Unravelling the complete genome sequence of *Advenella mimigardefordensis* strain DPN7ᵀ and novel insights in the catabolism of the xenobiotic polythioester precursor 3,3’-dithiodipropionate

Jan Hendrik Wübbeler,¹ Sebastian Hiessl,¹ Jörg Schuldes,² Andrea Thürmer,² Rolf Daniel² and Alexander Steinbüchel¹,³

¹Institut für Molekulare Mikrobiologie und Biotechnologie, Westfälische Wilhelms-Universität Münster, 48149 Münster, Germany
²Department of Genomic and Applied Microbiology and Göttingen Genomics Laboratory, Institut für Mikrobiologie und Genetik, Georg-August-Universität Göttingen, Göttingen, Germany
³Faculty of Biology, King Abdulaziz University, Jeddah, Saudi Arabia

*Advenella mimigardefordensis* strain DPN7ᵀ is a remarkable betaproteobacterium because of its extraordinary ability to use the synthetic disulfide 3,3’-dithiodipropionic acid (DTDP) as the sole carbon source and electron donor for aerobic growth. One application of DTDP is as a precursor substrate for biotechnically synthesized polythioesters (PTEs), which are interesting non-degradable biopolymers applicable for plastics materials. Metabolic engineering for optimization of PTE production requires an understanding of DTDP conversion. The genome of *A. mimigardefordensis* strain DPN7ᵀ was sequenced and annotated. The circular chromosome was found to be composed of 4 740 516 bp and 4112 predicted ORFs, whereas the circular plasmid consisted of 23 610 bp and 24 predicted ORFs. The genes participating in DTDP catabolism had been characterized in detail previously, but knowing the complete genome sequence and with support of Tn5::mob-induced mutants, putatively involved transporter proteins and a transcriptional regulator were also identified. Most probably, DTDP is transported into the cell by a specific tripartite tricarboxylate transport system and is then cleaved by the disulfide reductase LpdA, sulfoxygenated by the 3-mercaptopropionate dioxygenase Mdo, activated by the CoA ligase SucCD and desulfinated by the acyl-CoA dehydrogenase-like desulfinase AcdA. Regulation of this pathway is presumably performed by a transcriptional regulator of the xenobiotic response element family. The excessive sulfate that is inevitably produced is secreted by the cells by a unique sulfate exporter of the CPA (cation : proton antiporter) superfamily.

INTRODUCTION

*Advenella mimigardefordensis* strain DPN7ᵀ (formerly *Tetrathiobacter mimigardefordensis*, Wübbeler et al., 2006) is a betaproteobacterium with the extraordinary capacity to utilize the organosulfur compound (OSC) 3,3’-dithiodipropionic acid (DTDP; Fig. 1) as the sole carbon source for growth (Wübbeler et al., 2008) and as a precursor for the biosynthesis of polythioester (PTE; Fig. 1) (Xia et al., 2012).

The genus *Advenella* was proposed in 2005 (Coene et al., 2005) and currently comprises four species: the type species *Advenella incenata* (Coene et al., 2005), *Advenella kashmirensis* (Ghosh et al., 2005; Gibello et al., 2009), *A. mimigardefordensis* (Wübbeler et al., 2006; Gibello et al., 2009) and *Advenella faeciporci* (Matsuoka et al., 2012). Species of *Advenella* belong to the family Alcaligenaceae...
Microbiology

affiliated to the genus and physiological characteristics of the four species degraders of xenobiotics. The most important biochemical trophs, sophisticated parasites, as well as pathogens and include versatile heterotrophs, facultative chemolithotrophs, and small-sized morphotypes (De Ley et al., 1986). Strains of this family (Fig. 2) have been detected in a variety of habitats, e.g. soil, sewage sludge, and also veterinary and clinical samples. Moreover, Alcaligenaceae perform diverse metabolic reactions and include versatile heterotrophs, facultative chemolithotrophs, sophisticated parasites, as well as pathogens and degraders of xenobiotics. The most important biochemical and physiological characteristics of the four species affiliated to the genus Advenella are introduced in the following paragraphs.

A. incenata LMG 22250T was isolated from human sputum and described in 2005, together with some further strains, which were identified in cystic fibrosis patients and veterinary samples (Coene et al., 2005). Apparently, some strains of A. incenata are opportunistic pathogens (Coene et al., 2005; Vanlaere et al., 2006), but most of them were isolated worldwide from soil samples of the rhizosphere from economic plants, such as corn, rice and tobacco plants (Espinosa-Victoria et al., 2009; Shimoyama et al., 2009; Christiaen et al., 2011; Jin et al., 2011; Shahi et al., 2011). Interestingly, the type strain has the ability to degrade the herbicide terbutylazine and could be applied in the future. Consequently, we sequenced and annotated the genome of A. incenata (Matsuoka et al., 2012).

The whole-genome of A. kashmirensis WT001T was published and is accessible (Gosh et al., 2011). The type strain was isolated from a temperate orchard soil (Gosh et al., 2005), and has been recognized as a thiosulfate- and tetraethionate-oxidizing facultative chemolithotroph (Dam et al., 2007). Several isolated soil-dwelling strains of A. kashmirensis were able to detoxify selenite by reducing it to insoluble elemental red selenium (Hunter & Manter, 2008). Furthermore, various bacterial isolates, which were affiliated to A. kashmirensis on the basis of their 16S rRNA gene sequence similarity, degrade alkanes or are involved in the biodegradation of thiodiglycol - the hydrolysis product of yperite, a highly hazardous derivative of mustard gas employed in chemical weapons (Dam et al., 2009).

A. mimigardefordensis strain DPN7T was employed initially for research on catabolism of PTE precursor substrates (Wübbeler et al., 2006, 2008) and is now successfully applied for the biotechnological production of PTE homopolymers (Xia et al., 2012). The sulfur-containing homo-PTEs are microbially synthesized, biologically persistent polymers (Lüttke-Eversloh et al., 2002a, b; Kawada et al., 2003; Elbanna et al., 2004; Lüttke-Eversloh & Steinbüchel, 2004; Kim et al., 2005). The chemical synthesis of PTEs was reported in 1951 (Marvel & Kotch, 1951), but PTEs were never technically produced for commercialization (Kricheldorf & Schwarz, 2007). The preparation procedures were laborious and difficult, due to the requirement of toxic reagents, and gave low yields, resulting in high costs (Sanda et al., 2000). Production of PTEs by the methods and principles of green chemistry was, and still is, desirable; for that reason, the first report in 2001 of biotechnically synthesized PTE attracted much attention (Lüttke-Eversloh et al., 2001). These sulfur-containing polymers possess some unique characteristics, especially auspicious for specialized applications of rare biomaterials (Steinbüchel, 2005). Until recently, synthesis of PTE homopolymers was limited, due to the dependency on a few expensive and toxic 3-mercaptoalkanoates (Lüttke-Eversloh & Steinbüchel, 2003, 2004; Xia et al., 2012). Alternative economic precursor substrates are essential for further enhancement of the large-scale biotechnical production of established and also novel PTEs (Lüttke-Eversloh & Steinbüchel, 2003). Therefore, the catabolic pathway of the non-toxic precursor substrate DTDP was studied in A. mimigardefordensis strain DPN7T (Wübbeler et al., 2008, 2010; Schürmann et al., 2011, 2013; Fig. 3). An understanding of the catabolism, including the regulation of cellular activities in the presence of DTDP and its degradation intermediates as well as the transport of these sulfur compounds, would be essential for applications of modern biochemical and genetic methods to influence the microbial metabolic networks. Knowledge of the complete genome sequence of A. mimigardefordensis strain DPN7T will be valuable to further improve the biotechnical production of PTE homopolymers from economic and distinct compounds in the future. Consequently, we sequenced and annotated the genome of A. mimigardefordensis strain DPN7T and performed Tn5::mob mutagenesis to elucidate the entire catabolism of DTDP.

