

**Description of *Labrys sedimenti* sp. nov., isolated from a diclofenac-degrading enrichment culture, and genome-based reclassification of *Labrys portucalensis* as a later heterotypic synonym of *Labrys neptuniae***

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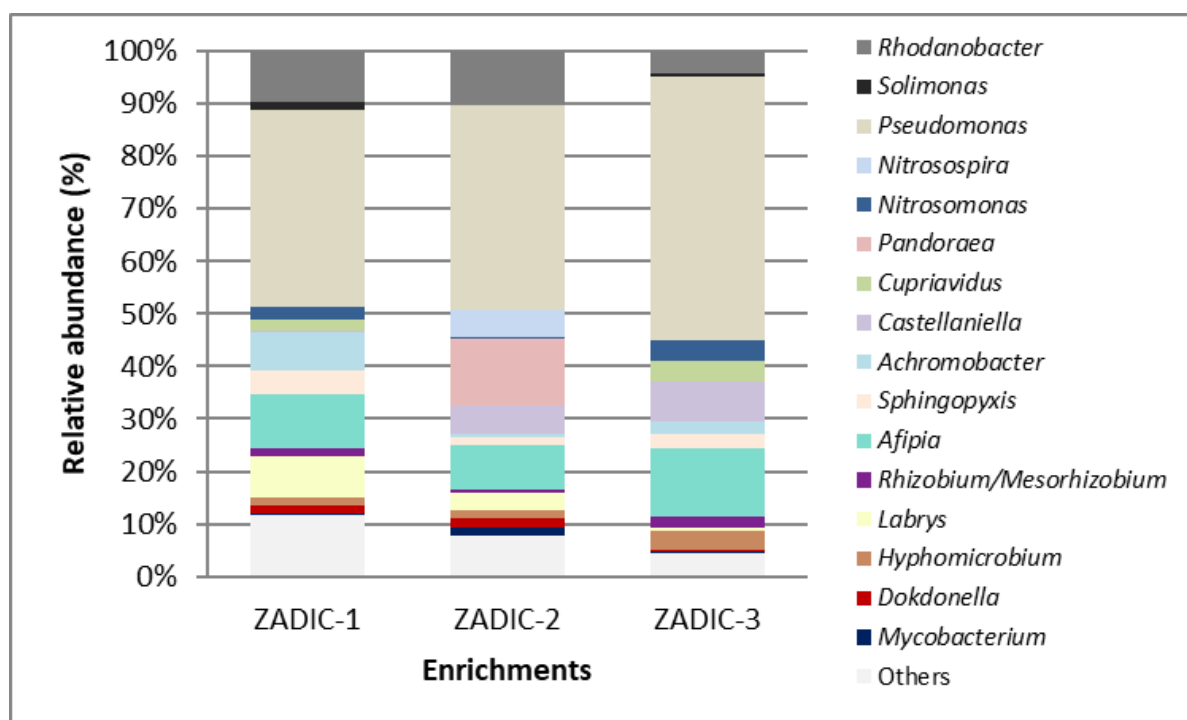
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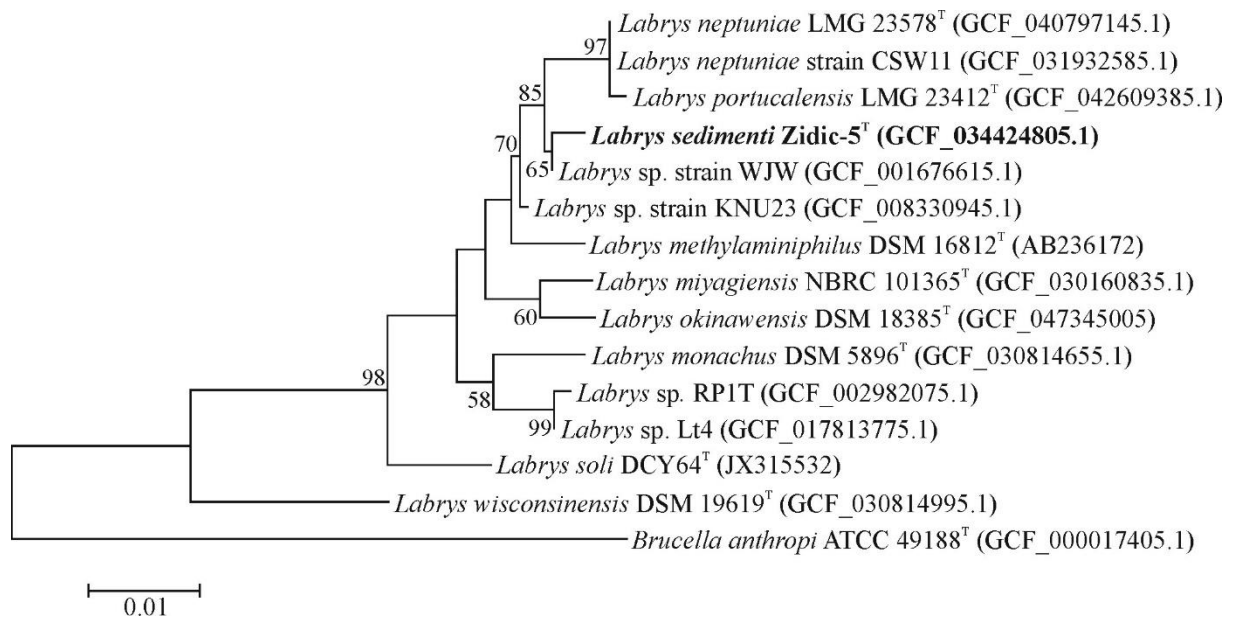
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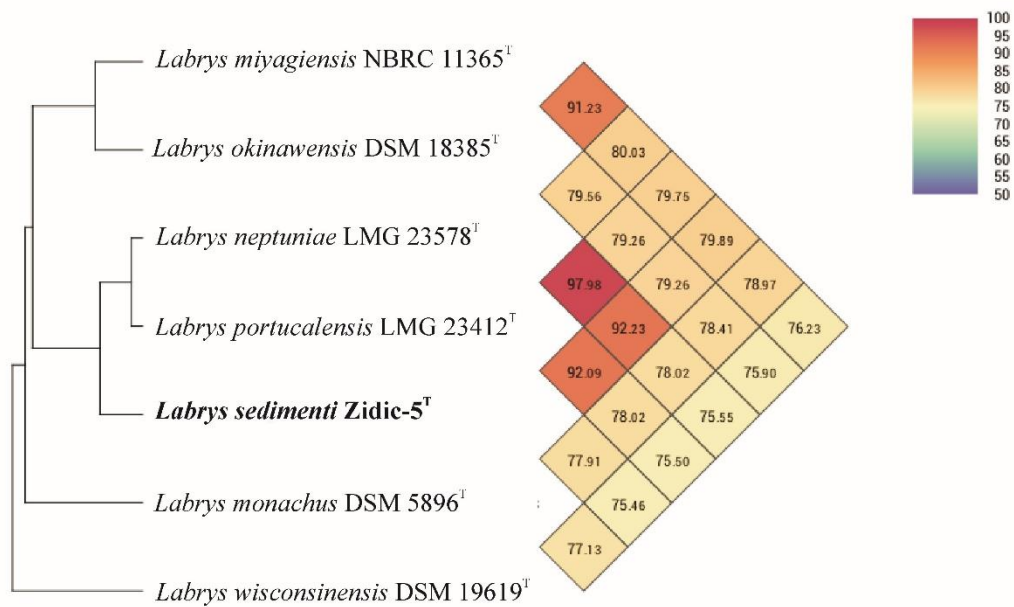
Supplementary material for publication in IJSEM online.



