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Draft whole-genome sequence of the antibiotic-producing soil isolate *Pseudomonas* sp. strain 250J

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Summary

Bacteria of the genus *Pseudomonas* are becoming increasing well known for their ability to produce a wide range of antimicrobial compounds. In a large-scale screening for antibiotic producers, we identified a soil isolate that uses 4-hydroxyphenylacetate as the sole carbon source, *Pseudomonas* sp. strain 250J, which produces cyclic lipodepsipeptides of the xantholysin family during the stationary phase of growth. The closest relatives of this strain are *Pseudomonas mosselii*, *Pseudomonas soli* and *Pseudomonas entomophila*. Sequencing of the 250J genome allowed us to find the genes relevant to antibiotic production, those which allow utilization of 4-hydroxyphenylacetate as a sole carbon source and a set of genes potentially involved in biocontrol.

Introduction

Strains of the genus *Pseudomonas* are known to be able to colonize a wide range of environmental niches due to their capacity to adapt and survive to changes in their habitats (Dos Santos *et al*., 2004). Behind this ability to survive under harsh conditions is that these microorganisms generally contain relatively large genomes (about 6 Mb), with a wide set of genes forming part of their core genome, which is in the range of 3500 genes (Zulema Udaondo and Juan Luis Ramos, unpublished) The core genes of the genus *Pseudomonas* define its basic physiological properties regarding the transport of a broad range of chemicals, central carbon metabolism, nitrogen and sulfur assimilation, alternative respiratory chains, and ability to produce biofilms and attach to plant surfaces, as well as chemotaxis (Duque *et al*., 2013; Roca *et al*., 2013; Molina *et al*., 2014). In addition, *Pseudomonas* strains have a set of expansive 'accessory genes' that contribute to the different strain-specific properties, such as bioremediation of pollutants, biocontrol, and pathogenicity of humans or plants (Silby *et al*., 2011; Wu *et al*., 2011).

Results and discussion

Pseudomonas sp. strain 250J is a Gram-negative, aerobic, soil bacterium we isolated in M9 minimal medium (Abril *et al*., 1989) using 4-hydroxyphenylacetate as the sole carbon source. Utilization of 4-hydroxyphenylacetate is restricted to a limited number of *Pseudomonas* strains, and therefore this metabolism is not part of the 'core' physiology of all *Pseudomonas* strains. We found that strain 250J is able to produce antibiotic compounds that inhibit growth of Gram-positive and Gram-negative microorganisms. These chemicals were then identified as cyclic lipodepsipeptides of the xantholysins family (Fig. 1). It is known that xantholysin A has antibacterial, antifungal and antitumoral activity (Li *et al*., 2013; Pascual *et al*., 2014).

These two properties make strain 250J of interest to learn further on the set of accessory genes; therefore, we deepen on the utilization of 4-hydroxyphenylacetate as a carbon source by 250J strain, and to determine the genes involved in the production of xantholysin. To this end, we sequenced the genome of 250J strain. Genomic DNA containing the chromosome was purified from *Pseudomonas* sp. strain 250J using the Wizard® Genomic DNA Purification Kit. Whole-genome sequencing was performed using 150-base, paired-end reads on the Illumina MiSeq Platform at the basic biology service of the University of Granada (Spain). A total of $2 \times 6\,838\,152$ paired sequences were generated and subsequently analysed and checked for quality using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The genomic sequence of *Pseudomonas* sp. strain 250J was obtained by assembly of the two paired-end datasets using Velvet

Received 5 August, 2014; accepted 16 October, 2014. *For correspondence. E-mail carlos.molina@eez.csic.es; Tel. (+34) 958 181 600 (Ext. 321); Fax (+34) 958 181 609. **Nucleotide sequence accession number.** This Whole Genome Shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number JHEE00000000. The version described in this paper is version JHEE01000000.

