SUPPLEMENTARY MATERIAL FOR

Dynamic response of *Mycobacterium vanbaalenii* PYR-1 to BP Deepwater Horizon crude oil

Seong-Jae Kim,[a] Ohgew Kweon,[a] John B. Sutherland,[a] Hyun-Lee Kim,[a] Richard C. Jones,[b] Brian L. Burback,[c] Steven W. Graves,[c] Edward Psurny,[c] and Carl E. Cerniglia[a]

Regulatory proteins

The genome of *M. vanbaalenii* PYR-1 is enriched with a high proportion of regulatory genes (about 473 genes), as generally found in free-living environmental bacteria, which encode a large variety of regulatory proteins, such as DNA-binding helix-turn-helix (HTH) transcriptional regulators, response regulators of two-component systems, and sigma factor subunits of RNA polymerase. Global response of this bacterium to BP crude oil may be more clearly observed in the analysis of transcriptional regulators. Various transcriptional factors, which control a variety of metabolic and physiological responses of the bacterium, were dynamically expressed. In the proteome analysis, we identified expression of 331 transcriptional regulatory genes (70.% of 473 genes), among which 367 proteins were STEM-categorized.

As shown in Data Set S6 in the supplemental material, we observed expression of various types of transcription regulators, including most of the major families of DNA-binding transcriptional regulators identified in bacterial genomes (1). Among them, the TetR family, which controls genes involved in multidrug resistance, catabolic pathways, biosynthesis of antibiotics, and osmotic stress (2), is the most abundant family of regulatory proteins identified in the proteome (114 proteins). It is not just because the TetR family of transcriptional regulators is the largest in the genome of *M. vanbaalenii* PYR-1 (32.4% of all transcriptional regulators), but, in fact, the proportion of expressed regulatory proteins was high, with 78.6% of the genome-encoded genes being identified. Many proteins belonging to the ArsR and MerR families of transcriptional repressors, acting as metal sensors, were identified. We also identified expression of a high proportion (25 out of 28 genes) of two-component systems, many of which control global cellular processes, including catabolic pathways (3). Several LuxR family regulators (20 out of 23 genes), acting as activators or repressors, were expressed as proteins, including two-component systems, most of which were downregulated. DNA-binding proteins belonging to this family were assumed to be, among several others, involved in the production of secondary metabolites, such as polyketides (4). We also observed differential expression of several proteins highly homologous with global transcriptional regulators reported in actinobacteria, such as *C. glutamicum*. For example, a Crp/Fnr family protein (Mvan_5435), highly homologous to the GlxR protein, considered as a regulatory-hub DNA-binding transcriptional regulator, was identified as downregulated at all points (5). Two proteins, Mvan_0096 and 0799, which are highly homologous to the global response-inducing regulators related to carbon metabolism, SugR and RamR, were downregulated.

Nine proteins encoding transcriptional regulators were identified, all of which are located closely with their regulated genes within catabolic islands in the genome of *M. vanbaalenii* PYR-1 (6). Expression of two proteins, Mvan_0462 and Mvan_0559, belonging to the IclR family,

were reduced. They regulate the phthalate (*pht*) and protocatechuate (*pca*) degradation pathways, respectively. The amount of a MarR type regulator, Mvan_0524, which regulated the fluoranthene pathway was increased. Expression of catabolic genes, on which these regulatory proteins exert their effects, was reduced.

Transport systems

Considering the composition of crude oil, which contains various kinds of nutrients as well as antimicrobial substances, it is crucial for the bacterium to efficiently regulate its transport systems. The proteome response of *M. vanbaalenii* to BP crude oil resulted in dynamic changes in the expression of transporter proteins with various specific roles. Upon exposure to BP crude oil, the permeability of the *M. vanbaalenii* PYR-1 outer membrane was probably increased with upregulation of porin proteins, causing increased intrusion of various small, hydrophilic solutes, including sugars, phosphate, metal ions and amino acids (7). We identified expression of 269 proteins out of 506 genome-predicted genes, encoding transporter-related proteins, in the proteome analysis (Fig. S7). Various types of transport proteins responded to BP crude oil exposure, since 210 proteins, out of 270 expressed proteins, were analyzed as STEM-filtered, showing their dynamic regulation. Among them, the largest number (172) of transporter proteins were class I primary active transporters, including the ATP-binding cassette (ABC) transporter superfamily and the P-type ATPase superfamily, followed by a group of transporters belonging to class 2 electrochemical potential-driven transporters. As shown in Fig. S7 (see also Data Set S7 in the supplemental material), we found 88 proteins to be upregulated following exposure to BP crude oil at least at one time point. Interestingly, these upregulated transporters were rich in 5 types of transporters, including some of the ABC transporters, resistance-nodulation-division (RND) type transporters, P-type transporters, and major facilitator superfamily (MFS)/ cation diffusion facilitator (CDF) family transporters. Many of these upregulation were analyzed as belonging to the STEM patterns 21, 23, 24, and 25, which indicates their actions are continuously required for *M. vanbaalenii* PYR-1 during crude oil incubation.

