Plasticizer degradation by marine bacterial isolates: a proteogenomic and metabolomic characterization

Robyn J. Wright[1,2*], Rafael Bosch[3,4], Matthew I. Gibson[5,6] and Joseph A. Christie-Oleza[1,3,4*]

[1] School of Life Sciences, University of Warwick, Coventry, UK

[2] School for Resource and Environmental Studies, Dalhousie University, Halifax, Canada

[3] University of the Balearic Islands, Palma, Spain

[4] IMEDEA (CSIC-UIB), Esporles, Spain

[5] Department of Chemistry, University of Warwick, Coventry, UK

[6] Medical School, University of Warwick, Coventry, UK

*corresponding authors: robyn.wright@dal.ca and Joseph.Christie@uib.eu.

Supplementary tables:

Supplementary Table S1: Screening of isolates.xlsx: Details of all isolates, which treatment they were obtained from and whether they grew on the plasticizers.

Supplementary Table S2: Genomic analysis of Mycobacterium sp. DBP42 and Halomonas sp. ATBC28.xlsx: Pathways identified in genomes, with all KEGG details and details of conserved domain searches for those identified here as being involved in plasticizer degradation.

Supplementary Table S3: Mycobacterium sp. DBP42 peptides and protein groups.xlsx: All proteins from MaxQuant.

Supplementary Table S4: Halomonas sp. ATBC28 peptides and protein groups.xlsx: All proteins from MaxQuant.

Supplementary Table S5: Mycobacterium sp. DBP42 proteomic analysis.xlsx: Relative abundances, T-tests and details of all proteins suggested to be involved in degradation that are mentioned in the text.

Supplementary Table S6: Halomonas sp. ATBC28 proteomic analysis.xlsx: Relative abundances, T-tests and details of all proteins suggested to be involved in degradation that are mentioned in the text.

Supplementary Table S7: Metabolomic analyses.xlsx: Full results for targeted and untargeted metabolomics.

