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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 configuration2,3. 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

Metabolic labelling
Mixing of cultures
Proteome extraction

Tandem MS/MS
In-gel digestion
1D-SDS PAGE

Peak picking & Quantification
Statistical analysis
Protein Lists

Fig. 1 A) Metabolic labelling was carried out in biological duplicate and proteins considered up-regulated (proteins with log2 ratio ≥ 0.9) were picked 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. B) The number of up-regulated proteins in the forward labelled replicate (14N + Ibuprofen) and reverse labelled replicate (14N + Ibuprofen) whereas Replicate 2 corresponds to the reverse labelled replicate (14N + Ibuprofen) and reverse labelled replicate (14N + Ibuprofen).

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. The number of Uniprot accession number of the closest protein homologue description of the closest protein homologue, 3Description of the closest protein homologue, 4Log2 ratio obtained from the quantitative proteomics analysis, 5Protein Score obtained from the quantitative proteomics analysis, 6The 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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References


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