Comparative genomics and functional analysis of niche-specific adaptation in *Pseudomonas putida*

Xiao Wu1,2, Sébastien Monchy1, Safiyh Taghavi1, Wei Zhu2, Juan Ramos3 & Daniel van der Lelie1

1Biology Department, Brookhaven National Laboratory, Upton, NY, USA; 2Department of Applied Mathematics & Statistics, State University of New York, Stony Brook, NY, USA; and 3EEZ-CSIC, Granada, Spain

**Abstract**

*Pseudomonas putida* is a gram-negative rod-shaped gammaproteobacterium that is found throughout various environments. Members of the species *P. putida* show a diverse spectrum of metabolic activities, which is indicative of their adaptation to various niches, which includes the ability to live in soils and sediments contaminated with high concentrations of heavy metals and organic contaminants. *Pseudomonas putida* strains are also found as plant growth-promoting rhizospheric and endophytic bacteria. The genome sequences of several *P. putida* species have become available and provide a unique tool to study the specific niche adaptation of the various *P. putida* strains. In this review, we compare the genomes of four *P. putida* strains: the rhizospheric strain KT2440, the endophytic strain W619, the aromatic hydrocarbon-degrading strain F1 and the manganese-oxidizing strain GB-1. Comparative genomics provided a powerful tool to gain new insights into the adaptation of *P. putida* to specific lifestyles and environmental niches, and clearly demonstrated that horizontal gene transfer played a key role in this adaptation process, as many of the niche-specific functions were found to be encoded on clearly defined genomic islands.

**Introduction**

The group of the pseudomonads comprises a heterogeneous set of microorganisms that can be isolated from many different niches (Clarke, 1982). They belong to the class Gammaproteobacteria and nearly 100 different strains have been described. Within this group of microorganisms, a number of strains have been associated to the species *Pseudomonas putida*, and in this review, we analyzed some of the most characteristic features derived from the analysis of their genome sequences. Bacteria of this species are known for their ability to colonize soils and for their capacity to degrade a wide variety of chemicals, including many natural and man-made compounds.

The rhizospheric bacterium *P. putida* KT2440 was isolated from garden soil in Japan based on its ability to use 3-methylbenzoate (Nakazawa, 2002). Since the publication of its complete 6.18 Mbp genome sequence in 2002, which represented the first sequenced *Pseudomonas* genome and that allowed a comparative analysis of the metabolically versatile *P. putida* KT2440 as a function of this strain’s ability to survive in marginal and polluted soils (Nelson et al., 2002), our knowledge of this strain has increased significantly. As a result, *P. putida* KT2440 represents the best-characterized strain from this species, and is considered as the workhorse for *Pseudomonas* research (Timmis, 2002; Dos Santos et al., 2004). *Pseudomonas putida* KT2440 is a microorganism generally recognized as safe (GRAS certified) for the cloning and expression of foreign genes, and although there is a high level of genome conservation with the pathogenic species *Pseudomonas aeruginosa* (85% of the predicted coding regions are shared), key virulence factors including exotoxin A and type III secretion systems were found to be lacking from its genome (Nelson et al., 2002). Its unusual wealth of determinants for high-affinity nutrient acquisition systems, mono- and di-oxygenases, oxidoreductases, ferredoxins and cytochromes, dehydrogenases, sulfur metabolism proteins, for efflux pumps and glutathione-S transferases and for the extensive array of extracytoplasmic function sigma factors, regulators and stress response systems constitutes the genomic basis for the exceptional nutritional versatility and opportunism of
*P. putida* KT2440. This metabolic diversity was successfully exploited for the experimental design of novel catabolic pathways as well as for its application in the production of high-added value compounds whose synthesis is not coupled to cell survival (Dos Santos et al., 2004). These include the hyperproduction of polymers (such as polyhydroxyalkanoates) (Huijberts et al., 1992; Huijberts & Eggink, 1996), industrially relevant enzymes, the production of epoxides, substituted catechols, enantiopure alcohols and heterocyclic compounds (Schmid et al., 2001). Biotechnology applications of *P. putida* KT2440 were further facilitated by the genome-scale reconstruction and analysis of metabolic network models (Nogales et al., 2008; Puchalka et al., 2008). Both models were used successfully to improve the production of polyhydroxyalkanoates from various carbon sources as examples of how central metabolic precursors of a compound of interest not directly coupled to the organism's growth function might be increased via the modification of global flux patterns. Because the species *P. putida* encompasses many strains with a wide range of metabolic features and numerous isolates with unique phenotypes, these models provide an important basic scaffold upon which future models of other *P. putida* strains, specifically addressing important properties for their niche-specific adaptation such as the growth of *P. putida* F1 on aromatic hydrocarbons, can be tailored as a function of strain-specific metabolic pathways.

*Pseudomonas putida* W619 is a plant growth-promoting endophytic bacterium, which was isolated from *Populus trichocarpa* × *deltoideae* cv. 'Hoogvorst,' and represents one of the most commonly found species among the poplar endophytes isolated so far (Taghavi et al., 2005, 2009). Very similar bacteria were isolated from the poplar rhizosphere, and as endophytes from root and stem material. Like *P. putida* KT2440, strain W619 is an excellent host for the expression of foreign genes. This property was exploited to successfully introduce and express the ability to degrade toluene and trichloroethylene (TCE). *In situ* bioaugmentation with *P. putida* W619-TCE reduced TCE evapotranspiration by 90% under field conditions. This encouraging result, which represents one of the few examples of successful bioaugmentation, was achieved after the establishment and enrichment of *P. putida* W619-TCE as a poplar root endophyte and by further horizontal gene transfer of TCE metabolic activity to members of the poplars endogenous endophytic population (Weyens et al., 2009). Because *P. putida* W619-TCE was engineered via horizontal gene transfer, its deliberate release is not restricted under European genetically modified organisms regulations.

*Pseudomonas putida* F1 was isolated from a polluted creek in Urbana, IL, by enrichment culture with ethylbenzene as the sole source of carbon and energy. F1 is considered a reference strain for the degradation and growth of *P. putida* on aromatic hydrocarbons, including benzene, toluene, ethylbenzene and *p*-cymene. Mutants of strain F1 that are capable of growing on *n*-propylbenzene, *n*-butylbenzene, isopropylbenzene and biphenyl as the sole carbon sources are easily obtained (Choi et al., 2003). In addition to aromatic hydrocarbons, the broad substrate toluene dioxygenase of strain F1 can oxidize TCE, indole, nitrotoluenes, chlorobenzenes, chlorophenols and many other substituted aromatic compounds (Spain & Gibson, 1988; Wackett & Gibson, 1988; Spain et al., 1989). Although *P. putida* F1 cannot use TCE as a source of carbon and energy, it is capable of degrading and detoxifying TCE in the presence of an additional carbon source. The ability of *P. putida* F1 to degrade benzene, toluene and ethylbenzene has a direct bearing on the development of strategies for dealing with environmental pollution.

*Pseudomonas putida* GB-1 was isolated from fresh water and characterized as a robust manganese (Mn^{2+}) oxidizer (Rosson & Nealson, 1982). When supplied with Mn^{2+}, the cells deposit manganese oxide outside the outer membrane during the early stationary growth phase, a process that is enzymatically catalyzed (Okazaki et al., 1997). This process was further studied at both the genetic and the physiological level, making GB-1, along with the closely related strain MnB1, the model organism for studies of Mn(II) oxidation (Okazaki et al., 1997; Caspi et al., 1998; de Vrind et al., 1998; Brouwers et al., 1999; Villalobos et al., 2003).

This review focuses on the diversity and adaptation of four *P. putida* strains (KT2440, W619, F1 and GB-1) in terms of their respective environments from a genome point of view, and provides further insights into their potential applications for the bioremediation of contaminated environments or as plant growth-promoting bacteria.

### Comparative genomics of *P. putida*

#### Structure and general features of the *P. putida* genomes

A whole-genome comparison using MEGAN (Huson et al., 2007) between *P. putida* W619 and the publicly available genome sequences of members of the genus *Pseudomonas* reveals the presence of 3708 shared coding sequences (CDS) (Fig. 1). An additional 684 CDS are shared between W619 and the genomes of the other three *P. putida* strains, plus 82, 47 and 108 CDS are uniquely shared between W619 and strains KT2440, GB-1 and F1, respectively. Among the *Pseudomonas* sp. outside the species *P. putida*, *Pseudomonas entomophila* L48 is the closest relative, sharing 110 additional genes with W619. Because this review focuses on the genomic analyses of representative strains of *P. putida*, *P. entomophila* L48 has not been included in this comparison. It should also be noticed that W619 contains 170 CDS that
have no hit (at \( E \text{ value of } 10^{-5} \)) to any CDS present among the sequenced *Pseudomonas* genomes, which could indicate that these genes potentially originate from organisms outside of the genus *Pseudomonas*.

The *P. putida* W619 genome was manually annotated using the MaGe annotation system (Vallenet et al., 2006) (http://www.genoscope.cns.fr/agc/website/), and compared with the well-annotated KT2440 and automatically annotated GB-1 and F1 genomes. The general genome features of the four *P. putida* strains are summarized in Table 1. Strains W619, F1 and GB-1 are predicted to encode 5471 CDSs with a coding density of 89%, 5300 CDSs with a coding density of 90% and 5417 CDSs with a coding density of 90%, respectively. These findings are comparable to the 5420 CDSs predicted for KT2440, with a coding percentage of 86%. The four strains share many general genome features. For example, all possess a single circular chromosome that displays a clear GC skew transition. Their chromosomal replication origin has the typical organization for *P. putida*, which is distinct from the enteric-type origins (Yee & Smith, 1990; Weigel et al., 1997). The oriC site locates between the *rpmH* and the *dnaA* genes and contains conserved DnaA-binding boxes (TTATCCACA). Figure 2 shows the Genome Atlas of *P. putida* W619 (Hallin et al., 2009; Ussery et al., 2009) with customized BLAST lanes added for F1, KT2440 and GB-1. The outer three lanes show genes of W619 shared with the other three *P. putida* strains. The numbers around the chromosomal atlas indicate the locations of the predicted genomic islands in W619 (see details in Supporting Information, Table S1). These regions are generally characterized by lack of synteny, along with a high intrinsic curvature and stacking energy, which is indicative of their higher recombination activity as compared with other chromosomal regions that seem to constitute the *P. putida*
core genome. The putative orthologous relations and synteny group arrangements of the four genomes are illustrated in the comparative synteny line plots (Fig. 3) and by the synteny statistics (Table S2). Based on the comparative synteny line plots (Fig. 3), *P. putida* W619 has considerable inverted alignments on both sides of the chromosomal replication origin as compared with the other three *P. putida* strains. This indicates that the W619 genome might have

### Table 1. Comparison of the general genome features of *Pseudomonas putida* strains W619, F1, GB-1 and KT2440

|                  | W619       | F1         | GB-1       | KT2440     |
|------------------|------------|------------|------------|------------|
| Number of bases  | 5,774,330  | 5,959,964  | 6,078,430  | 6,181,860  |
| Number of CDSs   | 5,471      | 5,300      | 5,417      | 5,420      |
| CDSs with predicted function (%) | 70.0       | 73.5       | 74.9       | 76.9       |
| CDSs without function with a homolog (%) | 25.6       | 23.6       | 22.7       | 19.1       |
| CDSs without function without a homolog (%) | 4.4        | 0.65       | 0.7        | 4.0        |
| Pseudogenes      | 26         | 49         | 8          | ND         |
| Coding %         | 89         | 90         | 90         | 86         |
| %GC              | 61         | 61         | 62         | 62         |
| rRNAs            | 75         | 76         | 74         | 73         |
| rRNA genes (clusters) | 22 (7)    | 20 (6)     | 22 (7)     | 22 (7)     |
| Putative orthologous relations (%) | 100        | 77         | 75         | 75         |
| W619             | 100        | 77         | 75         | 75         |
| F1               | –          | 100        | 80         | 82         |
| GB-1             | –          | –          | 100        | 77         |
| KT2440           | –          | –          | –          | 100        |

The numbers of genome features have been retrieved from the NCBI site (http://www.ncbi.nlm.nih.gov/sites/entrez/) and the JGI site (http://img.jgi.doe.gov/cgi-bin/pub/main.cgi). ND, not determined; the absence of pseudogenes in KT2440 is based on information provided by NCBI, which might be out of date. Orthologous relations are predicted using the RefSeq synteny statistic in MaGe platform (Vallenet et al., 2006).
undergone a major DNA rearrangement that involved swapping two DNA segments that are flanking the chromosomal replication origin. Members of the genus *Pseudomonas* are characterized by their ability to grow on defined minimal media using a huge variety of organic compounds as energy and carbon sources. The biosynthetic pathways for proteinogenic amino acids and vitamins have been solved recently for *P. putida* KT2440, and this information is well conserved in the other analyzed strains of this species (Molina-Henares et al., 2009, 2010). Using the COGs functional categories in the NCBI database, the *P. putida* strains were compared with the Gammaproteobacteria and total bacteria, and were found to share a similar distribution for most COG classes (Table S3) (Tatusov et al., 2000). However, all four *P. putida* strains displayed a higher percentage of genes putatively coding for signal transduction mechanisms (T), pointing to the presence of sophisticated regulatory networks to control gene expression in function of a highly variable environment, and inorganic ion transport and metabolism (P). On the other hand, the *P. putida* genomes were less dense in genes putatively involved in carbohydrate transport and metabolism (G) and replication, recombination and repair (L).