We identified upregulation of the channel-forming porin proteins, Mvan_0621 and MspA-like Mvan_4840. *M. vanbaalenii* PYR-1 upregulated expression of various transport systems to control metal and inorganic ions. We identified upregulation of CtpH (Mvan_1651), CtpD (Mvan_3729), and CtpV (Mvan_3670/3678), belonging to P-type ATPases, involved in transport of copper, cadmium, and zinc, respectively. Four ABC-type transporters (Mvan_2537/2538, 3370/3371, 5319/5320, and 5324/5325/5326), which appear to be involved in transport of molybdenum, cobalt, manganese, and zinc, respectively, were upregulated. A transport system involved in iron uptake (Mvan_4773/4775) was upregulated too. Proteins belonging to the CDF transporters (Mvan_3225/3726/5864), were also upregulated. A sulfate uptake permease, similar to CysZ (Mvan_3271), and CysHI proteins (Mvan_3857/3858), involved in assimilatory sulfate reduction of inorganic sulfate compounds, were upregulated. Two additional sulfate permease proteins, belonging to SulT transporters (SubI-CysTWA1, Mvan_3872-3875) and SulP transporter (Mvan_5693), were also upregulated. Proteins specifically involved in transport of potassium (KdpB, Mvan_4759), calcium (CtpE, Mvan_4989), and ammonium (AmtB, Mvan_2178), were increased. Quite a number of transport systems belonging to multidrug resistance (MDR) efflux pumps, involved in extrusion of toxic compounds, showed increased expression. These included ABC-type transporters (Mvan_2729/3121/3163/4499/5005), RND

efflux systems (Mvan_0271/0312/0683/1057/1197/1936/3189/3833/4573), and MFS-type transporters (Mvan_0726/2691/3561). A group of ABC-efflux transporters (Mvan_0362/0365/0368/0515/4606), implicated in solvent tolerance, were also upregulated. Expression of ABC type proteins, which are involved in the transport of (branched-chain) amino acids (Mvan_0436/2427), was also induced.

We identified expression of 6 clusters of genes based on similarity to those of *M. smegmatis*, encoding proteins involved in carbohydrate transporters, such as ribose, xylose, fructose, and glucose. Expressions of these proteins, however, mostly had no changes or were downregulated during BP crude oil exposure. Sugar transport systems, such as the glucose transporter, GlcP (Mvan_3472), along with a group of transporters involved in the fructose phosphotransferase system (PTS, Mvan_0093/0094/0095/0096/0097), and MusK (Mvan_2541), showed a downregulation upon exposure to crude oil. They indicated a decrease in the activity related to carbohydrate metabolism, which is reasonable considering the composition of crude oil.

M. vanbaalenii appeared to accomplish iron uptake by mycobactin (Mvan_3842/3843/3844). This extracellular siderophore protein, shown to be involved in iron chelation in *M. smegmatis*, was significantly upregulated during incubation with BP crude oil, except for day 30. No expression of another siderophore protein, Mvan_3380, was identified.

Biosurfactant

Trehalose is a disaccharide, freely present in mycobacterial cytoplasm, whose conjugates with fatty acids produce a type of surfactant, trehalose lipid, located in the cell envelope. Formation of this surface-active compound is assumed to be important because it enhances solubility and biodegradation of hydrocarbon contaminants in the environment (8). Previous study with rhodococci showed that this surfactant was induced by *n*-alkanes (9). *M. vanbaalenii* PYR-1 appeared to produce trehalose-containing glycolipids, a type of biosurfactant, as seen in several members of actinobacteria. Out of three pathways for trehalose biosynthesis known in mycobacteria, the genome of *M. vanbaalenii* PYR-1 possesses two, OtsAB (Mvan_5192/4107) and TreYZ (Mvan_2785/2784), and we identified expression of both. Among them, Mvan_4107 was highly expressed during BP crude oil degradation. TreYZ proteins were also slightly upregulated during crude oil incubation compared to day 0.