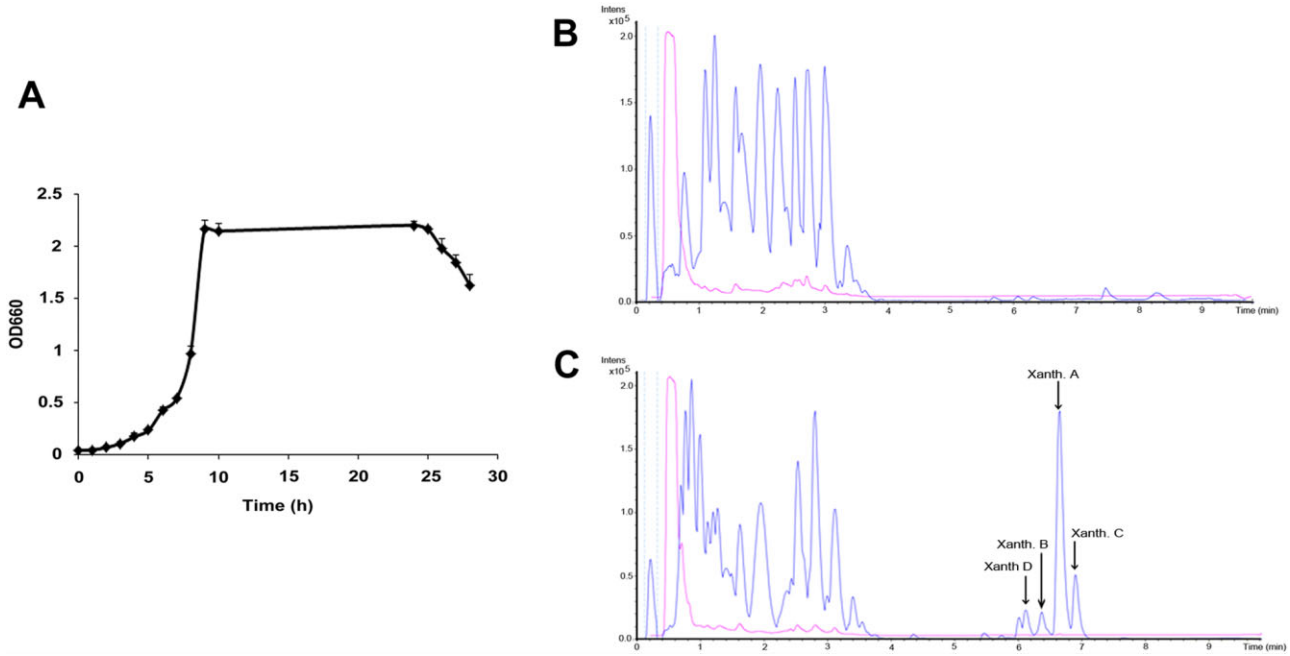

Fig. 1. Growth curve of *Pseudomonas* sp. 250J growing on LB (A), and xantholysin production in exponential phase (B) and in stationary phase (C). Xantholysin compounds are indicated in the chromatogram. Pink line indicates UV signal and blue line indicates ionized masses. The high pink peak at the beginning of the chromatogram is due to the dimethylsulphoxide used to dissolve the supernatant, which is not retained in the column.

(1.2.10 version) (Zerbino and Birney, 2008) with parameters determined by Velvet Optimizer 2.2.5 (http://bioinformatics.net.au/software.velvetoptimiser.shtml) resulting in 219 contigs with N_{50} of 62 Mbp and a read depth of 50×. The draft whole-genome sequence of *Pseudomonas* sp. strain 250J is made up of a 62.20%-GC circular chromosome of 5 906 591 bp with no plasmid (GenBank accession no. JHEE01000000). This genome encodes 5226 putative genes, including 9 rRNA and 73 tRNA genes that were annotated with the National Center for Biotechnology Information (NCBI) Prokaryotic Genome Annotation Pipeline and RAST server (Aziz *et al.*, 2008). We show a circular representation of the 250J genome in Fig. 2, where we represent the ordered draft genome generated with the BRIG software, and the percent identity that it shares with other *Pseudomonas* species, such as *P. entomophila*, *P. monteilii* and *P. putida* KT2440.