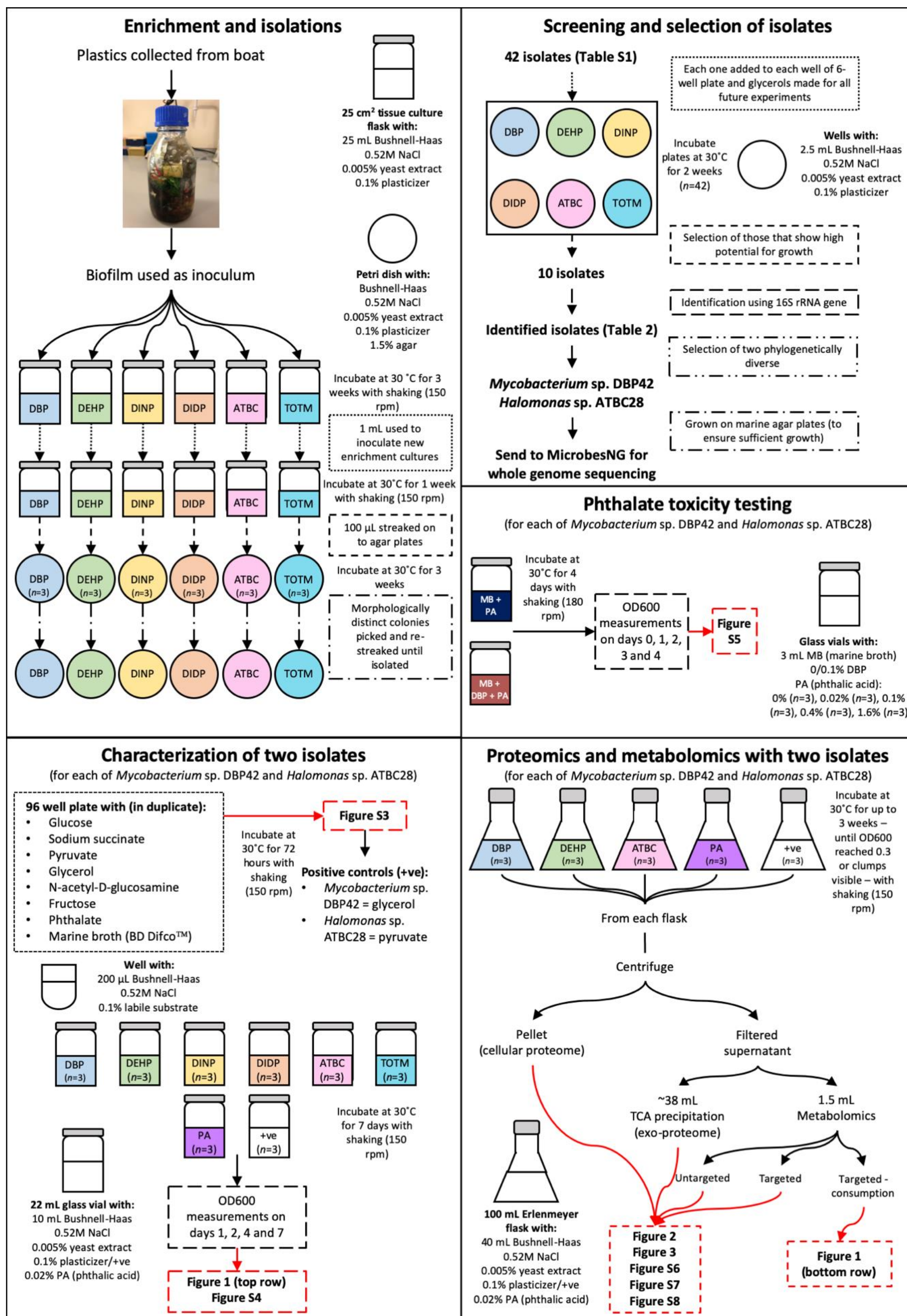

Figure S1. Schematic of experimental design used in this study showing the medium used for each set of experiments, the number of replicates, the analyses that these samples were used for and the figures that these data led to. For the enrichment, isolation, screening and selection of isolates and characterization of two isolates, six plasticizers were used: dibutyl phthalate (DBP), bis(2-ethyl hexyl) phthalate (DEHP), diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), acetyl tributyl citrate (ATBC) and trioctyl trimellitate (TOTM; chemical structures are shown in Table 1). For the proteomic and metabolomic characterization, a subset of three plasticizers were chosen to further characterize the mechanisms of plasticizer degradation by these two microorganisms: DBP and DEHP, two of the most abundantly used PAE plasticizers, both with differing chain lengths and degrees of branching, and ATBC, a new non-phthalic eco-friendly plasticizer for which no biodegradation pathway has been described to date.

Figure S2. Picture of plastics that the inoculum used for this study came from showing a mixture of plastics, *e.g.* expanded polystyrene and crisp packet.

Figure S3. Growth of *Mycobacterium* sp. DBP42 and *Halomonas* sp. ATBC28 on a range of different substrates (0.1% w/v in supplemented Bushnell-Haas mineral media), as well as marine broth, over 72 hours. Measurements were taken every half an hour. Panels 1 and 2 show biological replicates.