Stress response

Many bacteria express stress proteins in response to environmental changes, such as exposure to organic solvents or toxic compounds (10, 11). In *M. vanbaalenii* PYR-1, expression of genes, related to chaperones, heat/cold shock proteins, and SOS response proteins, were identified but most didn't respond following BP crude oil treatment. Only two heat shock proteins (Mvan_0296/1374) were upregulated. An increase in the expression of 6 universal stress proteins (UspA) was also observed, with 5 of them being highly upregulated at all the time points after BP crude oil exposure (STEM pattern 21). It indicates that, although BP crude oil might have created adverse conditions to *M. vanbaalenii* PYR-1, it probably did not cause strong stress responses. Stress response to environmental changes or adaptation to adverse conditions is usually achieved at the level of transcription through the use of sigma (σ) factors (12). Within the

genome of *M. vanbaalenii* PYR-1, as in many environmental bacteria (12), we identified a comparatively high proportion of σ factors (27 proteins) for its genome size. Among 27 σ factors which the *M. vanbaalenii* genome encodes, we identified expression of 12 proteins, with 8 proteins changed in abundance during BP crude oil incubation. Whereas the amount of the principal (housekeeping) σ factor SigA (Mvan_2461) was almost constant during cultivation with BP crude oil, suggesting constitutive expression, the nonessential principal-like σ factor SigB (Mvan_2451), which is known to be induced under various stress conditions, exhibited a tight repression at all points following crude oil treatment (STEM pattern 4). Expression of SigF (Mvan_1660), which is also known to be induced under a variety of stress conditions, was downregulated. The genome of *M. vanbaalenii* PYR-1 harbors 22 σ factors, belong to an extracytoplasmic function (ECF) subfamily, which controls transcription of the genes for various environmental signaling processes (13). Most of these alternative σ factors, including SigD, SigE, SigH, SigG, and SigM, showed no response or downregulation. All these results showing that most stress-related proteins, whose functions are known to be important, had no significant level of upregulation, indicate that, although some stress proteins and sigma factors of *M. vanbaalenii* PYR-1 are involved in cellular adaptation, the components of BP crude oil didn't appear to act as strong stresses to *M. vanbaalenii* PYR-1.

TABLE S1 Chemical standards and internal standards used in this study

Chemical	Manufacturer	Purity
Phenanthrene	Supelco	nl[a]
Phenanthrene-d_{10}	Sigma-Aldrich	98 atom % D
fluoranthene	Supelco	nl[a]
Fluoranthene-d_{10}	Sigma-Aldrich	98 atom % D
pyrene	Supelco	nl[a]
Pyrene-d_{10}	Sigma-Aldrich	98 atom % D
benzo(a)pyrene	Sigma-Aldrich	≥96%
benzo(a)pyrene-d_{12}	Sigma-Aldrich	98 atom % D
n-pentacosane	Sigma-Aldrich	≥98.5%
Phenanthrene-d_{52}	CDN Isotopes	99.3 atom % D
n-Dodecane	Sigma-Aldrich	≥99.6%
n-Tetradecane	Sigma-Aldrich	≥99.3%
n-Hexadecane	Sigma-Aldrich	≥99.1%
n-Octadecane	Sigma-Aldrich	≥99.1%
n-Eicosane	Sigma-Aldrich	≥99.8%
n-Henicosane	Sigma-Aldrich	≥99.7%
n-Docosane	Supelco	≥99.9%
n-Tricosane	Sigma-Aldrich	≥99.4%
n-Tetracosane	Sigma-Aldrich	≥99.9%
n-Pentacosane	Sigma-Aldrich	≥99.5%
n-Hexacosane	Sigma-Aldrich	≥99.7%
n-Heptacosane	Sigma-Aldrich	≥99.6%
n-Octacosane	Sigma-Aldrich	≥99.7%

[a] From an EPA 610-N PAH analytical standard kit. Purity not listed.

FIG S1 GC/MS chromatograms showing degradation of *n*-alkanes from C_{12} (dodecane) through C_{28} (octacosane), with C_{25} (pentacosane) and its d52 labeled internal standard (A) and showing PAHs, phenanthrene (Phn) and its d10 labeled internal standard (*m*/*z* 178 and 188), pyrene (Pyr) and fluoranthene (Fla) and their d10 labeled internal standards (*m*/*z* 202 and 212), and benzo[*a*]pyrene and its d12 labeled internal standard (B) in liquid cultures of *M. vanbaalenii* PYR-1 incubated with BP crude oil. GC/MS chromatograms only show peaks at days 0 and 30 are shown in red and black, respectively.

