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Comparative analysis of the complete genome of an *Acinetobacter calcoaceticus* strain adapted to a phenol-polluted environment

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Abstract

The complete genome sequence of *Acinetobacter calcoaceticus* PHEA-2, a non-pathogenic phenol-degrading bacterium previously isolated from industrial wastewater of an oil refinery in China, has been established. This is the first sequence of an *A. calcoaceticus* strain. We report here a comparative genomic analysis of PHEA-2 with two other *Acinetobacter* species having different lifestyles, *Acinetobacter baumannii* AYE, a pathogenic human-adapted strain, and *Acinetobacter baylyi* ADP1, a soil-living strain. For a long time, *A. calcoaceticus* could not be easily distinguished from *A. baumannii* strains. Indeed, whole-genome comparison revealed high synteny between *A. calcoaceticus* and *A. baumannii* genomes, but most genes for multiple drug resistance as well as those presumably involved in pathogenicity were not present in the PHEA-2 genome and phylogenetic analysis showed that *A. calcoaceticus* differed from *A. baumannii* antibiotic-susceptible strains. It also revealed that many genes associated with environmental adaptation were acquired by horizontal gene transfer, including an 8-kb phenol degradation gene cluster. A relatively higher proportion of transport-related proteins were found in PHEA-2 than in ADP1 and AYE. Overall, these findings highlight the remarkable capacity of *A. calcoaceticus* PHEA-2 to effectively adapt to a phenol-polluted wastewater environment.

Keywords: *Acinetobacter calcoaceticus*; Phenol degradation; Comparative genomics

1. Introduction

The *Acinetobacter* genus, which belongs to the gamma subgroup of *Proteobacteria*, has received particular attention because of its metabolic versatility and, in the case of clinical isolates, of its multidrug resistance (MDR) pattern (Juni, 1978; Munoz-Price and Weinstein, 2008; Towner, 2009). Acinetobacter have the ability to take up extracellular DNA from the environment, a mechanism probably used for the acquisition of new functions by horizontal gene transfer (HGT) (Barbe et al., 2004; Palmen et al., 1993). Strain PHEA-2, which was isolated from phenol polluted wastewater in China for its ability to degrade phenol, has been identified as an *Acinetobacter calcoaceticus* on the basis of molecular methods (Xu et al., 2003; Zhan et al., 2008). Physiological properties of strain PHEA-2 rendered genome analysis of this strain of particular interest (Zhan et al., 2009). Firstly, the taxonomic status of *A. calcoaceticus* was long controversial, since it could not be easily distinguished using classical phenotypic tests from *Acinetobacter baumannii* (Towner, 2009). Both species are often quoted as part of the *A. calcoaceticus*–*A. baumannii* complex (Gerner-Smidt et al., 1991; Towner, 2009). *A. baumannii* appears to be a significant pathogen in hospital environments, as it is a major cause of nosocomial infection due to its MDR pattern (Diancourt et al., 2010; Munoz-Price and Weinstein, 2008; van Dessel et al., 2004). Most MDR clinical isolates belong to the *A. baumannii* species, while *A. calcoaceticus* isolated from

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environmental samples are considered non-pathogenic and "sensitive" strains because they do not show extensive antibiotic resistance (Towner, 2009). In that respect, strain PHEA-2 is sensitive to most antibiotics. Secondly, *Acinetobacter* sp., such as the extensively studied soil bacterium *Acinetobacter baylyi* ADP1 (formerly known as *Acinetobacter* sp. ADP1), displayed the ability to degrade a wide variety of organic compounds (Barbe et al., 2004; Young et al., 2005), but did not show the ability to use phenol. Thus, the 8-kb DNA cluster that carries the genetic information responsible for phenol degradation in PHEA-2 (Xu et al., 2003) was probably acquired by HGT.

The complete genome sequencing of *A. calcoaceticus* PHEA-2 was undertaken because of the importance of this bacterium for bioremediation of phenol-polluted wastes (Zhan et al., 2011). To our knowledge, this is the first strain of *A. calcoaceticus* whose genome has been sequenced. We report here a comparison with the genome of the human pathogen *A. baumannii* AYE and with that of the soil-living bacterium *A. baylyi* ADP1. This permitted identification of traits specific to PHEA-2 or common among the three species, and of catabolic islands in the PHEA-2 genome determinant for its survival in hostile environments.

2. Materials and methods

2.1. Media and growth conditions used

The *Acinetobacter* strain was grown in Luria-Bertani (LB) medium (bacto-tryptone 10 g, bacto-yeast extract 5 g, NaCl 10 g) or mineral salt (MS) medium ($NaNO_3$ 0.5 g, K_2HPO_4 0.65 g, KH_2PO_4 0.17 g, $MgSO_4$ 0.10 g) with phenol or benzoate at 30 °C. When necessary, ampicillin (Amp) (20 μg/ml) was added to the media. Genomic DNA from *A. calcoaceticus* PHEA-2 was extracted from cells grown overnight at 30 °C in LB liquid cultures using the TIANamp Bacterial DNA kit (Tiangen). The complete genome sequence of *A. calcoaceticus* PHEA-2 had been determined by the Beijing Genomics Institute (BGI) using Solexa sequencing following standard Solexa protocols (Illumina, USA).

2.2. Genomic comparison

Sequence data for comparative analyses were obtained from the NCBI genbank (ftp://ftp.ncbi.nlm.nih.gov/genbank/genomes/Bacteria). Genomic comparisons were carried out by bidirectional BLASTP comparisons of whole-genome protein databases. Homology searches were conducted both at the nucleotide and amino acid sequence levels using BLAST with e-value $\leq 1 \times 10^{-5}$. Sequence families were built according to the BLASTP results, using Treebest (http://treesoft.svn.sourceforge.net/viewvc/treesoft/trunk/treebest/). Orthologous relations were supported by synteny detection using MUMmer (http://mummer.sourceforge.net/manual/).

2.3. 16S rRNA gene phylogenetic analysis

16S rRNA sequences of *Acinetobacter* strains were from the NCBI database (http://www.ncbi.nlm.nih.gov/nuccore/). Nucleotide sequence alignment was carried out by using ClustalW. The multiple alignments were then manually checked and trimmed using BioEdit. The dataset of the 27 concatenated sequences was fed to Molecular Evolutionary Genetics Analysis (MEGA) (Tamura et al., 2007) software to construct the maximum likelihood (ML) tree using the Neighbor-Joining (NJ) method. The distances between DNA sequences used for building the NJ tree were computed using Jukes–Cantor corrections. The NJ method produced a unique final tree based on the assumption of minimum evolution with the correct tree topology. Bootstrap values for the consensus tree were calculated by using 1000 replications.

3. Results and discussion

3.1. General features

A. calcoaceticus PHEA-2 has a single circular chromosome, 3,862,530 base pairs (bp) in length with an average G + C content of 38.8% (Fig. 1, Table 1) (Zhan et al., 2011). Comparison of the *A. calcoaceticus* PHEA-2 genome with those of *A. baumannii* AYE and *A. baylyi* ADP1 (Table 1, Fig. S1, Supplementary material) revealed the highest synteny, as well as the highest percentage of identity for protein-deduced sequences, between PHEA-2 and AYE genomes compared to ADP1. This reflects the fact that, for a long time, *A. calcoaceticus* isolates could not be differentiated from *A. baumannii* (Diancourt et al., 2010; Dolzani et al., 1995; Gerner-Smidt et al., 1991; van Dessel et al., 2004). PHEA-2 and AYE have genomes of similar size and both contain a similar number of putative coding sequences (CDS), 3599 in PHEA-2 versus 3590 in AYE. Strain ADP1 has the smallest chromosome. The different degree of synteny of the three strains observed led us to determine how many genes are homologous between the three species (Fig. 2). The three strains had in common 3029 CDS; a high level of conservation was observed for the genes devoted to nucleotide transport, energy production and conversion, lipid transport and metabolism, secondary metabolites biosynthesis, transport and catabolism. Compared to AYE and ADP1, 235 putative accessory genes were unique to PHEA-2 (Table S1), including a set of genes for the phenol catabolic pathway and a large percentage of genes for hypothetical proteins. A total of 216 genes are unique to AYE, including those for multidrug resistance, and 291 genes are unique to strain ADP1. It is thus tempting to conclude, as noted previously (Metzgar et al., 2004; Vallenet et al., 2008), that the genes reflect the lifestyle in distinct ecological niches: wastewater (PHEA-2), soil (ADP1) and hospital environments (AYE).

A 16S rRNA-based neighbor-joining tree clearly established that *A. calcoaceticus* and *A. baumannii* strains were in different phylogenetic clusters (Fig. S2). This tree included *A. baumannii* antibiotic-susceptible strains, such as strain SDF and strain ATCC17978 (Adams et al., 2008). This supports the hypothesis that *A. baumannii* antibiotic-susceptible strains and *A. calcoaceticus* have different evolutionary origins.

The genome of PHEA-2, similarly to what was observed in *A. baumannii* and *A. baylyi* species, carried DNA regions with

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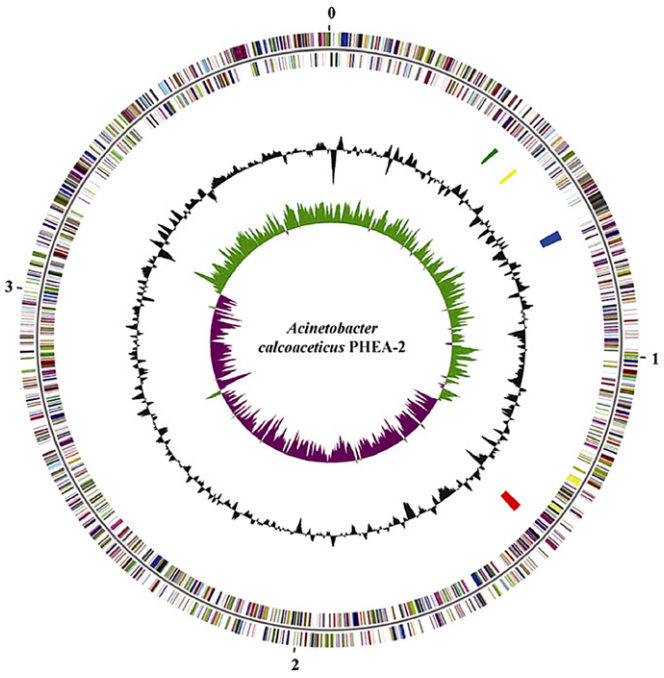

Fig. 1. Circular representation of the chromosome of *A. calcoaceticus* PHEA-2. The outer scale is marked in Mb. From outside to inside: Circles 1 and 2: predicted forward and reverse-strand gene products, coded according to the COG classification with color code for functions: salmon, translation, ribosomal structure and biogenesis; light blue, transcription; cyan, DNA replication, recombination and repair; turquoise, cell division; deep pink, posttranslational modification, protein turnover and chaperones; olive drab, cell envelope biogenesis; purple, cell motility and secretion; forest green, inorganic ion transport and metabolism; magenta, signal transduction; red, energy production; sienna, carbohydrate transport and metabolism; yellow, amino acid transport; orange, nucleotide transport and metabolism; gold, coenzyme transport and metabolism; dark blue, lipid metabolism; blue, secondary metabolites, transport and catabolism; gray, general function prediction only; black, function unclassified or unknown. Circle 3: genomic catabolic islands: green, Island I (BDGL000387-000401, 18 kb), vanillate and caffeate clusters; yellow, Island II (BDGL000460-000477, 17 kb), benzoate and phenol clusters; blue, Island III (BDGL000681-000741, 65 kb), 4-hydroxybenzoate and phenylacetate clusters; red, Island IV (BDGL001322-001371, 52 kb), catechol, protocarechuate and quinate clusters. Circles 4 and 5: G + C content and GC skew (G − C/G + C), respectively, window size 10 kb. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Features of the three *Acinetobacter* genomes.