The 4-hydroxyphenylacetate degradation pathway involves the conversion of this aromatic compound to homoprotocatechuate, which subsequently undergoes ring cleavage to eventually yield succinate and pyruvate as shown in Fig. S1A. In *P. entomophila* and *P. putida U*, the set of genes encoding the pathway enzymes are grouped in a cluster (*hpaCBXIHFDEG2G1AR*) (Vodovar *et al.*, 2006; Arcos *et al.*, 2010), while in the 250J strain the *hpa* pathway genes are distributed in three unlinked operons. In contig 89, we identified the *hpaIHFDEG2G1AR* cluster, which is similar to the genes found in *P. fluorescens* SBW25 (Paliwal *et al.*, 2014) (Fig. S1B), while the 250J genes *hpaBC*, which encode the 4-hydroxyphenylacetate 3-hydroxylase that performs the first reaction of the pathway, and a potential 4-hydroxyphenylacetic acid transporter, *hpaX*, were found in contigs 90 and 110 respectively.

It is known that cyclic lipodepsipeptides are generally produced by non-ribosomal peptides synthetase (NRPS) gene clusters. By using the ANTISMASH software for rapid identification, annotation and analysis of secondary metabolite biosynthesis genes (Blin *et al.*, 2013), we were able to predict 24 PKS/NRPS gene clusters for putative biosynthetic secondary metabolites. These clusters can be organized into three groups that correspond to three gene clusters specifically: three clusters involved in bacteriocin biosynthesis, nine NRPS and 12 clusters predicted as putative NRPS of unknown function. Close analysis of the nine NRPS allowed identification of five clusters that are involved in the biosynthesis of four different xantholysins. These four cyclic lipodepsipeptides have been identified in the culture supernatant of *Pseudomonas* sp. strain 250J, and their molecular masses and chemical formula have been determined (Fig. 1C): xantholysin A (1775.08 Da, $C_{84}H_{146}N_{18}O_{23}$), xantholysin B (1761.07 Da, $C_{83}H_{144}N_{18}O_{23}$), xantholysin C