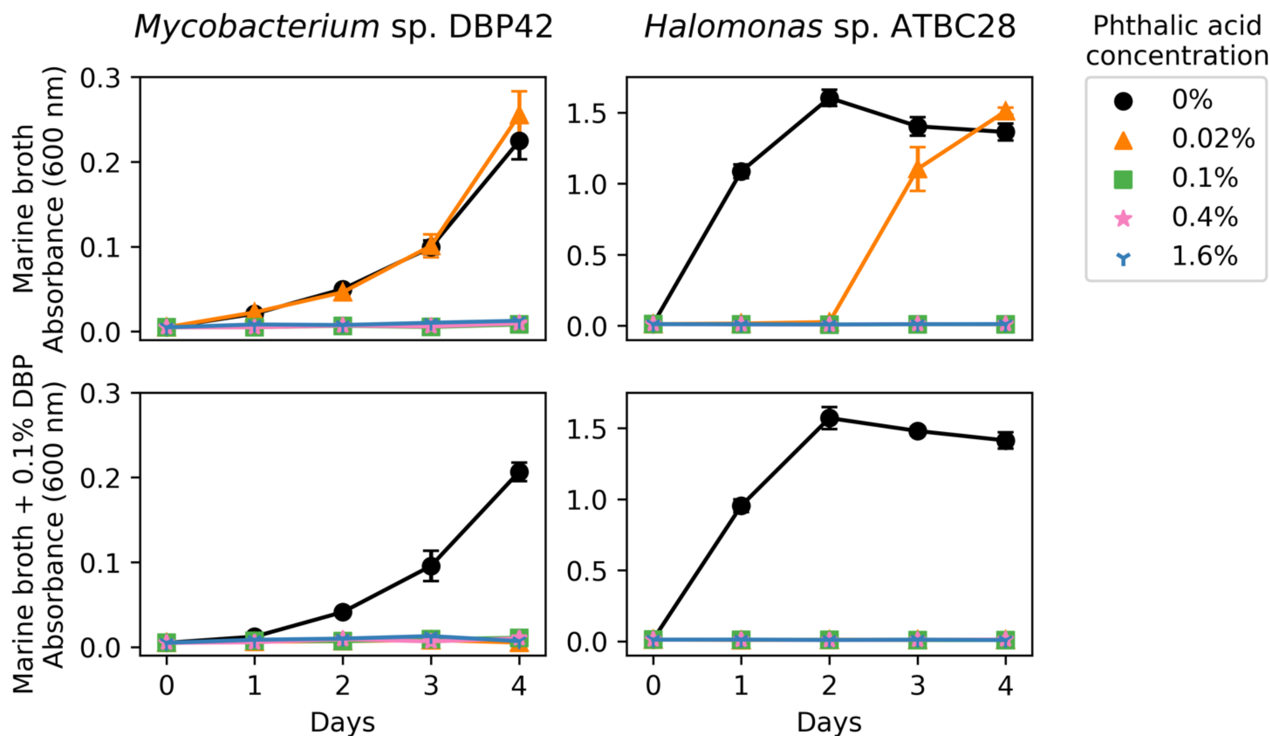

Figure S4. Growth of *Mycobacterium* sp. DBP42 (left) and *Halomonas* sp. ATBC28 (right) with marine broth (top) or marine broth with 0.1% DBP (w/v; bottom) with variable concentrations of phthalic acid (w/v): 0% (black circles), 0.02% (orange triangles), 0.1% (green squares; no growth), 0.4% (pink stars; no growth) or 1.6% (blue downwards triangle; no growth). Points and error bars represent the means and standard deviations, respectively, of three biological replicates.