**Mobile elements in *P. putida***

Conserved IS elements between the *P. putida* strains were identified using the IS Finder (http://www-is.biotoul.fr/) database and blast searches with a stringent E value threshold (below e^{-100}). Complete putative IS elements identified in W619, KT2440, F1 and GB-1 are listed in Table 2A. As can be seen in the table, the KT2440 genome contains 36 IS elements, a very high number especially in comparison with strain F1 (only 2 IS elements of the IS3 and IS5 families, respectively), with the majority of the IS elements in KT2440 being present in multicopies. In comparison, strains W619 and GB-1 contain eight and nine IS elements, respectively. The high number of IS elements may be related to the versatile catabolic ability of KT2440, which also requires an increased level of genome plasticity in order to adapt to various environments, this in comparison with the other strains that thrive in more specialized niches. When applying a higher threshold E score, several incomplete or truncated remnants of IS elements were identified among the various genomes (Table S4).

It should also be noted that several of the IS elements were unique to a *P. putida* genome, including seven copies of both ISPpu9 and ISPpu10, six copies of ISPpu8, two copies of both ISPpu13 and ISPpu11 and a copy of IS1246 found in KT2440, copies of ISPpu16, ISPpu27 and IS1382 in W619 and a copy of IS2000 in F1. None of the identified IS elements was present on more than two of the genomes.

IS elements are often associated with resistance and accessory functions that bacteria have acquired via horizontal gene transfer, or with DNA rearrangements in order to affect the expression or the stability of the newly acquired functions (Mahillon & Chandler, 1998). For example in *P. putida* W619, the unique mhp aromatic degradation operon was found between one incomplete copy of IS1182 (PputW619_1976-1977) and the copy of IS1382 (PputW619_1990), indicating that this operon was acquired via horizontal gene transfer. Also, a mercury resistance operon was found adjacent to the unique copy of ISPpu12 (PputW619_2325-2328) (Williams et al., 2002), while in its
Table 2. Mobile elements’ comparison for the Pseudomonas putida strains W619, KT2440, F1 and GB-1

| Family/Group | Name | Origin | Length | IR | DR | W619 ORF ID (PputW619) | KT2440 ORF ID (Pput_) | F1 ORF ID (Pput_) | GB-1 ORF ID (PputGB1_) |
|--------------|------|--------|--------|----|----|----------------------|----------------------|------------------|----------------------|
| (A) IS elements |      |        |        |    |    |                      |                      |                  |                      |
| IS3/IS551    | ISPu22 | P. putida GB-1 | 1232   | 29/45 | 3 – |                      |                      |                  | 0419-0415          | 1613-1614 |
| IS4/IS48     | ISPu8  | P. putida KT2440 | 1419   | 18 | 11 |                      |                      |                  |                      | 2972-2971 |
| IS5/IS5     | ISPa16 | P. aeruginosa | 1192   | 12 | 4 | 3354 |                      | – | – | 1395-1397 |
| IS5/IS5     | IS1246 | P. putida mt-2 (pWWO) | 1275 | 15/16 | ND | – | 2971 | – | – | 5424-5425 |
| (B) Phage |      |        |        |    |    |                      |                      |                  |                      |
| PputW6191301-1357 | PP1536-1584 |                      | Pput,3359-3401 | PputGB1_1181-1221 |
| PputW6193919-3971 | PP2266-2297 |                      | Pput,4096-4150 | PputGB1_1720-1762 |
| PputW6194030-4047 | PP3026-3066 |                      | – | PputGB1_3388-3473 |
| IS elements were identified using IS Finder: http://www-is.biotoul.fr/ | | | | | | | | | |
| IR, the length(s) of the terminal IR(s) in base pairs. A single number refers to two IRs with the same length; DR, the number of target base pairs duplicated on insertion; ND, not determined. | | | | | | | | | |
| (C) Other transposable elements |      |        |        |    |    |                      |                      |                  |                      |
| Tn7 family protein (tnsA) | 5195 | – | 5406 | – | 5405 | – | – | – | – |
| Tn7 family protein (tnsB) | 5194 | – | 5405 | – | 5405 | – | – | – | – |

*Phages with the same label inserted in the same position on the genomes of respective strains. The presence of phages was predicted using the PROPHINDER (Lima-Mendez et al., 2008) tool.
proximity, other transposable elements were identified, including a putative Tn3 family transposon (PputW619_2321-2322), a recombinase and a truncated transposase. Therefore, this gene segment might represent a composite mercury resistance transposon in W619, acquired via horizontal gene transfer and absent in P. putida F1, KT2440 and GB-1. Additional transposable elements are summarized in Table 2C. Among them, tnsABCD from the Tn7 family was found in association with a gene cluster encoding heavy metal resistance in W619 and KT2440.

The PROPHINDER (Lima-Mendez et al., 2008) tool was used for prophage prediction in the P. putida genomes. Prophages were identified in all four genomes (Table 2B), with their numbers ranging between 2 and 4. Several prophages were found to be inserted at the same location among different strains. For example, of the three prophages found in strain W619, one was found at the same location in strain GB-1, while another prophage (PputW619_4030-4047) was found inserted adjacent to a ferredoxin-related gene in both GB-1 (PputGB1_1181-1221) and F1 (Pput_4096-4150). This points to the existence of an insertion hotspot for this particular prophage in the P. putida core genome (Manna et al., 2004).

### Genomic islands in P. putida

We predicted specific genomic regions for each of the four P. putida strains in comparison with the other three strains using the MaGe annotation system (Vallenet et al., 2006). Based on the automatic prediction algorithm, 61, 54 and 66 putative regions were identified in P. putida KT2440, F1 and GB-1, respectively; along with the manual annotation of W619, 31 putative genomic islands were identified for P. putida W619 (Table S1). Different from 105 predicted genomic islands of KT2440 as described by Weinel et al. (2002), a number that was based on the compositional bias of the GC, di- and tetranucleotides of the chromosome itself, the 61 putative genomic islands in KT2440 that we identified in our analysis were predicted based on the lack of synteny against the genomes of the other three P. putida strains, GC bias and the existence of mobile genetic elements. For example, genomic island 19 (coordinates 922000–945000) predicted by Weinel et al. (2002) was not included in our analysis because this region is conserved in the other three P. putida strains. On the other hand, region 31 (coordinates 3121963–3224467) of KT2440, which was not considered as a genomic island by Weinel et al. (2002), lacks synteny to the genomes of W619 and GB-1, and carries genes that are involved in sugar transport (PP_2757-PP_2761).

Among the various putative genomic islands, many are suggested to have been acquired via horizontal gene transfer due to the presence of integrases or mobile genetic elements (transposons, IS elements) and an alternative matrix of codon usage compared with the rest of the chromosome. As shown in the following sections, these putative genomic islands have conferred the P. putida species with many accessory capabilities, including heavy metal resistance, aromatic compound degradation and stress responses, all
of which are highly relevant for the specific niche adaption of *P. putida*.

**Adaption of *P. putida* to a polluted soil environment**

**Heavy metal resistances in *P. putida***

Based on its genome sequence, *P. putida* KT2440 was predicted to tolerate various heavy metals (Canovas et al., 2003), and this strain's metal resistance properties were experimentally confirmed. *Pseudomonas putida* W619 strain was originally isolated from the roots of poplar cuttings that were obtained from trees growing on a field site aimed to remediate groundwater contaminated with various compounds, including BTEX compounds (benzene, toluene, ethylbenzene and xylene), TCE and Ni (Taghavi et al., 2005). It was therefore expected that strain W619 also possesses the capacity to deal with heavy metals. Based on the strain's genome sequence, 78 genes were identified that encode proteins putatively involved in heavy metal resistance and homeostasis, a number that exceeds the number of genes identified on the KT2440 genome (Canovas et al., 2003). Furthermore, when comparing the MIC values of strains W619 and KT2440 for various heavy metals, we found that W619 displayed an increased resistance to Cd(II) (MIC values of 0.5 and 0.25 mM for W619 and KT2440, respectively), Cu and Ni (see details below) and similar resistance to Zn(II) (MIC 0.5 mM) and Co(II) (MIC 0.1 mM). We therefore used the W619 genome as the reference to compare the different *P. putida* genomes for functions putatively involved in heavy metal resistance.

The majority of the *P. putida* W619 heavy metal resistance genes are found on two genomic regions, referred to as region 1 and 31 that are located on opposite sites of the chromosomal origin of replication. A similar organization is observed for F1 and GB-1 and in some way for KT2440. Most of the heavy metal resistance genes located in region 1 (coordinates 7447–75941) are conserved among all four *P. putida* strains (Table 3 and Fig. 4a). On the 5'-end, this region is delimited by an integrase gene (PputW619_0006). This integrase is also found in the equivalent regions of F1 and GB-1, but is absent in KT2440. Interestingly, region 1 contains three different systems for putative copper resistance: copRSABMG (PputW619_0018-11) for periplasmic detoxification, with CopA being a multicopper oxidase (MCO) that is thought to oxidize Cu(I) to Cu(II) in the periplasm (Rensing & Grass, 2003), and CopB being an outer membrane protein (Cha & Cooksey, 1991); a P-type ATPase gene copF (PputW619_0029) for the cytoplasmic detoxification of monovalent Cu(I); and the copper/silver resistance operon *cusFcuABC* (PputW619_0023-0020) for the periplasmic detoxification of Cu(I) and Ag(I) via expulsion by the three-component efflux system (cusABC) across the outer membrane. The accessory protein CusF is believed to be a periplasmic copper chaperon delivering Cu(I) to the CusABC complex (Franke et al., 2003). Region 1 also seems to encode for resistance to cadmium, zinc and cobalt based on the presence of the *czcABC* (a three-component efflux system for the periplasmic detoxification of Cd, Zn and Co) and the *czcDRS* gene (including the cation diffusion facilitator (CDF) protein CzcD) clusters with some rearrangement (PputW619_0060-62, 0043, 0046-0047). In addition, a *cadA* gene (PputW619_0058), encoding a CadA P-type ATPase, is present as well as a *grtABM* (PputW619_0052-0050) locus putatively involved in heavy metal resistance by repairing the membrane, whose integrity may have been altered by the precipitation of heavy metal on the bacterial surface. The clustering of the *czc* efflux system, the membrane maintenance genes *grtABM* and a putative porin (PputW619_0063) (Fig. 4a) are features that resemble those described for the Cd, Zn and Co resistance cluster (ORF82-106) of the *Cupriavidus metallidurans* CH3 plasmid pMOL30, but with some rearrangements (Monchy et al., 2007). It should also be noted that a *czcN* homolog, described to play a role in the regulation of the *czc* operon (Dong & Mergaey, 1994; Grosse et al., 1999), is located 18 kb upstream of the *czc* cluster in between the putative *cusF* and *copF* genes.

In between *copF* and *czcDRS*, a copy of *ISPpu14* (PputW619_0037-0039) was found, separating the copper/silver resistance cluster from the cadmium/zinc/cobalt resistance cluster. The region 1 counterparts of F1 and GB-1 seem to be identical in organization with very close synteny to W619, the only difference being the absence of *ISPpu14*. In the case of KT2440, region 1 is divided into two sections that are separated by the chromosomal replication origin: section 1, containing the copper/silver resistance cluster that ends with a copy of *ISPpu14*, and section 2, which starts with a putative integrase and contains the cadmium/zinc/cobalt resistance cluster.