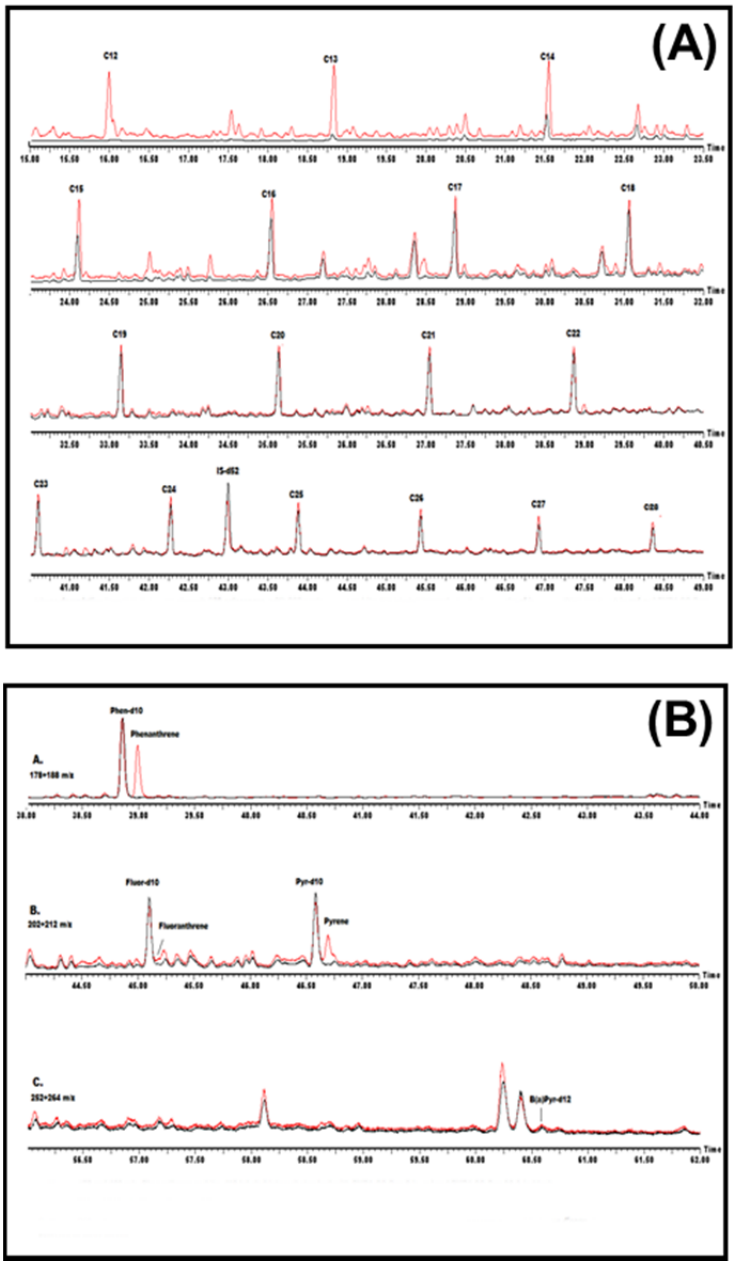


FIG S2 Pie charts showing the COG functional distribution based on *M. vanbaalenii* PYR-1 genome (A) and proteome (B)

