Introduction

Ralstonia is a newly designated genus that includes former members of Burkholderia species (Burkholderia pickettii and Burkholderia solanacearum). These organisms have been renamed as Ralstonia pickettii and Ralstonia solanacearum, respectively (Yabuuchi et al. 1995). Ralstonia pickettii is an aerobic Gram-negative, oxidase-positive, nonfermentative rod and is a ubiquitous micro-organism found in water and soil (Gilligan et al. 2003). Ralstonia pickettii has been identified in biofilm formation in plastic water piping (Anderson et al. 1990). The bacterium has been identified in ultrapure water in industrial systems (Kulakov et al. 2002), in the Space Shuttle water system (Koenig and Pierson 1997) and in laboratory-based purified water systems (Adley et al. 2005). The organism has the ability to survive and thrive in low nutrient (oligotrophic) conditions (McAlister et al. 2002); it is theorized that in ultrapure water systems, the bacteria may be able to scavenge from the polymers in plastic piping. In addition, R. pickettii has been shown to have biodegradative abilities, demonstrating its large metabolic diversity (Table 1). Many different species of bacteria are being investigated for bioremediation capabilities; three of the best characterized are Burkholderia vietnamiensis, Pseudomonas putida and Pseudomonas fluorescens. However, these species have drawbacks that may limit their use. Burkholderia vietnamiensis strain G4 (O’Sullivan and Mahenthiralingam 2005) is part of the Burkholderia cepacia complex (Bcc) (Genomovar V), which in cystic fibrosis (CF) patients leads to a deterioration of prognosis and an increased risk of death (Isles et al. 1984; Tablan et al. 1985; Corey and Farewell 1996). In a study carried out by LiPuma et al. (2001), B. vietnamiensis accounted for 5±1% of all Bcc cases found in 606 CF patients. Burkholderia vietnamiensis can also cause a phenomenon called plant tissue water soaking, which can cause disease and tissue damage in onions. As a result of the clinical relevance of Bcc species and their close interspecies relatedness, the biotechnological applications of all Bcc species have been severely restricted by the US EPA (Anon 2003). Pseudomonas fluorescens has been demonstrated to cause fin rot in fish (Sakai et al. 1989) and P. putida has also been shown to cause disease in fish (Nakatsugawa and Iida 1996; Wakabayashi et al. 1996). The use of these bacteria as bioremediators is, therefore, not advisable as environmental release could lead to environmental damage, such as disease in plants and depletion of fish stocks, and the potential cause of disease in humans. The use of these organisms could also cause public concern. Ralstonia pickettii demonstrates many advantages over these bacteria. Though the bacterium has been shown to be involved in...
Aromatic hydrocarbons

Aromatic hydrocarbons are volatile organic compounds (VOC's), which include phenol (C₆H₅OH), cresol (C₇H₈O), benzene (C₆H₆) and toluene (C₇H₈). They are found in a variety of household and industrial products such as germicides, antiseptics and several different household cleaners. Many of the aromatic hydrocarbons are toxic, carcinogenic and otherwise hazardous compounds that are frequently found as contaminants of soil and ground water (Siegrist 1992; Anon 2002). Strains of R. pickettii are capable of degrading many of these aromatic hydrocarbons and using them as both a carbon and energy source (Kukor and Olsen 1990, 1992). This is achieved by means of multi-enzyme pathways such as the Tbu pathway of R. pickettii PKO1, a soil isolate, which can convert aromatic hydrocarbons into catechols (Kukor and Olsen 1990, 1991; Olsen et al. 1994). The tbu pathway of R. pickettii PKO1 has been cloned as a 26.5-kbp DNA fragment designated pRO1957 and expressed in Pseudomonas aeruginosa (Olsen et al. 1994). The genes encoding enzymes for this catabolic pathway have been shown to be organized into three operons. The first is the tbuA1UBVA2C operon encoding the initial toluene-3-monoxygenase (T3MO) enzyme (Fig. 1) (Kukor and Olsen 1990, 1992; Olsen et al. 1994). Toluene-3-monooxygenase (T3MO) was first reported to hydroxylate toluene at the para position (Fishman et al. 2004). Therefore, the enzyme T3MO was renamed tolulene para-monoxygenase (TpMO). TpMO is regulated by the tbuT locus and is induced by other compounds such as ethylbenzene (C₆H₄(CH₃)), xylene (C₆H₄(CH₃)₂) and trichloroethylene (C₂HCl₂) (Byrne and Olsen 1996). The phenol p-cresol is then hydroxylated into catechol by a phenol/cresol hydroxylase (Fig. 1). The tbuD operon encodes phenol hydroxylase
This phenol hydroxylase is a flavoprotein that is capable of degrading a wide assortment of phenols. It exists in the form of a dimer and uses NADPH as a co-substrate (Kukor and Olsen 1992). The final component of the tbu pathway consists of the tbuWEFGKIHJ operon that encodes seven enzymes responsible for the meta cleavage of catechol (Fig. 1). These are catechol 2, 3-dioxygenase (Genbank accession number U20258, EC Number 1.13.11.2), 2-hydroxymuconate semialdehyde hydrolase (EC Number 3.7.1.9), 2-hydroxymuconate semialdehyde dehydrogenase (EC Number 1.2.1.45), 4-hydroxy-2-oxovalerate aldolase (EC Number 4.1.3.39), 4-oxalocrotonate decarboxylase (EC Number 4.1.1.77), 4-oxalocrotonate isomerase and 2-hydroxypent-2, 4-dienoate hydratase, respectively (Kukor and Olsen 1991). The steps of this pathway can be seen in Fig. 1. The tbuWEFGKIHJ operon is controlled by tbuS, which represses the operon in the absence of phenol and activates it when the effector molecules are present by forming a transcription activator complex. *Ralstonia pickettii* also has the ability to metabolize toluene and other aromatic hydrocarbons under hypoxic conditions. *Ralstonia pickettii* PKO1 can metabolize toluene when oxygen levels are 25% of air-saturated water which distinguishes it from other toluene metabolizing bacteria. PKO1 can degrade toluene at oxygen levels as low as 2 mg of dissolved O2 per litre (Kukor and Olsen 1996); this can be attributed to kinetic and binding differences within the enzyme catechol 2, 3-dioxygenase. The *R. pickettii* enzyme has a higher turnover rate and a two-fold greater affinity for the substrate than non-hypoxic strains (Kukor et al. 1993; Kukor and Olsen 1996). PKO1 adapts to hypoxic environments by its ability to use nitrate as an alternative electron acceptor to oxygen for the catabolism of aromatic hydrocarbons (Kukor and Olsen 1996). The bioremediation of polluted groundwater and toxic waste sites requires that the bacteria come into close physical contact with pollutants. This can be accomplished by chemotaxis, where *R. pickettii* PKO1 is attracted to toluene, but the response is dependent on induction by growth with toluene (Parales et al. 2000). *Ralstonia pickettii* PKO1 was shown to degrade 99-9% of benzene, toluene, ethylbenzene, ortho-, meta- and para-xylene, and styrene, and 75-9% of 1, 2, 4-trimethylbenzene at a concentration of 0.2 mg l⁻¹ after 48 h in a hydrocarbon degradation assay indicating that this organism has a wide range and high activity against many potential substrates (Leahy et al. 2003).