	A. calcoaceticus PHEA-2	*A. baylyi* ADP[a]	*A. baumannii* AYE[a]
Size (Mb)	3.86	3.60	3.94
Plasmids	None	None	4
GC content (%)	38.8	40.4	39.4
CDSs	3599	3279	3590
Protein coding density (%)	87.5	87.3	86.1
Conserved hypothetical proteins	504	895	1077
Insertion sequences	26	13	33
tRNAs	69	76	72
rRNA operons	2	7	6

[a] Data from Vallenet et al. (2008).

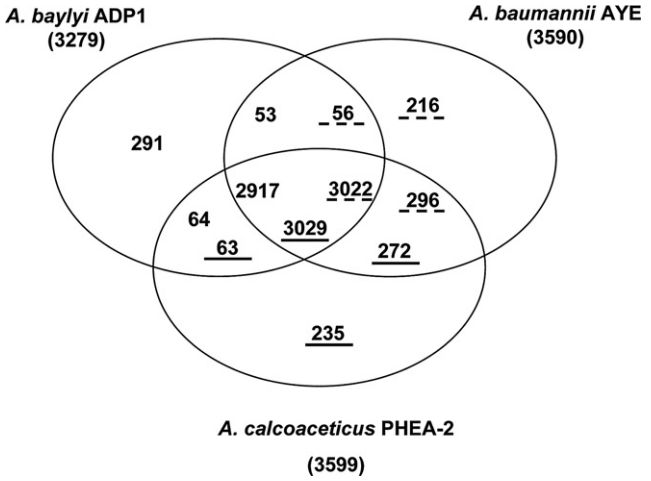

Fig. 2. Overlapping gene content comparisons of the *A. calcoaceticus* PHEA-2, *A. baumannii* AYE and *A. baylyi* ADP1 genomes. Putative orthologs are defined as genes showing at least 30% identity and 70% coverage. Not underlined, *A. baylyi* ADP1; straight underlining, *A. calcoaceticus* PHEA-2; dotted underlining, *A. baumannii* AYE.

much higher G + C content than the rest of the genome. It represented 5.5% of all genes in PHEA-2 (Fig. 1). Most genes associated with these putative genomic islands in PHEA-2 encode proteins that may be related to better adaptation to wastewater environment, suggesting acquisition by HGT. The PHEA-2 genome carries catabolic islands for degradation of aromatic compounds, as also reported previously in *A. baylyi* ADP1 and also found in AYE. Four of these regions with G + C content of 47.4% (Island I), 50.8% (Island II), 51.4% (Island III) and 49.1% (Island IV) are localized in Fig. 1. Degradation of benzoate is carried by island II which also contains the phenol degradation gene cluster (Fig. 3, Fig. S3). Two DNA regions for iron acquisition also displayed enriched in G + C content of 49.6% and 50.3%.

Bacterial competence for natural genetic transformation involves uptake of naked exogenous DNA from the environment and its integration into the genome, and requires the presence of a considerable number of gene products (Barbe et al., 2004; Friedrich et al., 2001; Soledad Ramirez et al., 2010). Indeed, *Acinetobacter* bacteria are known to be naturally competent. This trait has been extensively studied in *A. baylyi* ADP1 (Metzgar et al., 2004). The PHEA-2 genome has 30 natural competence-related genes (Table 2) including *pilBC*, *comEA* and *comEC* that had been previously described in *Pseudomonas putida* and other *Acinetobacter* species. ComEA is a transmembrane protein which binds external DNA and delivers it to the ComEC transporter (Smith et al., 2007). The 30 genes are organized into five clusters with high G + C contents of 47.8%, 50.1%, 51.3%, 51% and 51.9%, respectively.

3.2. Lack of pathogenicity and MDR determinants

Consistent with its sensitivity to most antibiotics, PHEA-2 lacks most of the MDR such as tetracyclines, chloramphenicol and other pathogenicity determinants found in *A. baumannii*

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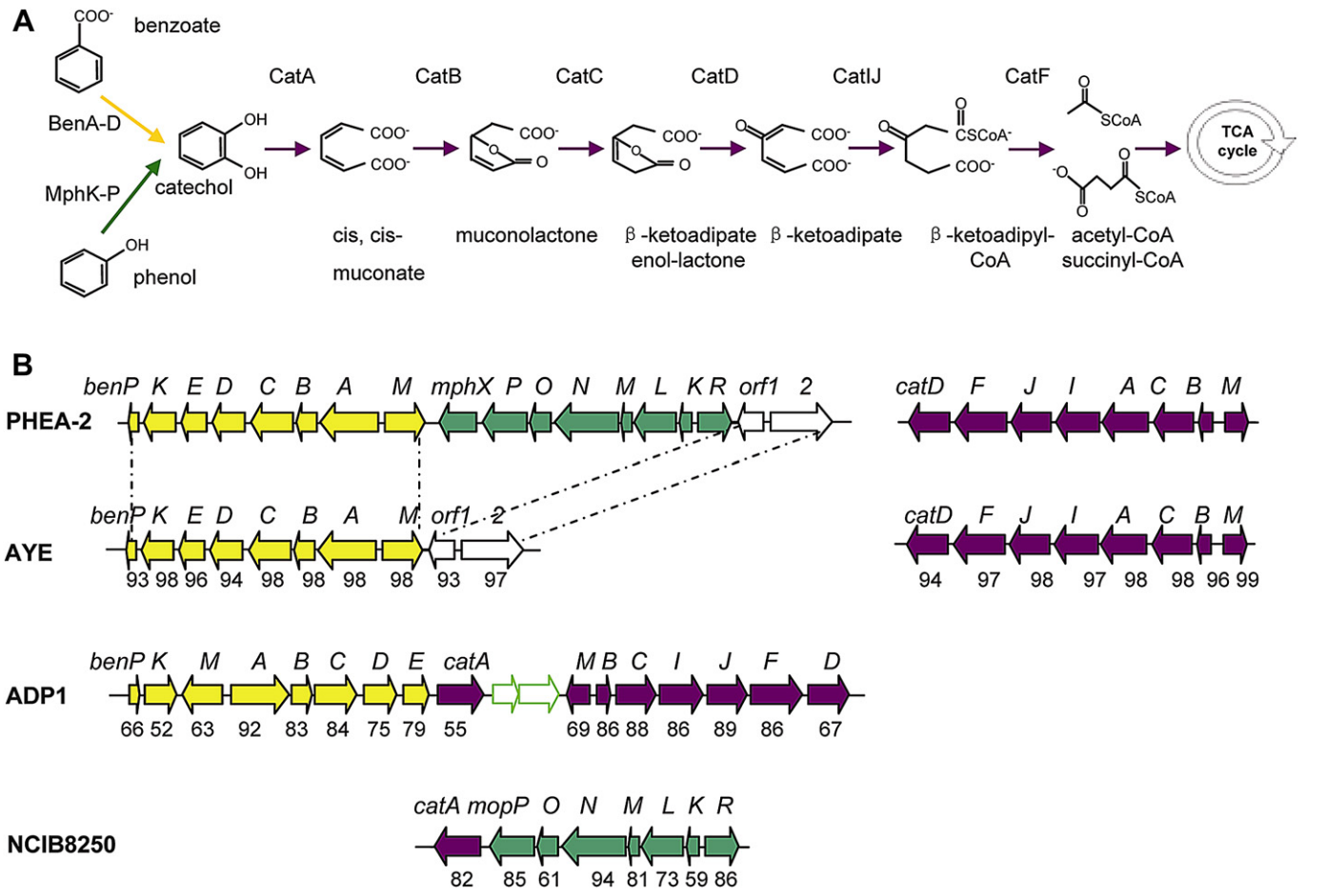

Fig. 3. Catabolic pathways for phenol and benzoate degradation in *Acinetobacter* strains. (A) Predicted biochemical steps of the catechol pathway in *A. calcoaceticus* PHEA-2. (B) Gene clusters for the degradation of phenol and benzoate in *A. calcoaceticus* PHEA-2, *A. baumannii* AYE, *A. calcoaceticus* NCIB8250 and *A. baylyi* ADP1, respectively. The *mph* gene cluster is inserted upstream of ORF1, a putative copper chaperone gene, in the highly conserved region which contains the *ben* gene cluster. The *mph* gene cluster is flanked by 6-bp direct repeats (CTCAAA), suggesting horizontal acquisition. Numbers beneath the arrows indicate the percentage of amino acid sequence identity between gene product and the equivalent protein of PHEA-2.

AYE (Adams et al., 2008; Fournier et al., 2006) (Table 3). No genes coding for toxins are detected in *A. calcoaceticus* or *A. baumannii*. PHEA-2 lacks genes for practically all known host-related virulence traits, such as type III and type IV secretion systems or hydrolytic enzymes while, as reported before, *A. baumannii*, contains a Type IV secretion apparatus similar to those proven to be important for virulence in the Legionella/Coxiella system (Smith et al., 2007).

A. baumannii AYE was found to carry an 86-kb island containing 45 genes conferring resistance to different classes of antibiotics (Fournier et al., 2006). In contrast, PHEA-2 was sensitive to most antibiotics. Thus, as expected, most of the resistance genes identified in *A. baumannii* were absent in the PHEA-2 genome except for *ampC* (resistance to beta-lactams), *parC* and *gyrA* (resistance to fluoroquinolones) and the putative aminoglycoside adenylyltransferase (resistance to aminoglycosides) (Table 3) (Fournier et al., 2006). Furthermore, the AYE resistance island encodes a complete operon associated with arsenic resistance (Fournier et al., 2006), which is found in many Gram-negative clinical strains and in environmental isolates (Misra, 1992; Saltikov and Olson, 2002). PHEA-2 was found to carry a defective arsenic resistance operon that lacks the essential *arsH* gene (Table 4). While genes associated with acriflavin-resistance are found in both PHEA-2 and AYE, no corresponding genes are detected in ADP1. Although PHEA-2 is sensitive to most antibiotics, we identified 12 genes conferring resistance to heavy metals including copper, cobalt, tellurite and arsenate. Resistance to heavy metal toxicity is important for microbes inhabiting contaminated sites.