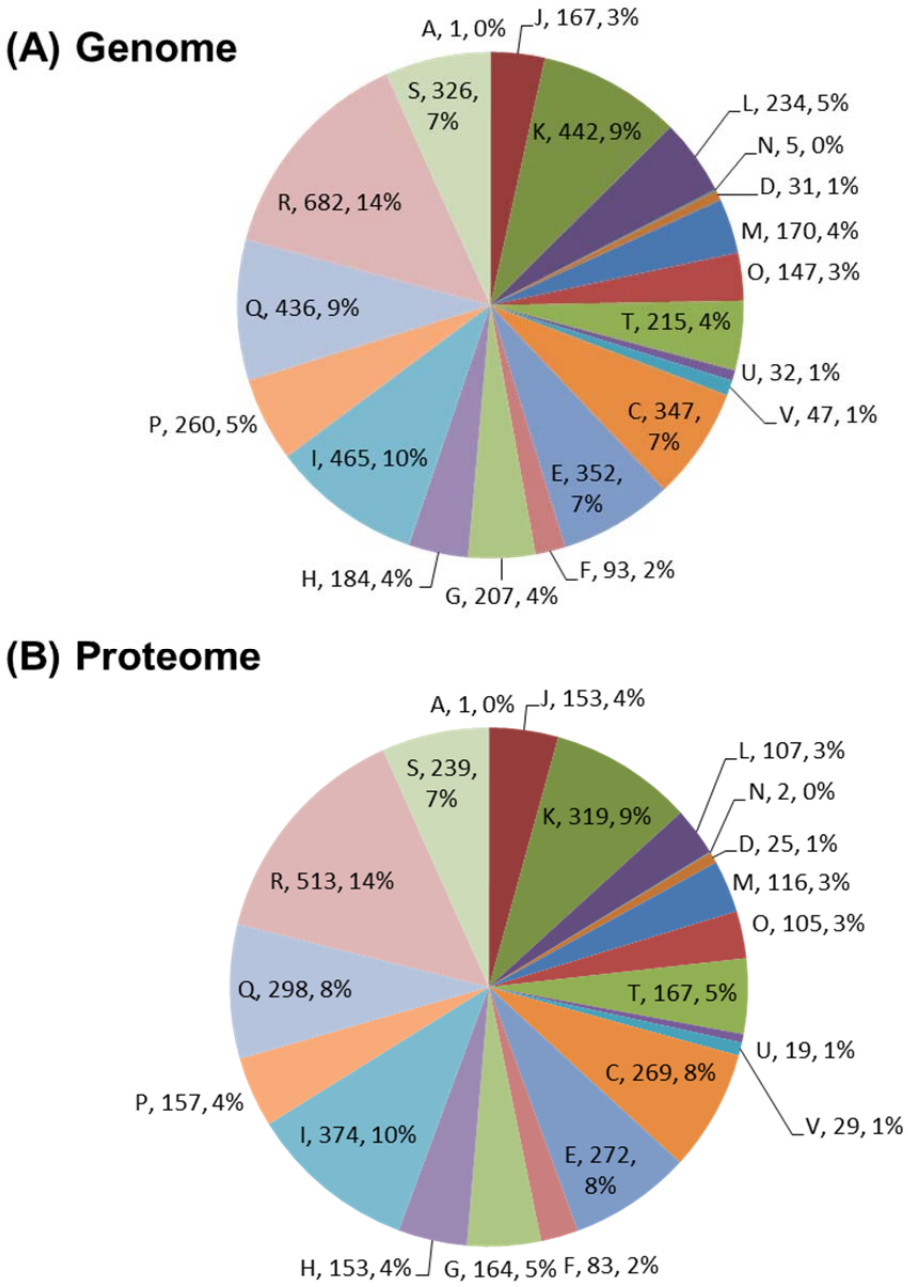


FIG S3 Pie charts showing the COG functional distributions of up- (A) and downregulated proteins (B)