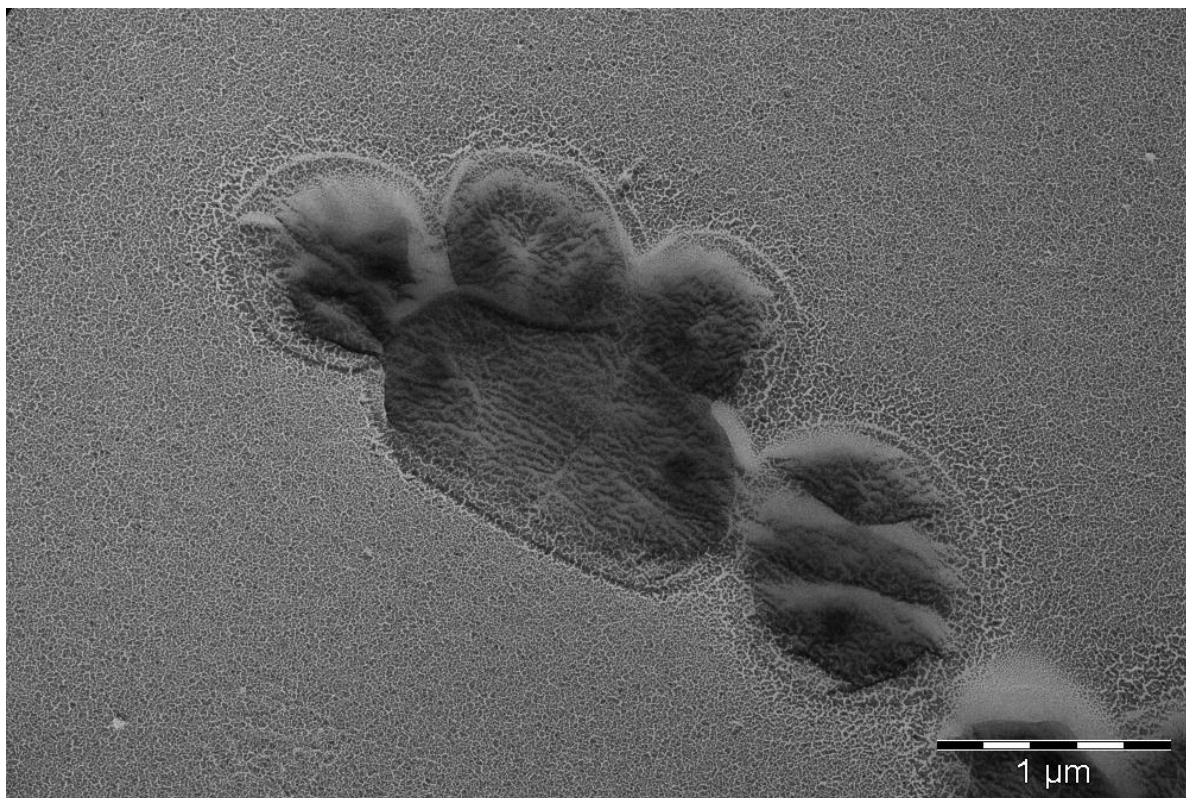
**Figure S1.** Genus level bacterial community structure of the diclofenac-degrading enrichment cultures as revealed by Illumina paired-end 16S rRNA gene amplicon sequencing. Only taxa contributing more than 1% abundance are depicted