3.3. Phenol degradation

The main routes for degradation of aromatic compounds found in PHEA-2 (i.e. the catechol (*cat*), protocatechuate (*pca*), phenylacetate (*pha*) or benzoate (*ben*)), are similar to those previously described in the soil-living *A. baylyi* ADP1 (Barbe et al., 2004) (Fig. S3). The clustering of these catabolic genes and their high G + C (47.4%, 50.8%, 51.4% and 49.1%) support the hypothesis that the four catabolic regions in the PHEA-2 genome were acquired by HGT (Fig. 1).

Phenol degradation is an unusual feature of *Acinetobacter* species and this raises a question of the origin of genes responsible for phenol catabolism. Only one other strain, *A. calcoaceticus* NCIB8250, was reported to utilize phenol

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Table 2
Competence genes of *A. calcoaceticus* PHEA-2.

Gene	Predicted function	PHEA-2 locus	ADP1 locus	AYE locus
comL	DNA uptake lipoprotein	BDGL000107	ACIAD2898	ABAYE2974
pilU	Twitching motility protein	BDGL000160	ACIAD0911	ABAYE2919
pilT	Twitching motility protein	BDGL000161	ACIAD0912	ABAYE2918
pilZ	Type IV pilus biogenesis protein	BDGL000949	ACIAD2360	ABAYE2074
comEC	DNA internalization-related competence protein	BDGL002047	No	ABAYE2047
pilJ	Type IV pilus biogenesis protein	BDGL002264	ACIAD0789	ABAYE0670
pilI	Twitching motility protein	BDGL002265	ACIAD0788	ABAYE0669
pilH	Twitching motility protein	BDGL002266	ACIAD0787	ABAYE0668
pilG	Twitching motility protein	BDGL002267	ACIAD0786	ABAYE0667
filmT	Putative type IV fimbrial biogenesis protein	BDGL002303	No	ABAYE0639
comF	Putative DNA transformation protein	BDGL002557	ACIAD2898	ABAYE0393
pilE	Tfp pilus assembly protein	BDGL002629	ACIAD3314	ABAYE0320
comE	Pilin a like competence factor	BDGL002630	ACIAD3315	ABAYE0319
comC	Putative competence factor involved in DNA binding and uptake	BDGL002631	ACIAD3316	ABAYE0318
pilX	Putative type IV fimbrial biogenesis protein	BDGL002632	ACIAD3317	ABAYE0317
comB	Possible pilus assembly protein	BDGL002633	ACIAD3318	ABAYE0316
pilV	Type IV fimbrial biogenesis protein	BDGL002634	ACIAD3319	ABAYE0315
pilA	Tfp pilus assembly protein	BDGL002647	No	No
comQ	Fimbrial assembly protein	BDGL002657	ACIAD3355	ABAYE0294
comL	Putative lipoprotein	BDGL002658	ACIAD3356	ABAYE0293
comO	Putative lipoprotein	BDGL002659	ACIAD3357	ABAYE0292
comN	Putative lipoprotein	BDGL002660	ACIAD3359	ABAYE0291
comM	Putative lipoprotein	BDGL002661	ACIAD3360	ABAYE0290
pilD	Type 4 prepilin-like proteins leader peptide processing enzyme	BDGL003237	ACIAD0360	ABAYE3446
pilC	Type IV fimbrial assembly protein	BDGL003239	ACIAD0361	ABAYE3445
pilB	Type IV-A pilus assembly	BDGL003240	ACIAD0362	ABAYE3444
pilF	Type IV fimbrial biogenesis protein	BDGL003412	ACIAD0558	ABAYE3265
smf	Putative protein involved in DNA uptake	BDGL002087	ACIAD0209	ABAYE3707
comEA	Putative DNA uptake protein	BDGL003489	No	No
comM	Magnesium chelatase, competence related protein	BDGL003129	ACIAD0242	ABAYE3668

(Schirmer et al., 1997). The general organization of phenol degradation genes in PHEA-2 shows a high degree of similarity to that of *A. calcoaceticus* NCIB8250 (Xu et al., 2003), except that the *mphX* gene downstream from the *mphP* gene in PHEA-2 is absent in NCIB8250 (Fig. 3). It is likely that the phenol degradation gene cluster was acquired by horizontal transfer from a common ancestor and that the *mphX* gene was subsequently lost by NCIB8250. The *mphX* gene is transcribed in the same direction as *mphKLMNOP* and encodes a protein with 293 amino acid residues showing weak identity with some unknown proteins encoded in the meta-cleavage pathway gene clusters for aromatic compound degradation. Our results indicated involvement of a novel repressor protein MphX in transcriptional regulation of phenol hydroxylase genes caused by a XylR/DmpR-type regulator MphR (Yu et al., 2011).

The *mph* catabolic island in PHEA-2 was inserted into a pre-existing catabolic island carrying the *ben* cluster, encoding for benzoate degradation. Interestingly, *ben* cluster regions in both PHEA-2 and AYE were very similar. Insertion of the 8-kb *mph* gene cluster, flanked by a 6-bp direct repeat, occurred between genes encoding the transcriptional activator BenM and a putative copper chaperone (Fig. 3B). As previously pointed out, soil is presumed to be the primary niche of Acinetobacter (Munoz-Price and Weinstein, 2008). Data reported here support this hypothesis and strongly suggest that the three *Acinetobacter* species evolved from a soil-living ancestor and consecutively acquired the genes responsible for aromatic compound degradation via HGT. We inferred that the *mph* gene cluster of PHEA-2 emerged as a result of adaptation to extreme and hostile modern wastewater environments.

Hence, the *mph* gene cluster may be an interesting case for further study of the evolutionary and regulatory mechanisms of phenol degradation.

3.4. Multiplicity of transport systems

Consistent with its metabolic versatility and environmental adaptability, *A. calcoaceticus* PHEA-2 possesses extensive transport capabilities. At least 443 genes encode transport-related proteins for aromatic compounds, amino acid, nucleotides, carbohydrates, lipid and inorganic ions in PHEA-2. Most of these transport systems also exist in *A. baylyi* ADP1 and *A. baumannii* AYE. However, a relatively higher proportion of transport-related proteins (12% of total proteins) were found in PHEA-2 than in ADP1 (10%) and AYE (9.5%) (Vallenet et al., 2008).

At least 32 transporters belonging to five families plausibly associated with drug resistance were identified in the PHEA-2 genome, including major facilitator superfamily (MFS), drug/metabolite transporters (DMT), resistance-nodulation-cell division (RND), the ATP binding cassette (ABC), and

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Table 3
Various antibiotic resistance genes in *A. baumannii* AYE and *A. calcoaceticus* PHEA-2.

Antibiotic class	Gene	Predicted specificity	AYE locus	PHEA-2 locus (identity)
Beta-lactams	*veb-1*	All bla except carb	ABAYE3623	No
	ampC	All bla except ctx, caz, fep	ABAYE1110	BDGL001854 (95%)
	oxa-10	All bla except esc, carb	ABAYE3619	No
	oxa-69	Unknown	ABAYE2122	No
Aminoglycosides	*aadA1*	Stre, spe	ABAYE3618	No
	aadDA1	Stre, spe	ABAYE3570	No
	aadB	Gen, kan, tob	ABAYE3622	No
	aadA	Unknown	ABAYE3739	BDGL003056 (95%)
	aphA1	Amikacin	ABAYE3578	No
	strA	Stre	ABAYE3648	No
	strB	Stre	ABAYE3647	No
Fluoroquinolones	*parC*	All flu	ABAYE3679	BDGL003117 (96%)
	gyrA	All flu	ABAYE0867	BDGL002058 (97%)
Tetracyclines	*tetA*	All tet	ABAYE3597	No
	tetR	All tet	ABAYE3598	No
	tetA	All tet	ABAYE3637	No
	tetR	All tet	ABAYE3639	No
	tetA	All tet	ABAYE0369	No
DHFR inhibitor	*dhfrI*	Tri	ABAYE3644	No
	dhfrX	Tri	ABAYE3614	No
Chloramphenicol	*cmlA*	Clo	ABAYE3620	No
	cmlA5	Clo	ABAYE3640	No
Rifampin	*arr-2*	Rifampin	ABAYE3621	No
Sulfonamides	*sulI*	All sulfonamides	ABAYE3612	No

Abbreviation: bla, beta-lactams; carb, carbapenems; caz, ceftazidime; clo, chloramphenicol; ctx, ceftriaxone; esc, extended-spectrum cephalosporines; fep, cefepime; flu, fluoroquinolones; gen, gentamicin; kan, kanamycin; spe, spectionmycin; stre, streptomycin; tet, tetracyclines; tob, tobramycin; tri, trimethoprim; DHFR, dihydrofolate reductase.

Table 4
Genes of *A. baumannii* AYE and *A. calcoaceticus* PHEA-2 associated with resistance to antiseptics.

Antiseptic class	Gene	Predicted specificity	AYE locus	PHEA-2 locus (identity)
Heavy metals (arsenic, mercury)	Arsenic resistance operon	Arsenic, antimony		
	arsB		ABAYE3659	BDGL000837 (89%)
	arsC		ABAYE3658	BDGL000835 (93%)
	arsH		ABAYE3660	No
	arsR		ABAYE3657	BDGL000836 (59%)
	Mercury resistance operon	Mercury		
	merA		ABAYE3605	No
	merC		ABAYE3604	No
	merD		ABAYE3606	No
	merE		ABAYE3607	No
	merR		ABAYE3601	No
Other heavy metals	*czcD* (Co/Zn/Cd efflux system)		ABAYE0272	BDGL002678 (97%)

membrane fusion proteins (MFP) (Fig. S4). PHEA-2 also carried a number of habitat-related transport systems. They include three Na^+-driven multidrug efflux pumps, as well as several systems for the detoxification of compounds like arsenate, cadmium, copper and other heavy metals. The PHEA-2 genome encoded at least three systems for the uptake and biosynthesis of osmoprotectants such as glycine-betaine, carnitine, choline, and betaine (*betA*, and *betB*). Presumably, these osmoprotectants accumulate in the cytoplasm in response to osmotic stress. They cannot be used as carbon sources due to the lack of the genes necessary for their catabolism. In the PHEA-2 genome, at least one ABC transporter (*ttgABCD*) is similar to efflux pumps for organic solvents in *P. putida* DOT-T1E, suggesting a physiological role in the efflux of toxic substrates or metabolites that may accumulate in the cell (Rojas et al., 2001).