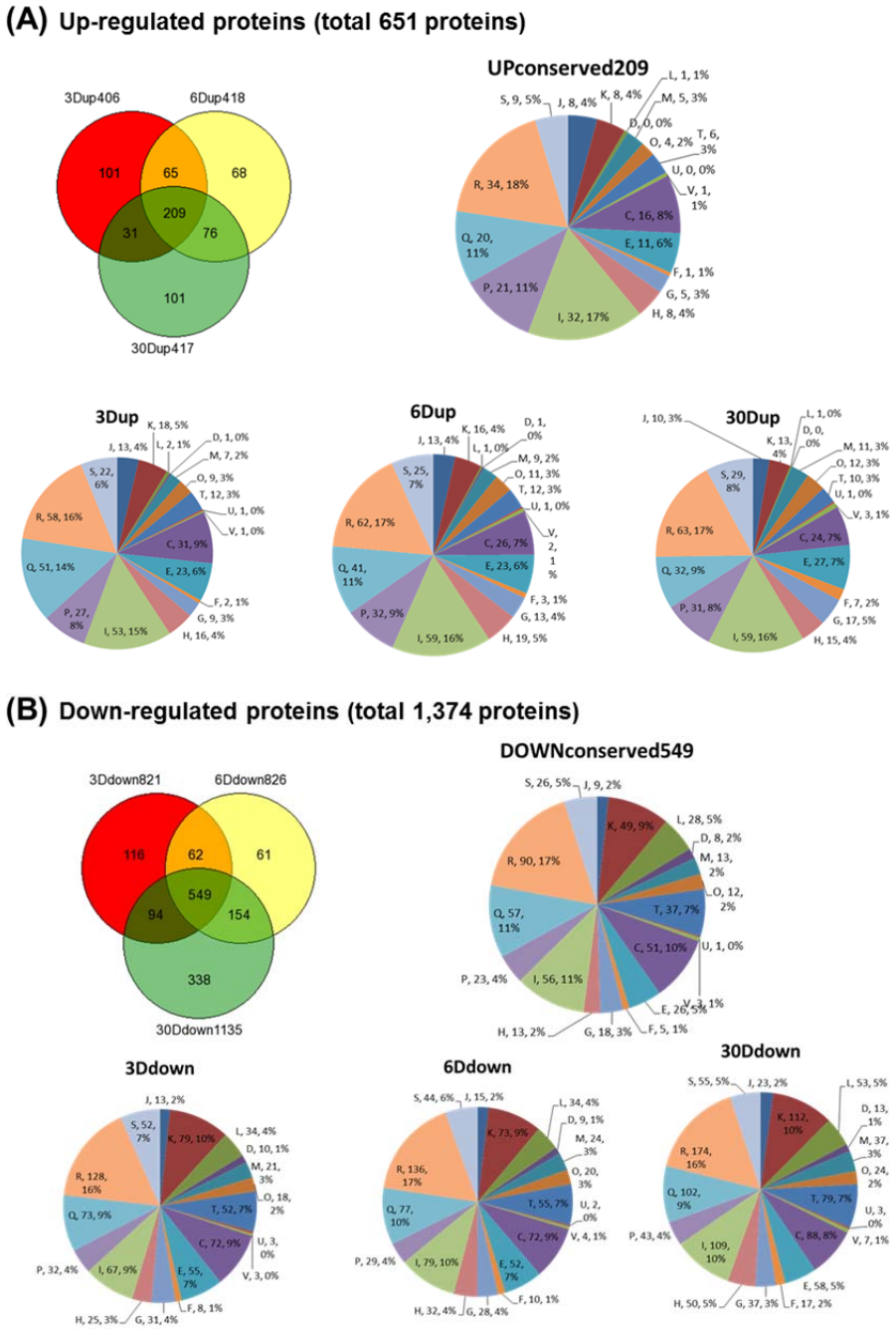


FIG S4 Snapshot of differential protein expression at each time point. Up- and down-regulated proteins can be simultaneously viewed on multiple pathway maps of KEGG. Red and blue correspond to up- and down-regulated pathways, respectively.