**Trichloroethylene**

*Ralstonia pickettii* strain PKO1 is one of the most extensively studied degraders of trichloroethylene (TCE) (C₂HCl₃), which is a suspected carcinogen (Anon 1976) and US EPA priority pollutant (Anon 1980). It is one of the most commonly detected VOC’s in groundwater.
been shown to degrade 50 mg l\(^{-1}\) at a rate of 0.2 mg l\(^{-1}\) after 48 h in a hydrocarbon degradation assay (Leahy et al. 2003).

1, 4-Dioxane

1, 4-Dioxane (C\(_4\)H\(_8\)O\(_2\)) is likely a human carcinogen (DeRosa et al. 1996) and a significant emerging water contaminant. It is extensively used as a stabilizer for chlorinated solvents such as 1, 1, 1-trichloroethane (TCA). It is used in manufacturing several organic chemicals including polystyrene, wood stains, varnishes and SBR latex production (Zenker et al. 2003) and as a wetting agent in paper and textile processing industries. It has been detected as a contaminant in both surface waters and groundwaters (Johns et al. 1998; Abe 1999; Jackson and Dwarkanath 1999). Ralstonia pickettii PK01 has been shown to degrade 50 mg l\(^{-1}\) of 1, 4-Dioxane in the presence of a hydrocarbon inducer at a rate of 0.31 ± 0.007 mg h\(^{-1}\) mg\(^{-1}\) protein (Mahendra and Alvarez-Cohen 2006).

Chlorinated phenolic compounds

Chlorophenol compounds are used widely as pesticides and biocides, and some, especially monochlorophenols, can be formed during the chlorination of wastewaters and by the breakdown of chlorinated aromatic compounds (Pritchard et al. 1987). Chlorinated compounds have a high degree of environmental persistence as well as a high solubility in water (Smith and Novak 1987). Finding ways to remove chlorophenol compounds presents an important challenge. Ralstonia pickettii strain LD1 (purified from a bacterial biofilm) can utilize monochlorophenols as carbon sources and catabolize these compounds without the use of supplemental nutrients or cofactors (Fava et al. 1995). LD1 can utilize 2-chlorophenol (1.51 mmol l\(^{-1}\)), 3-chlorophenol (0.57 mmol l\(^{-1}\)) and 4-chlorophenol (0.75 mmol l\(^{-1}\)) (C\(_6\)H\(_5\)ClO). The chlorophenol compounds are metabolized into chlorocatechols. The mechanism of degradation, however, is still unclear (Fava et al. 1995). The LD1 strains’ ability to mineralize monochlorophenols can be enhanced with the addition of vitamins to the culture medium. The addition to culture medium of a vitamin solution [containing biotin, folic acid, pyridoxine hydrochloride, riboflavin, thiamine hydrochloride, niacin, pantothenic acid, cyanocobalamin, p-aminobenzoic acid and thioctic acid (total final concentration ≤ 600 ppb)] resulted in a 7–16% increase in the amount of target compounds degraded over the incubation period required for the concentration of the compound in the cultures to drop to approximately zero (Kafkewitz et al. 1996).

2, 4, 6-Trichlorophenol (2, 4, 6-TCP) (C\(_6\)H\(_5\)Cl\(_3\)O) is used widely as a biocide, a wood preservative, an antiseptic, a glue preservative, in anti-mildew treatment and in manufacturing other chemicals (Anon 1990). The US EPA has classified 2, 4, 6-TCP as a possible human carcinogen. These compounds are toxic and can accumulate in the environment as they are highly resistant to degradation (Anon 1999). The ability of R. pickettii and other organisms, e.g. Rhodococcus chlorophilicus and Streptomyces rochei, to degrade this CP compound has been examined (Apajalahti and Salkinoja-Salonen 1987; Zabolina et al. 1995). Three known strains of R. pickettii (DTP0309, DTP0405, DTP0602), all isolated from soil, are capable of using 2, 4, 6-TCP as a carbon source, with strain DTP0602 being the most rapid degrader (Kiyohara et al. 1992). These soil isolates were able to use 0.5 mmol l\(^{-1}\) 2, 4, 6-TCP as a growth substrate and resting cell suspensions degraded up to 2.5 mmol l\(^{-1}\) 2, 4, 6-TCD. These bacteria consume 2, 4, 6-TCP and leave residual chloride ions in the culture media. They can also degrade other isomers of TCP as well as pentachlorophenol (PCP) (Kiyohara et al. 1992). In R. pickettii, the had locus encodes the genes responsible for degradation of 2, 4, 6-TCP. Moreover, 2, 4, 6-trichlorophenol-4-dechlorinase (hydroxylase) (Genbank accession number: D86544) isolated from the strain DTP0602 is responsible for the first step in the degradation of 2, 4, 6-TCP (Takizawa et al. 1995). The pathway is shown in Fig. 2. A hydroxyquinol 1, 2-dioxygenase enzyme (Genbank accession number: D86544, EC Number 1.13.11.37) then degrades the intermediate products created as shown in Fig. 2 (Hatta et al. 1999). Homology between the 2, 4, 6-trichlorophenol-4-dechlorinase in R. pickettii and the 2, 4, 6-trichlorophenol-4-monoxygenase in Azotobacter sp. GP1 has been found, particularly in the NH2-terminal amino region (Wieser et al. 1997). The genes responsible for the degradation of 2, 4, 6-Trichlorophenol from
R. pickettii have been cloned in both *Escherichia coli* and *P. putida* using a transposon tagging strategy (Takizawa *et al.* 1995).