Region 31 (coordinates: 5706900–5753604) of *P. putida* W619 is less conserved among the other three *P. putida* strains, and contains genes involved in arsenite/arsenate, chromium, nickel and copper resistances (Fig. 4b). The arsenite/arsenate resistance operon (*arsRCBH*; PputW619_5146-5149) has homologs on the KT2440, F1 and GB-1 genomes, except that their *arsB* genes are different: the gene from W619 is closely related to the *arsB* gene from *Acinetobacter baumannii* AYE, while the *arsB* genes from the other *P. putida* strains are closer to the *Escherichia coli* K12 *arsB*. Still, *P. putida* W619 and KT2440 showed similar growth and resistance to As(III) and As(V) in the range of concentration we tested (both viable in the presence of NaAsO2 (0.01 to ~2 mM) and Na2HAsO4 (0.01 to ~5 mM). Besides this...
Table 3. Summary and comparison of genes found in *Pseudomonas putida* that might be involved in metal resistance and homeostasis based on genomic analysis

| Gene | Metal | W619 (region)* | F1 | K2440 | GB-1 | Product |
|------|-------|---------------|----|-------|------|---------|
| copG | Cu    | PputW619_0011 | 0011 | 5377  | 0013 | Involved in survival in the presence of high bioavailable Cu(II) |
| copM | Cu    | Pput_0012     | 0012 | 5378  | 0014 | Copper resistance protein |
| copB' | Cu   | Pput_0013     | 0013 | 5379  | 0015 | Copper resistance protein |
| copB* | Cu   | Pput_0014     | 0014 | –     | 0016 | Putative CopB (frameshifted) |
| copA | Cu    | Pput_0015     | 0015 | 5380  | 0017 | Copper resistance protein A |
| copR | Cu    | Pput_0017     | 0017 | 5383  | 0019 | Transcriptional activator CopR |
| copS | Cu    | Pput_0018     | 0018 | 5384  | 0020 | Sensor protein CopS |
| cusC | Cu/Ag | Pput_0020     | 0020 | 5385  | 0022 | Cu/Ag tricomponent efflux outer membrane porin |
| cusB | Cu/Ag | Pput_0021     | 0021 | 5386  | 0023 | Cu/Ag tricomponent efflux membrane fusion protein |
| cusA | Cu/Ag | Pput_0022     | 0022 | 5387  | 0024 | Copper transporter, RND family |
| cusF | Cu/Ag | Pput_0023     | 0023 | 5388  | 0025 | Periplasmic copper-binding protein |
| -    | ?     | Pput_0024     | 0024 | 5389  | 0026 | S-isoprenylcysteine methyltransferase (czcN homolog) |
| copF | Cu    | Pput_0029     | 0029 | 030   | –    | Copper P-type ATPase |
| czcD | Cd/Zn/Co | Pput_0043  | 0043 | 0026  | 0040 | Cation Diffusion Facilitator (CDF) |
| czcR | Cd/Zn/Co | Pput_0046  | 0046 | 0029  | 0043 | DNA-binding response regulator |
| cztS | Cd/Zn/Co | Pput_0047  | 0047 | 0030  | 0044 | Sensory histidine kinase |
| gtrM | –     | Pput_0050     | 0050 | 0033  | 0047 | Glycosyltransferase (protein o-glycosylation) |
| gtrB | –     | Pput_0051     | 0051 | 0034  | 0048 | Glycosyltransferase (bactoprenol) |
| gtrA | –     | Pput_0052     | 0052 | 0035  | 0049 | Bactoprenol-linked glucose translocase (Flippase) |
| cadA | Cd/Zn | Pput_0058     | 0058 | 0041  | 0055 | Cadmium translocating P-type ATPase |
| czaC | Cd/Zn/Co | Pput_0061  | 0061 | 0044  | 0058 | Cobalt/zinc/cadmium efflux RND transporter |
| czbB | Cd/Zn/Co | Pput_0061  | 0061 | 0044  | 0058 | Cobalt/zinc/cadmium efflux RND transporter |
| czeC | Cd/Zn/Co | Pput_0062  | 0062 | 0045  | 0059 | Cobalt/zinc/cadmium resistance protein |
| -    | Cd/Zn/Co | Pput_0063  | 0063 | 0046  | 0060 | Putative porin, OprD family |
| czeR | Cd/Zn/Co | Pput_0064  | 0064 | 0047  | 0061 | DNA-binding heavy metal response regulator |
| cadR | Cd/Zn | Pput_0325     | 0325 | 5013  | 5140 | Cadmium translocating P-type ATPase |
| cadA | Cd/Zn | Pput_0326     | 0326 | 5012  | 5139 | Cadmium translocating P-type ATPase |
| arsC | As    | Pput_1207     | 1207 | 4072  | 1645 | Arsenate reductase |
| cinA | Cu    | Pput_1676     | 1676 | 3583  | 2159 | Copper-containing azurin-like protein |
| cinQ | Cu    | Pput_1677     | 1677 | 3584  | 2160 | Pre-Q2 reductase |
| copB | Cu    | Pput_1712     | 1712 | (8)   | –    | Copper resistance protein B |
| copA | Cu    | Pput_1713     | 1713 | (8)   | –    | Copper resistance protein A |
| -    | Zn    | Pput_1714     | 1714 | (8)   | –    | Cation efflux protein (Putative Zinc transporter ZitB) |
| arsB | As    | Pput_1715     | 1715 | (8)   | –    | Arsenite efflux pump |
| merR | Hg    | Pput_2323     | 2323 | (11)  | –    | Mercuric resistance operon regulatory protein |
| merB | Hg    | Pput_2324     | 2324 | (11)  | –    | Alkylmercury lyase (organomercurial lyase) |
| merR | Hg    | Pput_2325     | 2325 | (11)  | –    | Transcriptional regulator, MerR-family |
| -    | ?     | Pput_2326     | 2326 | (11)  | –    | Heavy metal/ H+ antiporter, CDF family |
| merE | Hg    | Pput_2336     | 2336 | (11)  | –    | Mercuric resistance protein |
| merD | Hg    | Pput_2337     | 2337 | (11)  | –    | HTH-type transcriptional regulator |
| merA | Hg    | Pput_2338     | 2338 | (11)  | –    | Mercuric Hg(II) reductase |
| merP | Hg    | Pput_2339     | 2339 | (11)  | –    | Mercuric transport protein periplasmic component |
| merT | Hg    | Pput_2340     | 2340 | (11)  | –    | Mercuric transport protein |
| merR | Hg    | Pput_2341     | 2341 | (11)  | –    | Mercuric resistance operon regulatory protein |
| nikR | Ni    | Pput_3004     | 3004 | 18    | 3341 | Putative nickel-responsive regulator |
| nikA | Ni    | Pput_3005     | 3005 | 18    | 3342 | Nickel ABC transporter, periplasmic nickel-binding protein |
| nikB | Ni    | Pput_3006     | 3006 | 18    | 3343 | Nickel transporter permease NikB |
| nikC | Ni    | Pput_3007     | 3007 | 18    | 3344 | Nickel transporter permease NikC |
| nikD | Ni    | Pput_3008     | 3008 | 18    | 3345 | Nickel import ATP-binding protein NikD |
| nikE | Ni    | Pput_3009     | 3009 | 18    | 3346 | Nickel import ATP-binding protein NikE |
| chrA | Cr    | Pput_3017     | 3017 | 18    | –    | Chromate transporter |
| modA | Mo    | Pput_3197     | 3197 | 1942  | 3828 | Mo ABC transporter, periplasmic Mo-binding protein |
| modB | Mo    | Pput_3198     | 3198 | 1941  | 3829 | Mo ABC transporter, permease protein |
| modC | Mo    | Pput_3199     | 3199 | 1940  | 3830 | Mo ABC transporter, ATP-binding protein |
arsRCBH operon, two additional arsC genes encoding the arsenate reductase were present on the *P. putida* chromosomes, as well as an additional copy of *arsRCBH* in KT2440 (PP_1927-1930).

The chromate resistance genes *chrFAB* (PputW619_5156-5158) and the nickel resistance gene *nreB* (PputW619_5159) are located downstream of the *ars* operon, and are incomplete or absent in KT2440, F1 and GB-1. They are followed by a second copy of the copper resistance genes *copSRABMG* (PputW619_5177-5178, 5180-5184), which is flanked by a copy of ISPpu14 (PputW619_5173-5175) and by a copy of a Tn7-like transposon (*tnsABCD*; PputW619_5195-5192). Remnants of the *cop* operon are found in KT2440, F1 and GB-1. As for region 1, the region 31 *cop* operon seems to be part of a composite mobile element. Although the organization of both *cop* operons is very similar, they showed only 80% protein identity, which rules out a recent duplication.

The *cop* determinant in *Pseudomonas syringae* pv. *tomato* contains six genes (*copABCDRS*) (Mellano & Cooksey, 1988). However, the presence of *copAB* was sufficient to

---

**Table 3. Continued.**

| Gene | Metal | ORF ID | ORF ID | ORF ID | ORF ID |
|------|-------|--------|--------|--------|--------|
| **arsC** | As     | 4088   | 4193   | 1531   | 1140   | Arsenate reductase (glutaredoxin family) |
| -       | Cu     | 4576   | 0627   | 0588   | 0633   | Putative copper-binding protein |
| **merE** | Cu     | 4578   | 0625   | 0586   | 0631   | Putative copper-translocating P-type ATPase |
| **znuA** | Zn     | 5108   | 0137   | 0120   | 0135   | Zinc uptake ABC transporter, periplasmic binding protein |
| **zur** | Zn     | 5109   | 0136   | 0119   | 0134   | Transcriptional repressor of Zn transport system |
| **znuC** | Zn     | 5110   | 0135   | 0118   | 0133   | Zinc ABC transporter, ATP-binding protein |
| **znuB** | Zn     | 5111   | 0134   | 0117   | 0132   | Zinc ABC transporter, permease protein |
| **arsR** | As     | 5146   | 3034   | 2718/1930 | 3077 | Arsenical resistance operon represser |
| **arsC** | As     | 5147   | 3036   | 2716/1928 | 3079 | Arsenite reductase |
| **arsB** | As     | 5148   | 3035   | 2717/1929 | 3078 | Arsenite efflux transporter |
| **arsH** | As     | 5149   | 3037   | 2715/1927 | 3080 | Arsenical resistance protein |
| **chrF** | Cr     | 5156   | 3159   | 2556   | 3384   | Chromate resistance regulator |
| **chrA** | Cr     | 5157   | 3158   | 2556   | 3383   | Chromate transporter |
| **chrB** | Cr     | 5158   | 3157   | 2556   | 3382   | Chromate resistance protein |
| **nreB** | Ni     | 5159   | 3156   | 2555   | 3381   | Major facilitator superfamily (nickel efflux family) |
| -       | ?      | 5161   | 3155   | 2554   | 3380   | Putative cation diffusion facilitator (CDF) |
| **copS** | Cu     | 5177   | 0574   | 2205   | 1828   | Sensor protein Cps |
| **copR** | Cu     | 5178   | 0575   | 2204   | 1827   | Transcriptional activator CopR |
| **copA** | Cu     | 5180   | 0576   | 2203   | 1826   | Copper resistance protein A |
| **copB** | Cu     | 5181   | 0577   | 2202   | 1825   | Copper resistance protein B |
| **copM** | Cu     | 5182   | 0578   | 2201   | 1824   | Cytochrome c family protein |
| **copG** | Cu     | 5183   | 0579   | 2200   | 1823   | Metal resistance-associated cytoplasmic protein |

*For the reference genome W619, the numbers in brackets indicate the genomic region of the gene, which was omitted if the gene was not located in a genomic region. ?, the metal specificity remains to be determined. The gene annotations and comparisons were obtained using the MaGe annotation platform (Vallenet et al., 2006).*
render the cells copper resistant (Mellano & Cooksey, 1988; Cha & Cooksey, 1993). CopAB are known to be involved in the detoxification and efflux of copper ions from the periplasm (Rensing et al., 2000; Monchy et al., 2006). In *P. syringae*, the CopA and CopB proteins contain the MXXMXXHM (MDH) motif repeated several times throughout the sequence. This motif also appears in CopA1 (PputW619_0015), where it is repeated 12 times. However,
this repeat is truncated for CopA2 (PputW619_5180) and is only present five times. A similar organization was described for the two copA genes in KT2440 (Canovas et al., 2003), having this motif repeated 14 and five times in CopA1KT2440 and CopA2KT2440 respectively, and was also observed for F1 and GB-1.

Both copies of CopB in W619 contained a frame shift mutation. For instance, in the case of CopB1, if this frame shift was ignored, the resulting complete protein would contain six copies of the MDH motif and 21 histidine residues, which are known to bind copper efficiently. The truncated CopB1 protein, however, only contains three MDH motifs and 13 histidine residues. A similar frame shift was also found in KT2440, F1 and GB-1, but only in one of the two CopB copies. In addition to the two copies of copSRAB, an additional copAB locus was identified on the W619 genome (PputW619_5169, located in region 8; coordinates: 1875105–1910820), which might make up for the frame shift mutations. In addition, W619 has a copF gene putatively encoding a P1-type ATPase, which is located in the inner membrane and involved in Cu(I) efflux from the cytoplasm to the periplasm (Rensing et al., 2000; Mergeay et al., 2003). The copF gene is absent in KT2440, which might explain the higher level of copper resistance for W619 compared with KT2440, with MIC values of 0.5 and 0.1 mM Cu, respectively.

Because P. putida W619 was isolated from a nickel-contaminated site, it was no surprise that it showed elevated levels of nickel resistance compared to KT2440, with MIC values of 1 and 0.1 mM, respectively. The only putative nickel resistance protein identified in W619 was a copy of nreB (PputW619_5159), located in region 31, which is absent in KT2440, F1 and GB-1. The presence of nreB is consistent with the observation that in A. xylosoxidans and E. coli, nreB expression was sufficient for nickel resistance (Grass et al., 2001). In KT2440, a novel metal resistance...
determinant mrdH (PP_2968) was identified recently, which encodes a protein with a chimeric domain organization from RcnA and CzcB (Haritha et al., 2009). MrdH was found to be involved in nickel, cadmium, and zinc resistance. The mrdH gene and mreA (PP_2969), the latter showing similarity to NreA-like proteins, lack homologs in P. putida W619, GB-1 and F1, and are located on genomic island 34 (GI 55 according to Weinil et al. 2002) of KT2440. The presumably higher specificity for nickel resistance of mreB compared with mrdH may explain strain W619’s much higher MIC value for Ni(II) as compared to KT2440. It should also be noted that the activities of the mobile genetic elements Tn4652 (PP_2964-65) and IS1246 (PP_2971), which are uniquely found on the KT2440 chromosome flanking mrdH and mreA, were found to be induced by the presence of Cd, Ni and Zn.