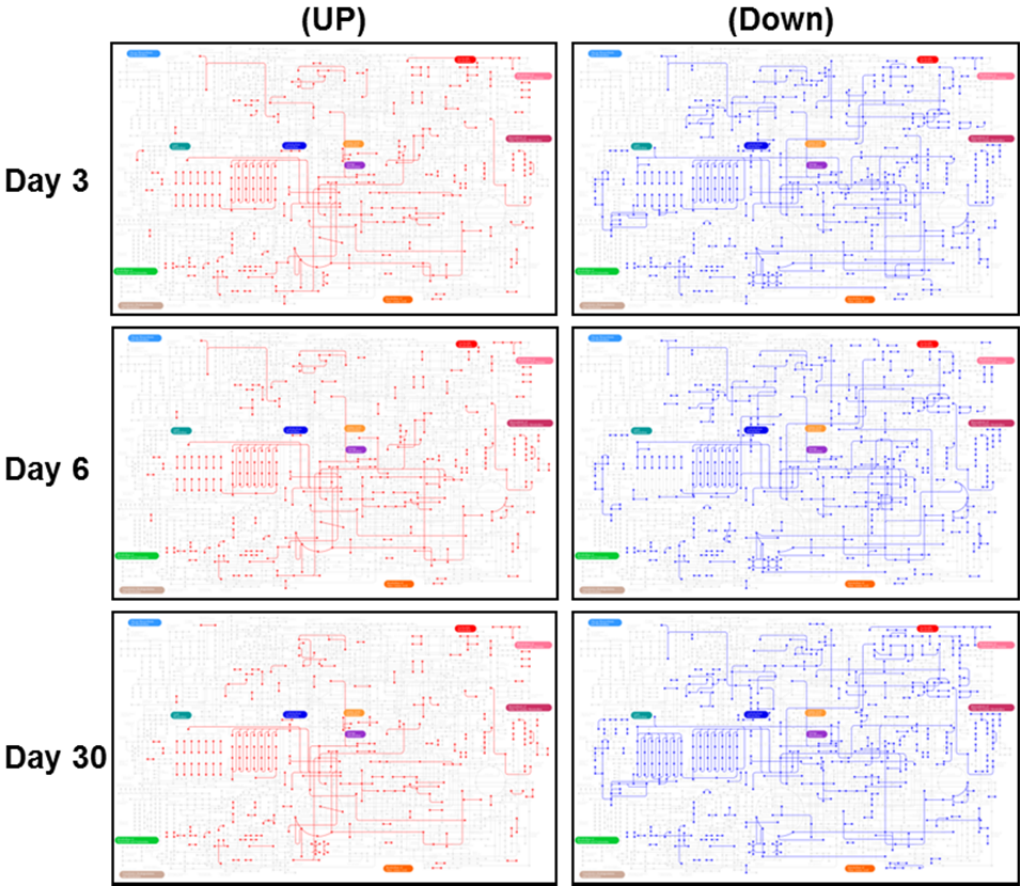


FIG S5 Enriched coexpression profiles clustered by the short time-series expression miner (STEM) (see Data Set S2C in the supplementary material). The differential expression of proteins can be divided into 26 STEM patterns. A total of 2,675 proteins were filtered, resulting in patterns 0 to 11 and 13 to 25, for first decrease and increase, respectively, then further changed in the level of expression.

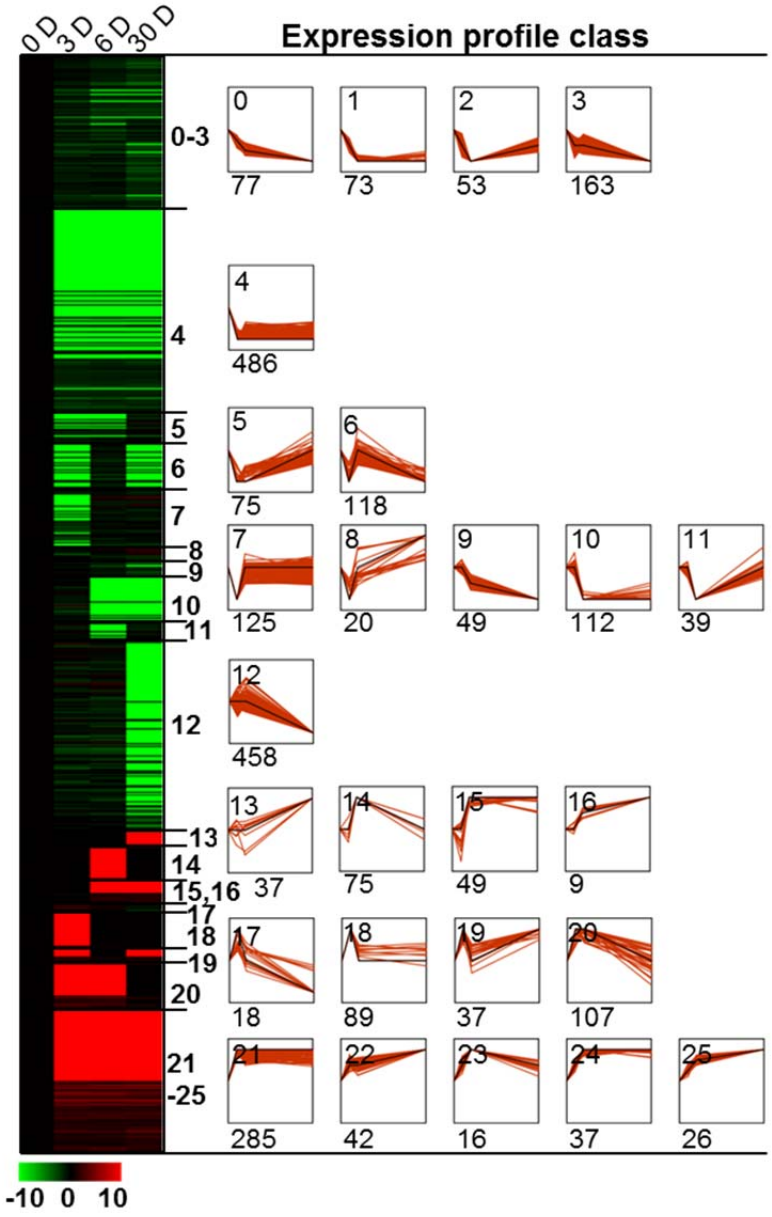


FIG S6 Cluster analysis of the 163 expressed proteins involved in PAH degradation, based on the functional modules of the PAH-MN. The colors indicate: blue, enzymes for RCP; red, enzymes for SCP; and green, enzymes for CAP, respectively.