**Lantadenes**

The lantana plant has encroached on many forests, orchards and pastureland in many parts of the world (Sharma *et al.* 1988; Pass 1991). Lantadenes are pentacyclic triterpenoid compounds derived from the lantana plant. If consumed by grazing animals, they cause cholestasis, hepatotoxicity and photosensitisation (Sharma *et al.* 1988). These cyclic triterpenoids are difficult compounds to degrade (Krasnobajew 1984). They have shown promise as pharmacological compounds as they inhibit the Epstein Barr virus (Inada *et al.* 1995) and demonstrate anti-HIV, anti-tumour, anti-bacterial and anti-inflammatory activities (Li *et al.* 1993; Fujioka *et al.* 1994; Pengsuparp *et al.* 1994). A strain of *R. pickettii* isolated from soil sampled proximal to lantana plants has demonstrated the ability to use lantadene A as a carbon source; however, this utilization was inhibited when other carbon-containing compounds were added to the growth medium (Sharma *et al.* 1997). This may lead to the exploitation of this bacterium in combating toxicity in grazing animals and in biotransforming lantadene into useful bioactive compounds.

**3, 4-Dichloropropionanilide**

3, 4-Dichloropropionanilide (C9H9Cl2NO) is the chemical name for a family of herbicides known as amides. It is used extensively in rice production for the control of grasses, especially barnyard grass and broadleaf weeds in rice (Barnes *et al.* 1987). It has been shown to have neurotoxic and immunotoxic effects in mice (Cuff *et al.* 1996). Approximately 70% of the US rice crop is treated with propanil, accounting for more than 95% of the 2.7–3.6 million kilogram of this active ingredient (AI) produced annually (Gianessi and Anderson 1995a,b; Aspelin and Grube 1999). A soil isolate of *R. pickettii* found demonstrated resistance to 3, 4-dichloropropionanilide because of the enzyme propanil hydrolase (Hirase and Matsunaka 1991). Research is being carried out on the mechanism of resistance of *R. pickettii* to these herbicides with the aim of using the genes responsible to produce herbicide-resistant transgenic rice plants (Piruzian *et al.* 1988).

**2, 4-Dichlorophenoxyacetic acid**

2, 4-Dichlorophenoxyacetic acid (C8H6Cl2O3) is a systemic chlorophenoxy herbicide used widely in Canada (more than 4 million kilograms annually) to control broadleaf weeds in cereal cropland and in industrial property, lawns, turf, pastures, non-cropland and aquatic weeds (Anon 1986a). Commercial 2, 4-Dichlorophenoxyacetic acid products are marketed as alkali salts, amine salts and ester formulations. Many micro-organisms, including the members of the *Pseudomonas* family, can degrade 2, 4-Dichlorophenoxyacetic acid (Ka and Tiedje 1994; Ka *et al.* 1994a, 1994b). The structural gene *tfdA* from plasmid pJP4 encoding the first of the functional enzymes necessary for the transformation of chlorocatechols into 3-oxoadipate (Don and Pemberton 1981) has been used as a probe to detect bacterial populations with 2, 4-Dichlorophenoxyacetic acid degrading capability. In *R. pickettii*, homologous *tfd* genes have been shown to be plasmid borne (Ka *et al.* 1994a). One *R. pickettii* isolate (designated 712) contains a 40–9-kb plasmid that
hybridizes to a 2, 4-Dichlorophenoxyacetic acid tfdA gene probe and shares the features in common with pKA2 from *Variovorax paradoxus* (formally *Alcaligenes paradoxa*) (Willems *et al.* 1991). pKA4 is self-transmissible, strongly hybridizes to pKA2 and has a similar restriction pattern. It is thought that these micro-organisms may have exchanged plasmids by intergeneric transfer (Ka and Tiedje 1994). The 2, 4-Dichlorophenoxyacetic acid degrading genes from the pKA2 plasmid may undergo recombination between the chromosome and the plasmid. This is well documented for aromatic hydrocarbon-degradative determinates on the *Pseudomonas* TOL plasmid that integrate into the host chromosome (Jeenes and Williams 1982; Sinclair *et al.* 1986; Ka and Tiedje 1994) and has been demonstrated with pKA2, which was transferred to *Burkholderia cepacia* and found to integrate into the chromosome of that organism.