**Figure S2.** Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences highlighting the phylogenetic position of *Labrys sedimenti* Zidic-5<sup>T</sup> strain relative to other members of the genus *Labrys*. The tree includes those non-type strains, in which case the whole-genome sequence is available. Bootstrap values (> 50%) based on 1000 bootstrap replicates are shown at branch nodes. Whole-genome sequence accession numbers are given in parenthesis. *Brucella anthropi* NBRC 15819<sup>T</sup> was used as an out-group. Bar, 0.01 substitutions per nucleotide position.

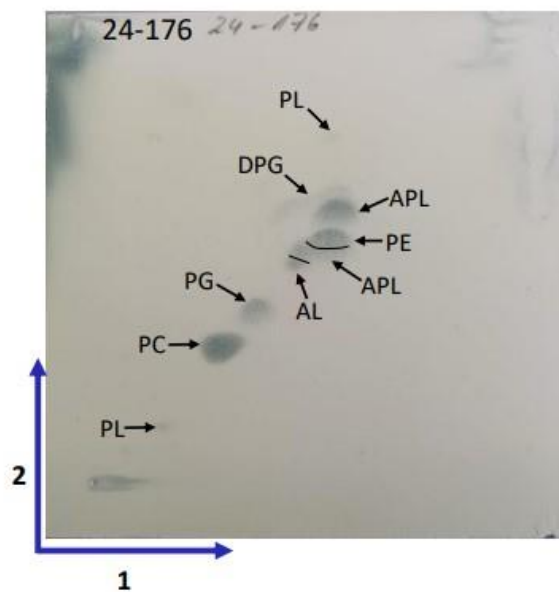


**Figure S3.** Heatmap generated with OrthoANI values calculated using the OAT software between strain Zidic-5<sup>T</sup> and other closely related *Labrys* species. The color code indicates the closest species in red to the farthest in blue.