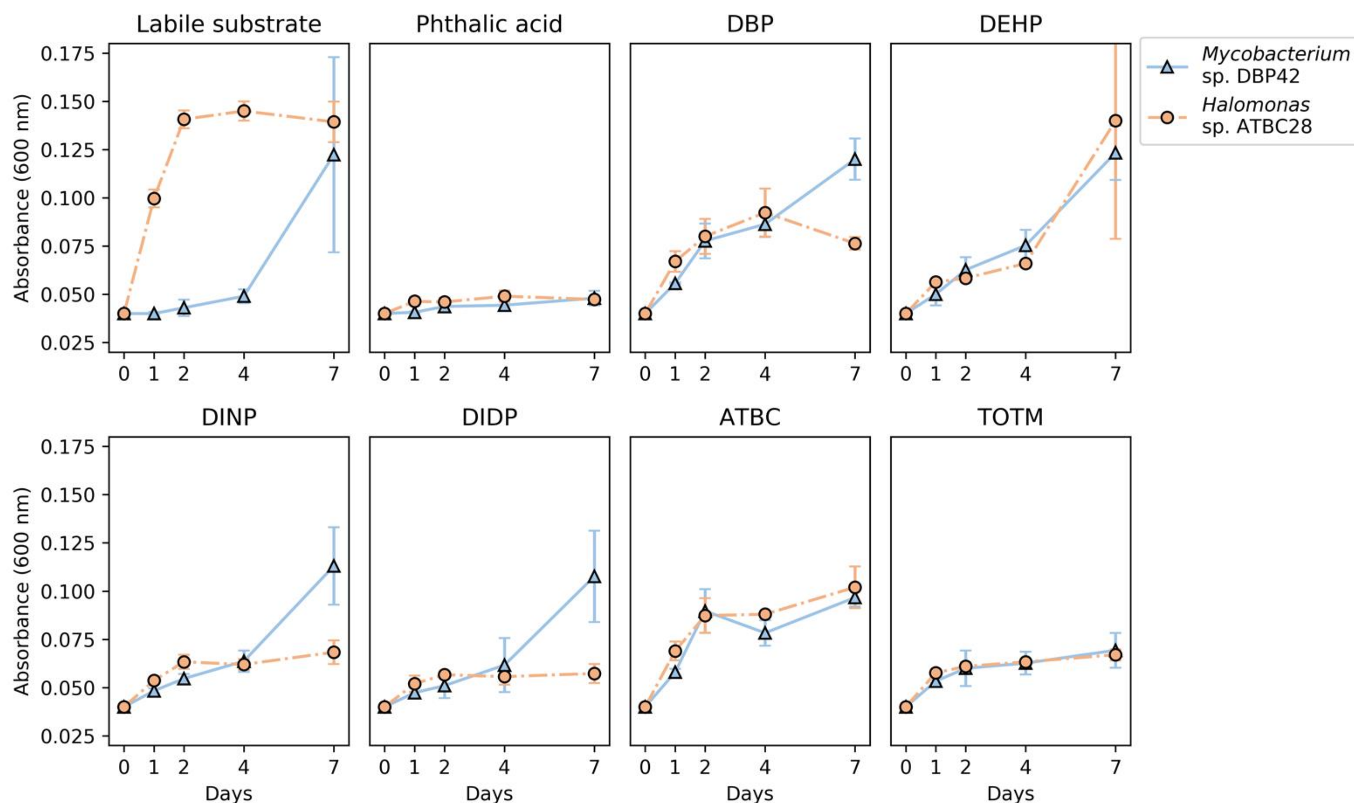

Figure S5. Growth of *Mycobacterium* sp. DBP42 (blue triangles with solid lines) and *Halomonas* sp. ATBC28 (orange circles with dashed lines) on a labile substrate (0.1% w/v of glycerol and 0.1% v/v of pyruvate, respectively, as tested in Supplementary Fig. S3) and six different plasticizers (0.1% v/v), as well as phthalate (0.02% w/v; Supplementary Fig. S4). Points and error bars represent the means and standard deviations, respectively, of three biological replicates. Curves for labile substrates, phthalic acid, DBP, DEHP and ATBC are as shown in Fig. 1.