**Nitroaromatics**

While nitrobenzene (C₆H₅NO₂) is primarily used in the production of aniline and aniline derivatives, such as methyl diphenyl diisocyanate (MDI), it also finds use in manufacturing rubber chemicals, pesticides, dyes and pharmaceuticals (Anon 1991). Nitrobenzene is also used in shoe and floor polishes, leather dressings, paint solvents and other materials in order to mask unpleasant odours. Substitution reactions with nitrobenzene are used to form m-derivatives (Anon 1991; Sittig 1991). Redistilled, as oil of mirbane, nitrobenzene has been used as an inexpensive perfume for soaps. A significant market for nitrobenzene is its use in the production of analgesic acetaminophen (Anon 1991). In 1992, releases of nitrobenzene to the environment reported to the Toxic Chemical Release Inventory by certain types of US industries totaled to about 917 000 pounds (Anon 1992). Because of its toxicity, nitrobenzene has been listed as a priority pollutant by the US EPA as far back as 1979 (Keith and Spain 1991) and has been added to the list of compounds regulated under the Resource Conservation and Recovery Act (Hanson 1990). Two different pathways of degradation of nitroaromatics have been found in two different strains of *R. pickettii*. In *R. pickettii* strain YH105 (sludge isolate), degradation of nitroaromatics occurs via a two-step enzymatic process that uses two chromosomally encoded genes: *p*-nitrobenzoate reductase and *p*-hydroxylaminobenzoate lyase as outlined in Fig. 3 (Genbank accession number AF187879). YH105 is able to degrade up to 15 mmol l⁻¹ *p*-nitrobenzoate to protocatechuate via *p*-hydroxylaminobenzoate; protocatechuate then enters the TCA cycle (Yabannavar and Zylstra 1995). The genes responsible have been cloned and expressed in *Escherichia coli*. Similar nitroreductases have been found in *Comamonas acidovorans* NBA-10 (Groenewegen *et al.* 1992) and *Pseudomonas pseudoalcaligenes* JS45 (Somerville *et al.* 1995); the enzyme hydroxylaminoloyase; however; has only been purified from *C. acidovorans* NBA-10 (Groenewegen and DeBont 1992). *Ralstonia pickettii* PKO1 degrades nitrobenzene, using TpMO, to 3- and 4-nitrocatechol via 3- and 4-nitrophenol, and these nitrocatechols are then slowly degraded to unidentified metabolites. Haigler and Spain (1991) had not identified the enzymes responsible for addition of the second hydroxyl group to the nitrophenols to form nitrocatechols; however, it has subsequently been shown that this reaction proceeds down the *thu* pathway (Parales *et al.* 1997; Fishman *et al.* 2004).

**Quinoline**

Quinoline (C₉H₇N) and its derivatives occur widely in coal tar, bone oil, oil shale and plant alkaloids, and serve as intermediates and solvents in the chemical industry. Quinoline and some of its derivatives are reported to be toxic, carcinogenic and mutagenic (Minako *et al.* 1997; Sideropoulos and Secht 1984). The widespread use of quinoline and its derivatives entails that these compounds, together with many other environmental chemicals, are distributed in the environment, thus polluting soil and water (Miethling *et al.* 1993; Sutton *et al.* 1996). Degradation of quinoline by microbial processes has attracted much interest in recent years. In 2002, a pure strain identified as *B. pickettii* was isolated from the activated sludge of a coke-oven wastewater treatment plant through enrichment using quinoline as sole source of carbon and nitrogen. The isolate was then used to test quinoline biodegradation by free cells, and this was shown to remove 500 mg l⁻¹ of quinoline after 9 h (Jianlong *et al.* 2002).

![Figure 3 Pathway for the degradation of *p*-nitrobenzoate. TCA, tricarboxylic acid.](image_url)
N-Nitrosodimethylamine

N-nitrosodimethylamine (NDMA) \((\text{C}_2\text{H}_6\text{N}_2\text{O})\) is considered a probable human carcinogen (Anon 1986b). This compound is regulated in USA waters with an US EPA cleanup level of 0.7 ng \(\text{l}^{-1}\) (Anon 2001). Its presence in the environment has been linked to aerospace facilities through the decomposition of hydrazine-based rocket fuels (MacDonald 2002) and more generally to the discharge of water and wastewater disinfected with chlorine (Njam and Trussell 2001; Mitch et al. 2003). In the latter case, it appears that secondary amines react with chlorine to form a hydrazine intermediate that is in turn oxidized to NDMA (Mitch and Sedlak 2002). Its persistence in groundwater aquifers has been responsible for the closure of municipal drinking water wells and its listing as a priority pollutant (Mitch et al. 2003). *Ralstonia pickettii* PKO1 has been found to degrade this compound in the presence of toluene at a rate of 1 ng \(\text{mg}^{-1} \text{min}^{-1}\) (Sharp et al. 2005). It was not stated in the paper whether NDMA was completely degraded or just transformed.

Conclusions

*Ralstonia pickettii* has the ability to survive and prosper in oligotrophic environment and use a variety of compounds as energy and carbon sources. The organism already has demonstrated its capacity to degrade a number of toxic substances (Table 1), making it an excellent candidate for bioremediation. It has several advantages over other candidate strains being studied such as *B. vietnamiensis* G4 [which is currently undergoing intense study (O’Sullivan and Mahenthiralingam 2005)] or *P. putida*, in that it is only weakly pathogenic with no phytopathogenic or animal pathogenic incidents being reported. Several areas of application have the potential to use *R. pickettii*, which include treatment of contaminated groundwater and municipal and industrial waste and sewage. Examples include the removal of toluene from groundwater of which a successful test was carried out in Hanahan, South Carolina. The use of bioremedicators prevented the contamination of residential areas from a massive fuel leak from a nearby military installation (Vroblesky et al. 1997). Natural microbial communities in the area were stimulated with nutrients to increase the biodegradation of toluene. Through the use of nutrient addition (Kafkewitz et al. 1996), bioremediation of compounds such as chlorophenols and pesticides found in sewage effluent and groundwater could be increased. The degradation of toxic compounds by micro-organisms that are part of the microflora of wastewater treatment plants could be augmented if plasmids and genes responsible for these properties in *R. pickettii* were transferred from *R. pickettii* to the indigenous micro-organisms. When genome sequence data of *R. pickettii* is available, the analysis of degradative processes will potentially allow optimization of the physiological state of *R. pickettii* strains during bioremediation applications and could potentially lead to the construction of novel or more proficient pathways for degradation. *Ralstonia pickettii* strain PKO1 could have the potential to be a super biodegrader with the introduction of plasmids bearing other degradative enzymes, e.g. pK4, and integrating other genes from different bacteria into the chromosome to assist in the breakdown of toxic compounds. An example of the potential of genome analysis can be seen in the alteration of TpMO so that the enzyme hydroxylates all three positions of toluene as well as both positions of naphthalene. The mutation in the enzyme produced a toluene para-monoxygenase variant that formed 75% \(m\)-cresol from toluene and 100% \(m\)-nitrophenol from nitrobenzene. This was the first time a true *meta*-hydroxylating toluene monooxygenase was created (Fishman et al. 2005). The demand for a safe organism makes *R. pickettii* a natural choice for bioremediation applications.

Acknowledgements

Thanks to Carol Ruddle for her help in using Chemdraw to render the diagrams.