METHODS

Bacterial strains, plasmids and growth conditions. Bacterial strains and plasmids used in this study are listed in Table S1 (available
in the online Supplementary Material). Escherichia coli strains were cultivated aerobically in lysogeny broth (LB) medium (Sambrook et al., 1989; Berlyn et al., 1996) at 37 °C with the addition of applicable antibiotics, if necessary. E. coli S17-1 harbouring the suicide plasmid pSUP5011 was used for Tn5::mob mutagenesis of A. mimigardeforsensis strain DPN77. Cells of A. mimigardeforsensis were cultivated aerobically in 0.8 % (v/w) nutrient broth (NB) or in mineral salts medium (MSM; Schlegel et al., 1961) at 30 °C containing the indicated carbon source. Carbon sources were prepared as filter-sterilized 1 M stock solutions and adjusted to pH 6.9–7.4. Solid media contained 1.8–2.0 % (v/w) purified agar-agar. Antibiotics were added to growth media at the following concentrations: 75 μg ampicillin ml−1 and 50 μg kanamycin ml−1.

**Tn5::mob mutagenesis.** For Tn5::mob mutagenesis, the suicide plasmid technique (Simon et al., 1983; Simon, 1984) was used by transferring the vector pSUP5011 from E. coli S17-1 to the kanamycin-susceptible A. mimigardeforsensis strain DPN77 by conjugation applying the spot-agar mating technique (Friedrich et al., 1981). Tn5::mob-induced mutants were selected on MSM agar plates containing 50 μg kanamycin ml−1 (MSMmob) and 20 mM sodium propionate or 20 mM sulfinopropionate (master plates). Putative mutants were transferred in a coordinated pattern on MSMmob agar plates containing 30 mM DTDP (selection plates) and on corresponding master plates for further analysis, and were otherwise treated as described previously (Wübbeler et al., 2008; Schürmann et al., 2011).

**Thiochemicals.** Sulfur-containing chemicals were purchased from Acros Organics. 3-Sulfinopropionate (3SP) was not available for purchase; therefore, it was synthesized as the disodium salt according to the method defined by Jolles-Bergeret (1974); the procedure was slightly modified by one repetition of the alkaline cleavage of the intermediate bis-(2-carboxyethyl)sulfone, as described in previous studies (Wübbeler et al., 2008; Schürmann et al., 2011). Success of synthesis and purity of the synthesized compound were confirmed by HPLC and GC-MS analyses.

**GC-MS analyses.** Purity of 3SP was determined upon methylation after lyophilization in the presence of 15 % (v/v) sulfuric acid (H2SO4) by GC analyses, as described previously (Schürmann et al., 2013).

**HPLC analyses.** HPLC analysis was carried out in a LaChrom Elite HPLC apparatus (VWR-Hitachi International) consisting of a Metacarb 67H advanced C column (Varian; Bio-Rad Aminex equivalent) and a 22350 VWR-Hitachi column oven. The column (300 mm × 6.5 mm) consisted of sulfonated polystyrene resin in the protonated form. The primary separation mechanism included ligand exchange, ion exclusion and adsorption. The column temperature was maintained at 30 °C with a 2350 VWR-Hitachi column oven. An L-2490 VWR-Hitachi refractive index detector was used for detection. Aliquots of 20 μl samples were injected and eluted with 0.005 N sulfuric acid in double-distilled water at a flow rate of 0.8 ml min−1. Online integration and analysis was done with EZ Chrome Elite Software (VWR International).

**DNA extraction and molecular techniques.** Chromosomal DNA of A. mimigardeforsensis strain DPN77 and the respective Tn5::mob-induced mutants was isolated using the NucleoSpin Tissue kit (Macherey-Nagel) according to the instructions of the manufacturer. To identify the Tn5::mob insertion loci, the two-step gene walking method (Pilhofer et al., 2007) was used and subsequent sequence analysis was performed by Seqlab. Oligonucleotides used in this study were synthesized by Eurofins and are listed in Table S1. PCR was done by applying Biomix (Bioline) and the resulting DNA fragments were purified using the peqGOLD Gel Extraction Kit I (Peqlab).

**Genome sequencing, assembly and gap closure.** A combination of Sanger sequencing and pyrosequencing was used for whole-genome sequencing of A. mimigardeforsensis strain DPN77. Isolated DNA from strain DPN77 was used to generate a 454 shotgun library according to the GS Rapid Library protocol (454 Life Sciences). The 454 DNA library was sequenced with the Genome Sequencer FLX system (454 Life Sciences) using titanium chemistry. A total of 195,897 shotgun reads were generated and assembled de novo into 30 large contigs (>500 bp) using Roche Newbler assembler software 2.0 FLX (454 Life Sciences). Sequence editing was done by using GAP4 as part of the Staden software package (Staden et al., 2000), and final gap closure was performed by PCR and primer walking using a Bio-X-Act kit (Bioline) and the 5 Prime Extender polymerase system (5 Prime) as described by the manufacturers.

**RESULTS AND DISCUSSION**

**General features**

**Genome.** The complete genome of A. mimigardeforsensis strain DPN77 comprises 4,764,126 bp and is distributed on two replicons: one circular chromosome consisting of 4,740,516 bp and a circular plasmid of 23,610 bp, which are composed of 4112 and 24 predicted ORFs, respectively. A putative function was assigned to 3692 (89.26 %) of all protein-coding sequences and 3681 (89.00 %) ORFs could be assigned to COGs categories (Fig. 4). The mean GC content was 54.22 %. The genome harboured seven pseudogenes, 39 tRNA genes and two copies of rRNA operons. The general features are listed in Table 1. Homologues of dnaA, dnaN and gyrB (MIM_c00010–MIM_c00030) are localized adjacent to each other. Furthermore, single copies of parA and parB, responsible for DNA partitioning, were located on the chromosome (MIM_c40760–MIM_c40750), and a ParA-like encoding gene was also detected on the plasmid (MIM_24p00230). The genome of A. mimigardeforsensis strain DPN77 harbours some ORFs presumably derived from mobile genetic elements or of viral origin. Seven putative integrases and 14 putative transposases were identified (for more details, see Table S2 and the ‘Supplementary sections’ in the online Supplementary Material).