**Figure S4.** Transmission electron microscopic image of Zidic-5<sup>T</sup>, grown on nutrient agar at 28°C for 2 days. Scale Bar: 1 μm.

DPG = Diphosphatidylglycerol  
 PE = Phosphatidylethanolamine  
 PC = Phosphatidylcholine  
 PG = Phosphatidylglycerol  
  
 APL = Aminophospholipid  
 AL = Aminolipid  
 PL = Phospholipid



**Figure S5.** Two-dimensional thin layer chromatography (TLC) profile of the polar lipids of strain Zidic-5<sup>T</sup>.

**Table S1.** Basic characteristics of the *Labrys* whole-genome sequences obtained in the present study. 1, strain Zidic-5<sup>T</sup>; 2, *Labrys neptuniae* LMG 23578<sup>T</sup>; 3, *Labrys portucalensis* LMG 23412<sup>T</sup>

| Characteristics                   | 1                | 2                | 3                |
|-----------------------------------|------------------|------------------|------------------|
| GenBank accession                 | JAXOUF0000000000 | JBFNQD0000000000 | JBHGPK0000000000 |
| No. of contigs                    | 109              | 49               | 168              |
| Size (Mbp)                        | 7.64             | 7.59             | 8.00             |
| Contig N50                        | 368.6 kb         | 349.7 kb         | 273.8 kb         |
| DNA G+C content                   | 63.5%            | 64 %             | 63.5%            |
| No. of CDSs                       | 7052             | 6915             | 7427             |
| No. of complete 16S<br>rRNA genes | 1                | 1                | 1                |
| No. of CRISPRs                    | 2                | 1                | 2                |
| Completeness (%)                  | 99.24            | 99.24            | 99.01            |
| Contamination (%)                 | 2.34             | 1.72             | 1.78             |

**Table S2.** Complete pathway modules identified by BlastKOALA analysis in the whole-genome sequences of 1, strain Zidic-5T; 2, *Labrys portucalensis* LMG 23412<sup>T</sup>; 3, *Labrys neptuniae* LMG 23578<sup>T</sup>; 4, *Labrys okinawensis* DSM 18385<sup>T</sup>.

| Pathway modules                                                                    | 1 | 2 | 3 | 4 |
|------------------------------------------------------------------------------------|---|---|---|---|
| <b>Carbohydrate metabolism</b>                                                     |   |   |   |   |
| <i>Central carbohydrate metabolism</i>                                             |   |   |   |   |
| Glycolysis (Embden-Meyerhof pathway), glucose => pyruvate                          | + | + | + | + |
| Glycolysis, core module involving three-carbon compounds                           | + | + | + | + |
| Gluconeogenesis, oxaloacetate => fructose-6P                                       | + | + | + | + |
| Pyruvate oxidation, pyruvate => acetyl-CoA                                         | + | + | + | + |
| Citrate cycle (TCA cycle, Krebs cycle)                                             | + | + | + | + |
| Citrate cycle, first carbon oxidation, oxaloacetate => 2-oxoglutarate              | + | + | + | + |
| Citrate cycle, second carbon oxidation, 2-oxoglutarate => oxaloacetate             | + | + | + | + |
| Pentose phosphate pathway (Pentose phosphate cycle)                                | + | + | + | + |
| Pentose phosphate pathway, oxidative phase, glucose 6P => ribulose 5P              | + | + | + | + |
| Pentose phosphate pathway, non-oxidative phase, fructose 6P => ribose 5P           | + | + | + | + |
| Pentose phosphate pathway, archaea, fructose 6P => ribose 5P                       | - | - | - | + |
| PRPP biosynthesis, ribose 5P => PRPP                                               | + | + | + | + |
| Entner-Doudoroff pathway, glucose-6P => glyceraldehyde-3P + pyruvate               | + | + | + | - |
| <i>Other carbohydrate metabolism</i>                                               |   |   |   |   |
| D-Glucuronate degradation, D-glucuronate => pyruvate + D-glyceraldehyde 3P         | - | + | - | - |
| D-galactonate degradation, De Ley-Doudoroff pathway, D-galactonate => glycerate-3P | + | + | + | + |
| Glycogen biosynthesis, glucose-1P => glycogen/starch                               | + | + | + | + |
| Nucleotide sugar biosynthesis, glucose => UDP-glucose                              | + | + | + | + |
| UDP-N-acetyl-D-glucosamine biosynthesis, prokaryotes, glucose => UDP-GlcNAc        | + | + | + | + |
| dTDP-L-rhamnose biosynthesis, glucose-1P => dTDP-L-Rha                             | + | + | + | + |
| Glyoxylate cycle                                                                   | + | + | + | + |