References

Abe, A. (1999) Distribution of 1, 4-dioxane in relation to possible sources in the water environment. *Sci Total Environ* 227, 41–47.

Adley, C.C., Ryan, M.P., Pembroke, J.T. and Saieb, F.M. (2005) *Ralstonia pickettii* in high purity water. In *Biofilms: Persistence and Ubiquity* ed. Mc Bain, A., Alison, D., Pratten, J., Spratt, D., Upton, M. and Verran, J. pp. 261–272. Cardiff: Biofilm Club.

Anderson, R.L., Holland, B.W., Carr, J.K., Bond, W.W. and Favero, M.S. (1990) Effect of disinfectants on *Pseudomonas* colonized on the interior surface of PVC pipes. *Am J Public Health* 80, 17–21.

Anon (1976) National Cancer Institute. *Carcinogenesis Bioassay of Trichloroethylene*. Washington DC:CAS no. 79–016. US Department of Health, Education, and Welfare publication (NIH) 76–802. US Department of Health, Education, and Welfare.

Anon (1980) *Ambient Water Quality Criteria for Trichloroethylene*. US EPA 440/5–80-073. Springfield, VA: National Technical Information Service.

Anon (1986a) Environment Canada/Agriculture Canada. Pesticide Registrant Survey, 1986 Report. Ottawa: Commercial Chemicals Branch, Environment Canada.
Anon (1986b) IARC (International Agency for Research on Cancer). Relevance of n-nitroso compounds to human cancer: exposures and mechanisms. In Proceedings of the IXth International Symposium on N-Nitroso Compounds ed. Bartsch, H. Baden, Austria, 1–5 September, 1986, pp. 244–253. New York: Oxford University Press.

Anon (1990) Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for 2, 4, 6-Trichlorophenol. US Public Health Service, US Department of Health and Human Services: Atlanta, GA.

Anon (1991) Mannsville Chemical Products Corporation. Nitrobenzene. Chemical Products Synopsis. February 1991. Asbury Park: US Dept. of Health and Social Services.

Anon (1992) TRI92. Toxics Release Inventory, Public Data Release. P. 244. Washington, DC: Office of Pollution Prevention and Toxics, US EPA.

Anon (1999) Integrated Risk Information System (IRIS) on 2, 4, 6-Trichlorophenol. Washington, DC: US EPA National Center for Environmental Assessment, Office of Research and Development.

Anon (2001) Record of decision for the western groundwater operable unit OU-3, Aerojet Sacramento Site. Washington, DC: US EPA.

Anon (2002) National Primary Drinking Water Regulations. Washington, DC: US EPA.

Anon (2003) Burkholderia cepacia Complex: Significant New Use Rule. Section 5(a) (2) of the Toxic Substances Control Act (TSCA), Vol. 68, N.114, pp. 35 315–35 320. http://www.epa.gov/fedrgstr/EPA-TOX/2003/June/Day-13/t15010.htm.

Apajalahti, J.H. and Salkinoja-Salonen, M.S. (1987) Dechlorination and para-hydroxylation of polychlorinated phenols by Rhodococcus chlorophenolicus. J Bacteriol 169, 675–681.

Aspelin, A.L. and Grube, A.H. (1999) Pesticides industry sales and usage: 1996 and 1997 market estimates. Report 733-R-99-001. Washington, DC: Office of Pesticide Programs, US Environmental Protection Agency.

Barnes, C.J., Lavy, T.L. and Mattice, J.B. (1987) Exposure of non-appicator personnel and adjacent areas to aerial applied propanil. Bull Environ Contam Toxicol 39, 126–133.

Byrne, A.M. and Olsen, R.H. (1996) Cascade regulation of the toluene-3-monoxygenase operon (tbaAUBVA2C) of Burkholderia picketti PKO1: role of the tbuA1 promoter (PtbuA1) in the expression of its cognate activator, TbuT. J Bacteriol 178, 6327–6337.

Corey, M. and Farewell, V. (1996) Determinants of mortality from cystic fibrosis in Canada, 1970–1989. Am J Epidemiol 143, 1007–1017.

Cuff, C.F., Zhao, W., Nukui, T., Schauer, R. and Barnett, J.B. (1996) 3, 4-Dichloropropionanilide-induced atrophy of the thymus: mechanisms of toxicity and recovery. Fundam Appl Toxicol 33, 83–90.

DeRosa, C.T., Wilbur, S., Holler, J., Richter, P. and Stevens, Y.W. (1996) Health evaluation of 1,4-dioxane. Toxicol Ind Health 12, 1–43, 171.

Don, R.H. and Pemberton, J.M. (1981) Properties of six pesticide degradation plasmids isolated from Alcaligenes paradoxis and Alcaligenes eutrophus. J Bacteriol 145, 681–686.

Fava, F., Armenante, P.M. and Kalkewitz, D. (1995) Aerobic degradation and dechlorination of 2-chlorophenol, 3-chlorophenol and 4-chlorophenol by a Pseudomonas pickettii strain. Lett Appl Microbiol 21, 307–312.

Fishman, A., Tao, Y. and Wood, T.K. (2004) Toluene 3-monoxygenase of Ralstonia picketti PKO1 is a para-hydroxylating enzyme. J Bacteriol 186, 3117–3123.

Fishman, A., Tao, Y., Rui, L. and Wood, T.K. (2005) Controlling the regiospecific oxidation of aromatics via active site engineering of toluene para-monoxygenase of Ralstonia picketti PKO1. J Biol Chem 280, 506–514.

Fujioka, T., Kashiwada, Y., Kilikuskie, R.E., Cosentino, L.M., Ballas, L.M., Jiang, J.B., Janzen, W.P., Chen, I.S., et al. (1994) Anti-AIDS agents, 11. Betulinic acid and platanic acid as anti-HIV principles from Syzygium claviflorum, and the anti-HIV activity of structurally related triterpenoids. J Nat Prod 57, 243–247.

Gianessi, L.P. and Anderson, J.E. (1995a) Pesticide Use in US Crop Production – National Summary Report. Washington, DC: National Centre for Food and Agricultural Policy.

Gianessi, L.P. and Anderson, J.E. (1995b) Pesticide use in Arkansas Crop Production – National Summary Report. Washington, DC: National Centre for Food and Agricultural Policy.