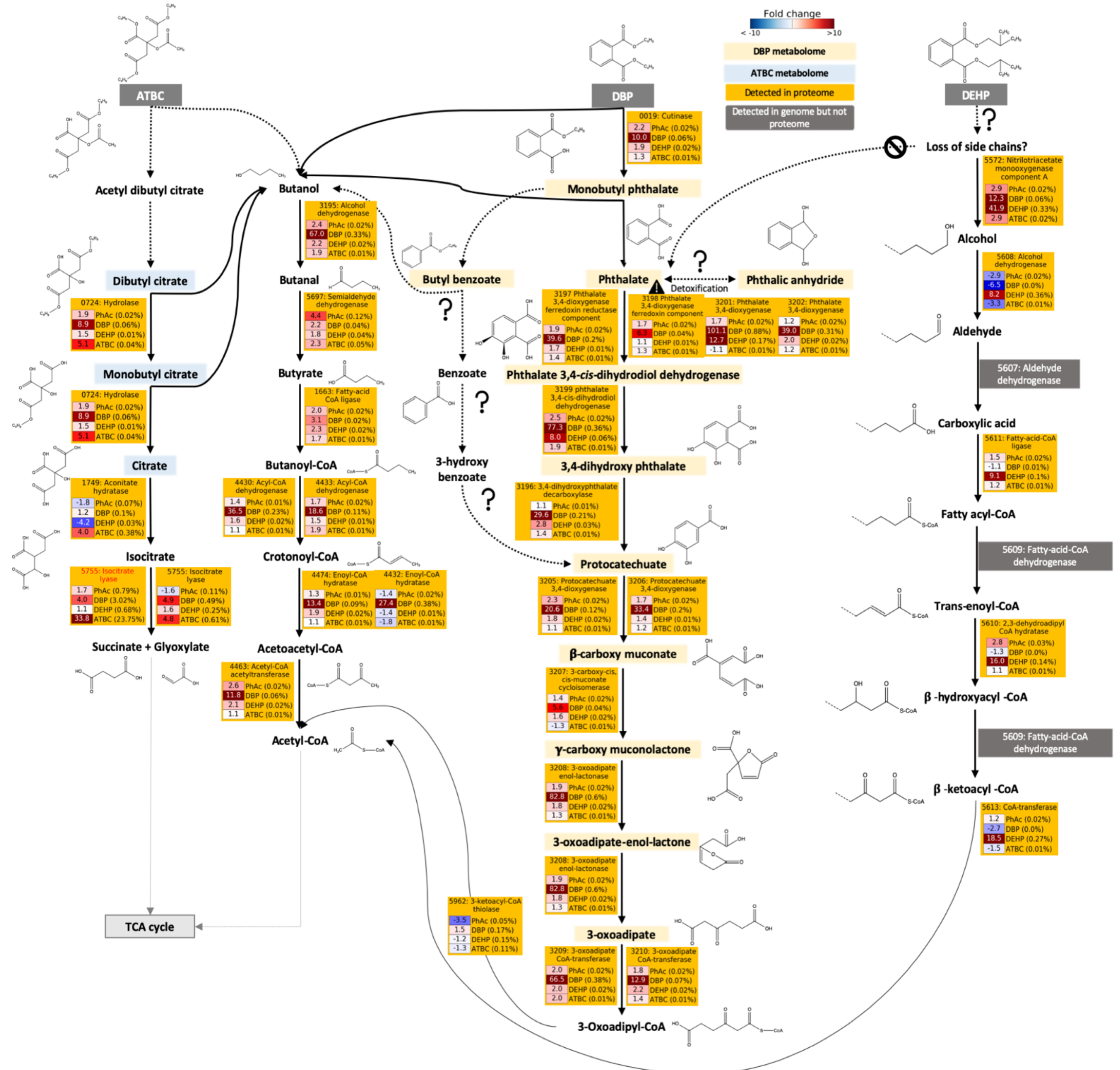

Figure S6. Catabolic pathways informed by genomic, proteomic and metabolomic analyses for DBP, DEHP and ATBC degradation by *Mycobacterium* sp. DBP42. Initial plasticizer substrates are shown in grey boxes, while degradation intermediates that were detected by metabolomics are shown with yellow or blue boxes if they were identified in the DBP or ATBC metabolomes, respectively. Dashed black arrows show reactions inferred by metabolomics, although no enzyme catalyzing the reaction could be confidently assigned by proteogenomics. Dashed grey arrows indicate that this substrate enters a known pathway (not detailed here). Solid arrows indicate reactions catalyzed by enzymes that were detected either in the genomes (dark grey) or proteomics (yellow). Enzyme ID number and fold change in each treatment (DBP, DEHP and ATBC *vs* control) is shown for each reaction. All enzymes shown here were detected in the cellular proteome, aside from the isocitrate lyase 5755 (name shown in red), which was detected in the exo-proteome. Dashed lines in chemical structures indicate uncertainty on composition.

