	26	(21)
	D3EZ72	Putative uncharacterized protein	-			(881)		(46)
	D3FEE9	Putative uncharacterized protein	-			(252)		(8)
	D3FEF4	PpiC-type peptidyl-prolyl cis-trans isomerase		-	958		64	
●	D3FBI3	Daunorubicin resistance ABC transporter ATPase subunit			509	(473)	8	(10)
●	D3FBH9	Extracellular solute-binding protein family 1		-	369		8	
●	D3FBI2	ABC-2 type transporter		-	161		4	

Table 1 Differentially expressed proteins of the biological replicates of *Patulibacter sp.* I11 grown in presence/absence of ibuprofen. Only up-regulated proteins (Log2 ratio ≥ 0.9) are shown in the table. No major influence of the ^{14}N- and ^{15}N- medium on the protein expression levels was observed.
[A]UniProt accession number of the closets protein homologue Description of the closets protein homologue, [B]Description of the closest protein homologue, [C]Log2 ratio obtained from the quantitative proteomics analysis, [D]Protein Score obtained from the quantitative proteomics analysis, [E]The number of quantitated peptides upon which the quantitative value (Log2 ratio) was determined.

Conclusion

- Several proteins related to uptake and degradation of aromatic acids as well as compound transport-related proteins were found among the proteins up-regulated in response to Ibuprofen.
- The high number of up-regulated putative uncharacterised proteins might suggest a novel pathway for the degradation of Ibuprofen in *Patulibacter sp.* Strain I11.

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