PHEA-2 carries two systems for iron transport, one based on siderophore-binding and the other on heme binding. Both systems were probably acquired by HGT, since they had a higher G + C content. The histamine-derived siderophore is also present in *A. baumannii* AYE but not in *A. baylyi* ADP1 (Vallenet et al., 2008). As shown in Fig. 4A, the 26 kb region (BDGL001860-001879) from strain PHEA-2 had the same gene order and very similar protein sequences as the corresponding region of *A. baumannii* (ABAYE1085-1104) (Vallenet et al., 2008). This siderophore is composed of histamine (*bas* and *hdc* genes) and siderophore transporters (*bar* and *bau* genes). The histamine moiety of the siderophore results from histidine decarboxylation which is catalyzed by the histidine decarboxylase encoded by the *hdc* gene in PHEA-2 and AYE. *A. baylyi*, which lacks the histamine siderophore, carries a transposase-flanked gene cluster encoding a siderophore composed of 2, 3-dihydroxybenzoic acid, serine, threonine or cysteine and possibly other unidentified elements, and therefore obviously different from the histamine siderophore of PHEA-2 (Fig. 4A) (Vallenet et al., 2008). PHEA-2 also contains a second iron acquisition system (BDGL000189-000196) similar to the heme acquisition system already described in *A. baumannii* strain SDF (ABSDF2280-2288), but absent from *A. baumannii* AYE and *A. baylyi* ADP1 (Fig. 4B) (Vallenet et al., 2008).

The multiplicity of transport systems noted in *A. calcoaceticus* PHEA-2 is probably of importance for its persistence in an industrial wastewater environment where nutrients are scarce and xenobiotic pollutants are diverse.

3.5. Genes possibly involved in the colonization of sewage environments

Strain PHEA-2 carries several genes that seem to play a role in biofilm formation, which is important for the colonization at the organic–water interfaces in sewage environments. We identified 38 genes including three genes encoding type I fimbriae assembly proteins, and 23 genes encoding type IV pili assembly proteins. It has been reported that type IV pili, flagella,

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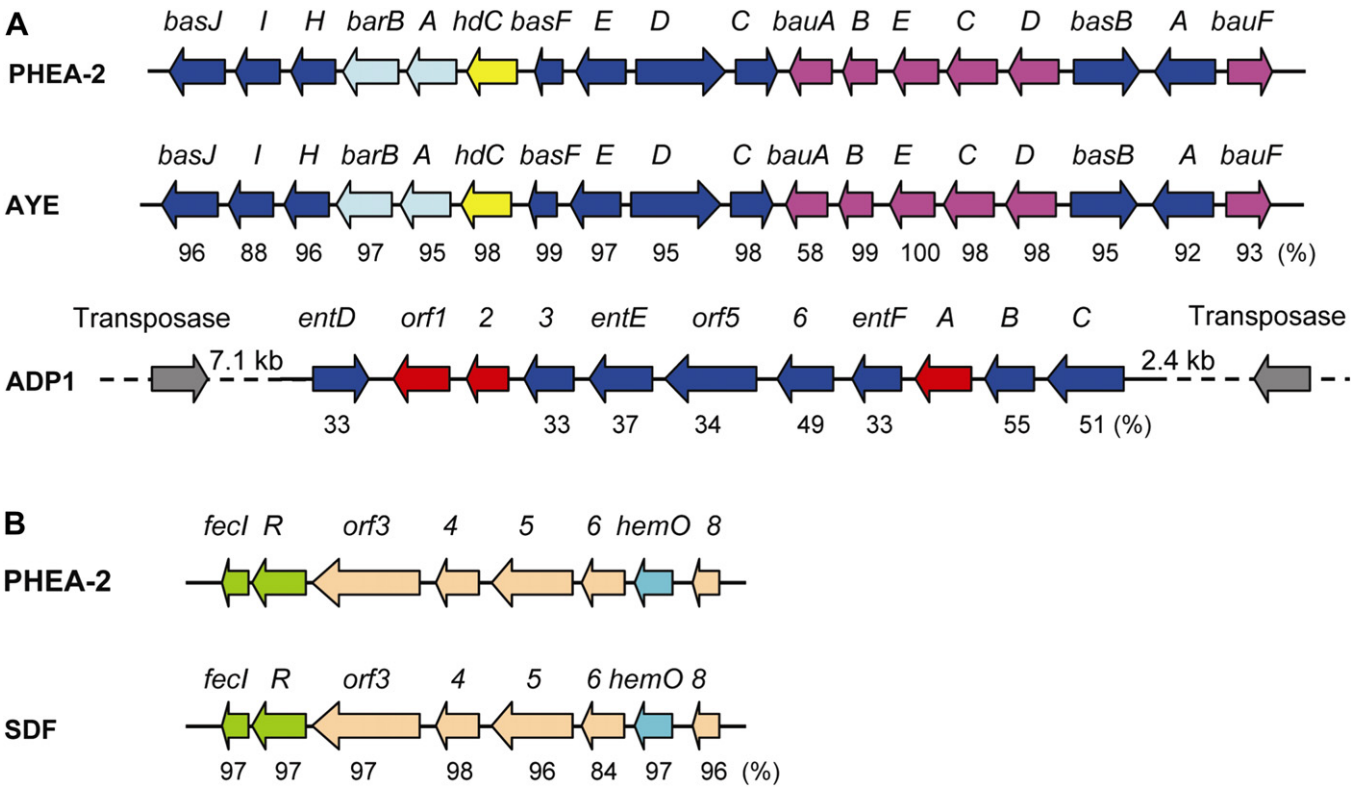

Fig. 4. Genetic organization of the gene clusters for (A) siderophore biosynthesis and transport and (B) haeme acquisition in *A. calcoaceticus* PHEA-2 in comparisons with the equivalent clusters from the other *Acinetobacter* strains. Numbers beneath the arrows indicate the percentage of amino acid sequence identity compared to the equivalent protein from PHEA-2. Corresponding operons have the same color.

or fimbriae is important for biofilm formation and type I fimbriae is also necessary for early biofilm formation (Pratt and Kolter, 1998; Vidal et al., 1996). Expression of chaperone usher secretion (*csu*) systems is required for pili formation and the concomitant attachment to plastic surfaces ensuring the formation of biofilm in *A. baumannii* (Tomaras et al., 2003). Three *csu* groups of genes have also been identified in PHEA-2. Apart from the *csu* systems, two-component systems involved in biofilm formation and diverse regulation networks (such as catabolite repression control) are also present in PHEA-2 (Bleichrodt et al., 2010).

Strain PHEA-2 shares in common with *A. baumannii* an operon encoding an AHL synthase and a LuxR family transcriptional regulator that could be involved in quorum sensing and biofilm formation (Kuchma and O'Toole, 2000; Rashid et al., 2000). This gene cluster does not exist in *A. baylyi* ADP1. Interestingly, PHEA-2 and *A. baumannii* AYE also contain an AHL lactonase gene that may be involved in the degradation of AHL signals.

3.6. Conclusion

In general, industrial wastewater is an extreme and hostile habitat where various xenobiotic compounds, including heavy metals, have toxic effects on microbial activity (Hu et al., 2005). Analysis of the complete genome sequence of PHEA-2 revealed the presence of genes whose functions are compatible with tolerance to various stresses. The *A. calcoaceticus* PHEA-2 genome shares high synteny with *A. baumannii*. It carries several laterally acquired regions for survival in hostile environments, but lacks most of the MDR and other pathogenicity determinants found in *A. baumannii*. Significant differences were noted among the genes that encode proteins involved in catabolic pathways, transport systems, surface appendages biosynthesis. The PHEA-2 genome carries a set of genes sharing similarity with known habitat-related stress-response genes and which give an advantage for the survival in phenol-polluted, low-nutrient wastewater. These findings may have significant potential for biotechnological applications.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.resmic.2011.10.006.

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References

Adams, M.D., Goglin, K., Molyneaux, N., Hujer, K.M., Lavender, H., Jamison, J.J., MacDonald, I.J., Martin, K.M., Russo, T., Campagnari, A.A., Hujer, A.M., Bonomo, R.A., Gill, S.R., 2008. Comparative genome sequence analysis of multidrug-resistant *Acinetobacter baumannii*. J. Bacteriol. 190, 8053–8064.

Barbe, V., Vallenet, D., Fonknechten, N., Kreimeyer, A., Oztas, S., Labarre, L., Cruveiller, S., Robert, C., Duprat, S., Wincker, P., Ornston, L.N., Weissenbach, J., Marliere, P., Cohen, G.N., Medigue, C., 2004. Unique features revealed by the genome sequence of *Acinetobacter* sp. ADP1, a versatile and naturally transformation competent bacterium. Nucleic Acids Res. 32, 5766–5779.

Bleichrodt, F.S., Fischer, R., Gerischer, U.C., 2010. The ß-ketoadipate pathway of *Acinetobacter baylyi* undergoes carbon catabolite repression, cross-regulation and vertical regulation, and is affected by Crc. Microbiology 156, 1313–1322.

Diancourt, L., Passet, V., Nemec, A., Dijkshoorn, L., Brisse, S., 2010. The population structure of *Acinetobacter baumannii*: expanding multiresistant clones from an ancestral susceptible genetic pool. PLoS One 5, e10034.

Dolzani, L., Tonin, E., Lagatolla, C., Prandin, L., Monti-Bragadin, C., 1995. Identification of *Acinetobacter* isolates in the *A. calcoaceticus-A. baumannii* complex by restriction analysis of the 16S-23S rRNA intergenic-spacer sequences. J. Clin. Microbiol. 33, 1108–1113.

Fournier, P.E., Vallenet, D., Barbe, V., Audic, S., Ogata, H., Poirel, L., Richet, H., Robert, C., Mangenot, S., Abergel, C., Nordmann, P., Weissenbach, J., Raoult, D., Claverie, J.M., 2006. Comparative genomics of multidrug resistance in *Acinetobacter baumannii*. PLoS Genet. 2, e7.

Friedrich, A., Hartsch, T., Averhoff, B., 2001. Natural transformation in mesophilic and thermophilic bacteria: identification and characterization of novel, closely related competence genes in *Acinetobacter* sp. strain BD413 and *Thermus thermophilus* HB27. Appl. Environ. Microbiol. 67, 3140–3148.

Gerner-Smidt, P., Tjernberg, I., Ursing, J., 1991. Reliability of phenotypic tests for identification of *Acinetobacter* species. J. Clin. Microbiol. 29, 277–282.