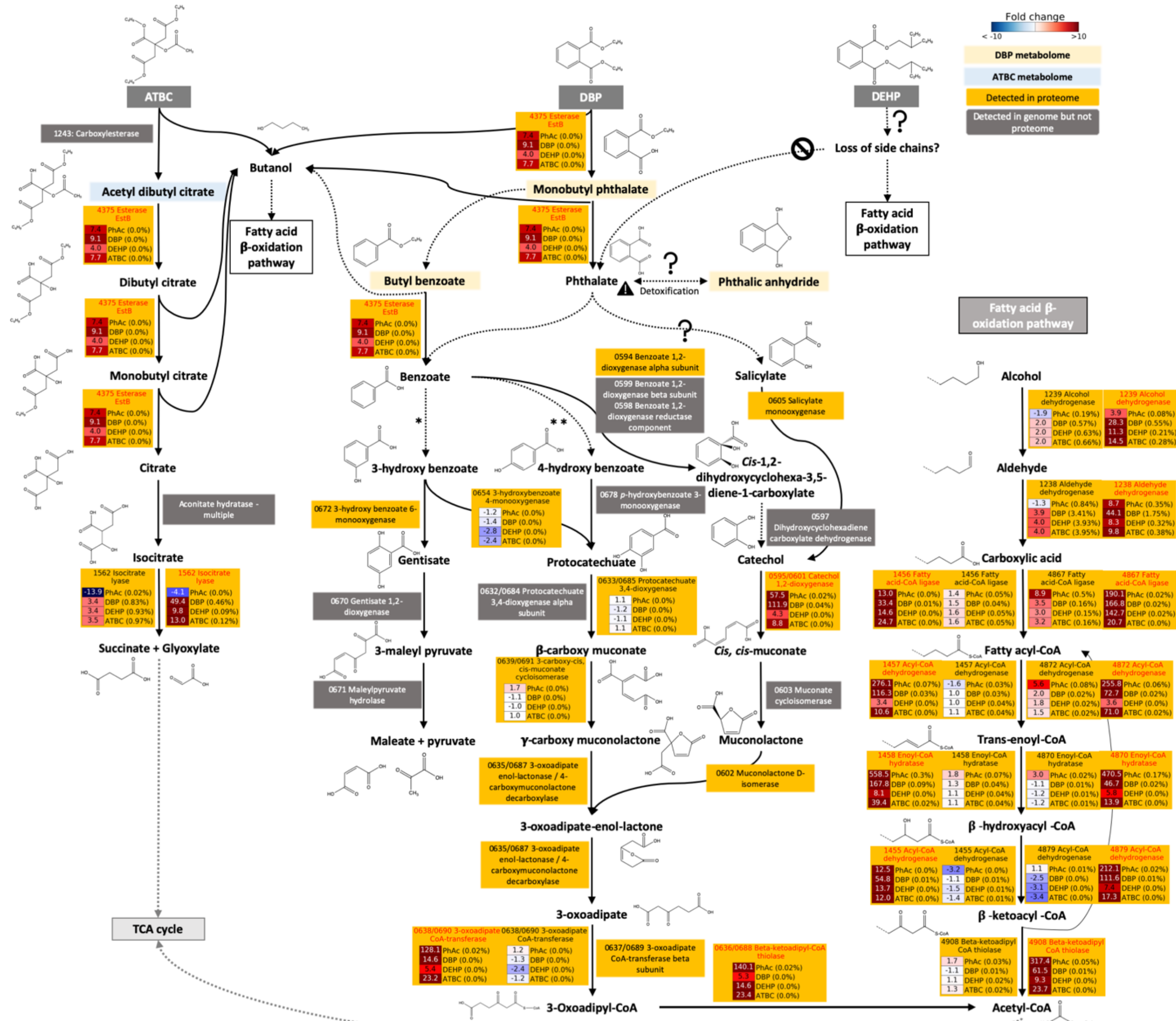

Figure S7. Catabolic pathways informed by genomic, proteomic and metabolomic analyses for DBP, DEHP and ATBC degradation by *Halomonas* sp. ATBC28. Initial plasticizer substrates are shown in grey boxes, while degradation intermediates that were detected by metabolomics are shown with yellow or blue boxes if they were identified in the DBP or ATBC metabolomes, respectively. Dashed black arrows show reactions inferred by metabolomics, although no enzyme catalyzing the reaction could be confidently assigned by proteogenomics. Dashed grey arrows indicate that this substrate enters a known pathway (not detailed here). Solid arrows indicate reactions catalyzed by enzymes that were detected either in the genomes (dark grey) or proteomics (yellow). Enzyme ID number and fold change in each treatment (DBP, DEHP and ATBC *vs* control) is shown for each reaction for which this enzyme was found in all three biological replicates of at least