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

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Introduction

The increase in diversity and quantity of Pharmacologically 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 PhACs1. 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

Condenser V.1.1

Protein Lists

Results

Fig. 2 A) Metabolic labelling was carried out in biological duplicate and proteins considered up-regulated (proteins with Log2 ratio > 0.9) were 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, 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).

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 log2 value of the 15N and 14N medium on the protein expression level was obtained.UniProt accession number of the closets protein homologue Description of the closets protein homologue, Log2 ratio obtained from the quantitative proteomics analysis, Protein Score obtained from the quantitative proteomics analysis. 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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References