Additional putative heavy metal-responsive genes, many of which are absent in KT2440, F1 and GB-1 (Table 3), can be found on putative genomic islands on the W619 chromosome. A gene coding for a CDF putatively involved in zinc transport (PputW619_1714) and an additional copy of arsB (PputW619_1715) are located on region 8 (coordinates: 1875105–1910820). This region is located next to genes encoding proteins putatively involved in iron uptake (PputW619_1716-1719). On region 11 (coordinates: 2520458–2644620), two clusters of genes involved in mercury resistance (Fig. 4c) were found: a merRB locus (PputW619_2323-2324) locus, which contains a merB organomercurial lyase required for cleavage of mercury-alkyl bonds, and that is flanked by a copy of Tn3 (PputW619_2321-2322) and an IS element from the ISL3 family (ISPpu12; PputW619_2325-2328); mercury resistance is completed by a merRTPADE operon (PputW619_2341-2346). This operon is flanked by a truncated transposase and a complete recombinase gene, and a copy of ISPsl. This organization suggests the acquisition of mercury resistance via two events of horizontal gene transfer, followed by DNA rearrangements. On the putative genomic island region 18 (coordinates 3322597–3400760), a copy of nikABCDE (PputW619_3005-3009) encoding an ABC nickel uptake transporter, which was also found in KT2440, and a copy of chrA (PputW619_3017) involved in the transport of chromate were found. Additional heavy metal resistance or homeostasis genes were also identified outside putative genomic islands and include cadAR (PputW619_0325-0326), modAABC (PputW619_3197-3199), znuABC (PputW619_5108-5111) and cinAQ (PputW619_1676-1677). These genes are common for all four P. putida strains. Among them, the copper-inducible genes cinAQ encode a copper-containing azurin-like protein and a pre-Qa reductase, respectively, and are located adjacent to their two-component regulatory system cinRS. Gene disruptions of cinA and cinQ did not lead to a significant increase in the copper sensitivity of P. putida KT2440, which might result from the redundancy of copper resistance systems (Quaranta et al., 2007). In addition, KT2440, F1 and GB-1 have a duplicated Cd/Zn/Cu resistance system, czeABC, which is lacking in W619.

### Degradation of organic solvents and aromatic compounds by P. putida

Previous genome analysis of P. putida KT2440 revealed many metabolic pathways for the transformation of aromatic compounds (Jimenez et al., 2002; Nelson et al., 2002). Putative genes coding for the degradation of aromatic compounds were compared among the four P. putida strains (see Table 4 and Table 55). Some of these aromatic compounds (ferulate, coumaryl alcohols, aldehydes and acids, p-hydroxybenzoate) may arise from the decomposition of plant materials, as can be found in the rhizosphere.

Although KT2440 has versatile metabolic pathways to degrade aromatic compounds, it is not able to grow on any aromatic hydrocarbon as a sole carbon source. In contrast, with a significantly wider range of growth substrates, P. putida F1 is best known for its capability of growth on several aromatic hydrocarbons, including benzene, toluene, ethylbenzene and p-cymene. Its toluene degradation (tod) pathway is featured by the toluene dioxygenase operon todABCDE (Zylstra et al., 1988; Gibson et al., 1990) and the corresponding two-component regulatory system, todST (Lau et al., 1997). Besides the ability to use toluene, ethylbenzene or benzene as the sole carbon source, F1 is also able to grow on p-cymene (p-isopropyltoluene) and its acid derivative, p-cumate (Eaton, 1996, 1997). The mechanism uses two pathways: the cymAaAbBCDER pathway (Pput_2900-2905, 2908), responsible for the oxidation of p-cymene to p-cumate, and the adjacent located cmtAaAbAcAdBCDEFGHI pathway (Pput_2888-2899) to take p-cumate to isobutyrate, pyruvate and acetyl coenzyme A (Eaton, 1996, 1997). In F1, the cym/cnt and the tod pathways are located less than 3 kb apart on a putative genomic island (3260962–3302713), which is featured by a lack of synteny with other P. putida strains and surrounded by phage-related genes, an organization that is indicative that this region was acquired via transduction. A sepABCDE gene cluster is located upstream of this genomic island, encoding for solvent efflux or multidrug pumps (Phoenix et al., 2003). KT2440, W619 and GB-1 all have conserved homologs for this cluster, referred to as tgtABC (Duque et al., 2001). In addition, genomic sequence comparisons predicted the universal existence of other aromatic catabolic pathways in all four P. putida strains, including the protocatechuate (pha) genes and catechol (cat) genes branches of the β-ketoadi- pate pathway, and the phenylacetate pathway (pha genes) (see Table 55).

The genome of W619 contains two mph operons for the degradation of 3-hydroxyphenylpropionate (Ferrandez et al., 2001).
et al., 1997): one complete mhpRABCDFET operon encoding enzymes for the conversion from 3-HTT to acetyl coenzyme A (CoA) and another incomplete cluster consisting of mhpEFD. The mhpEFD genes encode three enzymes that are conserved with part of the cym/cmt pathway: a 4-hydroxy-2-oxovalerate aldolase, an acetaldehyde dehydrogenase and a 2-hydroxypenta-2,4-dienoate hydratase (Table 4), which are respectively able to catalyze the conversion of 2-keto-4-pentenoate to 4-hydroxy-2-ketovalerate, to pyruvate and acetaldehyde and finally to acetyl CoA. This indicates that W619 has a broader potential for the degradation of aromatic compounds as compared with KT2440 and GB-1, but is less versatile than F1. For instance, unlike F1, P. putida W619 was unable to metabolize toluene or TCE, a property that could be complemented via acquisition of the pTOM plasmid of Burkholderia cepacia BU61 (Taghavi et al., 2005; Weyens et al., 2009). The W619 mhpRABCDFET operon is flanked by a truncated copy of IS1182 (PputW619_1976-1977) and an IS element from the IS30 family (IS1382; PputW619_1990), which are absent in

| Gene          | Product                                      | F1 ORF ID | KT2440 ORF ID | GB-1 ORF ID | W619 ORF ID |
|---------------|----------------------------------------------|-----------|---------------|-------------|-------------|
| sepA/ttgA     | RND family efflux transporter                | 2867      | 1386          | 4427        | 1026        |
| sepB/ttgB     | Hydrophobe/amphiphile efflux                | 2868      | 1385          | 4428        | 1025        |
| sepC/ttgC     | RND efflux outer membrane lipoprotein       | 2869      | 1384          | 4429        | 1024        |
| todT          | Response regulator                           | 2871      | –             | –           | –           |
| todS          | Signal transduction histidine kinase         | 2872      | –             | –           | –           |
| todE          | 3-methylcatechol 2,3-dioxygenase             | 2876      | –             | –           | –           |
| todD          | cis-Toluene dihydrodiol dehydrogenase       | 2877      | –             | –           | –           |
| todA          | Aromatic-ring-hydroxy-lyzing dioxygenase    | 2878      | –             | –           | –           |
| todB          | Ferredoxin                                   | 2879      | –             | –           | –           |
| todC2         | Toluene dioxygenase                          | 2880      | –             | –           | –           |
| todC1         | Toluene dioxygenase                          | 2881      | –             | –           | –           |
| todF          | 2-Hydroxy-6-oxo-2,4-heptadienoate hydrolase  | 2882      | –             | –           | –           |
| todX          | Membrane protein                             | 2883      | –             | –           | –           |
| -             | Enoly-coenzyme A hydrolase                  | 2887      | –             | –           | –           |
| mhpT          | 3-Hydroxyphenylpropionate acid transporter   | –         | –             | –           | 1985        |
| cmtG (mhpE)   | 4-Hydroxy-2-oxovalerate aldolase             | 2888      | –             | –           | 1984        |

The gene annotations and comparisons were obtained using the MaGe annotation platform (Vallenet et al., 2006).

The aromatic compound degradation pathway in Pseudomonas putida F1, and comparison with KT2440, W619 and GB-1.
KT2440, GB-1 and F1. The presence of these mobile genetic elements points toward the acquisition of this catabolic region via horizontal gene transfer.

**Mn(II) oxidation by P. putida**

In the environment, Mn cycles between the soluble reduced form Mn(II) and the insoluble oxidized forms Mn(III and IV) that can adsorb other trace metals from the environment and react as potent oxidizing agents. Thus, the Mn redox cycle has beneficial effects on the bioavailability and geochemical cycling of many essential or toxic elements (Tebo et al., 2005). *Pseudomonas putida* GB-1 is a Mn(II)-oxidizing model bacterium (Corstjens et al., 1992). Because Mn(III, IV) oxides are able to bind trace metals, this feature makes *P. putida* GB-1 a good candidate for bioremediation of heavy metal-contaminated sites. However, the mechanism for Mn(II) oxidation still remains to be elucidated. Several random transposon mutagenesis experiments led to the identification of genes important for Mn oxidation: the *ccm* operon coding for c-type cytochrome synthesis genes (Caspì et al., 1998; de Vrind et al., 1998), the MCO *cumA* (Brouwers et al., 1999) and genes encoding a general secretory pathway (de Vrind et al., 2003). A recent paper showed that the MCO CumA is dispensable for Mn(II) oxidation. Instead, a two-component regulatory system (Mnxs/S) was found to be essential for Mn(II) oxidation (Geszvain & Tebo, 2009). This is consistent with the fact that *cumA* is present in both Mn(II)-oxidizing and non-Mn(II)-oxidizing *Pseudomonas* strains (Francis & Tebo, 2001). Moreover, through transposon mutagenesis of other Mn(II) oxidases, such as *mnxG* (*PputGB1_2447*) and *PputGB1_2665*, Mn(II) oxidation was only slowed, but not lost in the mutant strains (Yamaguchi, 2009). These findings suggest that *P. putida* GB-1 may have multiple Mn(II) oxidases, with the expression of alternate enzymes dominating under different environmental conditions. Comparative genomics further indicates that genes related to Mn(II) oxidation in GB-1 have homologs in *P. putida* KT2440, W619 and F1, except that W619 lacks the homolog for *PputGB1_2665* (Table S6). The presence of these genes points to the potential for Mn(II) oxidation by KT2440, F1 and W619. Also, a link between Mn(II) oxidation and pyoverdine siderophore synthesis was reported for *P. putida* MnB1 (Parker et al., 2004). Thus, Mn(II) oxidation may influence the pyoverdine-mediated iron uptake by Mn(II)-oxidizing fluorescent *P. putida* strains.

**Response to oxidative stress by P. putida**

*Pseudomonas putida* strains thrive in environments that are characterized by oxidized stress including the rhizosphere, soils and sediments containing reactive metal species generated during the Mn-redox cycle, or reactive intermediates generated during the oxidative breakdown of aromatic hydrocarbons. As part of their adaptation, *P. putida* strains possess various enzymes including catalases, peroxidases and superoxide dismutases for defense against reactive oxygen species (ROS) (including superoxide, hydroperoxyl radical and hydrogen peroxide species), nitric oxide and phytoalexins (Hammond-Kosack & Jones, 1996; Zeidler et al., 2004). Comparative genomic analysis shows that many of the enzymes involved in the oxidative stress response are common among the *P. putida* species (Table S7). For example, the W619 genome encodes three superoxide dismutases: SodA, a Mn superoxide dismutase (*PputW619_4269*), SodB, an Fe superoxide dismutase (*PputW619_0981*), and SodC, a Cu/Zn superoxide dismutase (*PputW619_2485*). The sodC gene is located on a putative genomic island (region 12) and it is absent from the other *P. putida* strains. *Pseudomonas putida* W619 is also predicted to contain five catalases, KatA (*PputW619_4722*), KatB (*PputW619_2032*), KatE (*PputW619_5113*), KatG (*PputW619_2235*) and one putative catalase (*PputW619_2390*); two alkyl hydroperoxide reductases, AhpF and AhpC (*PputW619_3186-3187*), four additional putative alkyl hydroperoxide reductases (one putative AhpC, *PputW619_1113* and three having an AhpD domain, *PputW619_3104*, *PputW619_3108*, *PputW619_3238*); a chloroperoxidase (*PputW619_1849*); two thiol peroxidases (*PputW619_2803* and *PputW619_3977*); and two putative glutathione peroxidases (*PputW619_1244*, *PputW619_1483*), a glutathione oxidoreductase (*Gor, PputW619_3188*) and a glutaredoxin (*PputW619_3239*). Among these enzymes, the KatB and AhpD domain proteins, whose genes are located on three genomic islands (regions 9, 19 and 20), are unique to *P. putida* W619. We also identified an organic hydroperoxide resistance protein (Ohr, *PputW619_1469*) located adjacent to its organic hydroperoxide resistance transcriptional regulator (OhrR, *PputW619_1470*). Moreover, *P. putida* W619 is likely able to detoxify free radical nitric oxide by the presence of a flavohemoprotein nitric oxide dioxygenase (*PputW619_4378*) with its anaerobic nitric oxide reductase transcription activator NorR (*PputW619_4379*) (Tucker et al., 2004).