Gilligan, P.H., Lum, G., Vadamme, P.A.R. and Whittier, S. (2003) Burkholderia, Stenotrophomonas, Ralstonia, Brevundimonas, Comamonas, Delftia, Pandoraea and Acidovorax. In Manual of Clinical Microbiology, 8th edn ed. Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfäffer, M.A. and Yolken, R.H. pp. 729–748. Washington, DC: ASM.

Groenewegen, P.E.J. and DeBont, J.A.M. (1992) Degradation of 4-nitrobenzoate via 4-hydroxylaminobenzoate and 3, 4-dihydroxybenzoate in Comamonas acidovorans NBA-10. Arch Microbiol 158, 381–386.

Groenewegen, P.E.J., Breeuwer, P., van Helvoort, J.M.L.M., LangeHoff, A.A.M., de Vries, F.P. and deBont, J.A.M. (1992) Novel degradative pathway of 4-nitrobenzoate in Comamonas acidovorans NBA-10. J Gen Microbiol 138, 1599–1605.

Haigler, B.E. and Spain, J.C. (1991) Biotransformation of nitrobenzene by bacteria containing toluene degradative pathways. Appl Environ Microbiol 57, 3156–3162.

Hanson, D. (1990) Hazardous wastes: EPA adds 25 organics to RCRA list. Chem Eng News 68, 4.

Hatta, T., Nakano, O., Imai, N., Takizawa, N. and Kiyohara, H. (1999) Cloning and sequence analysis of hydroxyquinol 1, 2-dioxygenase gene in 2, 4, 6-trichlorophenol-degrading Ralstonia picketti DTP0602 and characterization of its product. J Biosci Bioeng 87, 267–272.

Hirase, K. and Matsunaka, S. (1991) Purification and properties of propanil hydrolase in Pseudomonas pickettii. Biochem Physiol 39, 302–308.
Inada, A., Nakanishi, T., Tokuda, H., Nishino, H., Iwahima, A. and Sharma, O.P. (1995) Inhibitory effects of lantadene and related triterpenoids on Epstein-Barr virus activation. Planta Med 61, 558–559.

Isles, A., Maclusky, I., Corey, M., Gold, R., Prober, C., Fleming, P. and Levison, H. (1984) Pseudomonas cepacia infection in cystic fibrosis: an emerging problem. J Pediatr 104, 206–210.

Jackson, R.E. and Dwarkanath, V. (1999) Chlorinated degrading solvents: physical-chemical properties affecting aquifer contamination and remediation. Ground Water Monit Rem 19, 102–110.

Jeenes, D.J. and Williams, P.A. (1982) Excision and integration of degradative pathway genes from TOL plasmid pWWO. J Bacteriol 150, 188–194.

Jianlong, W., Xiangchun, Q., Liping, H., Yi, Q. and Heegemann, W. (2002) Microbial degradation of quinoline by immobilized cells of Burkholderia pickettii. Water Res 36, 2288–2296.

Johns, M.M., Marshall, W.E. and Toles, C.A. (1998) Agricultural by-products as granular activated carbons for adsorbing dissolved metals and organics. J Chem Technol Biotechnol 71, 131–140.

Ka, J.O. and Tiedje, J.M. (1994) Integration and excision of a 2, 4-dichlorophenoxyacetate acid-degradative plasmid in Alcaligenes paradoxus and evaluation of its natural intergeneric transfer. J Bacteriol 176, 5284–5289.

Ka, J.O., Holben, W.E. and Tiedje, J.M. (1994a) Analysis of competition in soil among 2, 4-dichlorophenoxyacetic acid-degrading bacteria. Appl Environ Microbiol 60, 1121–1128.

Ka, J.O., Holben, W.E. and Tiedje, J.M. (1994b) Use of gene probes to aid in recovery and identification of functionally dominant 2, 4-dichlorophenoxyacetic acid-degrading populations in soil. Appl Environ Microbiol 60, 1116–1120.

Kafkewitz, D., Fava, F. and Armenante, P.M. (1996) Effect of vitamins on the aerobic degradation of 2-chlorophenol, 4-chlorophenol, and 4-chlorobiphenyl. Appl Microbiol Biotechnol 46, 414–421.

Kahng, H.Y., Byrne, A.M., Olsen, R.H. and Kukor, J.J. (2000) Characterization and role of tbuX in utilization of toluene by Ralstonia pickettii PKO1. J Bacteriol 182, 1232–1242.

Keith, L.Y. and Telliardi, W.A. (1979) Priority pollutants. I. A perspective view. Environ Sci Technol 13, 416–423.

Kiyohara, H., Hatta, T., Ogawa, Y., Kakuda, T., Yokoyama, H. and Takizawa, N. (1992) Isolation of Pseudomonas pickettii strains that degrade 2, 4, 6-trichlorophenol and their dechlorination of chlorophenols. Appl Environ Microbiol 58, 1276–1283.

Koenig, D.W. and Pierson, D.L. (1997) Microbiology of the space shuttle water system. Water Sci Technol 35, 59–64.

Krasnobajew, V. (1984) Terpenoids. In Biotransformations, vol. 6A ed. Leslich, K. pp. 97–126. Weinheim: Verlag Chemie.

Kukor, J.J. and Olsen, R.H. (1990) Molecular cloning, characterization, and regulation of a Pseudomonas pickettii PKO1 gene encoding phenol hydroxylase and expression of the gene in Pseudomonas aeruginosa PAO1c. J Bacteriol 172, 4624–4630.

Kukor, J.J. and Olsen, R.H. (1991) Genetic organization and regulation of a meta cleavage pathway for catechols produced from catabolism of toluene, benzene, phenol, and cresols by Pseudomonas pickettii PKO1. J Bacteriol 173, 4587–4594.

Kukor, J.J. and Olsen, R.H. (1992) Complete nucleotide sequence of tbuD, the gene encoding phenol/cresol hydroxylase from Pseudomonas pickettii PKO1, and functional analysis of the encoded enzyme. J Bacteriol 174, 6518–6526.