|                                                                           |   |   |   |   |
|---------------------------------------------------------------------------|---|---|---|---|
| Propanoyl-CoA metabolism, propanoyl-CoA => succinyl-CoA                   | + | + | + | + |
| <b>Energy metabolism</b>                                                  |   |   |   |   |
| <i>Carbon fixation</i>                                                    |   |   |   |   |
| Reductive pentose phosphate cycle (Calvin cycle)                          | + | + | + | - |
| CAM (Crassulacean acid metabolism), dark                                  | + | + | + | + |
| CAM (Crassulacean acid metabolism), light                                 | + | + | + | + |
| Phosphate acetyltransferase-acetate kinase pathway, acetyl-CoA => acetate | + | + | + | - |
| <i>Nitrogen metabolism</i>                                                |   |   |   |   |
| Assimilatory nitrate reduction, nitrate => ammonia                        | + | + | + | + |
| <i>Sulfur metabolism</i>                                                  |   |   |   |   |
| Assimilatory sulfate reduction, sulfate => H <sub>2</sub> S               | + | + | + | + |
| <i>ATP synthesis</i>                                                      |   |   |   |   |
| NADH:quinone oxidoreductase, prokaryotes                                  | + | + | + | + |
| Succinate dehydrogenase, prokaryotes                                      | + | + | + | + |
| Cytochrome c oxidase, prokaryotes                                         | + | + | + | + |
| Cytochrome bd ubiquinol oxidase                                           | + | + | + | + |
| Cytochrome o ubiquinol oxidase                                            | + | + | + | + |
| Cytochrome c oxidase, cbb3-type                                           | + | + | + | + |
| F-type ATPase, prokaryotes and chloroplasts                               | + | + | + | + |
| <b>Lipid metabolism</b>                                                   |   |   |   |   |
| <i>Fatty acid metabolism</i>                                              |   |   |   |   |
| Fatty acid biosynthesis, initiation                                       | + | + | + | + |
| Fatty acid biosynthesis, elongation                                       | + | + | + | + |
| beta-Oxidation, acyl-CoA synthesis                                        | + | + | + | + |
| <i>Lipid metabolism</i>                                                   |   |   |   |   |
| Phosphatidylcholine (PC) biosynthesis, PE => PC                           | + | + | + | + |
| Phosphatidylethanolamine (PE) biosynthesis, PA => PS => PE                | + | + | + | + |
| <b>Nucleotide metabolism</b>                                              |   |   |   |   |
| <i>Purine metabolism</i>                                                  |   |   |   |   |
| De novo purine biosynthesis, PRPP + glutamine => IMP                      | + | + | + | + |
| Adenine ribonucleotide biosynthesis, IMP => ADP,ATP                       | + | + | + | + |
| Guanine ribonucleotide biosynthesis, IMP => GDP,GTP                       | + | + | + | + |
| Deoxyribonucleotide biosynthesis, ADP/GDP/CDP/UDP => dATP/dGTP/dCTP/dUTP  | + | + | + | - |
| Adenine ribonucleotide degradation, AMP => Urate                          | + | + | + | + |
| Guanine ribonucleotide degradation, GMP => Urate                          | + | + | + | + |