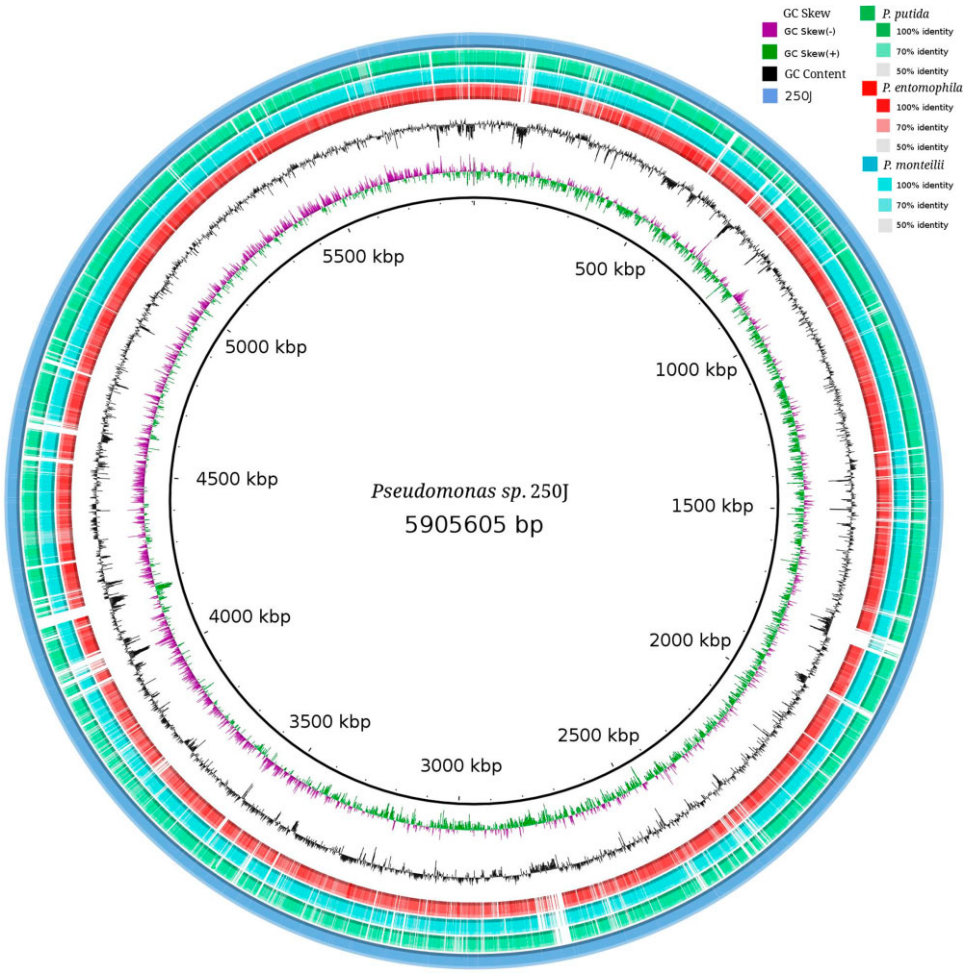

Fig. 2. BLAST comparison of draft genome of *Pseudomonas* sp. 250J against *Pseudomonas entomophila*, *Pseudomonas monteilii* and *Pseudomonas putida* KT2440 using BRIG. The innermost rings depict the GC content (black) and GC skew (purple/green) rings of *Pseudomonas* sp. 250J, followed by the query sequences of *P. entomophila* (red), *P. monteilii* (light blue), *P. putida* KT2440 (green) and *Pseudomonas* sp. 250J (blue), coloured according to BLAST identity.

(1802.0, $C_{86}H_{148}N_{18}O_{23}$) and xantholysin D (1775.09 Da, $C_{84}H_{146}N_{18}O_{23}$), with xantholysin A being the majoritarian one. The high number of PKS/NRPS sequences that could be producing secondary metabolites related with antimicrobial, antifungal and insecticidal compounds demonstrate the great armory that this strain presents to defend against other microorganisms and that could use to colonize other niches.

BLAST analyses were then done to pinpoint the *xtlA*, *xtlB* and *xtlC* genes, and the transcriptional regulator *xtlR*, which are related with xantholysin production in *P. putida* BW11M1. These genes are also conserved in *Pseudomonas* sp. 250J genome sequence (92%, 94%, 95% and 97% of identity sequence respectively). The xantholysin transporter is encoded by the *xtlD*, *xtlE* and *xtlF* genes and exhibits a percent of sequence identity with those of BW11M1 of 97%, 94% and 94% respectively. The xantholysin genes are organized in two clusters, one made of *xtlF*, *xtlR* and *xtlA*, and the other one performed by the *xtlD*, *xtlE*, *xtlB and xtlC* genes.

Multilocus sequence analysis (MLSA) comparison of the 250J strain with numerous other *Pseudomonas* strains revealed that the closest relatives to 250J are *P. mosselii*, *P. soli* and *P. entomophila*. *Pseudomonas* sp. 250J forms a monophyletic cluster with *P. mosselii*, *P. soli* and *P. entomophila* (Fig. 3). Furthermore, Eztaxon analysis of 16S rRNA genes supported the results obtained by MLSA analysis. *Pseudomonas entomophila* is known as a microorganism with insecticidal activity and which has the ability to kill a number of other invertebrates. A comparison between the genomes of *P. entomophila* and 250J was carried out in order to identify genes potentially related to pathogenicity. We found a number of proteases

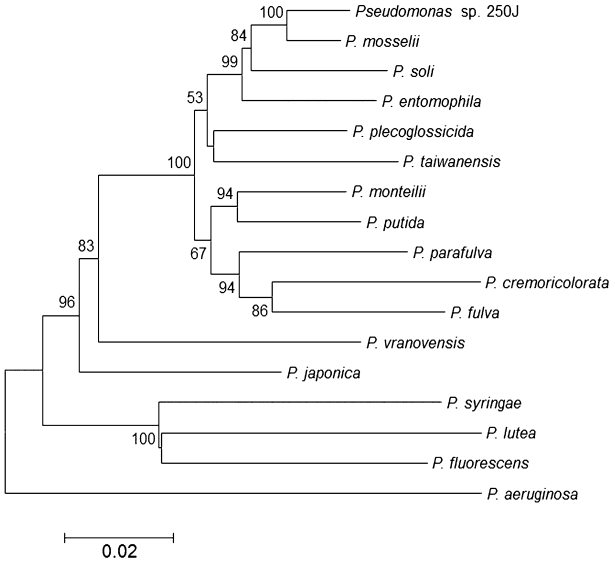

Fig. 3. Neighbour-joining tree illustrating the phylogenetic position of strain *Pseudomonas* sp. 250J and related members of the genus *Pseudomonas* based on partial concatenated gene sequences. Bar, 0.01 expected nucleotide substitution *per site*. *Pseudomonas aeruginosa* was used as outgroup. Only bootstrap values above 50% are indicated (1000 re-samplings) at branchings.