Kukor, J.J. and Olsen, R.H. (1996) Catechol 2, 3-dioxygenases functional in oxygen-limited (hypoxic) environments. Appl Environ Microbiol 62, 728–740.

Kukor, J.J., Mikesell, M.D. and Olsen, R.H. (1993) Catechol dioxygenase kinetic parameters for BTEX degradation in oxygen-limited (hypoxic) environments, abstr. K-85. In Abstracts of the 93rd General Meeting of the American Society for Microbiology 1993. p. 275. Washington, DC: ASM.

Kulakow, L.A., McAlister, M.B., Ogden, K.L., Larkin, M.J. and O’Hanlon, J.F. (2002) Analysis of bacteria contaminating ultrapure water in industrial systems. Appl Environ Microbiol 68, 1548–1555.

Leahy, J.G., Byrne, A.M. and Olsen, R.H. (1996) Comparison of factors influencing trichloroethylene degradation by toluene-oxidizing bacteria. Appl Environ Microbiol 62, 825–833.

Leahy, J.G., Tracy, K.D. and Eley, M.H. (2003) Degradation of mixtures of aromatic and chloroaliphatic hydrocarbons by aromatic hydrocarbon-degrading bacteria. FEMS Microbiol Ecol 43, 271–276.

Li, H.Y., Sun, N.J., Kashiwada, Y., Sun, L., Snider, J.V., Cosentino, L.M. and Lee, K.H. (1993) Anti-AIDS agents, 9. Suberosol, a new C31 lanostane-type triterpene and anti-HIV principle from Polyalthia suberosa. J Nat Prod 56, 1130–1133.

LiPuma, J.J., Spilker, T., Gill, L.H., Campbell, I.P.W., Liu, L. and Mahenthiralingam, E. (2001) Disproportionate distribution of Burkholderia cepacia complex species and transmissibility factors in cystic fibrosis. Am J Respir Crit Care Med 164, 92–96.

MacDonald, A. (2002) Perchlorate and NDMA in groundwater: occurrence, analysis and treatment. Presentation at the Fourth Symposium in the Series on Groundwater Contaminants. Baldwin Park, CA: Groundwater Resources Association of California.

Mahendra, S. and Alvarez-Cohen, L. (2006) Kinetics of 1, 4-dioxane biodegradation by monoxygenase-expressing bacteria. Environ Sci Technol 40, 5435–5442.

Massol-Deya, A., Weller, R., Rios-Hernandez, L., Zhou, J.Z., Hickey, R.F. and Tiedje, J.M. (1997) Succession and convergence of biofilm communities in fixed-film reactors
treating aromatic hydrocarbons in groundwater. *Appl Environ Microbiol* **63**, 270–276.

McAlister, M.B., Kulakov, L.A., O’Hanlon, J.F., Larkin, M.J. and Ogden, K.L. (2002) Survival and nutritional requirements of three bacteria isolated from ultrapure water. *J Ind Microbiol Biotechnol* **29**, 75–82.

McCay, K., Fox, B.G. and Steffan, R.J. (1996) Chloroform mineralisation by toluene-oxidizing bacteria. *Appl Environ Microbiol* **62**, 2716–2722.

Miethling, R., Hecht, V. and Deckwer, W.D. (1993) Microbial degradation of quinoline: kinetic studies with *Comamonas acidovorans* DSM 6426. *Biotechnol Bioeng* **42**, 589–595.

Minako, N., Takio, Y., Yuko, S. and Takashi, S. (1977) Mutagenicities of quinoline and its derivatives. *Mutat Res* **42**, 335–342.

Mitch, W.A. and Sedlak, D.L. (2002) Formation of N-nitrosodimethylamine (NDMA) from dimethylamine during chlorination. *Environ Sci Technol* **36**, 588–595.

Mitch, W.A., Sharp, J.O., Trussell, R.R., Valentine, R.L., Alvarez-Cohen, L. and Sedlak, D.L. (2003) N-nitrosodimethylamine (NDMA) as a drinking water contaminant: a review. *Environ Eng Sci* **20**, 389–404.

Nakatsuigawa, T. and Iida, Y. (1996) *Pseudomonas* sp. isolated from diseased ayu, *Plecoglossus altivelis*. *Fish Pathol* **31**, 221–227.

Njam, I. and Trussell, R.R. (2001) NDMA formation in water and wastewater. *J AWWA* **9**, 92–99.

Olsen, R.H., Kukor, J.J. and Kaphammer, B. (1994) A novel toluene-3-monoxygenase pathway cloned from *Pseudomonas pickettii* PKO1. *J Bacteriol* **76**, 3749–3756.

O’Sullivan, L.A. and Mahenthiralingam, E. (2005) Biotechnological potential within the genus *Burkholderia*. *Lett Appl Microbiol* **41**, 8–11.

Parales, R.E., Ontl, T.A. and Gibson, D.T. (1997) Cloning and sequence analysis of a catechol 2, 3-dioxygenase gene from the nitrobenzene-degrading strain *Comamonas sp JS765*. *J Ind Microbiol Biotechnol* **19**, 385–391.

Parales, R.E., Ditty, J.L. and Harwood, C.S. (2000) Toluene-degrading bacteria are chemotactic towards the environmental pollutants benzene, toluene, and chloroethene. *Appl Environ Microbiol* **66**, 4098–4104.

Park, J., Kukor, J.J. and Abriola, L.M. (2002) Characterization of the adaptive response to trichloroethene-mediated stresses in *Ralstonia pickettii* PKO1. *Appl Environ Microbiol* **68**, 5231–5240.

Pass, M.A. (1991) Poisoning of livestock by *Lantana* plants. In: *Toxicology of Plant and Fungal Compounds, Handbook of Natural Toxins*, Vol. 6 ed. Keeler, R. and Tu, A.T. pp. 297–311. New York: Marcel Dekker.

Pengsuparp, T., Cai, L., Fong, H.H., Kinghorn, A.D., Pezzuto, J.M., Wani, M.C. and Wall, M.E. (1994) Pentacyclic triterpenes derived from *Maprounea africana* are potent inhibitors of HIV-1 reverse transcriptase. *J Nat Prod* **57**, 415–418.