one treatment. Enzymes with names shown in black were detected in the cellular proteome while those in red were detected in the exo-proteome. Dashed lines in chemical structures indicate uncertainty on composition. * denotes that this step is described in KEGG, but no enzyme capable of this reaction is currently known, while ** denotes that this step can be carried out by a Cytochrome P450 that was not detected in the genome of *Halomonas* sp. ATBC28.

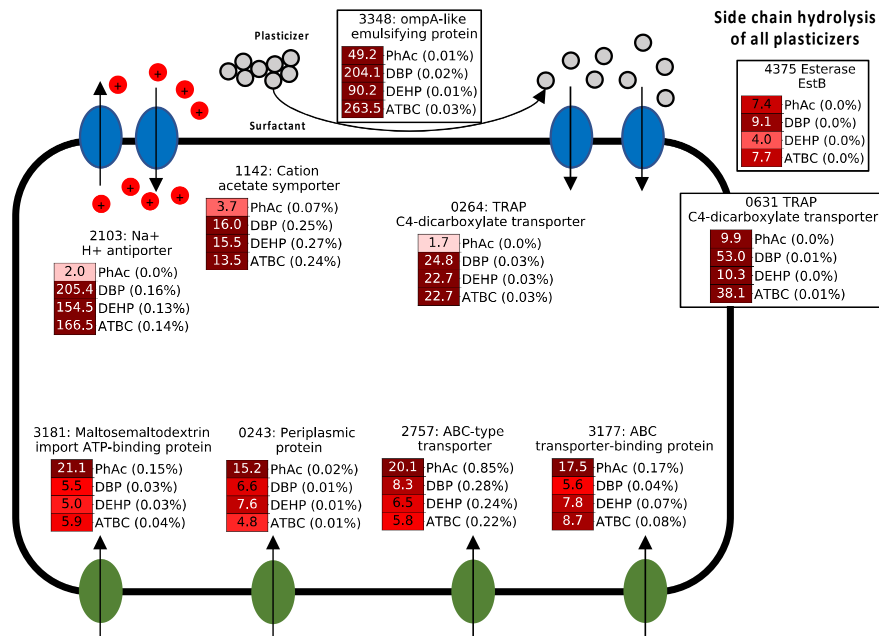

Figure S8. Proteins in *Halomonas* sp. ATBC28 that may be involved in the solubilization, hydrolysis or transport into the cell of plasticizers and phthalate. Black outlines around the boxes indicate that this protein was detected in the exo-proteome. Boxes show the genome position of the enzyme used for that step alongside the fold-change (when compared with the positive control, *Halomonas* sp. ATBC28 grown with pyruvate) and the percentage relative abundance within the proteomes.