Hu, P., Brodie, E.L., Suzuki, Y., McAdams, H.H., Andersen, G.L., 2005. Whole-genome transcriptional analysis of heavy metal stresses in *Caulobacter crescentus*. J. Bacteriol. 187, 8437–8449.

Juni, E., 1978. Genetics and physiology of Acinetobacter. Annu. Rev. Microbiol. 32, 349–371.

Kuchma, S.L., O'Toole, G.A., 2000. Surface-induced and biofilm-induced changes in gene expression. Curr. Opin. Biotechnol. 11, 429–433.

Metzgar, D., Bacher, J.M., Pezo, V., Reader, J., Doring, V., Schimmel, P., Marliere, P., de Crecy-Lagard, V., 2004. *Acinetobacter* sp. ADP1: an ideal model organism for genetic analysis and genome engineering. Nucleic Acids Res. 32, 5780–5790.

Misra, T.K., 1992. Bacterial resistances to inorganic mercury salts and organomercurials. Plasmid 27, 4–16.

Munoz-Price, L.S., Weinstein, R.A., 2008. Acinetobacter infection. N. Engl. J. Med. 358, 1271–1281.

Palmen, R., Vosman, B., Buijsman, P., Breek, C.K., Hellingwerf, K.J., 1993. Physiological characterization of natural transformation in *Acinetobacter calcoaceticus*. J. Gen. Microbiol. 139, 295–305.

Pratt, L.A., Kolter, R., 1998. Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol. Microbiol. 30, 285–293.

Rashid, M.H., Rumbaugh, K., Passador, L., Davies, D.G., Hamood, A.N., Iglewski, B.H., Kornberg, A., 2000. Polyphosphate kinase is essential for biofilm development, quorum sensing, and virulence of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. 97, 9636–9641.

Rojas, A., Duque, E., Mosqueda, G., Golden, G., Hurtado, A., Ramos, J.L., Segura, A., 2001. Three efflux pumps are required to provide efficient tolerance to toluene in *Pseudomonas putida* DOT-T1E. J. Bacteriol. 183, 3967–3973.

Saltikov, C.W., Olson, B.H., 2002. Homology of *Escherichia coli* R773 *arsA, arsB*, and *arsC* genes in arsenic-resistant bacteria isolated from raw sewage and arsenic-enriched creek waters. Appl. Environ. Microbiol. 68, 280–288.

Schirmer, F., Ehrt, S., Hillen, W., 1997. Expression, inducer spectrum, domain structure, and function of MopR, the regulator of phenol degradation in *Acinetobacter calcoaceticus* NCIB8250. J. Bacteriol. 179, 1329–1336.

Smith, M.G., Gianoulis, T.A., Pukatzki, S., Mekalanos, J.J., Ornston, L.N., Gerstein, M., Snyder, M., 2007. New insights into *Acinetobacter baumannii* pathogenesis revealed by high-density pyrosequencing and transposon mutagenesis. Genes Dev. 21, 601–614.

Soledad Ramirez, M., Don, M., Merkier, A.K., Soler Bistué, A.J., Zorreguieta, A., Centrón, D., Tolmasky, M.E., 2010. A naturally competent *Acinetobacter baumannii* clinical isolate as a convenient model for genetic studies. J. Clin. Microbiol. 48, 1488–1490.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Tomaras, A.P., Dorsey, C.W., Edelmann, R.E., Actis, L.A., 2003. Attachment to and biofilm formation on abiotic surfaces by *Acinetobacter baumannii*: involvement of a novel chaperone-usher pili assembly system. Microbiology 149, 3473–3484.

Towner, K.J., 2009. Acinetobacter: an old friend, but a new enemy. J. Hosp. Infect. 73, 355–363.

Vallenet, D., Nordmann, P., Barbe, V., Poirel, L., Mangenot, S., Bataille, E., Dossat, C., Gas, S., Kreimeyer, A., Lenoble, P., Oztas, S., Poulain, J., Segurens, B., Robert, C., Abergel, C., Claverie, J.M., Raoult, D., Medigue, C., Weissenbach, J., Cruveiller, S., 2008. Comparative analysis of Acinetobacters: three genomes for three lifestyles. PLoS One 3, e1805.

van Dessel, H., Dijkshoorn, L., van der Reijden, T., Bakker, N., Paauw, A., van den Broek, P., Verhoef, J., Brisse, S., 2004. Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. Res. Microbiol. 155, 105–112.

Vidal, R., Dominguez, M., Urrutia, H., Bello, H., Gonzalez, G., Garcia, A., Zemelman, R., 1996. Biofilm formation by *Acinetobacter baumannii*. Microbios 86, 49–58.

Xu, Y.Q., Chen, M., Zhang, W., Lin, M., 2003. Genetic organization of genes encoding phenol hydroxylase, benzoate 1,2-dioxygenase alpha subunit and its regulatory proteins in *Acinetobacter calcoaceticus* PHEA-2. Curr. Microbiol. 46, 235–240.

Young, D.M., Parke, D., Ornston, L.N., 2005. Opportunities for genetic investigation afforded by *Acinetobacter baylyi*, a nutritionally versatile bacterial species that is highly competent for natural transformation. Annu. Rev. Microbiol. 59, 519–551.

Yu, H.Y., Peng, Z.X., Zhan, Y.H., Wang, J., Yan, Y.L., Chen, M., Lu, W., Ping, S.Z., Zhang, W., Zhao, Z.L., Li, S.Y., Takeo, M., Lin, M., 2011. Novel regulator MphX represses activation of phenol hydroxylase genes caused by a XylR/DmpR-type regulator MphR in *Acinetobacter calcoaceticus*. PLoS One 6, e17350.

Zhan, Y.H., Yu, H.Y., Yan, Y.L., Chen, M., Lu, W., Li, S.Y., Peng, Z.X., Zhang, W., Ping, S.Z., Wang, J., Lin, M., 2008. Genes involved in the benzoate catabolic pathway in *Acinetobacter calcoaceticus* PHEA-2. Curr. Microbiol. 57, 609–614.

Zhan, Y.H., Yu, H.Y., Yan, Y.L., Ping, S.Z., Lu, W., Zhang, W., Chen, M., Lin, M., 2009. Benzoate catabolite repression of the phenol degradation in *Acinetobacter calcoaceticus* PHEA-2. Curr. Microbiol. 59, 368–373.

Zhan, Y.H., Yan, Y.L., Zhang, W., Yu, H.Y., Chen, M., Lu, W., Ping, S.Z., Peng, Z.X., Yuan, M.L., Zhou, Z.F., Elmerich, C., Lin, M., 2011. Genome sequence of *Acinetobacter calcoaceticus* PHEA-2, isolated from industry wastewater. J. Bacteriol. 193, 2672–2673.

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Supplementary materials for online publication

Fig. S1 Synteny between the three *Acinetobacter* strains. The program of MUMmer software was used to compare amino acid sequences of the predicted genes. Five syntenous genes in a row produced a single dot on the graph. The start of the PHEA-2 genome was adjusted to make it begin with *dnaA* in accordance with the others.

Fig. S2 Unrooted 16S rRNA gene sequence-based dendrogram, revealing the relationships between the *Acinetobacter* genome sequences in GenBank. Cluster analysis based on the neighbor-joining method was performed using Genebase (Applied Maths) using an open gap penalty of 100% and a unit gap cost of 25%. Underlining indicates completed genome sequences.

Fig. S3 Predicted biochemical steps for the catabolism of aromatic compounds in *A. calcoaceticus* PHEA-2. The three central aromatic intermediates, protocatechuate, catechol and phenylacetate, are boxed.

Fig. S4 Comparison of the numbers of predicted drug efflux proteins in *A. calcoaceticus* PHEA-2, *A. baumannii* AYE and *A. baylyi* ADP1. Transport proteins were classified using the TransAAP tool (http://www. membranetransport. org/) which is based on the TransportDB program. Genetically associated membrane fusion or outer membrane proteins were not included in the graph, and only homologues of known multidrug efflux transporters were counted. MFS, major facilitator superfamily; DMT, drug/metabolite transporter superfamily; ABC, ATP-binding cassette superfamily; RND, resistance-nodulation-cell division superfamily; MFP, membrane fusion protein superfamily. The total number of transport proteins is given in parentheses after the strain designation.

Table S1. Genes present in PHEA-2 but absent in ADP1 and AYE

Gene ID	Gene name	Size (bp)	Product	Best match (% nucleotide identity)
BDGL000001		2243	hypothetical protein	*A. baumannii* SDF (92%)
BDGL000002		1025	hypothetical protein	*A. baumannii* SDF (90%)
BDGL000006		623	hypothetical protein	No hits
BDGL000007		347	hypothetical protein	No hits
BDGL000008		371	hypothetical protein	No hits
BDGL000009	*tnpR*	632	hypothetical protein	*A. baumannii* SDF (88%)
BDGL000010		584	hypothetical protein	No hits
BDGL000012		134	hypothetical protein	*Acinetobacter* sp. DR1 (92%)
BDGL000038		170	hypothetical protein	*A. baumannii* SDF (86%)
BDGL000046		347	hypothetical protein	*Acinetobacter* sp. DR1 (91%)
BDGL000141		539	hypothetical protein	*A. baumannii* ACICU (93%)
BDGL000178		434	hypothetical protein	No hits
BDGL000179		911	hypothetical protein	No hits
BDGL000181		875	hypothetical protein	*A. baumannii* SDF (94%)
BDGL000182		527	hypothetical protein	No hits
BDGL000183		617	hypothetical protein	No hits
BDGL000237		119	hypothetical protein	*A. baumannii* AB0057 (99%)
BDGL000260	*fepA*	143	hypothetical protein	*Acinetobacter* sp. DR1 (98%)

BDGL000271		128	hypothetical protein	*Acinetobacter* sp. DR1 (92%)
BDGL000295		458	hypothetical protein	*A. baumannii* ATCC17978 (98%)
BDGL000306		167	hypothetical protein	*Acinetobacter* sp. DR1 (92%)
BDGL000316		113	hypothetical protein	*Acinetobacter* sp. DR1 (89%)
BDGL000349		317	hypothetical protein	*Acinetobacter* sp. DR1 (94%)
BDGL000351	*yjgN*	1088	hypothetical protein	*Acinetobacter* sp. DR1 (95%)
BDGL000353		923	hypothetical protein	*Acinetobacter* sp. DR1 (93%)
BDGL000354		470	hypothetical protein	*Acinetobacter* sp. DR1 (93%)
BDGL000358		389	hypothetical protein	*Acinetobacter* sp. DR1 (74%)
BDGL000363		707	hypothetical protein	*A. baumannii* AB307-0294 (95%)
BDGL000372		452	hypothetical protein	*A. baumannii* 17978 (93%)
BDGL000373		200	hypothetical protein	No hits
BDGL000374		554	hypothetical protein	No hits
BDGL000434		119	hypothetical protein	*Acinetobacter* sp. DR1 (95%)
BDGL000448		374	hypothetical protein	No hits
BDGL000467		128	hypothetical protein	*Acinetobacter* sp. DR1 (96%)
BDGL000469	*mphX*	881	hypothetical protein	*Acinetobacter* sp. *DR1* (94%)
BDGL000470	*mphP*	1061	phenol 2-monooxygenase	*A.calcoaceticus* NCIB8250 (84%)
BDGL000471	*mphO*	362	phenol hydroxylase P4 protein	*A.calcoaceticus* NCIB8250 (61%)
BDGL000472	*mphN*	1526	phenol hydroxylase P3 protein	*A.calcoaceticus* NCIB8250 (94%)

