

## Editorial

# *Pseudomonas putida*: a cosmopolitan opportunist *par excellence*

Bacteria of the genus *Pseudomonas* belong to the gamma subclass of the Proteobacteria and are chemo-organotrophic aerobic, Gram-negative rods with polar flagella and a respiratory rather than a fermentative metabolism (Palleroni, 1984). Some species are capable of denitrification and can use nitrate as an electron acceptor for the generation of energy. They are ubiquitous saprophytic bacteria found in most temperate, aerobic and semi-aerobic soil and water habitats, have simple nutritional requirements and grow rapidly on standard laboratory media (and therefore tend to overgrow other microorganisms that might be present in a sample). The genus *Pseudomonas* is a large taxonomic grouping of species that collectively exhibit a highly diverse range of activities: they are extremely versatile metabolically, physiologically and genetically and engage in many critically important activities, such as element cycling, degradation and recycling of biogenic and xenobiotic organic compounds, food spoilage, growth promotion and protection from pathogens of plants, parasitism of other bacteria and parasitism and disease production in plants and animals, etc. They are nutritionally omnivorous, some species being able to use more than 100 different sources of carbon and energy, and are particularly renowned for their ability to exploit toxic organics such as aliphatic and aromatic hydrocarbons. *Pseudomonas* strains are often resistant to antibiotics, disinfectants, detergents and heavy metals and are able to develop resistance to organic solvents that can disrupt the cell membranes of unadapted bacteria (Ramos *et al.*, 2002). Hydrocarbon metabolism and resistance to noxious agents are frequently encoded on transmissible plasmids and transposons (Jacoby, 1979; Boronin, 1992; Tsuda, 1996), and *Pseudomonas* isolates often contain multiple plasmids and transposons; as a consequence, such traits are readily exchanged among Pseudomonads and transmitted to other bacteria.

*Pseudomonas putida* is a rapidly growing bacterium

frequently isolated from most temperate soils and waters, particularly polluted soils. It is a nutritional opportunist *par excellence* and a paradigm of metabolically versatile microorganisms that recycle organic wastes in aerobic compartments of the environment, thus playing a key role in the maintenance of environmental quality. Its fascinating biochemistry and physiology, its robustness, rapid growth and ease of handling in the laboratory and its amenability to genetic analysis and manipulation have resulted in *P. putida* becoming a laboratory 'workhorse' for research on soil bacteria and bacteria-mediated soil processes. Its prominence has also been favoured by its frequent occurrence as the predominant organism found in selective enrichments in which an 'exotic' compound is offered as the sole source of carbon and energy, probably as a result of its rapid growth under the copiotrophic conditions of such selective enrichments (i.e. relatively high concentrations of substrates, non-limiting minerals, high aeration and incubation temperatures of 20–30°C). The fact that many of these exotic phenotypes are based on plasmid-encoded pathways that channel such substrates to metabolites that feed into central metabolic pathways has simplified their genetic and biochemical analysis. Well-studied examples of such catabolic plasmids are the TOL plasmid pWW0 (Williams and Murray, 1974), the NAH7 plasmid (Dunn and Gunsalus, 1973) and the CAM plasmid (Rheinwald *et al.*, 1973), encoding the catabolism of toluene/xylenes, naphthalene and camphor respectively. *P. putida* strains also have chromosomally encoded pathways for the catabolism of a variety of organic compounds, many of which occur as natural products or as fungal metabolites from the partial degradation of lignin. The fact that *P. putida* possesses such an arsenal of degradative functions presumably reflects its extensive spectrum of 'housekeeping' catabolic pathways and enzymes (Jimenez *et al.*, 2002; Nelson *et al.*, 2002), its tendency freely to acquire plasmids from other bacteria and its relaxed-specificity gene expression system, allowing the expression of genes derived from a wide variety of different bacteria.

There are no known strains of *P. putida* that are animal or plant pathogens, and this saprophytic species is con-

Dedicated to the memory of Rose Timmis (died 22. 4. 2002), who sowed and cultivated the curiosity underlying much of the *Pseudomonas* work with which K.N.T. has been associated.

sidered to be environmentally innocuous. For this reason, *P. putida* was recognized to be a promising candidate from which to develop a safety strain for recombinant DNA experiments, requiring a host with a versatile catabolic physiology, and for environmental applications. Strain KT2440 (Bagdasarian *et al.*, 1981) is a derivative of the best characterized toluene-degrading bacterium, originally isolated in Japan and designated *Pseudomonas arvilla* strain mt-2 (Nakazawa, 2002) and subsequently reclassified as *P. putida* mt-2 (Williams and Murray, 1974). Strain mt-2 harbours the archetypal TOL plasmid, pWWO, which specifies a pathway for the oxidative catabolism of toluene/xylenes. KT2440 is a restriction-negative, plasmid-free derivative of mt-2 that is a good recipient in gene transfer experiments, but does not act as a donor of introduced DNA (unless transfer functions are introduced). In 1982, it was certified by the Recombinant DNA Advisory Committee (RAC) of the United States National Institutes of Health as the host strain of the first host–vector bio-safety (HV1) system for gene cloning in Gram-negative soil bacteria (Federal Register, 1982). An extensive spectrum of versatile genetic tools, in particular mini-transposons and tools based on these, have since been developed for its analysis, manipulation and use as a host for cloned genes from other soil organisms (e.g. Mermod *et al.*, 1986; Herrero *et al.*, 1990; de Lorenzo *et al.*, 1990; de Lorenzo and Timmis, 1994). KT2440 and its rifampicin-resistant derivative, strain KT2442, are used world-wide as hosts of choice for the analysis, cloning and manipulation of genes from soil bacteria, particularly genes encoding enzymes that degrade hydrocarbons and aromatic xenobiotics, and their regulation. A detailed physical and genetic map was published in 1998 (Ramos-Diaz and Ramos, 1998).