The oxidative stress response system is controlled via complex regulatory networks (Storz & Imlay, 1999). One of the key regulators is the peroxide resistance protein PerR, which was identified as the major regulator of the inducible peroxide stress response in *Bacillus subtilis* (Mongkolsuk & Helmann, 2002). In *P. putida* W619, the PerR protein is encoded by *PputW619_2615*, a LysR family transcriptional regulator that shares 61.5% identity with PerR of *E. coli* K12. PerR regulates the expression of *dps* (DNA-binding stress protein, *PputW610_4005*), *fur* (the ferric uptake repressor, *PputW619_0702*), *ahpCF* and *katA* (Mongkolsuk & Helmann, 2002), all of which are present in *P. putida* W619.
In some cases, the regulation of ROS-responsive genes is iron dependent and coupled to that of iron-binding proteins, such as bacterioferritin (Bfr). As in KT2440, the bacterioferritin α subunit (bfrA, PputW619_4721) in *P. putida* W619 is located next to catalase A (katA) (Dos Santos et al., 2004), and the bfrB and Bfd-associated ferredoxin gene (PputW619_1111-1112) are located adjacent to *ahpC* (PputW619_1113). Furthermore, various oxidation-resistant metabolic enzymes were putatively identified in W619, including *acna* (encoding an aconitase, PputW619_1631), which is stable under oxidative stress, and *fumC* (PputW619_4271), located upstream of *sodA* (PputW619_4269), which encodes a hydroperoxide resistant isoform of fumarase. In *P. aeruginosa*, the *fumC*-sodA operon is shown to be negatively regulated in the presence of iron via the Fur protein (Polack et al., 1996).

**Siderophore production by *P. putida***

The bioavailability of iron in the soil is limited by the very low solubility of the Fe$^{3+}$ ion, and bacteria have developed diverse mechanisms for iron acquisition. One of the most important mechanisms is the production and release of siderophores to scavenge iron from the environment via the formation of soluble Fe$^{2+}$ complexes, which can subsequently be taken up by active transport systems (Neilands, 1995; Ravel & Cornelis, 2003). In addition to their own siderophores, bacteria may utilize a large number of heterologously produced siderophores that are taken up via various siderophore receptors (Dean et al., 1996). Many *Pseudomonas* strains show fluorescence under UV light, indicative of the production of the siderophore pyoverdine (PVD) (Meyer, 2000). In accordance with this observation, nonribosomal peptide synthetase (NRPS) genes for pyoverdine synthesis were found in all four *P. putida* strains (Table S8). A cluster of peptide NRPS genes was identified, together with the PVD ABC transporter gene *pvdE* and a TonB-dependent receptor gene *fpvA*. At a separate locus, the chromosomal NRPS gene *psvA* was found to be present in all four strains along with the σ factor *psvS* and, with the exception of strain F1, the siderophore biosynthesis gene *pvdZ* (Moon et al., 2008). In F1, the *pvdZ* gene is located next to the *pvdE* gene. It should also be noted that the four *P. putida* strains are lacking the homologs of pseudomonine synthesis genes as identified in *P. entomophila* L48 (PSEEK_2500-2507), and therefore seem to be unable to produce this secondary siderophore (Matthijs et al., 2009).

For the transport of pyoverdine, *P. putida* W619 possesses multiple copies (20) of genes encoding TonB-dependent outer-membrane receptors, as reported previously by Cornelis & Bodilis (2009). Out of these 20 TonB-dependent receptors, 17 are putative ferric siderophore receptors, including a putative ferric enterobactin receptor (*fepA*) and a FeCα protein for ferric citrate uptake (Table S8). Of the three remaining TonB-dependent receptors, one is a putative copper-regulated channel protein (OprC) (PputW619_4630) (Yoneyama & Nakae, 1996), while the specificity of the other two receptors remains unclear (PputW619_1083, PputW619_5001). As summarized in Cornelis & Bodilis (2009), the genomes of F1, KT2440 and *P. entomophila* L48 carry 29 to 31 putative TonB-dependent receptor genes, a number considerably higher than the 20 genes identified in W619. It has been shown experimentally that *P. entomophila* L48 is able to utilize a large variety of heterologous pyoverdines, but that in contrast, KT2440 can only use its own pyoverdine and the pyoverdine heterologously produced by *P. syringae* LMG 1247 (Matthijs et al., 2009). This observation is in accordance with the fact that L48 has the highest number of ‘orphan’ TonB-dependent receptor genes (11) that are not found on the genomes of the *P. putida* strains, while the *P. putida* KT2440, F1 and W619 genomes only contain 4, 3 and 5 ‘orphan’ genes, respectively. Thus, the capacity of W619 to utilize heterologous pyoverdine needs further investigation (Cornelis & Bodilis, 2009).

The TonB-dependent siderophore receptors genes in W619 are often located adjacent to genes coding a transmembrane anti-σ sensor (FecR family) and an ECF-σ70 transcriptional regulator of the FecI family, which might regulate the expression of receptors (Folschweiler et al., 2000; Braun & Endriss, 2007; Cornelis et al., 2009). It should be noted that in the genomes of *P. putida* strains, up to 13 ECF-σ factors were found that showed similarity to the FecI-σ factor of *E. coli* (Martínez-Bueno et al., 2002). The specificity of the receptors for various ferric siderophores is not well understood, but the presence of multiple TonB-dependent siderophore receptors might confer *P. putida* strains with the ability to use a broad spectrum of these heterologously synthesized compounds (Paulsen et al., 2005). For example, the *P. putida* strains possess an iron uptake system that involves the genes coding for the outer-membrane ferric enterobactin receptor, PfeA/FepA, and its corresponding two-component regulator, PfeRS (Dean et al., 1996). This system may allow *P. putida* to compete with *Enterobacteriaceae* for iron by utilizing the ferric-enterobactin complex. In addition, a universal TonB-dependent heme receptor gene (*phuR*) was found in all four *P. putida* strains (PP_1006, PputW619_4218, Pput_1043, PputGBL_1005), while two other heme uptake-related genes, *HasR* and *HxuC*, typically found in the pathogens *P. aeruginosa* (Ochsner et al., 2000) and *P. entomophila* (Cornelis & Bodilis, 2009), were absent in *P. putida*. The redundant presence of systems that scavenge iron from the environment argues for the importance of iron acquisition in the ecology of this bacterium and its ability to colonize multiple niches.
Association of \textit{P. putida} with plants

Both \textit{P. putida} KT2440 and W619 were found to live in association with plants, either as a rhizospheric strain or as an endophyte, respectively. Comparative genomics, using \textit{P. putida} W619 as the reference strain, was used to identify the functions that are important for the association of these bacteria and their host plant.

Motility

The \textit{P. putida} W619 genome contains a large cluster of fifty-two genes involved in flagella biosynthesis (PputW619_3655-3737) (Table S9). This cluster is similarly organized in all four \textit{P. putida} strains, except that W619 contains within the flagellar biosynthesis cluster a gene cluster involved in LPS biosynthesis and sporulation. Furthermore, the flagellar biosynthesis clusters in W619, KT2440 and F1 are interrupted by a region of atypical composition and lost synteny that contains \textit{d-Alanine} and cystathionine ligases (genomic region 22; PputW619_3671-3677). \textit{Pseudomonas putida} KT2440 is known to be a good swimmer; this in contrast to the solvent-tolerant \textit{P. putida} DOT-T1E (Segura et al., 2004). It was demonstrated that the impaired swimming capability of DOT-T1E resulted from a mutation in the \textit{flhB} gene, which resulted in higher solvent resistance (Segura et al., 2004). The \textit{P. putida} W619 \textit{flhB} gene is 91% identical to that of KT2440, suggesting a swimming capability similar to KT2440. In addition, it was demonstrated in KT2440 that mutations in the flagellar biosynthesis genes \textit{flgL} (PputW619_3721), \textit{fliA} (PputW619_3667), \textit{fleQ} (PputW619_3698), \textit{fliL} (PputW619_3691), \textit{fliN} (PputW619_3683) and \textit{fliD} (PputW619_3730) resulted in different degrees of impaired swimming motility, and swarming, and also affected the adhesion to seeds (Espinosa-Urgel et al., 2000; Yousef-Coronado et al., 2008). The preliminary microarray result obtained with \textit{P. putida} W619 showed that the transcription of genes encoding the flagellar proteins (PputW619_3724-3726) is induced when the cells are grown hydropically in the presence of poplar roots, pointing toward a role of mobility in the plant–bacterial recognition/interaction process (data not shown).

Pili and curli fibers

Type I pili assembly occurs in the periplasm and involves the chaperone–usher pili assembly system (Thanassi et al., 1998). The four \textit{P. putida} genomes all encoded a cluster of type I pili synthesis proteins, CsuABCDE, where CsuC and CsuD are predicted as the usher pathway chaperone and the usher protein, respectively (Table S10).

Type IV pili are typically 5-7 nm fibers that play a very important role in host colonization by a wide range of plant and animal pathogens (Mattick, 2002). The biogenesis and function of type IV pili are controlled by a large number of genes. On the \textit{P. putida} genomes, three clusters of type IV pili biosynthesis genes were identified, \textit{pilMNPQ}, \textit{pilACD} and \textit{pilEXW/fimT}, as well as four copies of the \textit{pilZ} gene for pili assembly. The pili assembly might be linked to the cell cycle, because \textit{pilZ} is transcriptionally coupled to \textit{holB}, which encodes the \textit{δ} subunit of DNA polymerase III (Alm et al., 1996). Also \textit{P. putida} contains a complex pili biosynthesis regulatory system, \textit{pilGHJLchpA}, where \textit{pilLchpA} encode a large fusion protein that acts as a very sophisticated signal transduction protein, ChpA (Alm & Mattick, 1997). Moreover, type IV pili are involved in natural DNA uptake (transformation). There are two additional putative \textit{traX} genes (PputW619_2699, PputW619_5167) in \textit{P. putida} W619, related to the conjugal DNA transfer that are absent in KT2440, F1 and GB-1.

Curli fibers are involved in adhesion to surfaces, cell aggregation and biofilm formation and play an important role in host cell adhesion and invasion (Barnhart & Chapman, 2006). The \textit{P. putida} strains possess two gene clusters for curli fiber biogenesis: \textit{csgEFG} and \textit{csgAB}. The \textit{CsgAB} proteins provide the two curli structural subunits, while \textit{CsgEFG} are accessory proteins required for curli assembly (Hämmer et al., 1995).

Colonization of seeds by \textit{P. putida}

In \textit{P. putida} KT2440, colonization of seeds was affected by mutations in various genes (Yousef-Coronado et al., 2008). As expected, homologs of these genes were identified in \textit{P. putida} W619: \textit{lapA} (PputW619_5060), \textit{lapBCD} (PputW619_5062-5064), \textit{hemN2} (PputW619_0364), \textit{coxE} (PputW619_0130), \textit{galU} (PputW619_3189) and a hypothetical protein (PputW619_0162) that was shown to be involved in the oxidative stress response. We hypothesize that these genes have the same functions in W619. For instance, the LapA protein involved in biofilm formation is exported to the outside of the cell via the type I LapBC secretion system (Hinsa et al., 2003). Furthermore, \textit{P. putida} W619 contains an additional gene coding for an outer membrane component of the type I secretion system (PputW619_5061). The genome of \textit{P. putida} W619 also encodes a putative adhesin (PputW619_3808) and a surface-adhesion calcium-binding outer membrane-like protein that was 24% identical to LapA. This large (3923 aa) protein is encoded next to its putative type I secretion system in a gene organization similar to the \textit{lapABC} cluster. Other genes on the \textit{P. putida} W619 genome putatively involved in adhesion include PputW619_1818 and PputW619_1489, both of which code for autotransporter proteins (secretion type V) with a pectin/lyase/pertactin domain. In contrast to the poplar endophyte \textit{Enterobacter} sp. 638, no genes encoding a hemagglutinin protein were identified on the \textit{P. putida} W619 genome. Among closely
related *Pseudomonas* strains, we only found hemagglutinin-like adhesion genes in the insect pathogen *P. entomophila* L48 (PSEEN_0141, PSEEN_2177, PSEEN_3946), where they are predicted to be important virulence factors and involved in adhesion and type I or two-partner secretion systems (Vodovar et al., 2006).

**Establishment of *P. putida* W619 in poplar**

The *ndvB* gene (PputW619_2133) encodes a protein (2881 aa) involved in the production of β-(1,2)-glucan. The membrane-bound NdV protein catalyzes three enzymatic activities: the initiation (protein glucosylation), elongation and cyclization in *situ* of β-(1,2)-glucan, which is then released into the periplasm (Castro et al., 1996). It has been reported that cyclic β-(1,2)-glucan is involved in the attachment of *Agrobacterium tumefaciens* to plant cells (Douglas et al., 1985). In *Rhizobium meliloti*, mutations of the *ndvB* gene reduced the amounts of periplasmic β-(1,2)-glucan, which resulted in altered phenotypes related to phage and antibiotic sensitivity, motility, and growth in low-osmolarity media. Bacteroids produced by two of the downstream mutants were morphologically abnormal, indicating that *ndvB* is involved not only in invasion but also in bacteroid development (Ielpi et al., 1990). The *ndvB* gene is not present in *P. putida* GB-1, F1 or KT2440, but was identified in the other two poplar endophytes: *Serratia proteamaculans* 568 and *Enterobacter* sp. 638.