**Plasmid.** The plasmid pMM24 harbours 24 ORFs, 18 (75 %) of which were assigned to a putative function. No genes associated with the utilization of OSCs such as DTDP were identified on pMM24. Two ORFs possibly coding for an addiction module consisting of a toxin (MIM_24p00210) and an antidote (MIM_24p00220) were found on this
plasmid. Furthermore, two genes encoding UmUCD (MIM_24p000020–MIM_24p 00030) involved in DNA repair were detected on the plasmid, but a copy of paralogue umuCD also exists on the chromosome (MIM_c07720–MIM_c07730). In addition, a transcriptional regulator of the LysR family (MIM_24p00130) and a putative extracytoplasmic solute-binding receptor of the TTT (tripartite tricarboxylate transporter family) (MIM_24p00110) are located on pMIM24. Many paralogues of these two genes exist on the chromosome (Table 2).

A total of five metabolic genes are localized on pMIM24, two of which code for proteins possibly participating in tyrosine metabolism: a fumarylacetoacetate hydrolase domain-containing protein (MIM_24p00140) and a putative gentisate 1,2-dioxygenase (MIM_24p00080). For the latter, no paralogues were identified on the chromosome. Another ORF on the plasmid encodes a putative 3-hydroxybenzoate 6-hydrolase (MIM_24p00150), which could be involved in polycyclic aromatic hydrocarbon degradation. Furthermore, a 3-isopropylmalate dehydratase (leuCD, MIM_24p00090–MIM_24p 00100) was detected on pMIM24, nonetheless four leuCD paralogues are also present on the chromosome.

**Heterotrophic carbon metabolism**

*A. mimigardefordensis* strain DPN7<sup>T</sup> was capable of using several amino acids, pentoses, hexoses and organic acids as carbon and energy sources for heterotrophic growth (Wübbeler et al., 2006; Matsuoka et al., 2012, C. Meinert, 2012; Shin et al., 2014).
unpublished). High specific growth rates were obtained with TCA cycle intermediates (e.g. succinate or malate), sugar acids (e.g. gluconate) and short-chain fatty acids (e.g. acetate, butyrate or propionate). Furthermore, the strain had the peculiar ability to utilize DTDP and 3SP as the sole carbon source for growth (see below).

**DTDP.** DTDP is a xenobiotic and is commonly used in different fields of application (Saxena & Gupta, 1984; Tsutsumi et al., 1998; Lütke-Eversloh & Steinbüchel, 2003; Codognoto et al., 2007; Xia et al., 2012). It is the structural analogue of the disulfide amino acid cystine; the only difference being the absence of amino groups in DTDP. Formation of DTDP in the natural environment has not yet been described, but it is possible as DTDP is the oxidation product of two molecules of 3-mercapto-propionic acid (3MP; Fig. 1) and 3MP is frequently detected in nature (Kiene & Taylor, 1988; Al-Farawati &

---

**Fig. 4.** Chromosome and plasmid map of *A. mimigardefordensis* strain DPN7T. Genes encoded by the leading and the lagging strand (circles 1 and 2, respectively) of the chromosome and plasmid (40-fold enlarged relative size compared with the chromosome) of *A. mimigardefordensis* strain DPN7T are marked in red and blue, respectively. Circle 3 represents protein-coding regions according to COGs categories. The two inner circles represent the GC content and the GC skew, respectively.
van den Berg, 2001; Bürgmann et al., 2007; Todd et al., 2007). In A. mimigardefordensis strain DPN7T, DTDP is most probably transported into the cell via the TTT family system (Table S3, Fig. S1A; MIM_c08710–MIM_c08730; C. Meinert, unpublished) and cleaved into two molecules of 3MP by the disulfide reductase LpdA (Fig. S1B; MIM_c19220; Wübberer et al., 2010). 3MP is then oxygenated by the 3MP dioxygenase Mdo (Fig. S1C; MIM_c31400) yielding 3SP (Bruland et al., 2009), which is activated to the corresponding CoA thioester by the CoA ligase SucCD (Fig. S1D; MIM_c18280–MIM_c18290; Schürmann et al., 2011). The next step is the abstraction of sulfite from 3-sulfinopropionyl-CoA by a reaction of the acyl-CoA dehydrogenase-like desulfinase AcdA (Fig. S1C; MIM_c31390; Schürmann et al., 2013) and subsequently propionyl-CoA enters the central metabolism via the methylcitric acid cycle (Wübberer et al., 2008). Regulation of this pathway is presumably realized by a transcriptional regulator of the XRE (xenobiotic response element) family (MIM_c31360), which was located on the chromosome in the vicinity of acd and mdo (Fig. S1C).

The cells of the taxonomically proximate type strain were incapable of using DTDP or its degradation intermediate 3SP as a carbon source (Wübberer et al., 2006). It is highly probable that the reason for this noticeable distinction between A. mimigardefordensis strain DPN7T and A. kashmirensis WT001T is the absence of operating paralogues to mdo (MIM_c31400) and acdA (MIM_c31390) – the two key enzymes in the catabolic pathway of DTDP. The detected genes with the highest sequence similarities in A. kashmirensis WT001T were TKWG_22195 and TKWG_25370, showing only 49 and 32% identities to mdo and acdA, respectively. The other two metabolic genes known to be involved in DTDP catabolism were present in the A. kashmirensis genome: the disulfide reductase (TKWG_11515; 87% identical aa to MIM_c19220) and the CoA ligase (TKWG_10860–TKWG_10865; >97% identical aa to MIM_c18280–MIM_c18290), which both showed very high sequence similarities to the orthologues in A. mimigardefordensis strain DPN7T.

Furthermore, an operon of the proposed transport system for uptake of DTDP could not be predicted for A. kashmirensis. However, the import of DTDP in A. mimigardefordensis strain DPN7T is presumably carried out by the TTT system (C. Meinert, unpublished) and the supposed procedure is described in the following (for a graphical overview, see Fig. S2). If DTDP is available, it binds most probably to the Bug-like extracytoplasmic solute receptor-binding protein TctC (MIM_c08730). Loaded TctC putatively partitions between the transport pathway via interactions with TctBA (MIM_c08720–MIM_c08710), and the signalling pathway via interactions with the periplasmic domain of one of the putative sensor kinases TctE (MIM_c39200, MIM_c22990, MIM_c17190), which were located immediately adjacent to three tctAB. Activation of the signal transduction cascade with involvement of a putative cytoplasmic response regulator TctD (MIM_c39210, MIM_c23000, MIM_c17200) could result in an upregulation of the tctCB transcription. tctC was designated originally as bug (Bordetella uptake gene) because it showed significant sequence similarities to a gene family that was found to be strongly over-represented in Bordetella (Antoine et al., 2003). However, Bug-like extracytoplasmic solute receptor-binding protein encoding genes (termed tctC) are also abundant in the genomes of many other betaproteobacteria (Table 2). The number of genes coding for TctC tremendously outnumbers the genes coding for the predicted membrane and sensor components of the TTT system (Huvent et al., 2006) (Table 2). Therefore, an additional function of this protein in signalling cascades was postulated. Solute receptor proteins that participate in such signal transduction cascades could be useful for activating transport or metabolic pathways in response to the presence of their specific ligands (Antoine et al., 2005). Interestingly, the majority of orphan tctC in A. mimigardefordensis strain DPN7T was often located in the direct vicinity of genes coding for transcriptional regulators, as was also reported for the abundant tctC in the genome of Cupriavidus necator H16 (Pohlmann et al., 2006), thus supporting the theory of a regulatory role of these Bug-like extracytoplasmic solute receptor-binding proteins. The low number of tctA in the genome of A. kashmirensis is a striking difference to A. mimigardefordensis strain DPN7T on the genomic level (Table 2). It should be noted that the relative number of genes coding for bacterial main transport systems is comparably high in the A. mimigardefordensis genome (Table 2). The possession of a convenient number of transporter-encoding genes is an important prerequisite for the ability to utilize xenobiotic compounds like DTDP and 3SP in the first place. However, not only is an uptake system necessary, but the existence of suitable exporters for disposal of toxic intermediates is mandatory for a successful adaptation of xenobiotics degradation. In addition to the putative sulfate exporter (MIM_c23530), which is responsible for the