KT2440 is being exploited in the development of a variety of biotechnological applications, including the design of new catabolic pathways for pollutants (e.g. Ramos *et al.*, 1986; 1987; Rojo *et al.*, 1987; Erb *et al.*, 1997), the production by biocatalysis of intermediates, including chiral synthons for chemical syntheses (Wubbolts and Timmis, 1990), and quality improvement of fossil fuels, for example by desulphurization (Galan *et al.*, 2000). KT2440 is also able to colonize the rhizosphere of a variety of crop plants, such as corn, wheat, strawberry, sugar cane and spinach (Espinosa-Urgel *et al.*, 2002), and is being used to develop new biopesticides and plant growth promoters that function in the plant rhizosphere.

Biotechnological applications in the environment involving the release of genetically modified microbes is (still) a controversial issue. Critical to both the safe application of recombinant microbes in the environment and the reassurance of public concerns is adequate information on safety-related properties of the microbes in question. In this regard, it is worth noting that a series of genetic

circuits for the containment of recombinant *P. putida* strains, and of their transgenes, has been developed and validated (e.g. Diaz *et al.*, 1994; Munthali *et al.*, 1996). In addition to available safety-relevant phenotypic information, whole-genome sequencing of pertinent microbes such as KT2440 provides unique opportunities to extract and exploit entirely new safety-related information that will lead to the design of a new generation of safety strains with enhanced environmental predictability.

The soil environment is characterized by extreme spatial heterogeneity in matrix structure, composition, chemistry, osmolarity, ionic strength, pH, buffering capacity, oxygen tension, etc., which provides an infinitely large number of microniches that vary substantially in the physico-chemical environments that they provide. It is equally characterized by extreme temporal variation, resulting from regular and irregular variations in temperature and water content. The ubiquity of *P. putida* reflects a highly developed ability to adapt to the various and varying physico-chemical conditions it faces in different unpolluted and polluted soil environments. This ability involves the monitoring of a variety of environmental signals, the integration of this information with information on the physiological status of the cell and the appropriate tuning of the complex regulatory network that controls cellular metabolism. Environmental adaptability is a key element in the physiological potency of *P. putida* and central to efforts to optimize its biotechnologically relevant activities (and those of other bacteria) in this environment. The sequencing of the *P. putida* genome provides a unique and timely opportunity to access its metabolic and regulatory secrets through genome-wide analyses of the components of the regulatory networks and other functions of this organism involved in its adaptation to conditions prevailing in unpolluted and polluted soils, and to elucidate the basis of its success in the soil environment and as an opportunist.