**Plant growth-promoting properties of *P. putida***

**Synthesis of plant growth-promoting hormones by *P. putida***

**Indole-3-acetic acid**

Many plant growth-promoting bacteria are capable of synthesizing phytohormones that affect the growth and development of their plant host. *In vitro*, *P. putida* W619 was the most efficient producer of IAA in comparison with other endophytic bacteria (Taghavi et al., 2009). Consistently, the *P. putida* W619 genome encodes two putative tryptophan-dependent IAA synthesis pathways. One is via tryptamine and indole-3-acetaldehyde and requires a tryptophan decarboxylase (PputW619_2223), an amine oxidase (PputW619_0482) and an indole-3-acetaldehyde dehydrogenase (encoded by many putative genes PputW619_0192/0213/0597/2257/2639/2872/2926/2546/3767). This pathway also exists in KT2440, F1 and GB-1. The alternate pathway is converting tryptophan to indole-3-acetamide via a tryptophan 2-monoxygenase (PputW619_1175/4820), which is subsequently converted into IAA by a putative deaminase (PputW619_2551). *Pseudomonas putida* KT2440, F1 and GB-1 lack homologs for PputW619_1175, but contain conserved genes for PputW619_4820. Furthermore, the step to convert indole-3-acetamide into IAA seems to be absent in KT2440; thus, this pathway might be incomplete and nonfunctional. Along with the high level of IAA production, *P. putida* W619 has three genes encoding putative auxin efflux carriers (PputW619_2484/2492/3829). *Pseudomonas putida* KT2440, F1 and GB-1 also have auxin efflux carriers, but only in two copies for F1 and KT2440.

**ACC deaminase**

A functional 1-aminocyclopropane-1-carboxylate deaminase (*acd*) (EC: 3.5.99.7), involved in counteracting the plant’s ethylene stress response, is absent on the genomes of *P. putida* W619, GB-1, F1 and KT2440, as was confirmed by the failure of these strains to grow on 1-aminocyclopropane-1-carboxylate as the sole carbon or nitrogen source. Although putative ACC deaminases were identified, all lack the particular amino acids E296 and L323 (respectively, replaced by a T or S and a T) that constitute part of the signature sequence of the active site of the true ACC deaminases and are involved in substrate specificity (Glick et al., 2007).

**Acetoin and 2,3-butanediol synthesis**

Volatile compounds such as 3-hydroxy-2-butanone (acetoin) and 2,3-butanediol are emitted by rhizobacteria to enhance plant growth (Ryu et al., 2003). In contrast to the poplar endophyte *Enterobacter* sp. 638, whose genome was sequenced recently (Taghavi et al., 2010), the *P. putida* W619 genome does not encode the *budABC* operon involved in the conversion of pyruvate to acetoin and 2,3-butanediol. The *P. putida* W619 genome carries the gene *poxB* (PputW619_2979) encoding a pyruvate dehydrogenase (genomic region 18). While the principal function of this enzyme is to convert pyruvate into acetaldehyde, a small fraction of the pyruvate can be converted into acetoin as a byproduct of the hydroxyethyl-thiamin diphosphate reaction intermediate. Although the production of acetoin by *P. putida* W619 is probably very low, especially at a low pyruvate concentration, this plant growth hormone can be utilized and converted into 2,3-butanediol, another plant growth hormone, by the poplar tree.

On the genomes of *P. putida* F1 (Pput_0595-0591), GB-1 (PputGB1_0601-0597) and KT2440 (PP_0556-0552) genes (*acoXABC adh*) involved in the catabolic conversion of acetoin and 2,3-butanediol to central metabolites were identified. Interestingly, these genes were not present in *P. putida* W619. Therefore, *P. putida* W619 does not have an antagonist effect on the production of the plant growth-
promoting phytohormones acetoin and 2,3-butanediol, which might be beneficial as these compounds stimulate root formation and indirectly the availability of carbon sources for KT2440 when residing in the plant rhizosphere.

Salicylic acid

The genome of \textit{P. putida} W619 encodes a salicylate 1-monoxygenase (\textit{mahG}, PputW619\_2140) involved in the conversion of salicylate into catechol. Homologues of this gene were also found on the genomes of \textit{P. putida} KT2440, F1 and GB-1. Salicylate is a phenolic phytohormone involved in plant growth and development, and plays a role in the resistance to pathogens by inducing the production of pathogenesis-related proteins (Van Huijsduijnen et al., 1986).

Defense against plant pathogens by \textit{P. putida}

Many pathogenic fungi secrete mannitol, a powerful reactive oxygen scavenger, into the apoplast during plant infection (Jennings, 1984; Velez et al., 2007). This process has been shown to be required for pathogenicity as it suppresses the reactive oxygen-mediated plant host defenses (Chaturvedi et al., 1996a,b; Velez et al., 2008). The production of mannitol dehydrogenase (MTD, EC 1.1.1.255) was hypothesized to increase the effectiveness of the plant defense response via the conversion of pathogen-secreted mannitol into mannose (Jennings et al., 2002). Interestingly, the gene encoding for MTD was found in \textit{P. putida} W619 (\textit{mltD}, PputW619\_2037) located on a putative genomic island (region 9), along with genes coding for a mannitol ABC transporter (\textit{mltKGF}, PputW619\_2038-2041), a transcriptional activator (\textit{mltR}, PputW619\_2042), a fructokinase (\textit{mltZ}, PputW619\_2035) and a xylulokinase (\textit{mltY}, PputW619\_2036). It is therefore hypothesized that \textit{P. putida} W619 can assist its plant host in its defense against pathogenic fungi, an important property for its commensal lifestyle. The \textit{mltZYDKGE} cluster, which is very similar to the operon found in \textit{P. fluorescens} DSM50106 (Brunker et al., 1998a,b), is absent in \textit{P. putida} KT2440, F1 and GB-1.

Virulence factors in \textit{P. putida}

Like for the granted biosafety strain \textit{P. putida} KT2440, strains W619, F1 and GB-1 also lack a number of key determinants required for virulence and virulence-associated traits (Nelson et al., 2002). There is no evidence in the four \textit{P. putida} genome sequences for putative functions required for the biosynthesis of exotoxin A, phospholipase C or pectin lyase that are frequently present in plant and animal pathogens. Additionally, the type III secretion pathway present in the plant pathogens \textit{P. syringae} pv. \textit{tomato} DC3000 or pv. \textit{phaseolicola} 1448A (Cornelis & Van Gijseghem, 2000; Feil et al., 2005; Vencato et al., 2006) is missing in all four \textit{P. putida} strains. Some individual homologs with putative functions were found, such as PputW619\_0806 coding for a putative type III HopPmaJ effector. However, this is in sharp contrast to the presence of a 20-kb gene cluster encoding a putative type III secretion system in the rhizosphere bacterium \textit{P. fluorescens} SBW25 (Preston et al., 2001). Components of the type VI secretion system (T6SS) were also identified in \textit{P. putida} strains and compared with the three T6SS clusters described in \textit{P. aeruginosa} (Filloux et al., 2008) (Table S11). The T6SS clusters are partially conserved among the \textit{P. putida} strains, including the ATPase ClpV, and the secreted VgrG and Hcp proteins. In W619, one of the T6SS-associated gene clusters (PputW619\_3242-43, 3245-46, 3255-56, 3260-61) is located in genomic region 20 (3574758–3603632). Although the majority of genomic region 20 appears to lack synteny with the other three \textit{P. putida} strains, the T6SS-associated genes seem to be conserved among KT2440, F1 and GB-1. Furthermore, in region 20, GAF/GGDEF and EAL signaling domains (Galperin, 2004) were identified that are hypothesized to play a role in regulating bacteria–host interactions. It also needs to be examined how far the functions of the putative T6SS in \textit{P. putida} differ from the systems described in other Pseudomonads. For instance, they do not seem to play a role in virulence, as they lack homologs of the serine–threonine protein kinase (STPK), a key virulence factor in \textit{P. aeruginosa} (Bingle et al., 2008).

Concluding remarks

The present inventory of genes and operons and the comparative genome analysis of the genome sequences of \textit{P. putida} KT2440, W619, F1 and GB-1 provided a powerful tool to gain new insights into the adaptation of this species to specific lifestyles and environmental niches. Although \textit{P. putida} strains are generally known for their versatile metabolic properties, this comparison also clearly demonstrated that horizontal gene transfer played an important role in this adaption process, as many of the niche-specific functions were found to be encoded on clearly defined genomic islands or on regions with lost synteny between the closely related strains. Often the genomic islands are delineated by mobile elements, many of which seem to be strain specific. This further supports the notion that these genomic islands were acquired rather than being part of the \textit{P. putida} core genome.

The presence of many incomplete pathways as well as the duplication of pathways on the genomes of all four \textit{P. putida} strains is striking. Examples include pathways involved in heavy metal resistances such as the copper resistance systems in \textit{P. putida} W619 or the duplication of \textit{czcCBA} in \textit{P. putida}
F1, KT2440 and GB-1, the breakdown of aromatic compounds, such as the presence of a complete plus an incomplete mph operon for the degradation of 3-hydroxyphenylpropionate (3-HP) in W619, or the presence of two alternative pathways for the synthesis of IAA in P. putida W619 compared with only one pathway in the other three strains. The presence of various complete and incomplete pathways indicates that these strains, before adapting to their current biotopes, have a rich history of colonizing various biotopes, each of which required specific adaptations that were facilitated via the acquisition and reshuffling of new pathways. These adaptations also required the dedicated management of incoming systems to avoid functional redundancy (i.e. under stress when resources are scarce), their optimization and the economical interplay between various pathways genes, supported by a large diversity of regulators typically found in P. putida. A similar duplication and interplay of functions was described recently for the adaptation of C. metallidurans CH34 to environments polluted by heavy metals (Janssen et al., 2010). Whole-genome expression studies combined with metabolite analysis and proteomics should help to better understand the mechanisms for the interplay and roles of functions essential for the niche adaptation of these strains, and when combined with genome-scale metabolic reconstruction models (Nogales et al., 2008; Puchalka et al., 2008), will provide a nonempirical basis for the development of biotechnological applications that can be further tailored as a function of strain-specific metabolic pathways.

As expected from the unique features of the niches from which the strains were isolated, P. putida F1 was the best adapted to degrade a variety of aromatic compounds, while W619 was more competent with regard to heavy metal resistances and beneficial effects on plant. A comparison between P. putida W619 and KT2440 is of special interest, as it could provide valuable clues to identify key functions for an endophytic lifestyle in comparison with survival in the rhizosphere. Noticeable differences include the presence of an endophytic lifestyle in comparison with survival in the rhizosphere. This could provide valuable clues to identify key functions for the niche adaptation of these strains, and when combined with genome-scale metabolic reconstruction models (Nogales et al., 2008; Puchalka et al., 2008), will provide a nonempirical basis for the development of biotechnological applications that can be further tailored as a function of strain-specific metabolic pathways.

Acknowledgements

This work was supported by the US Department of Energy, Office of Science, BER, project number KP1102010 under contract DE-AC02-98CH10886. X.W., S.M., S.T. and D.v.d.L. were supported by Laboratory Directed Research and Development funds (LDRD09-005) at the Brookhaven National Laboratory under contract with the US Department of Energy. Thanks are due to Claudine Médiégue and the team of the Genoscope annotation platform for their help and the use of the MAGE system for the annotation and comparison of P. putida W619. Sequencing of P. putida W619 was performed at the Joint Genome Institute (JGI) under the auspices of the US Department of Energy’s Office of Science, Biological and Environmental Research Program, and by the University of California, Lawrence Berkeley National Laboratory under contract No. DE-AC02-05CH11231, Lawrence Livermore National Laboratory under contract No. DE-AC52-07NA27344 and Los Alamos National Laboratory under contract No. DE-AC02-06NA25396. Thanks are due to Max Mergeay for providing precious feedback on heavy metal resistances and their organization in P. putida.

Authors’ contribution

X.W. and S.M. contributed equally to the manuscript.

Statement

Re-use of this article is permitted in accordance with the Terms and Conditions set out at: http://wileyonlinelibrary.com/onlineopen#OnlineOpen_Terms

References

Alm RA & Mattick JS (1997) Genes involved in the biogenesis and function of type-4 fimbriae in Pseudomonas aeruginosa. Gene 192: 89–98.
Alm RA, Bodero AJ, Free PD & Mattick JS (1996) Identification of a novel gene, pilZ, essential for type 4 fimbrial biogenesis in Pseudomonas aeruginosa. J Bacteriol 178: 46–53.
Barnhart MM & Chapman MR (2006) Curli biogenesis and function. Annu Rev Microbiol 60: 131–147.
Bingle LE, Bailey CM & Pallen MJ (2008) Type VI secretion: a beginner’s guide. Curr Opin Microbiol 11: 3–8.
Brouwers GJ, de Vrind JP, Corstjens PL, Cornelis P, Baysse C & de Vrind-de Jong EW (1999) *cuaA*, a gene encoding a multicopper oxidase, is involved in Mn$^{2+}$ oxidation in *Pseudomonas putida* GB-1. *Appl Environ Microb* **65**: 1762–1768.