Piruzian, E.S., Mett, V.L., Kobets, N.S. and Urmeeva, F.I. (1988) The use of bacterial genes encoding herbicide tolerance in constructing transgenic plants. *Microbiol Sci* **5**, 242–248.

Pritchard, P.H., O’Neill, E.J., Spain, C.M. and Ahearn, D.G. (1987) Physical and biological parameters that determine the fate of p-chlorophenol in laboratory test systems. *Appl Environ Microbiol* **53**, 1833–1838.

Rajagopal, R. (1986) Conceptual design for a groundwater quality monitoring strategy. *Environ Prof* **8**, 244–264.

Ryan, M.P., Pembroke, J.T. and Adley, C.C. (2006) *Ralstonia pickettii*: a persistent gram-negative nosocomial infectious organism. *J Hosp Infect* **62**, 278–284.

Sakai, M., Atsuta, S. and Kobayashi, M. (1989) *Pseudomonas fluorescens* isolated from the diseased rainbow trout, *Oncorhynchus mykiss*. *Kitasato Arch Exp Med* **62**, 157–62.

Sharma, O.P., Makkar, H.P. and Dawra, R.K. (1988) A review of the noxious plant *Lantana camara*. *Toxicon* **26**, 975–987.

Sharma, O.P., Dawra, R.K., Datta, A.K. and Kanwar, S.S. (1997) Biodegradation of lantadene A, the pentacyclic triterpenoid hepatotoxin by *Pseudomonas pickettii*. *Lett Appl Microbiol* **24**, 229–232.

Sharp, J.O., Wood, T.K. and Alvarez-Cohen, L. (2003) Aerobic biodegradation of *N* nitrosodimethylamine (NDMA) by axenic bacterial strains. *Biotechnol Bioeng* **89**, 608–618.

Sideropoulos, A.S. and Secht, S.M. (1984) Evaluation of microbial testing methods for the mutagenicity of quinoline and its derivatives. *Mutat Res* **11**, 59–66.

Siegrist, R.L. (1992) Volatile organic compounds in contaminated soils: the nature and validity of the measurement process. *J Hazard Mater* **29**, 3–15.

Sinclair, M.I., Maxwell, P.C., Lyon, B.R. and Holloway, B.W. (1986) Chromosomal location of TOL plasmid DNA in *Pseudomonas putida*. *J Bacteriol* **168**, 1302–1308.

Sittig, M. (1991) *Handbook of Toxic and Hazardous Chemicals and Carcinogens*, 3rd edn. New Jersey: Noyes Publications.

Smith, J.A and Novak, J.T. (1987) Biodegradation of chlorinated phenols in surface soil. *Water Air Soil Pollut* **33**, 29–42.

Somerville, C.C., Nishino, S.F. and Spain, J.C. (1995) Purification and characterization of nitrobenzene nitroreductase from *Pseudomonas pseudoalcaligenes* JS45. *J Bacteriol* **177**, 3837–3842.

Sutton, S.D., Pfaller, S.L., Shann, J.R., Warshawsky, D., Kinkle, B.K. and Vestal, J.R. (1996) Aerobic biodegradation of 4-methylquinoline by a soil bacterium. *Appl Environ Microbiol* **62**, 2910–2914.

Tablan, O.C., Chorba, T.L., Schidlow, D.V., White, J.W., Hardy, K.A., Gilligan, P.H., Morgan, W.M., Carson, L.A., et al. (1985) *Pseudomonas cepacia* colonization in patients with cystic fibrosis: risk factors and clinical outcome. *J Pediatr* **107**, 382–387.
Takizawa, N., Yokoyama, H., Yanagihara, K., Hatta, T. and Kiyohara, H. (1995) A locus of Pseudomonas pickettii DTP0602, *had*, that encodes 2, 4, 6-trichlorophenol-4-dechlorinase with hydroxylase activity, and hydroxylation of various chlorophenols by the enzyme. *J Ferment Bioeng* 80, 318–326.

Vroblesky, D.A., Robertson, J.F., Petkewich, M.D., Chapelle, F.H., Bradley, P.M. and Landmeyer, J.E. (1997) Remediation of Petroleum Hydrocarbon-Contaminated Ground water in the vicinity of a Jet-Fuel Tank Farm, South Carolina: Hanahan. US Geological Survey Water-Resources Investigations Report 96–4251, 61.

Wakabayashi, H., Sawada, K., Ninomiya, K. and Nishimori, Y. (1996) Bacterial hemorrhagic ascites of ayu caused by *Pseudomonas* sp. *Fish Pathol* 31, 239–240.

Wieser, M., Wagner, B., Eberspacher, J. and Lingens, F. (1997) Purification and characterization of 2, 4, 6-trichlorophenol-4-monoxygenase, a dehalogenating enzyme from *Azotobacter* sp. strain GP1. *J Bacteriol* 179, 202–208.

Willems, A., De Ley, J., Gillis, M. and Kersters, K. (1991) *Comamonadaceae*, a new family encompassing the acidovorans rRNA complex, including *Variovorax paradoxus* gen. nov., comb. nov., for *Alcaligenes paradoxus* (Davis 1969). *Int J Syst Bacteriol* 41, 445–450.

Yabannavar, A.V. and Zylstra, G.J. (1995) Cloning and characterization of the genes for p-nitrobenzoate degradation from *Pseudomonas pickettii* YH105. *Appl Environ Microbiol* 61, 4284–4290.

Yabuuchi, E., Kosako, Y., Yano, I., Hotta, H. and Nishiuchi, Y. (1995) Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. *Microbiol Immunol* 39, 897–904.

Zabolina, O., Latus, M., Eberspacher, J., Golovleva, L.A. and Lingens, F. (1995) Purification and characterization of 6-chlorohydroxyquinol 1, 2-dioxygenase from *Streptomyces rochei* 303: comparison with an analogous enzyme from *Azotobacter* sp. strain GP1. *J Bacteriol* 177, 229–234.

Zenker, M.J., Borden, R.C. and Barlaz, M.A. (2003) Occurrence and treatment of 1, 4-dioxane in aqueous environments. *Environ Eng Sci* 20, 423–432.