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Count</th>
<th>Per cent of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of the genome</td>
<td>4764 126 bp</td>
<td>100</td>
</tr>
<tr>
<td>chromosome</td>
<td>4740 516 bp</td>
<td>99.5</td>
</tr>
<tr>
<td>plasmid</td>
<td>23 610 bp</td>
<td>0.5</td>
</tr>
<tr>
<td>DNA G + C content</td>
<td>2583 218 bp</td>
<td>54.22</td>
</tr>
<tr>
<td>DNA coding sequence</td>
<td>4113 819 bp</td>
<td>86.35</td>
</tr>
<tr>
<td>Predicted protein-coding genes</td>
<td>4091</td>
<td>98.91</td>
</tr>
<tr>
<td>with putative function</td>
<td>3692</td>
<td>89.26</td>
</tr>
<tr>
<td>with unknown function</td>
<td>399</td>
<td>9.65</td>
</tr>
<tr>
<td>RNA genes</td>
<td>45</td>
<td>1.09</td>
</tr>
<tr>
<td>rRNA genes</td>
<td>6</td>
<td>0.15</td>
</tr>
<tr>
<td>tRNA genes</td>
<td>39</td>
<td>0.94</td>
</tr>
<tr>
<td>Pseudogenes</td>
<td>7</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 1. General features of the genome of A. mimigardefordensis strain DPN7T

http://mic.sgmjournals.org
Table 2. Heat map of abundant genes in the genome of *A. mimigardefordensis* strain DPN7 and comparison with elected proteobacteria

Colours of the heat map emphasize the comparative quantity of the gene in the particular column: green, most abundant; red, least abundant.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Genome size (protein)</th>
<th>ABC (ATPase)</th>
<th>TctC (TTT)</th>
<th>LysR</th>
<th>MFS</th>
<th>SDR</th>
<th>Acyl-CoA DH</th>
<th>Aldehyde DH</th>
<th>DctQ (TRAP-T)</th>
<th>Thiolase</th>
<th>TauE (exporter)</th>
<th>TctA (TTT)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Advenella mimigardefordensis</em> DPN7</td>
<td>4091</td>
<td>140</td>
<td>3.4</td>
<td>130</td>
<td>3.2</td>
<td>121</td>
<td>3.0</td>
<td>62</td>
<td>1.5</td>
<td>36</td>
<td>0.9</td>
<td>32</td>
</tr>
<tr>
<td><em>Advenella kashmirensis</em> WT001</td>
<td>3933</td>
<td>88</td>
<td>2.2</td>
<td>122</td>
<td>3.1</td>
<td>102</td>
<td>2.6</td>
<td>50</td>
<td>1.3</td>
<td>32</td>
<td>0.8</td>
<td>21</td>
</tr>
<tr>
<td><em>Burkholderia xenovorans</em> LB400</td>
<td>8951</td>
<td>204</td>
<td>2.3</td>
<td>13</td>
<td>0.1</td>
<td>186</td>
<td>2.1</td>
<td>151</td>
<td>1.7</td>
<td>101</td>
<td>1.1</td>
<td>56</td>
</tr>
<tr>
<td><em>Burkholderia mallei</em> ATCC 23344</td>
<td>5414</td>
<td>95</td>
<td>1.8</td>
<td>0</td>
<td>0</td>
<td>82</td>
<td>1.5</td>
<td>78</td>
<td>1.4</td>
<td>32</td>
<td>0.6</td>
<td>20</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em> Tohama I</td>
<td>3761</td>
<td>97</td>
<td>2.6</td>
<td>86</td>
<td>2.3</td>
<td>87</td>
<td>2.3</td>
<td>38</td>
<td>1.0</td>
<td>29</td>
<td>0.8</td>
<td>22</td>
</tr>
<tr>
<td><em>Ralstonia solanacearum</em> GM1000</td>
<td>5120</td>
<td>76</td>
<td>1.5</td>
<td>2</td>
<td>0.04</td>
<td>78</td>
<td>1.5</td>
<td>65</td>
<td>1.3</td>
<td>39</td>
<td>0.8</td>
<td>16</td>
</tr>
<tr>
<td><em>Roseobacter denitrificans</em> OCh 114</td>
<td>4146</td>
<td>117</td>
<td>2.8</td>
<td>4</td>
<td>0.1</td>
<td>28</td>
<td>0.7</td>
<td>17</td>
<td>0.4</td>
<td>26</td>
<td>0.6</td>
<td>13</td>
</tr>
<tr>
<td><em>Cupriavidus necator</em> H16</td>
<td>6629</td>
<td>125</td>
<td>1.9</td>
<td>156</td>
<td>2.4</td>
<td>176</td>
<td>2.7</td>
<td>89</td>
<td>1.3</td>
<td>63</td>
<td>1.0</td>
<td>69</td>
</tr>
<tr>
<td><em>Cupriavidus necator</em> N-1</td>
<td>7832</td>
<td>132</td>
<td>1.7</td>
<td>193</td>
<td>2.5</td>
<td>233</td>
<td>3.0</td>
<td>121</td>
<td>1.5</td>
<td>87</td>
<td>1.1</td>
<td>69</td>
</tr>
<tr>
<td><em>Variovorax paradoxus</em> B4</td>
<td>6590</td>
<td>190</td>
<td>2.9</td>
<td>172</td>
<td>2.6</td>
<td>190</td>
<td>2.9</td>
<td>67</td>
<td>1.0</td>
<td>66</td>
<td>1.0</td>
<td>33</td>
</tr>
<tr>
<td><em>Escherichia coli</em> K12 DH1</td>
<td>4160</td>
<td>77</td>
<td>1.9</td>
<td>0</td>
<td>0</td>
<td>53</td>
<td>1.3</td>
<td>62</td>
<td>1.5</td>
<td>14</td>
<td>0.3</td>
<td>4</td>
</tr>
</tbody>
</table>

ABC (ATPase), genes coding for the ATP-binding domain-containing protein of the ABC transport system (Pfam: 00005/12848/09821); TctC (TTT), genes coding for the putative Bug (*Bordetella* uptake gene)-like extracytoplasmic solute-binding receptor of the TTT family (Pfam: 03401); LysR, genes coding for transcriptional regulators of the LysR family (Pfam: 00126/03466); MFS, genes coding for major facilitator symporter (Pfam: 07690/13347/05977); SDR, genes coding for short-chain dehydrogenases/reductases (Pfam: 00106); Acyl-CoA DH, genes coding for acyl-CoA dehydrogenase-like proteins (Pfam: 02771/02770/00441/12186/08028/12418); Aldehyde DH, genes coding for aldehyde dehydrogenases (Pfam: 00171); DctQ (TRAP-T), genes coding for the membrane protein DctQ of the TRAP-T system (Pfam: 04290); thiolute, genes coding for acetyl-CoA acetyltransferases (thiolases II) and thiolute I (Pfam: 00108/02803); TauE (exporter), genes coding for a putative exporter of sulfate and OSCs (Pfam: 01925); TctA (TTT), genes coding for the large transmembrane protein of the TTT family (Pfam: 01970).

n, Number of encoding genes in the respective genome; %, per cent of the gene count in the specific genome.