|                                                                                   |   |   |   |   |
|-----------------------------------------------------------------------------------|---|---|---|---|
| <i>Pyrimidine metabolism</i>                                                      |   |   |   |   |
| Pyrimidine ribonucleotide biosynthesis, UMP => UDP/UTP,CDP/CTP                    | + | + | + | + |
| Pyrimidine deoxyribonucleotide biosynthesis, UDP => dTTP                          | + | + | + | - |
| Pyrimidine degradation, uracil => beta-alanine, thymine => 3-aminoisobutanoate    | + | + | + | + |
| <b>Amino acid metabolism</b>                                                      |   |   |   |   |
| <i>Serine and threonine metabolism</i>                                            |   |   |   |   |
| Serine biosynthesis, glycerate-3P => serine                                       | + | + | + | + |
| Threonine biosynthesis, aspartate => homoserine => threonine                      | + | + | + | + |
| Glycine cleavage system                                                           | + | + | + | + |
| Betaine biosynthesis, choline => betaine                                          | + | + | + | + |
| Betaine degradation, bacteria, betaine => pyruvate                                | + | + | + | + |
| Ectoine degradation, ectoine => aspartate                                         | + | + | + | - |
| <i>Cysteine and methionine metabolism</i>                                         |   |   |   |   |
| Cysteine biosynthesis, serine => cysteine                                         | + | + | + | + |
| Methionine biosynthesis, aspartate => homoserine => methionine                    | + | + | + | - |
| <i>Branched-chain amino acid metabolism</i>                                       |   |   |   |   |
| Valine/isoleucine biosynthesis, pyruvate => valine / 2-oxobutanoate => isoleucine | + | + | + | + |
| Isoleucine biosynthesis, threonine => 2-oxobutanoate => isoleucine                | + | + | + | + |
| Leucine biosynthesis, 2-oxoisovalerate => 2-oxoisocaproate                        | + | + | + | + |
| Leucine degradation, leucine => acetoacetate + acetyl-CoA                         | + | + | + | + |
| <i>Lysine metabolism</i>                                                          |   |   |   |   |
| Lysine biosynthesis, succinyl-DAP pathway, aspartate => lysine                    | + | + | + | + |
| <i>Arginine and proline metabolism</i>                                            |   |   |   |   |
| Ornithine biosynthesis, glutamate => ornithine                                    | + | + | + | + |
| Arginine biosynthesis, ornithine => arginine                                      | + | + | + | + |
| Proline biosynthesis, glutamate => proline                                        | + | + | + | + |
| Proline degradation, proline => glutamate                                         | + | + | + | + |
| <i>Polyamine biosynthesis</i>                                                     |   |   |   |   |
| Polyamine biosynthesis, arginine => ornithine => putrescine                       | + | + | + | + |
| <i>Histidine metabolism</i>                                                       |   |   |   |   |
| Histidine biosynthesis, PRPP => histidine                                         | + | + | + | + |

|                                                                                   |   |   |   |   |
|-----------------------------------------------------------------------------------|---|---|---|---|
| Histidine degradation, histidine => N-formiminoglutamate => glutamate             | + | + | + | + |
| <i>Aromatic amino acid metabolism</i>                                             |   |   |   |   |
| Shikimate pathway, phosphoenolpyruvate + erythrose-4P => chorismate               | + | + | + | + |
| Tryptophan biosynthesis, chorismate => tryptophan                                 | + | + | + | + |
| <i>Other amino acid metabolism</i>                                                |   |   |   |   |
| Glutathione biosynthesis, glutamate => glutathione                                | + | + | + | + |
| <b>Metabolism of cofactors and vitamins</b>                                       |   |   |   |   |
| <i>Cofactor and vitamin metabolism</i>                                            |   |   |   |   |
| NAD biosynthesis, aspartate => quinolinate => NAD                                 | + | + | + | + |
| Coenzyme A biosynthesis, pantothenate => CoA                                      | + | + | + | + |
| Biotin biosynthesis, pimeloyl-ACP/CoA => biotin                                   | + | + | + | - |
| Lipoic acid biosynthesis, plants and bacteria, octanoyl-ACP => dihydrolipoyl-E2/H | + | + | + | + |
| Molybdenum cofactor biosynthesis, GTP => molybdenum cofactor                      | + | + | + | + |
| PreQ1 biosynthesis, GTP => 7-Aminomethyl-7-deazaguanine                           | - | + | + | - |
| C1-unit interconversion, prokaryotes                                              | + | + | + | + |
| <b>Biosynthesis of other secondary metabolites</b>                                |   |   |   |   |
| <i>Biosynthesis of other bacterial compounds</i>                                  |   |   |   |   |
| Aerobactin biosynthesis, lysine => aerobactin                                     | + | + | + | - |
| <b>Xenobiotics biodegradation</b>                                                 |   |   |   |   |
| <i>Aromatics degradation</i>                                                      |   |   |   |   |
| Benzoate degradation, benzoate => catechol / methylbenzoate => methylcatechol     | + | + | + | - |
| Catechol ortho-cleavage, catechol => 3-oxoadipate                                 | + | + | + | - |