and toxins (Table S1), such as AprA (DA83_19105), an alkaline protease that is encoded together with its secretion system and involved in virulence in many species (Miyoshi and Shinoda, 2000); serine-proteases (DA83_13385 and DA83_13390); or the RTX toxin (DA83_05900) that are encoded in the 250J strain genome. Strain 250J bears the *hcnABC* genes (DA83_11195, DA83_11200, DA83_11205), which are related with hydrogen cyanide production, a toxic compound required for the virulence of *P. entomophila* against *Drosophila melanogaster*, killing of *Caenorhabditis elegans* by *P. aeruginosa* (Gallagher and Manoil, 2001) and in the suppression of soil-borne plant pathogens by certain *P. fluorescens* species (Haas and Defago, 2005). Experimental assays demonstrate the ability of 250J to produce proteases, lipases, hydrogen cyanide and siderophores, molecules with a known role in protection against pathogens that can affect growth of fungal and soil microorganisms, and as is the case of siderophores to improve the survival of 250J and its competitiveness against other microorganisms in the same environmental niche (Fig. 4). The production of this kind of molecules is of particular interest in biocontrol. On the other hand, we also tested the haemolytic activity of 250J and confirmed that this strain was able to produce haemolysis of goat blood. The genome of the 250J strain does not bear homologous of genes encoding for the insecticidal complex (TcdA, TcdB and TccC), which is present and characteristic of all the entomopathogens.

In summary, the present results show that the soil isolate *Pseudomonas* sp. strain 250J is a strain capable of naturally overproducing xantholysins and is able to use 4-hydroxyphenylacetic acid as a carbon source. Although strain 250J lacks known insecticidal genes, it is closely related to *P. entomophila* and bears a set of genes potentially involved in biocontrol.

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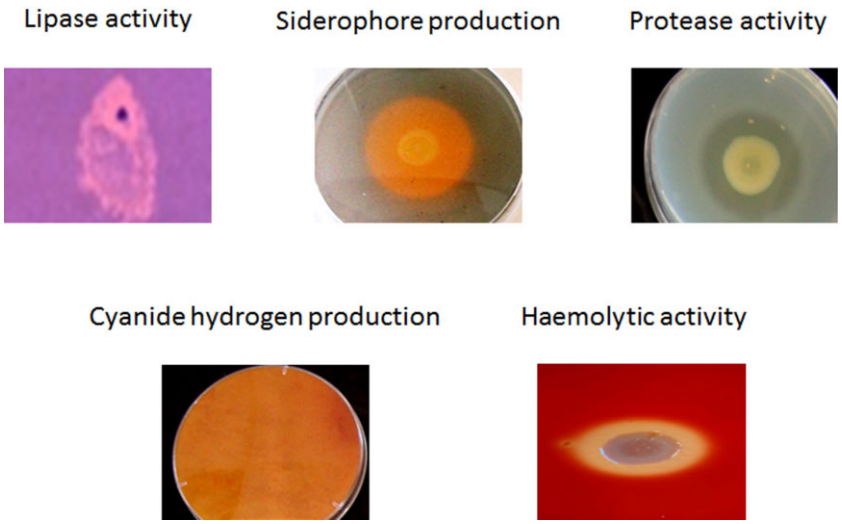

Fig. 4. Properties of 250J strain for biocontrol. Enzymatic assays were performed according to a set of protocols to test potential antimicrobial activities of microorganisms growing on solid plates. These assays have been described by Roca and colleagues (2013).

References

Abril, M.A., Michán, V., Timmis, K.N., and Ramos, J.L. (1989) Regulator and enzyme specificities of the TOL plasmid-encoded upper pathway for degradation of aromatic hydrocarbons and expansion of the substrate range of the pathway. *J Bacteriol* **171:** 6782–6790.

Arcos, M., Olivera, E.R., Arias, S., Naharro, G., and Luengo, J.M. (2010) The 3,4-dihydroxyphenylacetic acid catabolon, a catabolic unit for degradation of biogenic amines tyramine and dopamine in *Pseudomonas putida* U. *Environ Microbiol* **12:** 1684–1704.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., *et al*. (2008) The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* **9:** 75.