BDGL000473	*mphM*	266	phenol hydroxylase P2 protein	*A.calcoaceticus* NCIB8250 (81%)
BDGL000474	*mphL*	998	phenol 2-monooxygenase	*A.calcoaceticus* NCIB8250 (72%)
BDGL000475		116	hypothetical protein	*Acinetobacter* sp. DR1 (94%)
BDGL000476	*mphK*	287	phenol 2-monooxygenase	*A.calcoaceticus* NCIB8250 (59%)
BDGL000477	*mphR*	1670	activator of phenol-degradative genes	*A.calcoaceticus* NCIB8250 (86%)
BDGL000504		131	hypothetical protein	*Acinetobacter* sp. DR1 (97%)
BDGL000511		362	hypothetical protein	*Acinetobacter* sp. DR1 (90%)
BDGL000514		404	hypothetical protein	*Acinetobacter* sp. DR1 (83%)
BDGL000518		860	modification methylase	*A. baumannii* ACICU (90%)
BDGL000519		1430	hypothetical protein	No hits
BDGL000546		116	hypothetical protein	*A. baumannii* AB307-0294 (98%)
BDGL000550		146	hypothetical protein	*A. baumannii* AB307-0294 (100%)
BDGL000556		128	hypothetical protein	No hits
BDGL000572	*iolE*	1052	apurinic/apyrimidinic endonuclease	*Acinetobacter* sp. DR1 (94%)
BDGL000579		371	hypothetical protein	No hits
BDGL000584	*ytfG*	950	probable nucleoside-diphosphate-sugar epimerase	*Acinetobacter* sp. DR1 (85%)
BDGL000586		692	hypothetical protein	*A. baumannii* 17978 (88%)

BDGL000590		962	hypothetical protein	*Acinetobacter* sp. DR1 (89%)
BDGL000605		1910	hypothetical protein	No hits
BDGL000616		1868	hypothetical protein	No hits
BDGL000617		125	hypothetical protein	*Acinetobacter* sp. DR1 (97%)
BDGL000626		1433	hypothetical protein	*A. baumannii* 17978 (96%)
BDGL000627		1079	hypothetical protein	*A. baumannii* 17978 (85%)
BDGL000633		920	hypothetical protein	*Acinetobacter* sp. DR1 (97%)
BDGL000635		734	hypothetical protein	*Acinetobacter* sp. DR1 (99%)
BDGL000636		776	hypothetical protein	*Acinetobacter* sp. DR1 (98%)
BDGL000651		122	hypothetical protein	*Acinetobacter* sp. DR1 (97%)
BDGL000779		113	hypothetical protein	*Acinetobacter* sp. DR1 (97%)
BDGL000785		119	hypothetical protein	*A. baumannii* ACICU (98%)
BDGL000796		407	hypothetical protein	*Acinetobacter* sp. DR1 (94%)
BDGL000817		146	hypothetical protein	*Acinetobacter* sp. DR1 (95%)
BDGL000971	*hpA2*	404	similar to acetyltransferase	*Acinetobacter* sp. DR1 (90%)
BDGL000977		122	hypothetical protein	*A. baumannii* SDF (87%)
BDGL001025		116	hypothetical protein	No hits
BDGL001056		716	hypothetical protein	No hits
BDGL001099		245	hypothetical protein	No hits
BDGL001101		113	hypothetical protein	No hits
BDGL001102		191	hypothetical protein	No hits
BDGL001103		428	hypothetical protein	No hits

BDGL001104		131	hypothetical protein	No hits
BDGL001163		122	hypothetical protein	*Acinetobacter* sp. DR1 (99%)
BDGL001200		401	hypothetical protein	No hits
BDGL001201		599	hypothetical protein	No hits
BDGL001204		1562	hypothetical protein	No hits
BDGL001206		554	hypothetical protein	No hits
BDGL001221	*pinR*	581	sin; recombinase Sin	*A. baumannii* SDF (85%)
BDGL001226		248	hypothetical protein	No hits
BDGL001228		908	hypothetical protein	No hits
BDGL001267		545	hypothetical protein	No hits
BDGL001270		941	hypothetical protein	*Acinetobacter* sp. DR1 (91%)
BDGL001271	*frpA*	1445	bifunctional hemolysin-adenylate cyclase precursor	*Acinetobacter* sp. DR1 (88%)
BDGL001274		206	hypothetical protein	*A. baumannii* ACICU (98%)
BDGL001282		383	hypothetical protein	No hits
BDGL001284		113	hypothetical protein	No hits
BDGL001285		269	hypothetical protein	No hits
BDGL001286		140	hypothetical protein	*A. baunannii* AB307-0294 (99%)
BDGL001287		746	hypothetical protein	No hits
BDGL001289		611	hypothetical protein	No hits
BDGL001290		452	hypothetical protein	*A. baumannii* SDF (80%)

BDGL001291		4286	hypothetical protein	No hits
BDGL001304		368	hypothetical protein	No hits
BDGL001461		128	hypothetical protein	*Acinetobacter* sp. DR1 (93%)
BDGL001474		446	hypothetical protein	No hits
BDGL001496		116	hypothetical protein	*Acinetobacter* sp. DR1 (93%)
BDGL001498	*ocd2*	1013	ornithine cyclodeaminase	*Acinetobacter* sp. DR1 (94%)
BDGL001502	*garL*	674	HpcH/HpaI aldolase	*Acinetobacter* sp. DR1 (92%)
BDGL001528		632	hypothetical protein	*Acinetobacter* sp. DR1 (92%)
BDGL001529		1781	hypothetical protein	*Acinetobacter* sp. DR1 (92%)
BDGL001548		116	hypothetical protein	*Acinetobacter* sp. DR1 (88%)
BDGL001551		287	hypothetical protein	*Acinetobacter* sp. DR1 (91%)
BDGL001552		131	hypothetical protein	*Acinetobacter* sp. DR1 (91%)
BDGL001557		209	hypothetical protein	No hits
BDGL001558		572	putative lysozyme from bacteriophage	*A. baumannii* SDF (87%)
BDGL001596		230	hypothetical protein	*Acinetobacter* sp. DR1 (89%)
BDGL001685		1151	sterol desaturase	*Acinetobacter* sp. DR1 (93%)
BDGL001690		1829	hypothetical protein	*Acinetobacter* sp. DR1 (95%)
BDGL001693		1010	phosphoenolpyruvate carboxylase	*Acinetobacter* sp. DR1 (94%)
BDGL001697		1052	hypothetical protein	*Acinetobacter* sp. DR1 (95%)
BDGL001723		1685	aspartate/alanine exchanger	*A. baumannii* ACICU (94%)

			family protein	
BDGL001724	*aspC*	1598	aspartate aminotransferase	*Acinetobacter* sp. DR1 (92%)
BDGL001755		128	hypothetical protein	No hits
BDGL001779		170	hypothetical protein	*Acinetobacter* sp. DR1 (96%)
BDGL001801		161	hypothetical protein	*A. baumannii* SDF (99%)
BDGL001865		155	hypothetical protein	*A. baumannii* ACICU (98%)
BDGL001883		602	hypothetical protein	No hits
BDGL001884		1163	hypothetical protein	No hits
BDGL001885		938	hypothetical protein	No hits
BDGL001886		2027	hypothetical protein	No hits
BDGL001887		1355	hypothetical protein	No hits
BDGL001935		137	hypothetical protein	*Acinetobacter* sp. DR1 (92%)
BDGL001945		125	hypothetical protein	*Acinetobacter* sp. DR1 (96%)
BDGL001982		146	hypothetical protein	*Acinetobacter* sp. DR1 (95%)
BDGL002031		185	hypothetical protein	*Acinetobacter* sp. DR1 (94%)
BDGL002085		647	hypothetical protein	No hits
BDGL002087		743	hypothetical protein	*Acinetobacter* sp. DR1 (91%)
BDGL002088		338	hypothetical protein	No hits
BDGL002092		323	hypothetical protein	*Acinetobacter* sp. DR1 (96%)
BDGL002127		158	hypothetical protein	*Acinetobacter* sp. DR1 (90%)
BDGL002150		134	hypothetical protein	*Acinetobacter* sp. DR1 (99%)
BDGL002151		128	hypothetical protein	*Acinetobacter* sp. DR1 (99%)

BDGL002156		125	hypothetical protein	No hits
BDGL002170		632	hypothetical protein	*Acinetobacter* sp. DR1 (89%)
BDGL002171		809	hypothetical protein	*Acinetobacter* sp. DR1 (93%)
BDGL002172		1190	aspartate beta-hydroxylase	*Acinetobacter* sp. DR1 (92%)
BDGL002173		1493	hypothetical protein	*Acinetobacter* sp. DR1 (92%)
BDGL002194		1439	hypothetical protein	No hits
BDGL002195		671	hypothetical protein	No hits
BDGL002200		134	hypothetical protein	No hits
BDGL002206		191	hypothetical protein	*Acinetobacter* sp. DR1 (93%)
BDGL002232		113	hypothetical protein	No hits
BDGL002249		203	hypothetical protein	*A. baumannii* AB307-0294 (93%)
BDGL002284		116	hypothetical protein	*A. baumannii* AB307-0294 (78%)
BDGL002294	*lamB*	1181	maltoporin	*P. mendocina* YMP (52%)
BDGL002343	*yafK*	581	hypothetical protein	*Acinetobacter* sp. DR1 (89%)
BDGL002350		560	putative glutathione S-transferase	*A. baumannii* ACICU (93%)
BDGL002395		197	hypothetical protein	No hits
BDGL002404		401	hypothetical protein	No hits
BDGL002436		926	hypothetical protein	*Acinetobacter* sp. DR1 (90%)
BDGL002465		359	hypothetical protein	*Acinetobacter* sp. DR1 (92%)
BDGL002466		965	hypothetical protein	*Acinetobacter* sp. DR1 (88%)
BDGL002467		560	hypothetical protein	*Acinetobacter* sp. DR1 (92%)