Reasons for selection of bacteria included in this table in addition to *A. mimigardefordensis* DPN7: *A. kashmirensis* WT001 is the closest related type strain. *Burkholderia xenovorans* LB400 is a diverse and versatile non-pathogenic bacterium that possesses one of the largest annotated bacterial genomes. *Burkholderia mallei* ATCC 23344, *Bordetella pertussis* Tohama I and *Ralstonia solanacearum* GM1000 are well-studied pathogens with different specializations. *Roseobacter denitrificans* OCh 114 belongs to the metabolically diverse purple alphaproteobacteria that are abundant in marine environments. *C. necator* H16 is able to utilize DTDP and 3MP for the synthesis of hetero-PTEs. *C. necator* N-1 can grow with 3SP as the sole carbon source, and *Variovorax paradoxus* B4 was isolated and annotated due to its ability to use 2-mercaptosuccinic acid for growth as the sole carbon, energy and sulfur source. *E. coli* K12 DH1 serves as the best-studied representative of the gammaproteobacteria.
removal of excessive sulfate, an export system for the harmful intermediate 3MP must also exist in A. mimigardefordensis strain DPN7T. During growth with DTDP as the sole carbon source, 3MP was detectable in high concentrations in the culture supernatant (Wübbeler et al., 2008) and thus had to be somehow exported. Presumably, the cells exported 3MP to maintain the intracellular osmotic pressure and/or to prevent undesired side reactions of this thiol. The export system is unknown, but it could be supposed that a TauE exporter is involved, which was described recently as an exporter of sulfite and OSCs (Mayer et al., 2012), and whose corresponding encoding genes are abundant in the genome of A. mimigardefordensis strain DPN7T (Table 2).

Propionate. The catabolism of monocarboxylates such as propionate is of major importance for bacterial strains, both in their natural habitats as well as during biotechnical applications. As only little information is available about the transport of propionate in bacterial cells (Jolkver et al., 2009), it is merely supposed that a transporter of the SSS (solute:sodium symporter) family (TCDB 2.A.21; Table S3) is involved in propionate uptake in A. mimigardefordensis strain DPN7T. Inside the cells, propionate is presumably ligated with CoA by putative acyl-CoA synthetases (MIM_c14630, MIM_c23610, MIM_c38630) yielding propionyl-CoA. No genes encoding methylmalonyl-CoA mutase, pyruvate ferredoxin oxidoreductase or propionate-CoA transferase, which are the enzymes necessary for the methylmalonyl-CoA pathway, reductive carboxylation and the conversion of lactoyl-CoA into lactate, respectively, were detected. Nonetheless, all genes involved in the methylycitric acid cycle (Fig. S1C, E) and the β-oxidation of propionyl-CoA via acryloyl-CoA and 3-hydroxypropionyl-CoA are present in the genome of A. mimigardefordensis strain DPN7T; therefore, the metabolism of propionyl-CoA is most probably conducted via these two pathways. Propionyl-CoA formed as an intermediate in the DTDP degradation pathway was undoubtedly metabolized via the methylcitric acid cycle (Wübbeler et al., 2008). This assumption was supported by the finding that a Tn5::mob insertion in the gene coding for a transcriptional regulator of the XRE family (MIM_c31360), which was located between genes involved in the methylcitric acid cycle and the precedent catabolism of DTDP (Fig. S1C), impaired growth of the mutant jhw46 (Table S1) with DTDP or propionate as the sole carbon source. The utilization of DTDP as a source of sulfur was not impaired in this Tn5::mob-induced mutant. It was therefore assumed that the sulfur from DTDP could still be abstracted by the desulfitase AcdA and used by the cells of A. mimigardefordensis jhw46, but the propionyl-CoA was not further metabolized to support cell growth, most probably due to an inoperable methylcitric acid cycle caused by the disruption of the XRE transcriptional regulator (MIM_c31360; Fig. S1C).

Glycerol. The simple alcohol glycerol is an abundant substance found in nature: It is the structural constituent of several lipids and is frequently produced during osmoregulation in yeasts (Wang et al., 2001). Thus, many known bacteria are able to use glycerol as the sole source of carbon and energy, and consequently its application in some industrial processes is a good alternative to traditional carbohydrates (da Silva et al., 2009). In A. mimigardefordensis strain DPN7T, glycerol was applied successfully to improve the biotechnical production of PTEs (Xia et al., 2012). The stability of the pH in the cultivation medium was the main advantage for employing glycerol as the carbon source in this process. Transport of glycerol in the cell is usually conducted by passive diffusion through the cytoplasmic membrane and/or via a facilitated diffusion achieved by an integral membrane protein of the MIP (major intrinsic protein) family (Lin, 1976; Romano, 1986). A. mimigardefordensis strain DPN7T harbour one gene putatively encoding a MIP (MIM_c22510) and is therefore probably capable of facilitated uptake of glycerol. The catabolic pathway of glycerol in A. mimigardefordensis strain DPN7T could proceed via two ways. (i) It starts with activation by a glycerol kinase (MIM_c01080, MIM_c04710) into sn-glycerol 3-phosphate, continuing with conversion in dihydroxyacetonephosphate by a glycerol 3-phosphate dehydrogenase (MIM_c01190, MIM_c32210) and leads in the glycosylation or gluconeogenesis. (ii) Glycerol could putatively be converted into glyceraldehyde by an aldol/keto reductase (MIM_c13750, MIM_c17820, MIM_c21070). Afterwards, one of the 26 aldehyde dehydrogenases (Table 2) catalyses the transformation into glyceraldehyde, followed by the conversion into glyceraldehyde 3-phosphate by a glyceraldehyde kinase (MIM_c26670). Glycerate 3-phosphate is then further metabolized via the Embden–Meyerhof–Parnas pathway. In contrast, the closest related type strain A. kashmirensis WT001T was not able to grow with glycerol as the sole carbon source (Ghosh et al., 2005). The putative glycerol uptake facilitator of the MIP family is present in the A. kashmirensis genome (TKWG_13070), but a frame-shift in its only gene encoding a glyceraldehyde 3-phosphate dehydrogenase (TKWG_20235, TKWG_20230) is most probably the cause of the inability to use glycerol for growth. Curiously, the alternative pathway via glyceraldehyde, glycerate and glycylate 3-phosphate could also not be accomplished, because its gene encoding glyceraldehyde kinase contains two frame-shifts (TKWG_08145, TKWG_08150, TKWG_08155).