**Table S3.** Results of the API 50 CH and API ZYM tests. 1, strain Zidic-5<sup>T</sup>; 2, *Labrys portucalensis* LMG 23412<sup>T</sup>; 3, *Labrys neptuniae* LMG 23578<sup>T</sup>. +, positive reaction; -, negative reaction; W+, weak positive.

| API test results           | 1 | 2 | 3  |
|----------------------------|---|---|----|
| <i>API 50 CH</i>           |   |   |    |
| Glycerol                   | + | + | +  |
| Erythritol                 | - | - | +  |
| D-Arabinose                | - | - | -  |
| L-Arabinose                | + | + | +  |
| D-Ribose                   | + | + | +  |
| D-Xylse                    | + | + | +  |
| L-Xylose                   | - | - | W+ |
| D-Adonithol                | + | + | +  |
| Metil-β-D-xylopyranoside   | - | - | -  |
| D-Galactose                | + | + | +  |
| D-Glucose                  | + | + | +  |
| D-Fructose                 | + | + | +  |
| D-Mannose                  | + | + | +  |
| L-Sorbose                  | + | + | +  |
| L-Rhamnose                 | - | - | +  |
| Dulcitol                   | + | + | +  |
| Inositol                   | + | + | +  |
| D-Mannitol                 | + | + | +  |
| D-Sorbitol                 | - | + | +  |
| Methyl-α-D-mannopyranoside | - | - | -  |
| Methyl-α-D-glucopyranoside | - | - | -  |
| N-Acetyl-D-glucosamine     | - | - | -  |
| Amygdalin                  | - | - | -  |
| Arbutin                    | - | - | +  |
| Esculine                   | + | + | +  |
| Salicin                    | - | - | -  |
| D-Cellobiose               | + | + | +  |
| D-Maltose                  | - | - | +  |
| D-Lactose                  | - | - | +  |
| D-Melibiose                | + | + | +  |
| D-Sacharose                | - | - | +  |
| D-Tehalose                 | - | + | +  |
| Inulin                     | - | - | -  |
| D-Melezitose               | - | - | -  |
| D-Raffinose                | - | - | +  |
| Starch                     | - | - | -  |
| Glycogen                   | - | - | -  |
| Xylitol                    | + | + | +  |
| Gentiobiose                | + | - | +  |
| D-Turanose                 | + | - | +  |
| D-Lyxose                   | + | + | +  |
| D-Tagatose                 | + | + | +  |
| D-Fucose                   | + | + | +  |

|                                    |   |   |   |
|------------------------------------|---|---|---|
| L-Fucose                           | - | - | + |
| D-Arabitol                         | + | + | + |
| L-Arabitol                         | - | - | + |
| Potassium-gluconate                | + | + | + |
| Potassium-2-ketogluconate          | - | - | - |
| Potassium-5- ketogluconate         | - | - | - |
| <i>API ZYM</i>                     |   |   |   |
| Alkaline phosphatase               | + | + | + |
| Esterase (C4)                      | + | + | + |
| Esterase lipase (C8)               | - | - | - |
| Lipase (C14)                       | - | - | - |
| Leucine arylamidase                | + | + | + |
| Valine arylamidase                 | + | + | + |
| Cystine arylamidase                | - | - | - |
| Trypsine                           | - | - | - |
| $\alpha$ -Chymotrypsine            | - | - | - |
| Acid phosphatase                   | + | + | + |
| Naphtol-AS-BI-phosphohydrolase     | - | - | + |
| $\alpha$ -Galactosidase            | - | - | - |
| $\beta$ -Galactosidase             | - | - | - |
| $\beta$ -Glucuronidase             | - | - | - |
| $\alpha$ -Glucosidase              | - | - | - |
| $\beta$ - Glucosidase              | - | - | - |
| N-Acetyl- $\beta$ -glucosaminidase | - | - | - |
| $\alpha$ -Mannosidase              | - | - | - |
| $\alpha$ -Fucosidase               | - | - | - |