BDGL002468		419	hypothetical protein	*Acinetobacter* sp. DR1 (89%)
BDGL002478		314	hypothetical protein	*A. baumannii* ACICU (95%)
BDGL002501		152	hypothetical protein	No hits
BDGL002546		152	hypothetical protein	*Acinetobacter* sp. DR1 (91%)
BDGL002566		209	hypothetical protein	No hits
BDGL002580		416	hypothetical protein	*Acinetobacter* sp. DR1 (93%)
BDGL002581		425	hypothetical protein	*A. baumannii* ACICU (86%)
BDGL002614		788	hypothetical protein	*Acinetobacter* sp. DR1 (97%)
BDGL002615		1493	hypothetical protein	No hits
BDGL002635		599	hypothetical protein	*A. baumannii* ATCC17978 (84%)
BDGL002639		155	hypothetical protein	*A. baumannii* AB307-0294 (92%)
BDGL002642		128	hypothetical protein	No hits
BDGL002646		1313	hypothetical protein	No hits
BDGL002683		140	hypothetical protein	No hits
BDGL002684	*czcN*	617	protein-S-isoprenylcysteine methyltransferase	No hits
BDGL002695		122	hypothetical protein	*Acinetobacter* sp. DR1 (98%)
BDGL002714		164	hypothetical protein	*A. baumannii* AB307-0294 (93%)
BDGL002720		458	hypothetical protein	*A. baumannii* ATCC17978 (92%)
BDGL002721		956	hypothetical protein	*A. baumannii* ATCC17978 (94%)
BDGL002725		353	hypothetical protein	No hits
BDGL002726	*fosA*	407	glutathione transferase FosA	*A. baumannii* ATCC17978 (84%)

BDGL002735		140	hypothetical protein	*A. baumannii* ATCC17978 (92%)
BDGL002792		278	hypothetical protein	No hits
BDGL002807		695	hypothetical protein	No hits
BDGL002808		767	hypothetical protein	No hits
BDGL002830		173	hypothetical protein	*Acinetobacter* sp. DR1 (99%)
BDGL002831	*yfhB*	656	hypothetical protein	*A. baumannii* ATCC17978 (90%)
BDGL002832		689	hypothetical protein	*A. baumannii* ATCC17978 (92%)
BDGL002833		2360	hypothetical protein	No hits
BDGL002834		563	hypothetical protein	No hits
BDGL002849		341	hypothetical protein	No hits
BDGL002850		1217	FmdA2; formamidase	*A. baumannii* ACICU (94%)
BDGL002908		122	hypothetical protein	*Acinetobacter* sp. DR1 (98%)
BDGL002960		125	hypothetical protein	*A. baumannii* SDF (98%)
BDGL002972	*ycjS*	950	hypothetical protein	*Acinetobacter* sp. DR1 (92%)
BDGL002978	*amsK*	1106	amylovoran biosynthesis glycosyl transferase AmsK	*Acinetobacter* sp. DR1 (99%)
BDGL002980		1064	hypothetical protein	*Acinetobacter* sp. DR1 (97%)
BDGL002981		1100	hypothetical protein	*Acinetobacter* sp. DR1 (96%)
BDGL002982	*lsgF*	830	LsgF; putative UDP-galactose--lipooligosaccharide galactosyltransferase	*Acinetobacter* sp. DR1 (96%)
BDGL002989	*cgmA*	1661	sulfatase	*Acinetobacter* sp. DR1 (94%)

BDGL002996		134	hypothetical protein	*Acinetobacter* sp. DR1 (92%)
BDGL003001		1607	hypothetical protein	No hits
BDGL003003		509	acetyltransferase	*A. baumannii* SDF (91%)
BDGL003008		800	hypothetical protein	No hits
BDGL003009		296	hypothetical protein	No hits
BDGL003010		515	hypothetical protein	No hits
BDGL003011		353	hypothetical protein	*Acinetobacter* sp. DR1 (86%)
BDGL003012		476	hypothetical protein	*A. baumannii* ATCC17978 (96%)
BDGL003014		503	hypothetical protein	*Acinetobacter* sp. DR1 (89%)
BDGL003104		203	hypothetical protein	No hits
BDGL003105	*creD*	1403	hypothetical protein	*E. coli* K12 (24%)
BDGL003108		116	hypothetical protein	*Acinetobacter* sp. DR1 (97%)
BDGL003123		188	hypothetical protein	No hits
BDGL003166		146	hypothetical protein	*Acinetobacter* sp. DR1 (97%)
BDGL003213		116	hypothetical protein	No hits
BDGL003215		125	hypothetical protein	No hits
BDGL003221		173	hypothetical protein	*Acinetobacter* sp. DR1 (95%)
BDGL003225		131	hypothetical protein	*Acinetobacter* sp. DR1 (93%)
BDGL003257	*glod5*	386	putative glyoxalase	*Acinetobacter* sp. DR1 (92%)
BDGL003298		128	hypothetical protein	*Acinetobacter* sp. DR1 (96%)
BDGL003323		1439	hypothetical protein	No hits
BDGL003324		746	SpoOM family protein	No hits

BDGL003396		119	acetate kinase	*Acinetobacter* sp. DR1 (97%)
BDGL003465		122	hypothetical protein	*A. baumannii* SDF (87%)
BDGL003467		395	hypothetical protein	*Acinetobacter* sp. DR1 (91%)
BDGL003477		1262	hypothetical protein	No hits
BDGL003503		188	hypothetical protein	*A. baumannii* AB307-0294 (93%)
BDGL003511		125	hypothetical protein	*Acinetobacter* sp. DR1 (98%))
BDGL003543	*bag*	440	DNA-directed RNA polymerase II largest subunit	*Acinetobacter* sp. DR1 (86%)
BDGL003570		659	hypothetical protein	*Acinetobacter* sp. DR1 (93%)
BDGL003579		1052	uncharacterized protein	No hits
BDGL003580		446	membrane protein TctB	No hits
BDGL003581		1499	protein of unknown function	No hits
BDGL003582	*abrB*	1046	Putative ammonia monooxygenase	*E. coli* K12 (24%)
BDGL003592		233	hypothetical protein	No hits
BDGL003593		158	hypothetical protein	No hits

Fig. S1

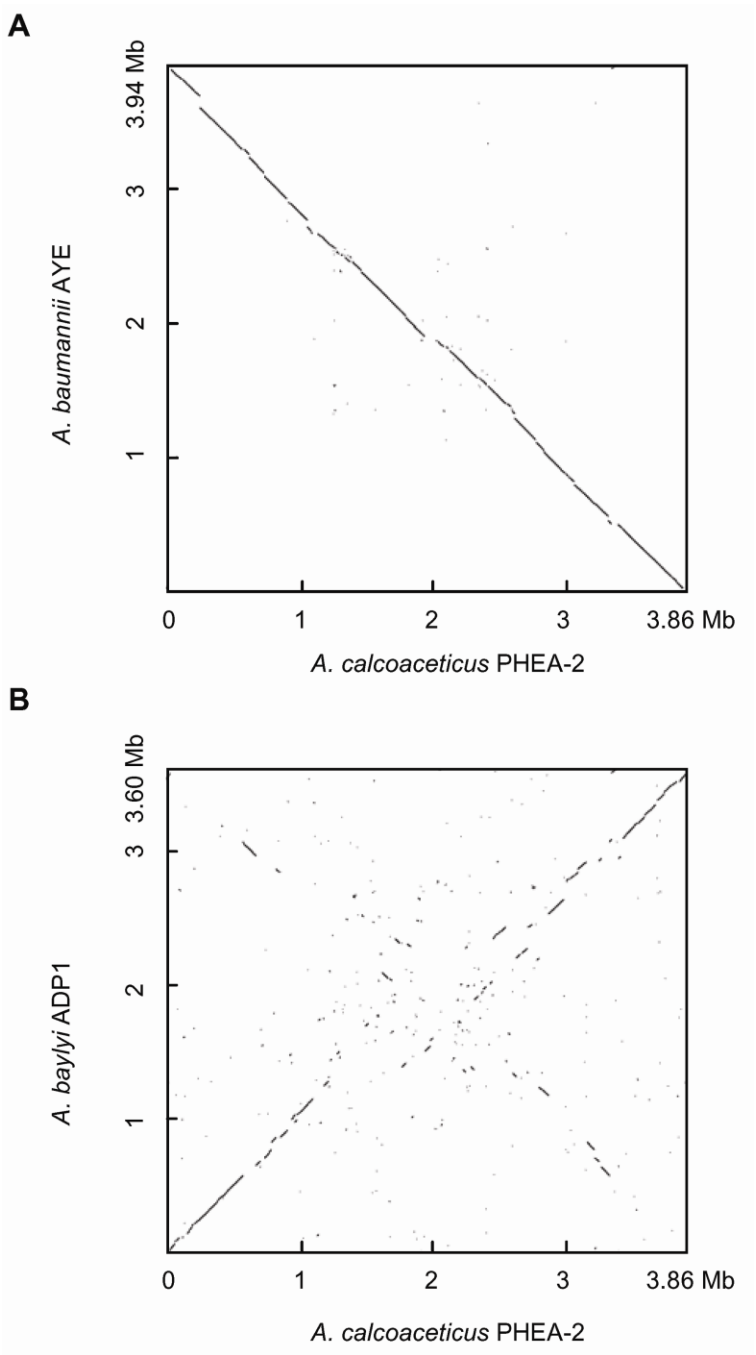

A
A. baumannii AYE
3.94 Mb
3
2
1
0
1
2
3
3.86 Mb
A. calcoaceticus PHEA-2
B
A. baylyi ADP1
3.60 Mb
3
2
1
0
1
2
3
3.86 Mb
A. calcoaceticus PHEA-2