Sugars and gluconate. Various sugars such as arabinose, D-fructose, D-galactose, D-glucose, D-ribose and D-xylene support growth, but A. mimigardefordensis was not able to grow with D-fructose, D-mannose and L-xylene, for example, as sole carbon sources (Wübbeler et al., 2006; Matsuoka et al., 2012; C. Meinert, unpublished). Interestingly, A. mimigardefordensis strain DPN7T does not have a complete phosphotransferase system (PTS); only the genes encoding Enzyme I (EI), EIia and the phosphocarrier protein HPr were detected (MIM_c21900–MIM_c21920). Transport of glucose by a PTS is the most common uptake system in Gram-negative bacteria, whereas the transport via glucose facilitators [e.g. major facilitator superfamily (MFS) type] and ATP-binding
cassette (ABC) transport systems is the exception (Jahreis et al., 2008). The genome of *A. mimigardefordensis* strain DPN7T harbours a putative monosaccharide ABC transport system (MIM_c39770–MIM_c39800), but the commonly adjacent sugar kinase regulator is missing and glucose transport was therefore most probably not conducted in this way. It is theoretically possible that some sugars could be transported into the cell by MFS-type proteins (Table 2), but recent data of our laboratory indicated the involvement of the TRAP-T (tripartite ATP-independent periplasmic transporter) family (Table S3; MIM_c39430–MIM_c39450) in the uptake of L-arabinose, D-fucose, D-galactose, D-glucose, D-gluconate and D-xylose (C. Meinert, unpublished). Apparently, there are no genes encoding hexokinases (COG5026), glucokinases (COG0837) or gluconokinases (COG3265) present in the genome, but a ribokinase was identified (MIM_c39760). Also, no gene coding for a 6-phosphofructokinase (COG0205) was detectable and accordingly glycolysis is not completely executable. However, gluconeogenesis is operable due to two recognized genes (MIM_c14350, MIM_c23250) encoding fructose 1,6-bisphosphatase. On account of these results and based on the in silico analyses, it was proposed that glucose and gluconate are catabolized via the semi-phosphorylated Entner–Doudoroff pathway (Conway, 1992).

**Taurine.** The abundant sulfonate taurine (2-aminoethanesulfonic acid) is frequently used as source of sulfur by micro-organisms (Cook & Denger, 2002). Taurine is a non-proteinogenic amino acid, and occurs as a major organic solute in all vertebrates and in a wide range of marine invertebrates. In *E. coli*, the use of taurine as a sulfur source was attributed to the *tauABCD* gene cluster that encodes a specialized sulfonate–sulfur utilization (*ssu*) system, which is in most cases exclusively involved in utilization of this substrate (van der Ploeg et al., 1996; Eichhorn et al., 2000; Cook et al., 2006). *A. mimigardefordensis* strain DPN7T was able to grow with taurine as the sole source of carbon and sulfur (Wübøler et al., 2008). The uptake of taurine is most probably catalysed by ABC-type transport systems encoded by *tauABC* (Table S3). The subsequent release of sulfite is supposedly catalysed by TauD, an Fe(II) α-ketoglutarate-dependent taurine dioxygenase (MIM_c03960, MIM_c04000, MIM_c14220, MIM_c38710). This enzyme is absolutely dependent on ferrous iron, has a tetrameric structure (Knauer et al., 2012), and converts taurine into sulfite and acetyl-CoA, which is then accessible for the central metabolism. Thus, the respective gene cluster responsible for uptake and degradation of taurine in *A. mimigardefordensis* strain DPN7T via aminoacetylehyde was identified as MIM_c38710–MIM_c38740 (Fig. S1F) and via sulfocetaldehyde as MIM_c36630–MIM_c36680 (Fig. S1G).

**Aliphatic sulfonates.** As a fundamental component for all life, sulfur appears in organisms as both inorganic and organic compounds. Excluding PTE-producing bacterial strains, for example, up to 1 % (w/v) of the typical cell dry weight consists of sulfur (Kertesz, 2000), mainly due to cysteine and methionine as components of proteins (Sievert et al., 2007). Additionally, sulfur plays a key role in iron–sulfur clusters of proteins. Examples of vital OSCs are CoA, biotin (vitamin H or B7), thiamine (vitamin B1), several sulfolipids, various secondary metabolites and the OSCs responsible for the maintenance of cellular redox potentials: coenzyme B, ergothiol, glutathione, mycothiol, ovothiol and trypanothione (Hand & Honek, 2005). The sulfur required for biosynthesis processes is generally derived from the assimilation of sulfate by plants and bacteria (Kertesz, 2000). In natural environments, only small amounts of sulfur are present as sulfate. Instead, the major amount of sulfur is only accessible as a heterogeneous mixture of diverse OSCs, e.g. sulfate esters, sulfonates and peptides (Mirleau et al., 2005). Therefore, numerous micro-organisms have developed a variety of pathways to make sulfur available for their central metabolism. The active inter-conversion of organic and inorganic sulfur forms in soil is due to microbial activities, and a plant-growth-promoting effect was demonstrated for desulphonating bacterial strains (Kertesz & Mirleau, 2004; Schmalenberger & Kertesz, 2007; Schmalenberger et al., 2008). The *ssu* genes are known to be responsible for uptake and degradation of aliphatic sulfonates other than taurine (Eichhorn et al., 2000); accordingly, nine putative *ssuABC* clusters, which code for ABC-type aliphatic sulfonate transport systems, were identified in the genome of *A. mimigardefordensis* strain DPN7T (Table S3). Interestingly, the *ssuABC* genes with the locus tag MIM_c03970–MIM_c03990 are encircled by two *tauD* (MIM_c03960, MIM_c04000). Furthermore, the *ssuABC* gene cluster with the locus tag MIM_c01510–MIM_c01530 (Fig. S1H) is flanked by all the three genes in the genome that encode alkanesulfonate monoxygenases SsuD (MIM_c01490, MIM_c01540, MIM_c01550) and another *ssuABC* cluster (MIM_c01580–MIM_c01560). SsuD is able to desulphonate a wide range of aliphatic, but not aromatic sulfonates, with the above-mentioned exception of taurine (Eichhorn et al., 2000). To perform this desulphonation reaction, the two-component SsuD needs FMNH₂ as a substrate, which is provided by the associated FMN reductase SsuE (MIM_c01500). In addition to the nine *ssuABC* paralogues, another ABC-type nitrate/sulfonate/bicarbonate transport system was identified (MIM_c16790–MIM_c16810) and also the
structurally related nitrate ABC transporters were identified (MIM_c09400–MIM_c09420; MIM_c34060–MIM_c34080).