Brouwers GJ, de Vrind JP, Corstjens PL, Cornelis P, Baysse C & de Vrind-de Jong EW (1999a) *cuaA*, a gene encoding a multicopper oxidase, is involved in Mn$^{2+}$ oxidation in *Pseudomonas putida* GB-1. *Appl Environ Microb* **65**: 1762–1768.

Brunker P, Altenbuchner J & Mattes R (1998a) Structure and function of the genes involved in mannnitol, arabitol and glucitol utilization from *Pseudomonas fluorescens* DSM50106. *Gene* **206**: 117–126.

Brunker P, Altenbuchner J & Mattes R (1998b) The mannitol utilization genes of *Pseudomonas fluorescens* are regulated by an activator: cloning, nucleotide sequence and expression of the *mtlR* gene. *Gene* **215**: 19–27.

Chaturvedi V, Flynn T, Niehaus WG & Wong B (1996a) Stress tolerance and pathogenic potential of a mannnitol mutant of *Pseudomonas putida* F1. *Appl Environ Microb* **62**: 3549–3555.

Chaturvedi V, Flynn T, Niehaus WG & Wong B (1996b) Stress tolerance and pathogenic potential of a mannnitol mutant of *Pseudomonas putida* F1: cloning and characterization of DNA encoding the 319-kilodalton protein intermediate. *J Bacteriol* **178**: 6043–6048.

Cha JS & Cooksey DA (1991) Copper resistance in *Pseudomonas syringae* mediated by periplasmic and outer membrane proteins. *P Natl Acad Sci USA* **88**: 8915–8919.

Cha JS & Cooksey DA (1993) Copper hypersensitivity and uptake in *Pseudomonas syringae* containing cloned components of the copper resistance operon. *Appl Environ Microb* **59**: 1671–1674.

Chaturvedi V, Flynn T, Niehaus WG & Wong B (1996a) Stress tolerance and pathogenic potential of a mannnitol mutant of *Cryptococcus neoformans*. *Microbiology* **142**: 937–943.

Chaturvedi V, Wong B & Newman SL (1996b) Oxidative killing of *Cryptococcus neoformans* by human neutrophils. Evidence that fungal mannnitol protects by scavenging reactive oxygen intermediates. *J Immunol* **156**: 3836–3840.

Choi EN, Cho MC, Kim Y, Kim CK & Lee K (2003) Expansion of growth substrate range in *Pseudomonas putida* F1 by mutations in both *cymR* and *tods*, which recruit a ring-fission hydrolase CmEt and induce the *tods* catabolic operon, respectively. *Microbiology* **149**: 795–805.

Clarke PH (1982) The metabolic versatility of pseudomonads. *Antonie Van Leeuwenhoek* **48**: 105–130.

Cornelis P & Baysse C (2009) A survey of TonB-dependent receptors in fluorescent pseudomonads. *Environ Microbiol Rep* **1**: 256–262.

Cornelis P, Matthijs S & Van Oeﬄeen L (2009) iron uptake regulation in *Pseudomonas aeruginosa*. *Biometals* **22**: 15–22.

Corstjens PL, de Vrind JP, Westbroek P & de Vrind-de Jong EW (1992) Enzymatic iron oxidation by *Leptothrix discophora*: identification of an iron-oxidizing protein. *Appl Environ Microb* **58**: 450–454.

Dean CR, Neshat S & Poole K (1996) PfeR, an enterobactin-responsive activator of ferric enterobactin receptor gene expression in *Pseudomonas aeruginosa*. *J Bacteriol* **178**: 5361–5369.

de Vrind J, De Groot A, Brouwers GJ, Tommassen J & de Vrind-de Jong E (2003) Identification of a novel Gsp-related pathway required for secretion of the manganese-oxidizing factor of *Pseudomonas putida* strain GB-1. *Mol Microbiol* **47**: 993–1006.

de Vrind JP, Brouwers GJ, Corstjens PL, den Dulk J & de Vrind-de Jong EW (1998) The cytochrome c maturation operon is involved in manganese oxidation in *Pseudomonas putida* GB-1. *Appl Environ Microb* **64**: 3556–3562.

Dong Q & Mergeay M (1994) Cac/cnr efflux: a three-component chemiosmotic antiport pathway with a 12-transmembrane-helix protein. *Mol Microbiol* **14**: 185–187.

Dos Santos VA, Heim S, Moore ER, Stratz M & Timmis KN (2004) Insights into the genomic basis of niche specificity of *Pseudomonas putida* KT2440. *Environ Microbiol* **6**: 1264–1286.

Douglas CJ, Staneloni RJ, Rubin RA & Nester EW (1985) Identification and genetic analysis of an *Agrobacterium tumefaciens* chromosomal virulence region. *J Bacteriol* **161**: 850–860.

Duque E, Segura A, Mosqueda G & Ramos JL (2001) Global and cognate regulators control the expression of the organic solvent efflux pumps TtgABC and TtgDEF of *Pseudomonas putida*. *Mol Microbiol* **39**: 1100–1106.

Eaton RW (1996) p-Cumate catabolic pathway in *Pseudomonas putida* F1: cloning and characterization of DNA carrying the *cmt* operon. *J Bacteriol* **178**: 1351–1362.

Eaton RW (1997) p-Cymene catabolic pathway in *Pseudomonas putida* F1: cloning and characterization of DNA encoding conversion of p-cymene to p-cumate. *J Bacteriol* **179**: 3171–3180.

Espinosa-Urgel M, Salido A & Ramos JL (2000) Genetic analysis of functions involved in adhesion of *Pseudomonas putida* to seeds. *J Bacteriol* **182**: 2363–2369.

Feil H, Feil WS, Chain P et al. (2005) Comparison of the complete genome sequences of *Pseudomonas aeruginosa* pv. *syringae* B728a and pv. *tomato* DC3000. *P Natl Acad Sci USA* **102**: 11064–11069.

Ferrandez A, Garcia JL & Diaz E (1997) Genetic characterization and expression in heterologous hosts of the 3-(3-hydroxyphenyl) propionate catabolic pathway of *Escherichia coli* K-12. *J Bacteriol* **179**: 2573–2581.

Filloux A, Schalk II, Celia H, Kieffer B, Abdallah MA & Pattus F (2000) The pyoverdin receptor FpvA, a TonB-dependent receptor involved in iron uptake by *Pseudomonas aeruginosa* (review). *Mol Membr Biol* **17**: 123–133.
Francis CA & Tebo BM (2001) *cumA* multicopper oxidase genes from diverse Mn(II)-oxidizing and non-Mn(II)-oxidizing *Pseudomonas* strains. *Appl Environ Microb* 67: 4272–4278.

Franke S, Grass G, Rensing C & Nies DH (2003) Molecular analysis of the copper-transporting efflux system CusCFBA of *Escherichia coli*. *J Bacteriol* 185: 3804–3812.

Galperin MY (2004) Bacterial signal transduction network in a genomic perspective. *Environ Microbiol* 6: 552–567.

Geszvain K & Tebo BM (2009) Identification of a two-component regulatory pathway essential for Mn(II) oxidation in *Pseudomonas putida* GB-1. *Appl Environ Microb* 1: 1–2.

Gibson D, Zylstra GJ & Zylstra GJ (1990) Biotransformations catalyzed by toluene dioxygenase from *Pseudomonas putida* F1. *Pseudomonas: Biotransformations, Pathogenesis, and Evolving Biotechnology* (Silver S, Chakrabarty AM, Iglewski B and et al. eds), pp. 121–132. American Society for Microbiology Press, Washington, DC.

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J & McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. *Crit Rev Plant Sci* 26: 227–242.

Grass G, Fan B, Rosen BP, Lemke K, Schlegel HG & Rensing C (2001) *NreB* from *Achromobacter xylosoxidans* 31A Is a nickel-induced transporter conferring nickel resistance. *J Bacteriol* 183: 2803–2807.

Grosse C, Grass G, Anton A et al. (1999) Transcriptional organization of the *czc* heavy-metal homeostasis determinant from *Alcaligenes eutrophus* *J Bacteriol* 181: 2385–2393.

Hallin PF, Stærfeldt H-H, Rotenberg E, Binnewies TT, Benham CJ & Ussery D (2009) GeneWiz browser: an interactive tool for visualization of sequenced chromosomes. *Stand Genomic Sci* 1: 204–215.

Hammar M, Arnvist A, Bian Z, Olsen A & Normark S (1995) Expression of two *csg* operons is required for production of fibronectin- and congo red-binding curli polymers in *Escherichia coli* K-12. *Mol Microb* 18: 661–670.

Hammond-Kosack KE & Jones JD (1996) Resistance gene-dependent plant defense responses. *Plant Cell* 8: 1773–1771.

Haritha A, Sagar KP, Tiwari A, Kiranmayi P, Rodrigue A, Mohan PM & Singh SS (2009) *MrdH*, a novel metal resistance determinant of *Pseudomonas putida* KT2440, is flanked by metal-inducible mobile genetic elements. *J Bacteriol* 191: 5976–5987.

Hinsa SM, Espinosa-Urgel M, Ramos JL & O’Toole GA (2003) Transition from reversible to irreversible attachment during biofilm formation by *Pseudomonas fluorescens* WCS365 requires an ABC transporter and a large secreted protein. *Mol Microb* 49: 905–918.

Huijberts GNM & Eggink G (1996) Production of poly(3-hydroxyalkanoates) by *Pseudomonas putida* KT2442 in continuous cultures. *Appl Microb Biot* 46: 233–239.

Huijberts GNM, Eggink G, Dewaard P, Huisman GW & Witholt B (1992) *Pseudomonas putida* KT2442 cultivated on glucose accumulates poly(3-hydroxyalkanoates) consisting of saturated and unsaturated monomers. *Appl Environ Microb* 58: 536–544.

Huson DH, Auch AF, Qi J & Schuster SC (2007) MEGAN analysis of metagenomic data. *Genome Res* 17: 377–386.

Ielpi L, Dylan T, Ditta GS, Helinski DR & Stanfield SW (1990) The nvdB locus of *Rhizobium meliloti* encodes a 319-kDa protein involved in the production of beta-(1,2)-glucan. *J Biol Chem* 265: 2843–2851.

Janssen PJ, Van Houdt R, Moors H et al. (2010) The complete genome sequence of *Capnocytophaga endodontalis* GB-1. *Appl Environ Microb* 1: 1–2.

Jennings DB, Daub ME, Pharr DM & Williamson JD (2002) Constitutive expression of a celery mannosidase dehydrogenase in tobacco enhances resistance to the mannosidase-secreting fungal pathogen *Alternaria alternata*. *Plant J* 32: 41–49.

Jennings DH (1984) Polyl metabolism in fungi. *Adv Microb Physiol* 25: 149–193.

Jimenez JL, Minambres B, Garcia JL & Diaz E (2002) Genomic analysis of the aromatic catabolic pathways from *Pseudomonas putida* KT2440. *Environ Microbiol* 4: 824–841.

Lau PC, Wang Y, Patel A et al. (1997) A bacterial basic region leucine zipper histidine kinase regulating toluene degradation. *P Natl Acad Sci USA* 94: 1453–1458.

Lima-Mendez G, Van Helden J, Toussaint A & Leplae R (2008) Prophinder: a computational tool for prophage prediction in prokaryotic genomes. *Bioinformatics* 24: 863–865.

Mahillon J & Chandler M (1998) Insertion sequences. *Microbiol Mol Biol R* 62: 725–774.

Manna D, Breier AM & Higgins NP (2004) Microarray analysis of transposition targets in *Escherichia coli*: the impact of transcription. *P Natl Acad Sci USA* 101: 9780–9785.

Martinez-Bueno MA, Tobias R, Rey M & Ramos JL (2002) Detection of multiple extracytoplasmic function (ECF) sigma factors in the genome of *Pseudomonas putida* KT2440 and their counterparts in *Pseudomonas aeruginosa* PA01. *Environ Microbiol* 4: 842–855.

Matthijs S, Laus G, Meyer JM (2000) *Pyoverdine* pigments, siderophores and their counterparts in *Pseudomonas aeruginosa* PA01. *Environ Microbiol* 265: 149–193.

Mellano MA & Cooksey DA (1988) Induction of the copper resistance operon from *Pseudomonas syringae*. *J Bacteriol* 170: 4399–4401.

Mergay M, Monchy S, Vallaeys T et al. (2003) *Ralstonia metalidurans*, a bacterium specifically adapted to toxic metals: towards a catalogue of metal-responsive genes. *FEMS Microbiol Rev* 27: 385–410.