Fig. S2

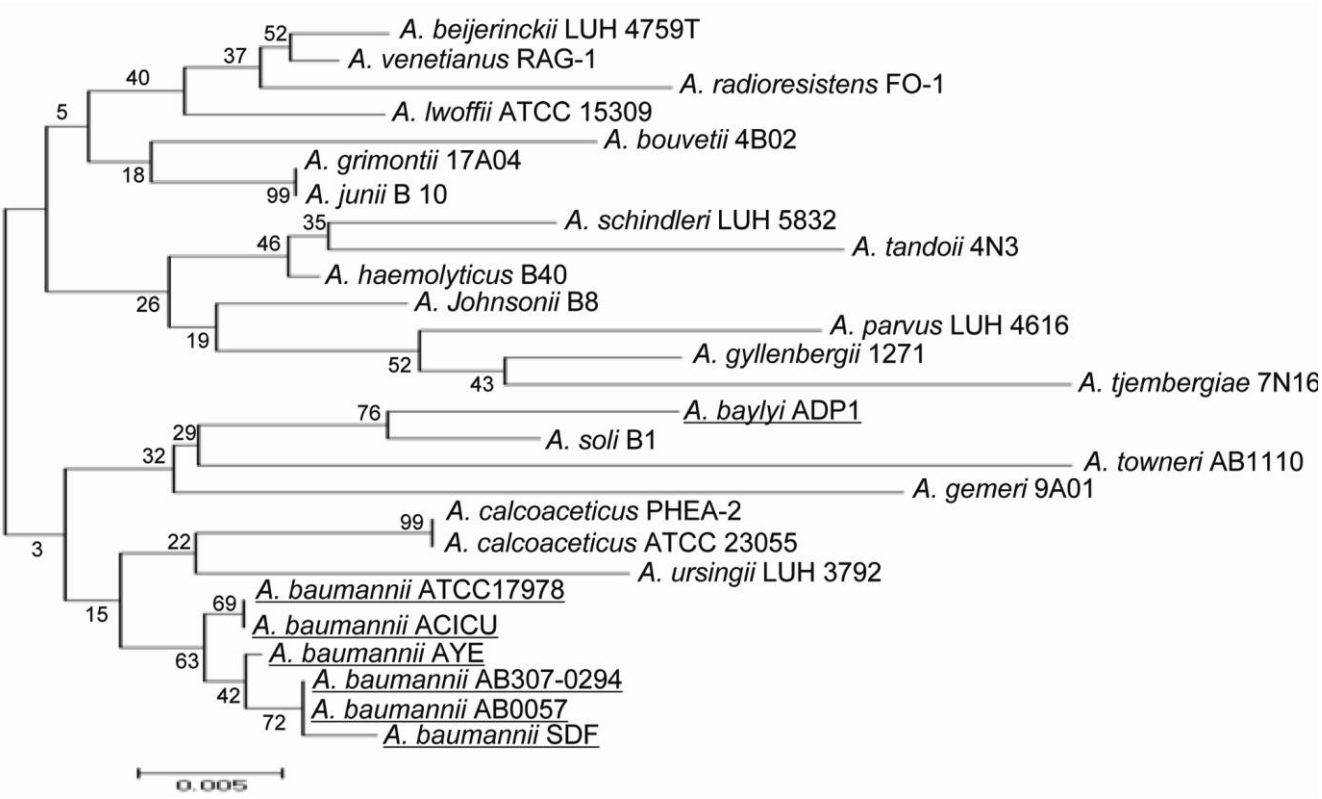

A. beijerinckii LUH 4759T
A. venetianus RAG-1
A. radioresistens FO-1
A. lwoffii ATCC 15309
A. bouvetii 4B02
A. grimontii 17A04
A. junii B 10
A. schindleri LUH 5832
A. tandoii 4N3
A. haemolyticus B40
A. Johnsonii B8
A. parvus LUH 4616
A. gyllenbergii 1271
A. tjembergiae 7N16
A. baylyi ADP1
A. soli B1
A. towneri AB1110
A. gemeri 9A01
A. calcoaceticus PHEA-2
A. calcoaceticus ATCC 23055
A. ursingii LUH 3792
A. baumannii ATCC17978
A. baumannii ACICU
A. baumannii AYE
A. baumannii AB307-0294
A. baumannii AB0057
A. baumannii SDF
0.005

Fig. S3

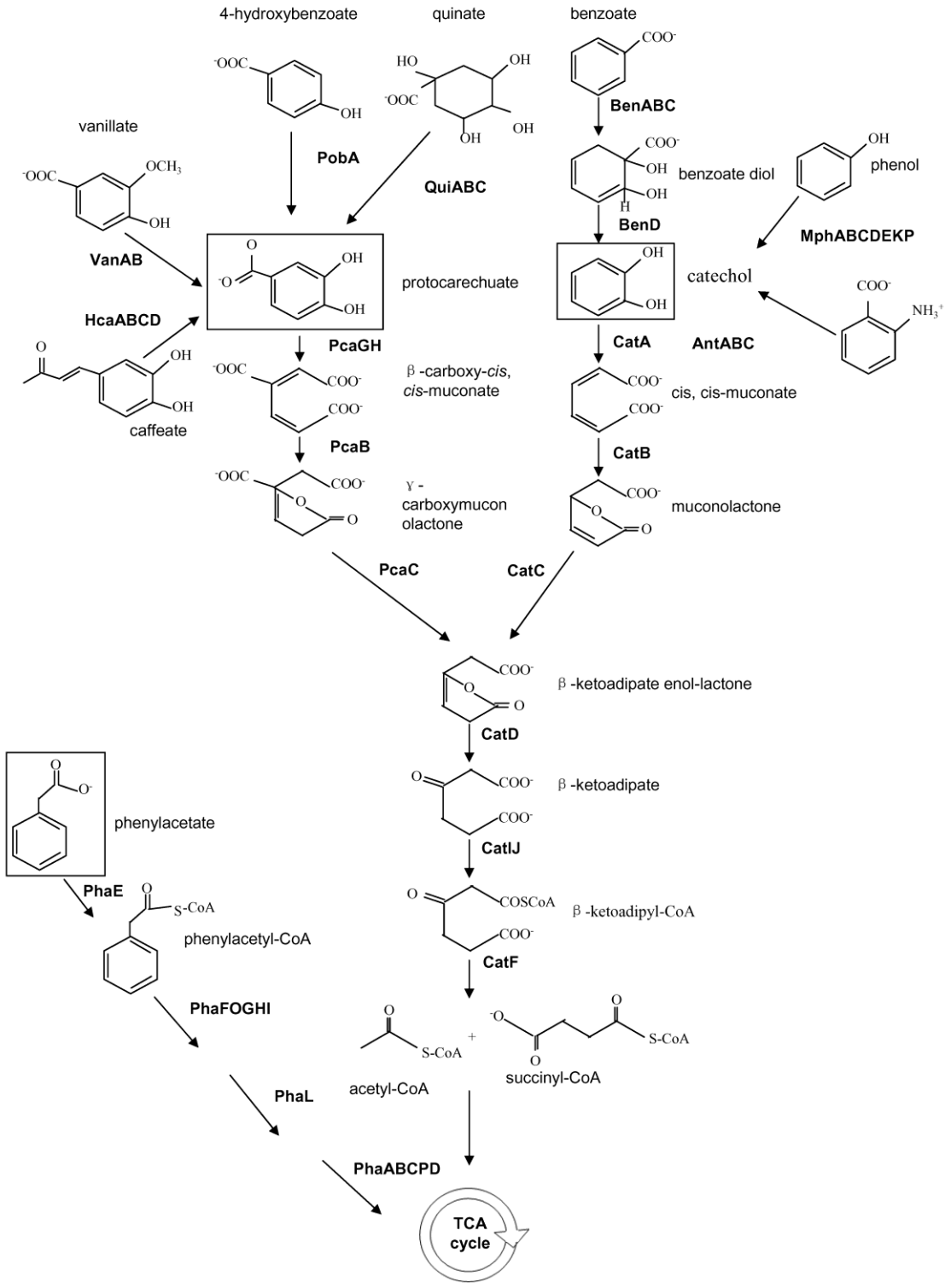
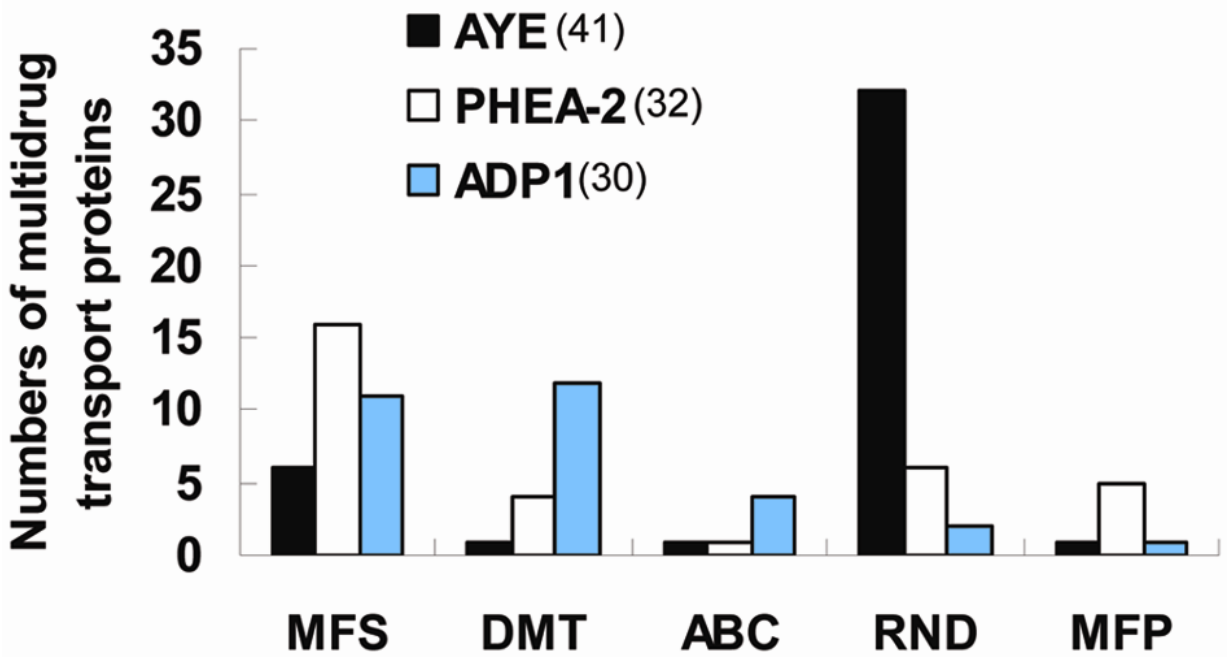

4-hydroxybenzoate
quinate
benzoate
vanillate
PobA
QuiABC
BenABC
benzoate diol
phenol
BenD
MphABCDEKP
VanAB
protocarechuate
catechol
HcaABCD
PcaGH
CatA
AntABC
caffeate
β -carboxy-cis, cis-muconate
cis, cis-muconate
PcaB
CatB
γ - carboxymuconolactone
muconolactone
PcaC
CatC
β -ketoadipate enol-lactone
CatD
β -ketoadipate
CatIJ
phenylacetate
PhaE
phenylacetyl-CoA
β -ketoadipyl-CoA
CatF
PhaFOGHI
acetyl-CoA
succinyl-CoA
PhaL
PhaABCPD
TCA cycle

Fig. S4


Numbers of multidrug transport proteins
35
30
25
20
15
10
5
0
AYE (41)
PHEA-2 (32)
ADP1(30)
MFS
DMT
ABC
RND
MFP