Blin, K., Medema, M.H., Kazempour, D., Fischbach, M.A., Breitling, R., Takano, E., and Weber, T. (2013) antiSMASH 2.0–a versatile platform for genome mining of secondary metabolite producers. *Nucleic Acids Res* **41:** W204–W212.

Dos Santos, V.A., Heim, S., Moore, E.R., Stratz, M., and Timmis, K.N. (2004) Insights into the genomic basis of niche specificity of *Pseudomonas putida* KT2440. *Environ Microbiol* **6:** 1264–1286.

Duque, E., de la Torre, J., Bernal, P., Molina-Henares, M.A., Alaminos, M., Espinosa-Urgel, M., *et al*. (2013) Identification of reciprocal adhesion genes in pathogenic and non-pathogenic Pseudomonas. *Environ Microbiol* **15:** 36–48.

Gallagher, L.A., and Manoil, C. (2001) Pseudomonas aeruginosa *PAO1 kills* Caenorhabditis elegans *by cyanide poisoning*. *J Bacteriol* **183:** 6207–6214.

Haas, D., and Defago, G. (2005) Biological control of soil-borne pathogens by fluorescent *pseudomonads*. *Nat Rev Microbiol* **3:** 307–319.

Li, W., Rokni-Zadeh, H., De Vleeschouwer, M., Ghequire, M.G., Sinnaeve, D., Xie, G.L., *et al*. (2013) The antimicrobial compound xantholysin defines a new group of *Pseudomonas* cyclic lipopeptides. *PLoS ONE* **8:** e62946.

Miyoshi, S., and Shinoda, S. (2000) Microbial metalloproteases and pathogenesis. *Microbes Infect* **2:** 91–98.

Molina, L., Udaondo, Z., Duque, E., Fernandez, M., Molina-Santiago, C., Roca, A., *et al*. (2014) Antibiotic resistance determinants in a Pseudomonas putida strain isolated from a hospital. *PLoS ONE* **9:** e81604.

Paliwal, V., Raju, S.C., Modak, A., Phale, P.S., and Purohit, H.J. (2014) *Pseudomonas putida* CSV86: a candidate genome for genetic bioaugmentation. *PLoS ONE* **9:** e84000.

Pascual, J., Garcia-Lopez, M., Carmona, C., Sousa Tda, S., de Pedro, N., Cautain, B., *et al*. (2014) Pseudomonas soli sp. nov., a novel producer of xantholysin congeners. *Syst Appl Microbiol* **37:** 412–416.

Roca, A., Pizarro-Tobias, P., Udaondo, Z., Fernandez, M., Matilla, M.A., Molina-Henares, M.A., *et al*. (2013) Analysis of the plant growth-promoting properties encoded by the genome of the rhizobacterium Pseudomonas putida BIRD-1. *Environ Microbiol* **15:** 780–794.

Silby, M.W., Winstanley, C., Godfrey, S.A., Levy, S.B., and Jackson, R.W. (2011) *Pseudomonas* genomes: diverse and adaptable. *FEMS Microbiol Rev* **35:** 652–680.

Vodovar, N., Vallenet, D., Cruveiller, S., Rouy, Z., Barbe, V., Acosta, C., *et al*. (2006) Complete genome sequence of the entomopathogenic and metabolically versatile soil bacterium *Pseudomonas entomophila*. *Nat Biotechnol* **24:** 673–679.

Wu, X., Monchy, S., Taghavi, S., Zhu, W., Ramos, J., and van der Lelie, D. (2011) Comparative genomics and functional analysis of niche-specific adaptation in Pseudomonas putida. *FEMS Microbiol Rev* **35:** 299–323.

Zerbino, D.R., and Birney, E. (2008) Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* **18:** 821–829.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Pathway and genes related with 4-hydroxyphenylacetate degradation. (A) Degradation pathway of 4-hydroxyphenylacetate. (B) Genes related with the synthesis of enzymes that are involved in the degradation pathway.

Table S1. Genes in *Pseudomonas* sp. 250J that are related to genes whose products may form part of virulence systems.