Meyer JM (2000) Pyoverdines: pigments, siderophores and potential taxonomic markers of fluorescent *Pseudomonas* species. *Arch Microbiol* 174: 135–142.

Molina-Henares MA, Garcia-Salamanca A, Molina-Henares J, de la Torre AJ, Herrera MC, Ramos JL & Duque E (2009)
Functional analysis of aromatic biosynthetic pathways in *Pseudomonas putida* KT2440. *Microbial Biotechnol* 2: 91–100.

Molina-Henares MA, de la Torre J, García-Salamanca A, Molina-Henares AJ, Herrera MC, Ramos JL & Duque E (2010) Identification of conditionally essential genes for growth of *Pseudomonas putida* KT2440 on minimal medium through the screening of a genome-wide mutant library. *Environ Microbiol* 12: 1468–1485.

Monchy S, Benotmane MA, Wattiez R et al. (2006) Transcriptomic and proteomic analyses of the pMOL30-encoded copper resistance in *Cupriavidus metallidurans* strain CH34. *Microbiology* 152: 1765–1776.

Monchy S, Benotmane MA, Janssen P, Vallaëys T, Taghavi S, van der Leie D & Mergey M (2007) Plasmids pMOL28 and pMOL30 of *Cupriavidus metallidurans* are specialized in the maximal viable response to heavy metals. *J Bacteriol* 189: 7417–7425.

Nakazawa T (2002) Travels of a *Pseudomonas* from Japan around the world. *Environ Microbiol* 4: 782–786.

Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. *J Biol Chem* 270: 26723–26726.

Nelson KE, Weinl C, Paulsen IT et al. (2002) Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. *Environ Microbiol* 4: 799–808.

Nogales J, Palsson BO & Thiele I (2008) A genome-scale metabolic reconstruction of *Pseudomonas putida* KT2440: iNJ746 as a cell factory. *BMC Syst Biol* 2: 7.

Nogales J, Palsson BO & Thiele I (2008) A genome-scale metabolic reconstruction of *Pseudomonas putida* KT2440: iNJ746 as a cell factory. *BMC Syst Biol* 2: 7.

Ochsner UA, Johnson Z & Vasil ML (2000) Genetics and regulation of two distinct haem-uptake systems, phu and has, in *Pseudomonas aeruginosa*. *Microbiology* 146 (Pt 1): 185–198.

Okazaki M, Sugita T, Shimizu M et al. (1997) Partial purification and characterization of manganese-oxidizing factors of *Pseudomonas fluorescens* GB-1. *Appl Environ Microbiol* 63: 4793–4799.

Parker DL, Sposito G & Tebo BM (2004) Manganese(III) binding to a pyoverdine siderophore produced by a manganese(II)-oxidizing bacterium. *Geochim Cosmochim Ac* 68: 4809–4820.

Paulsen IT, Press CM, Ravel J et al. (2005) Complete genome sequence of the plant commensal *Pseudomonas fluorescens* Pf-5. *Nat Biotechnol* 23: 873–878.

Phoenix P, Keane A, Patel A, Bergeron H, Ghoshal S & Lau PC (2003) Characterization of a new solvent-responsive gene locus in *Pseudomonas putida* F1 and its functionalization as a versatile biosensor. *Environ Microbiol* 5: 1309–1327.

Polack B, Dacheux D, Delic-Attree I, Toussaint B & Vignais PM (1996) *Pseudomonas aeruginosa* famC and sodA genes belong to an iron-responsive operon. *Biochem Biophys Res Co* 226: 555–560.

Preston GM, Bertrand N & Rainey PB (2001) Type III secretion in plant growth-promoting *Pseudomonas fluorescens* SBW25. *Mol Microbiol* 41: 999–1014.

Puchalka J, Oberhardt MA, Godinho M et al. (2008) Genomic-scale reconstruction and analysis of the *Pseudomonas putida* KT2440 metabolic network facilitates applications in biotechnology. *PLoS Comput Biol* 4: e1000210.

Quaranta D, McCarty R, Bandarian V & Rensing C (2007) The copper-inducible cin operon encodes an unusual methionine-rich azurin-like protein and a pre-Q0 reductase in *Pseudomonas putida* KT2440. *J Bacteriol* 189: 5361–5371.

Ravel J & Cornelis P (2003) Genomics of pyoverdine-mediated iron uptake in pseudomonads. *Trends Microbiol* 11: 195–200.

Rensing C & Grass G (2003) *Escherichia coli* mechanisms of copper homeostasis in a changing environment. *FEMS Microbiol Rev* 27: 197–213.

Rensing C, Fan B, Sharma R, Mitra B & Rosen BP (2000) CopA: An *Escherichia coli* CaiI–translocating P-type ATPase. *P Natl Acad Sci USA* 97: 652–656.

Rosson RA & Nealson KH (1982) Manganese binding and oxidation by spores of a marine *Bacillus*. *J Bacteriol* 151: 1027–1034.

Ryu CM, Farag MA, Hu CH, Reddy MS, WeiHX, Pare PW & Kleopper JW (2003) Bacterial volatiles promote growth in *Arabidopsis*. *P Natl Acad Sci USA* 100: 4927–4932.

Schmid A, Dordick JS, Hauer B, Kiener A, Wubbolts M & Witholt B (2001) Industrial biocatalysis today and tomorrow. *Nature* 409: 258–268.

Segura A, Hurtado A, Duque E & Ramos JL (2004) Transcriptional phase variation at the flhB gene of *Pseudomonas putida* DOT-T1E is involved in response to environmental changes and suggests the participation of the flagellar export system in solvent tolerance. *J Bacteriol* 186: 1905–1909.

Spain JC & Gibson DT (1988) Oxidation of substituted phenols by *Pseudomonas putida* F1 and *Pseudomonas* sp strain Js6. *Appl Environ Microbiol* 54: 1399–1404.

Spain JC, Zylstra GJ, Blake CK & Gibson DT (1989) Monoxygenation of phenol and 2,5-dichlorophenol by toluene dioxygenase in *Pseudomonas putida* F1. *Appl Environ Microbiol* 55: 2648–2652.

Storz G & Inlay JA (1999) Oxidative stress. *Curr Opin Microbiol* 2: 188–194.

Taghavi S, Barac T, Greenberg B, Borremans B, Vangronsveld J & van der Leie D (2005) Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. *Appl Environ Microbiol* 71: 8500–8505.

Taghavi S, Garafola C, Monchy S et al. (2009) Genome survey and characterization of endophytic bacteria exhibiting a beneficial effect on growth and development of poplar trees. *Appl Environ Microbiol* 75: 748–757.
Taghavi S, van der Lelie D, Hoffman A et al. (2010) Genome
enterobacter sp. 638. PLoS Genet 6: e1000943.
Tatusov RL, Galperin MY, Natale DA & Koonin EV (2000) The
cog database: a tool for genome-scale analysis of protein
functions and evolution. Nucleic Acids Res 28: 33–36.
Tebo BM, Johnson HA, McCarthy JK & Templeton AS (2005)
Geomicrobiology of manganese(II) oxidation. Trends
Microbiol 13: 421–428.
Thamassi DG, Saulino ET & Hultgren SJ (1998) The chaperone/
usher pathway: a major terminal branch of the general
secretory pathway. Curr Opin Microbiol 1: 223–231.
Timmis KN (2002) Pseudomonas putida: a cosmopolitan
opportunist par excellence. Environ Microbiol 4: 779–781.
Tucker NP, D’Autreaux B, Studholme DJ, Spiro S & Dixon R
(2004) DNA binding activity of the Escherichia coli nitric oxide
sensor NorR suggests a conserved target sequence in diverse
proteobacteria. J Bacteriol 186: 6656–6660.
Ussery DW, Kiil K, Lagesen K, Sicheritz-Ponten T, Bohlin J &
Wassenaar TM (2009) The Genus Burkholderia: analysis of 56
species. J Gen Virol 86: 107–157.
Vallenet D, Labarre L, Rouy Z et al. (2006) MaGe: a microbial
genome annotation system supported by synteny results.
Nucleic Acids Res 34: 53–65.
Van Huisdijuien RAMH, Alblas SW, De Rijk RH & Bol JF (1986)
Induction by salicylic acid of pathogenesis-related proteins
and resistance to alfalfa mosaic virus infection in various plant
species. J Gen Virol 67: 2135–2143.
Velez H, Glassbrook NJ & Daub ME (2007) Mannitol metabolism
in the phytopathogenic fungus Alternaria alternata. Fungal
Genet Biol 44: 258–268.
Velez H, Glassbrook NJ & Daub ME (2008) Mannitol
biosynthesis is required for plant pathogenicity by
Alternaria alternata. FEMS Microbiol Lett 285: 122–129.
Vencato M, Tian F, Alfano JR et al. (2006) Bioinformatics-enabled
identification of the HrpL regulon and type III secretion
system effector proteins of Pseudomonas syringae pv.
phascolartha 1448A. Mol Plant Microbe In 19: 1193–1206.
Villalbos M, Toner B, Bargá J & Sposito G (2003)
Characterization of the manganese oxide produced by
Pseudomonas putida strain MnB1. Geochim Cosmochim Ac 67:
2649–2662.
Vodovar N, Vallenet D, Cruveiller S et al. (2006) Complete
genome sequence of the entomopathogenic and metabolically
versatile soil bacterium Pseudomonas entomaphila. Nat
Biotechnol 24: 673–679.
Wackett LP & Gibson DT (1988) Degradation of
trichloroethylene by toluene dioxygenase in whole-cell studies
with Pseudomonas putida F1. Appl Environ Microbiol 54:
1703–1708.
Weigel C, Schmidt A, Ruckert B, Lurz R & Messer W (1997)
DnaA protein binding to individual DnaA boxes in the
Escherichia coli replication origin, oriC. EMBO J 16:
6574–6583.
Wein C, Nelson KE & Tummler B (2002) Global features of the
Pseudomonas putida KT2440 genome sequence. Environ
Microbiol 4: 809–818.
Weyens N, Van Der Lelie D, Artois T et al. (2009)
Bioaugmentation with engineered endothypic bacteria
improves contaminant fate in phytoremediation. Environ Sci
Technol 43: 9413–9418.
Williams PA, Jones RM & Shaw LE (2002) A third transposable
element, ISPpu12, from the toluene-xylene catalytic plasmid
pWW0 of Pseudomonas putida mt-2. J Bacteriol 184:
6572–6580.
Yamaguchi A (2009) Genetic analysis reveals complex Mn(II)
oxidation regulation in Pseudomonas putida GB1. PhD
Thesis, Oregon Health & Science University, Portland, OR.
Yee TW & Smith DW (1990) Pseudomonas chromosomal
replication origins: a bacterial class distinct from Escherichia
coli-type origins. P Natl Acad Sci USA 87: 1278–1282.
Yoneyama H & Nakae T (1996) Protein C (OprC) of the outer
membrane of Pseudomonas aeruginosa is a copper-regulated
channel protein. Microbiology 142 (Pt 8): 2137–2144.
Yousef-Coronado F, Travieso ML & Espinosa-Urgel M (2008)
Different, overlapping mechanisms for colonization of abiotic
and plant surfaces by Pseudomonas putida. FEMS Microbiol
Lett 288: 118–124.
Zeidler D, Zahringuer I, Gerber J et al. (2004) Innate immunity in
Arabidopsis thaliana: lipopolysaccharides activate nitric oxide
synthase (NOS) and induce defense genes. P Natl Acad Sci
USA 101: 15811–15816.
Zylstra GJ, McCombie WR, Gibson DT & Finette BA (1988)
Toluene degradation by Pseudomonas putida F1: genetic
organization of the tolu operon. Appl Environ Microbiol 54:
1498–1503.

Supporting Information
Additional Supporting Information may be found in the online
version of this article:
Table S1. Putative genomic islands identified on the
chromosomes of Pseudomonas putida W619, KT2440, F1
and GB-1.
Table S2. Putative orthologous relationships between the
chromosomes of the four Pseudomonas putida strains.
Table S3. Comparative analysis of the functional group
distribution among bacteria, Gammaproteobacteria, Pseudomonas,
and the four P.putida strains (KT2440, F1, GB-1 and
W619), based on Clusters of Orthologous Groups of proteins (COGs)
classification.
Table S4. Incomplete or truncated remnants of IS elements
identified among the genomes of Pseudomonas putida
KT2440, W619, F1 and GB-1.
Table S5. Complete overview of putative genes involved in
the degradation of aromatic compounds identified on the
genomes of Pseudomonas putida W619, KT2440, F1 and GB-1.
Table S6. Putative genes involved in Mn(II) oxidation identified on the genomes of Pseudomonas putida W619, KT2440, F1 and GB-1.

Table S7. Putative genes involved in the oxidative stress response in Pseudomonas putida W619, KT2440, F1 and GB-1.

Table S8. Putative genes involved in siderophore synthesis and iron uptake.

Table S9. Putative genes involved in the motility of Pseudomonas putida strains.

Table S10. Putative genes involved in the pili and curli fibers biosynthesis.

Table S11. Conserved genes of the type VI secretion system of Pseudomonas putida strains compared with the three T6SS clusters identified in Pseudomonas aeruginosa.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.