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## References

- Bagdasarian, M., Lurz, R., Rückert, B., Franklin, F.C.H., Bagdasarian, M.M., Frey, J., and Timmis, K.N. (1981) Specific-purpose plasmid cloning vectors. II. Broad host range, high copy number, RSF1010-derived vectors, and a host-vector system for gene cloning in *Pseudomonas*. *Gene* **16**: 237–247.
- Boronin, A. (1992) Diversity and relationships of *Pseudomonas* plasmids. In *Pseudomonas: Molecular Biology and Biotechnology*. Galli, E., Silver, S., and Witholt, B. (eds). Washington, DC: American Society for Microbiology Press, pp. 329–340.
- Diaz, E., Munthali, M., de Lorenzo, V., and Timmis, K.N. (1994) Universal barriers to lateral spread of specific genes among microorganisms. *Mol Microbiol* **13**: 855–861.
- Dunn, N.W., and Gunsalus, I.C. (1973) Transmissible plasmid coding early enzymes of naphthalene oxidation in *Pseudomonas putida*. *J Bacteriol* **114**: 974–979.
- Erb, R.W., Eichner, C.A., Wagner-Döbler, I., and Timmis, K.N. (1997) Bioprotection of microbial communities from toxic phenol mixtures by a genetically-designed pseudomonad. *Nature Biotechnol* **15**: 378–382.
- Espinosa-Urgel, M., Kolter, R., and Ramos, J.L. (2002) Root colonization by *Pseudomonas putida*: love at first sight. *Microbiology* **148**: 341–343.
- Federal Register (1982) Appendix E, Certified host-vector systems. 47: 17197.
- Galan, B., Diaz, E., and Garcia, J.L. (2000) Enhancing desulphurization by engineering a flavin reductase-encoding gene cassette in recombinant biocatalysts. *Environ Microbiol* **2**: 687–694.
- Herrero, M., de Lorenzo, V., and Timmis, K.N. (1990) Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in Gram-negative bacteria. *J Bacteriol* **172**: 6557–6567.
- Jacoby, G.A. (1979) Plasmids of *Pseudomonas aeruginosa*. In *Pseudomonas aeruginosa*. Doggett, R.G. (ed.). New York: Academic Press, pp. 271–309.
- Jiménez, J.I., Miñambres, B., García, J.L., and Díaz, E. (2002) Genomic analysis of the aromatic catabolic pathways of *Pseudomonas putida* KT2440. *Environ Microbiol* **4**: 824–841.
- de Lorenzo, V., and Timmis, K.N. (1994) Analysis and construction of stable phenotypes in Gram-negative bacteria with Tn5 and Tn10-derived mini-transposons. In *Methods in Enzymology*, Vol. 235. *Bacterial Pathogenesis*. Clark, V.L., and Bavoil, P.M. (eds). Orlando: Academic Press, pp. 386–405.
- de Lorenzo, V., Herrero, M., Jakubzik, U., and Timmis, K.N. (1990) Mini-Tn5 transposon derivatives for insertion mutagenesis, promoter probing, and chromosomal insertion of cloned DNA in Gram-negative eubacteria. *J Bacteriol* **172**: 6568–6572.
- Mermoud, N., Ramos, J.-L., Lehrbach, P.R., and Timmis, K.N. (1986) Vector for regulated expression of cloned genes in a wide range of Gram-negative bacteria. *J Bacteriol* **167**: 447–454.
- Munthali, M.T., Timmis, K.N., and Diaz, E. (1996) Restricting recombinant DNA dispersal: design of a contained biological catalyst. *Bio/Technology* **14**: 189–191.
- Nakazawa, T. (2002) Travels of a *Pseudomonas*, from Japan around the world. *Environ Microbiol* **4**: 782–786.
- Nelson, K.E., Weinl, C., Paulsen, I.T., Dodson, R.J., Hilbert, H., Martins dos Santos, V.A.P., et al. (2002) Complete genome sequence and comparative analysis of the metabolically versatile *Pseudomonas putida* KT2440. *Environ Microbiol* **4**: 799–808.
- Palleroni, N. (1984) Family I. Pseudomonadaceae. In *Bergey's Manual of Systematic Bacteriology*, Vol. 1. Kreig, N.R. (ed). Baltimore, MD: Williams & Wilkins, pp. 141–199.
- Ramos, J.L., Stolz, A., Reineke, W., and Timmis, K.N. (1986) Altered effector specificities in regulators of gene expression. TOL plasmid *xy/S* mutants and their use to engineer expansion of the range of aromatics degraded by bacteria. *Proc Natl Acad Sci USA* **83**: 8467–8471.
- Ramos, J.L., Wasserfallen, A., Rose, K., and Timmis, K.N. (1987) Redesigning metabolic routes: manipulation of TOL plasmid pathway for catabolism of alkylbenzoates. *Science* **235**: 593–596.
- Ramos, J.L., Duque, E., Gallegos, M.T., Godoy, P., Ramos-Gonzalez, M.I., Rojas, A., et al. (2002) Mechanisms of solvent tolerance in gram-negative bacteria. *Annu Rev Microbiol* **56**: 743–768.
- Ramos-Diaz, M.A., and Ramos, J.L. (1998) Combined physical and genetic map of the *Pseudomonas putida* KT2440 chromosome. *J Bacteriol* **180**: 6352–6363.
- Rheinwald, J.G., Chakrabarty, A.M., and Gunsalus, I.C. (1973) A transmissible plasmid controlling camphor oxidation in *Pseudomonas putida*. *Proc Natl Acad Sci USA* **70**: 885–889.
- Rojo, F., Pieper, D.H., Engesser, K.-H., Knackmuss, H.-J., and Timmis, K.N. (1987) Assemblage of *ortho*-cleavage route for simultaneous degradation of chloro- and methylaromatics. *Science* **238**: 1395–1398.
- Tsuda, M. (1996) Catabolic transposons in Pseudomonads. In *Molecular Biology of Pseudomonads*. Nakazawa, T., Furukawa, K., Haas, D., and Silver, S. (eds). Washington, DC: American Society for Microbiology Press, pp. 219–228.
- Williams, P.A., and Murray, K. (1974) Metabolism of benzoate and the methylbenzoates by *Pseudomonas putida* (arvilla) mt-2: evidence for the existence of a TOL plasmid. *J Bacteriol* **120**: 416–423.
- Wubbolts, M.G., and Timmis, K.N. (1990) Biotransformation of substituted benzoates to the corresponding *cis*-diols by an engineered strain of *Pseudomonas oleovorans* producing the TOL plasmid-specified enzyme toluate-1,2-dioxygenase. *Appl Environ Microbiol* **56**: 569–571.