Sulfate metabolism. The import of extracellular sulfate into cells of *A. mimigardefordensis* strain DPN7\(^T\) is performed by the specific ABC transport system CysPTWA (Table S3; MIM_c15530–MIM_c5500), which belongs to the SulF family (TC 3.A.1.6), and an MFS-type sulfate permease (Table S3; MIM_c24690) of the SulP family (TC 2.A.53) (Aguilar-Barajas et al., 2011). A CysZ-like sulfate permease (TC 9.B.7) was not identified in the genome of *A. mimigardefordensis* strain DPN7\(^T\). For the subsequent assimilation of sulfur from inorganic sulfate, which seems to be similar in all prokaryotes, activation by coupling to a nucleoside is mandatory and could be accomplished by a sulfate adenylyltransferase (*cysD*/cysN, MIM_c15480/MIM_c15490) yielding adenosine 5'-phosphosulfate (APS). The next step is the reduction of APS by the APS reductase (EC 1.8.4.10) (MIM_c15470) releasing sulfite (Bick et al., 2000). Apparently, phosphoadenosine 5'-phosphosulfate cannot be formed in *A. mimigardefordensis* strain DPN7\(^T\), as no gene encoding adenylylsulfate kinase was identified in the genome. Sulfite is converted into sulfide by the sulfite reductase (*cysl*/cysJ, MIM_c29690/MIM_c29700), which catalyses a six-electron reduction of sulfite to sulfide (Crane et al., 1995). Sulfide is then metabolized and usually utilized to generate cysteine, either by the cysteine synthase CysK (MIM_c16980) or CysM (MIM_c22720).

*A. mimigardefordensis* strain DPN7\(^T\) was also not able to oxidize sulfane sulfur species directly to sulfate, as is common for some alphaproteobacteria (Friedrich et al., 2005). Thus, an entire inorganic sulfur compound oxidation gene cluster (*sox* operon) was not detected in the genome of *A. mimigardefordensis* strain DPN7\(^T\), but part of the *sox* operon was reported to exist in *A. kashmirensis* WT001\(^T\) (Ghosh et al., 2011), enabling strain WT001\(^T\) to utilize tetrathionate and thiosulfate chemolithotrophically (Ghosh et al., 2005). Paralogues to *soxA*, *soxX*, *soxW*, *soxS* and *soxL* were not recognized in the genome of strain DPN7\(^T\). Therefore, *A. mimigardefordensis* strain DPN7\(^T\) was not able to oxidize completely reduced sulfur compounds and subsequently use the resulting electrons of this process for energy transformation. However, five sulfite oxidases were detected, which all showed homology to, and could possibly act and be annotated as, *soxC* (MIM_c01600, MIM_c03760, MIM_c12620, MIM_c24010, MIM_c36020). Downstream of two of these *soxC*-like genes, homologous genes putatively coding for SoxD were located (MIM_c01590, MIM_c36010), which are important in the detoxification process of sulfite acting as sulfite oxidases. Furthermore, a putative dimanganese-containing 5'-nucleotidase-encoding gene *soxB* (MIM_c17110), a putative regulator *soXR* (MIM_c02390) as well as a fused gene *soxYZ* (MIM_c36710) coding for the covalently sulfur-binding/chelating protein were detectable in *A. mimigardefordensis* strain DPN7\(^T\).

**Sulfite and sulfate export.** The microbial degradation of either naturally occurring or xenobiotic pollutant OSCs such as dibenzothiophene (McFarland, 1999), di-\(n\)-octylsulfide (Kirkwood et al., 2005), dimethylsulfiniopropionic acid (Yoch, 2002) or various C2- (Cook & Denger, 2002) and C3-sulfonates (Cook et al., 2006) has been studied in detail, and the metabolism of OSCs is an essential part of the global sulfur cycle (Sievert et al., 2007). During microbial utilization of DTDP as the sole carbon source, the excess sulfur was abstracted from 3SP-CoA as sulfite (Schürmann et al., 2013). Accumulation of charged sulfite species like HSO\(_3\)\(^-\) or SO\(_3\)\(^2-\) leads to reactions with cell constituents, such as coenzymes, cofactors and nucleic acids, and as a consequence most likely to irreversible inhibition of cell growth (Gunnison, 1981). Despite its toxicity, sulfite is a transient intermediate during the catabolism of cysteine and taurine, as has been known in the degradation of arylsulfonates and alkylsulfonates for a long time (Kondo & Ishimoto, 1972). However, sulfite is a strong nucleophile that may convert protein disulfides into sulfonated cysteine derivatives (Bailey & Cole, 1959). As the integrity of disulfide bonds is crucial to the tertiary structure of proteins, it may have strong negative effects on growth. If only DTDP, 3SP or taurine are available as the main carbon source, the occurrence of excessive sulfite is inevitable. The sulfite anion is usually not excreted in huge quantities into the medium (Cook et al., 2006) and thus a detoxification process performed by the cells is essential. Commonly, the resulting sulfite is oxidized to sulfate, either by sulfite oxidases (EC 1.8.3.1) or sulfate dehydrogenases (EC 1.8.2.1) (MIM_c01600, MIM_c03760, MIM_c12620, MIM_c24010, MIM_c36020), which both (COG2041) catalyse the direct oxidation of sulfite to sulfate in eukaryotes or prokaryotes, respectively (Feng et al., 2007; Kappler, 2007). The alternative AMP-dependent pathway with the formation of adenylyl phosphosulfate is not accomplished by *A. mimigardefordensis*, because no genes encoding adenylylsulfate reductase (EC 1.8.99.2) were detectable in the genome. Presumably, large amounts of sulfate were exclusively excreted by a putative membrane protein (Table S3; MIM_c23530; COG2855), which belongs to the CPA (cation: proton antiporter) superfamily, more precisely to the PSE (putative sulfate exporter) family (Transporter Classification Database 2.A.98; Brüggemann et al., 2004; Rein et al., 2005; Saier et al., 2009). The encoding gene in *A. mimigardefordensis* was disrupted by Tn5::mob insertions (Fig. S11), and the corresponding Tn5::mob-induced mutants JG7 and LR32 (Table S1) exhibited a clear phenotype: no growth with DTDP, 3SP or taurine as carbon source. This very interesting finding confirmed the exigency of an operable export system for the maintenance of a constant osmotic pressure in the cell, as also proposed previously for bacteria that were growing with an OSC as the sole carbon source (Rein et al., 2005; Cook et al., 2006). Apparently, the gene with the locus tag MIM_c23530 (Fig. S11) is the only executable sulfate exporter in *A. mimigardefordensis*, which was verified to be obligatory during utilization of DTDP, 3SP and taurine.
Protein secretion systems and biosynthesis of storage compounds

Protein secretion systems. Three complete systems were identified from the major translocation systems for the secretion of proteins across the cytoplasmic membrane known in bacteria (Desvaux et al., 2009) in A. mimigardefordensis strain DPN7T: the well-known TAT pathway is encoded in one gene cluster (Table S4), the general Sec pathway, whose encoding genes are distributed on the chromosome (Table S4), and the type VI secretion system (T6SS; Table S4). The T6SS is versatile and not yet completely understood (Silverman et al., 2006; Silverman et al., 2012), and is therefore commonly referred to as a virulence factor. The peculiar feature is that many pathogens possess at least one T6SS, but most bacteria with a recognized T6SS are not pathogenic (Jani & Cotter, 2010). T6SS plays undoubtedly an important role in virulence, e.g. it is required in Burkholderia mallei for the virulence of this pathogen, as demonstrated in a hamster model (Burtinick et al., 2010). Otherwise, the T6SS is involved in interbacterial interactions (Hood et al., 2010) and can also act as an antipathogenesis factor (Parsons & Heffron, 2005). The two proteins secreted in a T6SS-dependent manner are basically the haemolysin coregulated protein (Hcp) and the valine-glycine repeat protein G (VgrG), which both showed a codependency for export. Interestingly, the genome of A. mimigardefordensis strain DPN7T harboured nine distinct Vgr-encoding genes. Three vgrG were identified in the Vibrio cholerae O1 genome (Pukatzki et al., 2006) and 10 vgr paralogues were identified in the genome of Pseudomonas aeruginosa PAO1 (Hachani et al., 2011). Thus, the fact that some bacteria possess a large number of vgr genes is remarkable, but the reason is still the subject of research.