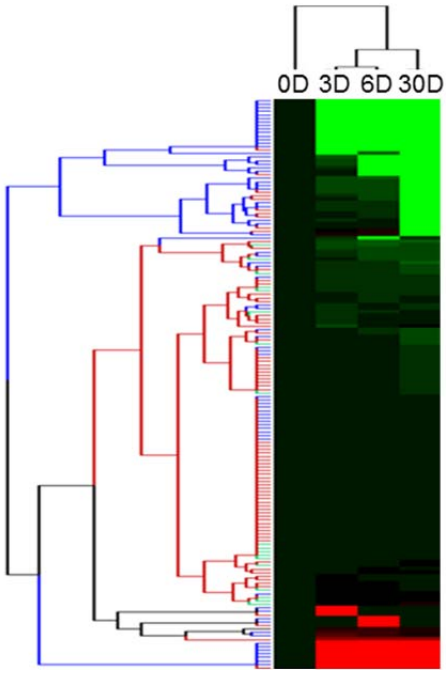
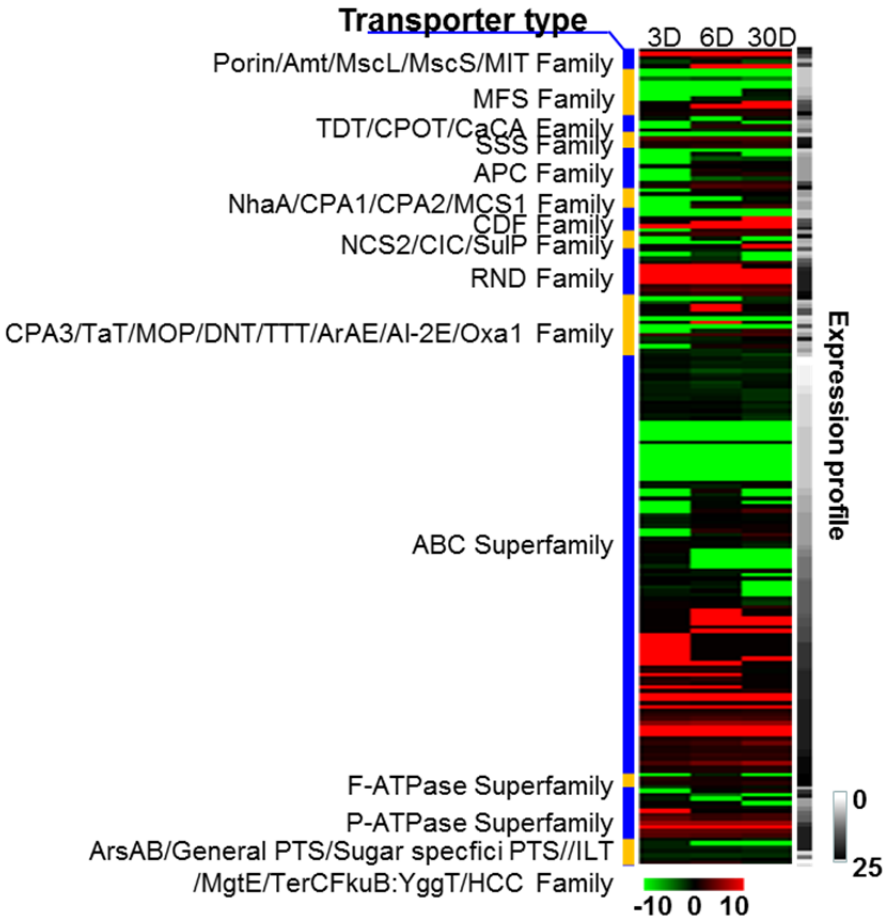


FIG S7 Expression profile (STEM) of 210 proteins involved in transport during incubation of *M. vanbaalenii* PYR-1 with BP crude oil.



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