Polyhydroxalkanoates (PHAs) and PTE synthesis. Cells of A. mimigardefordensis accumulated polyhydroxybutyrate (PHB) as an intracellular storage compound (Wöbbeler et al., 2006). They were also capable of synthesizing PTE, if cultivated under the appropriate conditions and treated with the necessary genetic modifications (Xia et al., 2012). PHAs (Fig. 1) are polyesters synthesized as storage compounds by a wide variety of micro-organisms (Steinbüchel, 1991) that can be biotechnologically produced by applying renewable primary products (Tan, 2004), such as low-cost carbon sources from agriculture. Synthesis and accumulation are implemented in bacterial strains if a carbon source is excessive for growth and another macroelement is restricted (Pötter & Steinbüchel, 2005).

Biosynthesis of PHB depends on three different enzymes (PhaABC) and an enhanced concentration of acetyl-CoA inside the cells. One phaC was identified in the genome of A. mimigardefordensis (MIM_c13530–MIM_c13550), but phaA was unexpectedly located elsewhere (MIM_c21300). The genes encoding the PHA synthesis repressor PhaR (MIM_c13550) and the PHA synthase PhaC (MIM_c13530) had no paralogues in the genome, whereas phaA and phaB homologues, β-thiolases, and acetoactetyl-CoA reductases were abundant (Table 2). The abundance of isologs of phaA and phaB is common for PHA-synthesizing bacteria, and was also described, for example, for C. necator (Pohlmann et al., 2006) – the model organism of PHA research (Reinecke & Steinbüchel, 2009).

Another important factor in the biosynthesis of PHB is the small non-catalytic amphiphilic phasin proteins (Pötter & Steinbüchel, 2005). Phasins (PhaP) are synthesized by PHB-accumulating bacteria, which are localized at, and interact with, the surface of PHB granules. The genome of A. mimigardefordensis strain DPN7T harbours two genes encoding PhaP. In silico analyses and comparison with sequence data of C. necator H16 revealed that one of these phaP (MIM_c10360) is an orthologue of the major phasin-encoding gene phaP1 (H16_A1381; Neumann et al., 2008) and the second phaP (MIM_c36280) is an orthologous gene of phaP5 (H16_B1934). PhaP5 of C. necator is not essential for granule formation (Pfeiffer & Jendrossek, 2011), but interacts with the multifunctional protein PhaM, which is responsible for anchoring PHB granules with the cell DNA prior to segmentation (Pfeiffer et al., 2011). The gene in A. mimigardefordensis strain DPN7T (MIM_c04510) with the highest sequence similarity to phaM (H16_A0141) has only low (33 %) deduced amino acid identities. Further important PHB granule-associated proteins involved in homeostasis (Brigham et al., 2012) and degradation are the PHB depolymerases PhaZ and the PHB oligomer hydrolases PhaY. Two phaZ homologues were detectable in A. mimigardefordensis strain DPN7T, one (MIM_c24170) is an orthologue to phaZ1 from C. necator and the other (MIM_c0120) to phaZ6. The identified gene encoding PhaY (MIM_c18780) showed the highest similarities to C. necator phaY2.

In addition to the ability to synthesize polyoxoesters such as PHB, cells of A. mimigardefordensis strain DPN7T are also able to activate and incorporate 3MP as a building block of PTE, which is accomplished by unknown enzymes and its own PhaC, if supplied with DTDP as precursor (Wöbbeler et al., 2006; Xia et al., 2012). As with C. necator (Lütke-Eversloh & Steinbüchel, 2003), WT A. mimigardefordensis only produces the heteropolymer poly(3HB-co-3MP). However, after engineering of the metabolism when (i) mdo (MIM_c31400) was deleted, (ii) the genes from Clostridium acetobutylicum coding for a butyrate kinase (buk) and a phosphotransbutyrylase (ptb) were introduced into the genome, and (iii) its own phaC was overexpressed on a suitable vector in addition to the copy on the genome, cells of A. mimigardefordensis produced poly(3MP) homopolymer up to 25 % (w/w) of the cell dry weight if cultivated in MSM with glycerol as carbon source and DTDP as sulfur-containing precursor (Xia et al., 2012). A. mimigardefordensis is at present the best choice for an optimized production of poly(3MP) applying non-toxic precursor substrates such as DTDP and/or to establish a PTE synthesis process using only economical alternative resources as carbon and sulfur sources.
Conclusions
The detailed information about the genome of *A. mimigardefordensis* strain DPN7<sup>T</sup> was beneficial for the complete elucidation of the DTDP catabolic pathway (Fig. 3). In addition, the three Tn5::mob-induced mutants presented in this study (Table S1) contributed to the identification of the only functioning exporter of sulfate (MIM_c23530) and of an important transcriptional regulator (MIM_c31360) in DTDP catabolism. The metabolic genes involved are not clustered in a single operon and are also not localized on the plasmid pMIM24, as presumed at the start of the research on DTDP degradation. Two of the gene products involved, i.e. the CoA ligase (SucCD; MIM_c18280–MIM_c18290) and the disulfide reductase (LpdA; MIM_c19220), were known to be involved in other essential metabolic pathways. The succinate-CoA ligase contributed to the TCA cycle and the disulfide reductase dihydrolipoamide dehydrogenase was part of the pyruvate dehydrogenase multienzyme complex (MIM_c19200–MIM_c19220). *mmd* and *acda* were in fact localized adjacent to each other (Fig. S1C), and both were postulated to be key enzymes of DTDP catabolism. It is therefore supposed that the ability of DTDP degradation evolved due to (i) a broad substrate specificity of pre-existing enzymes and (ii) the duplication of enzyme-coding genes followed by the subsequent accumulation of point mutations in the DNA sequence, resulting in a novel association with specialized functions of these proteins that led to the ability to degrade DTDP. It is well-known that promiscuous enzymes could be recruited to provide new functions when the catalysis of secondary reactions provides a selective advantage to the organism. The so-called patchwork combination was often employed in the evolution of degrading pathways for xenobiotic compounds (Copley, 2000; Schmidt et al., 2003; Wagner 2012; Mayer et al., 2012).

As the complete genome sequence of *A. mimigardefordensis* strain DPN7<sup>T</sup> is now available, it will be possible to investigate the proteome during growth on DTDP or other interesting OSCs by 2D gel electrophoresis and MALDI-TOF. This approach will provide further insights, and will greatly support and extend our current knowledge of DTDP catabolism.

ACKNOWLEDGEMENTS
We gratefully acknowledge the assistance of Lena Radamm, Jessica Grote, Madeleine Hamley and Rita Weyer in characterizing mutants of *A. mimigardefordensis*.

REFERENCES


Comamonadaceae in sulfur transformations by microbial wheat rhizosphere communities exposed to different sulfur fertilization regimes. Environ Microbiol 10, 1486–1500.


Edited by: G. Unden