Adaptive responses and cellular behaviour of biphenyl-degrading bacteria toward polychlorinated biphenyls

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Abstract

Polychlorinated biphenyls (PCBs) are one of the most widely distributed classes of chlorinated chemicals in the environment. For cleanup of large areas of PCB-contaminated environments, bioremediation seems to be a promising approach. However, the multitude of PCB congeners, their low bioavailability and high toxicity are important factors that affect the cleanup progression. Elucidating how the PCB-degrading microorganisms involved in the process adapt to and deal with the stressing conditions caused by this class of compounds may help to improve the bioremediation process. Also specific physiological characteristics of biphenyl-utilizing bacteria involved in the degradation of PCBs may enhance their availability to these compounds and therefore contribute to a better microbial mineralization. This review will focus in the stress responses caused in aerobic biphenyl-utilizing bacteria by PCBs and its metabolic intermediates and will also analyze bacterial properties such as motility and chemotaxis, adherence to solid surfaces, biosurfactant production and biofilm development, all properties found to enhance bacteria–pollutant interaction.

Keywords: PCB; Chemotaxis; Stress; Toxicity; Biosurfactant; Bioremediation; Biofilm; Polyphosphate

Contents

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1. Introduction

In the past decades, a vast range of xenobiotic compounds have been found to be vulnerable to microbial mineralization. In most instances where bioremediation has been demonstrated, the catabolic pathway and its regulation have also been characterized.

Biphenyl is an aromatic compound used as a fungicide for citrus in agriculture and as a chemical feedstock for organic syntheses in industry. It has been used as a model compound to study bioavailability of soil-sorbed chemicals and polychlorinated biphenyls (PCBs) degradation studies (Wu et al., 2003; Pieper, 2005).

PCBs can be produced by the direct chlorination of biphenyl and up to 209 different congeners, containing from 1 to 10 chlorine substitutions, can thus be produced. Usual commercial PCB mixtures, marketed as Aroclor, Kaneclor, Clophen or Delor, generally contain 20–60 congeners, mostly tri-hexachlorinated derivates. Because PCBs have been extensively used for a variety of industrial purposes, these recalcitrant compounds are recognized to be one of the most serious environmental pollutants worldwide (Kimbrough, 1995).

Biphenyl-utilizing bacteria are able to metabolize PCBs into chlorobenzoic acids by using biphenyl-catabolic enzymes via an oxidative route. Bacteria capable of degrading PCBs have been isolated from a range of sites and the pathways and encoding genes (*bph*) have been well studied (for a recent review see Furukawa, 2004). The genes encoding the degradative pathway are organized in an operon structure, *bphA1* to *bphA4*, *bphB*, *bphC* and *bphD*. Genes *bphA1* to *bphA4* encode a multicomponent dioxygenase enzyme complex that converts biphenyl to a dihydrodiol, which is transformed by the *bphB* gene product, a dihydrodiol dehydrogenase, to 2,3-dihydroxybiphenyl. Another dioxygenase enzyme, the *bphC* gene product, cleaves 2,3-dihydroxybiphenyl to yield a yellow colored meta-cleavage product, which is transformed subsequently to benzoate and a pentanoic acid derivative by the product of the *bphD* gene (Furakawa et al., 1990; Furukawa, 1994; Furukawa et al., 2004). *Burkholderia xenovorans* LB400 (formerly known as *B. cepacia* or *B. fungorum* LB400) and *Pseudomonas pseudoalcaligenes* KF707 have been the most extensively studied species with respect to the degradation of PCBs. These two microorganisms show distinct differences in the ranges of PCBs used as substrates. The range of PCB congeners oxidized by the LB400 enzymes is wider than that oxidized by KF707, which has a higher activity for several di-para-substituted PCBs (Bopp, 1986; Gibson et al., 1993). Also, novel isolates such as *Pseudomonas* sp. B4 (Elbe River) have been studied and compared at molecular and physiological levels with the model PCB-degraders (Bartels et al., 1999; Chavez et al., 2004).

Studies with different biphenyl- and PCB-degrading bacteria, including both gram-negative and gram-positive strains, have created the biochemical and genetic bases for PCB bioremediation (Abramowicz, 1990; Brenner et al., 1994; Furukawa, 1994). Recent extensive reviews are available concerning the microbial advantages, limitations and economics of PCB bioremediation (Abraham et al., 2002; Ohtsubo et al., 2004; Pieper, 2005).

Responding to changes in the environment is a fundamental property of a living cell. It is particularly important for unicellular organisms, which interact directly with the changing microenvironment. Through evolution, microorganisms have developed useful mechanisms that help them to regulate their cellular function in response to changes in their environment (Storz and Hengge-Aronis, 2000).

The high toxicity of the many PCB congeners and their low bioavailability, which are significant factors that influence the bioremediation process in the contaminated environments, have not been extensively addressed. Therefore, the present article reviews some of the specific physiological characteristics of biphenyl-utilizing bacteria that enhance the bioavailability of PCBs. Cell adherence and surface hydrophobicity, biosurfactant production, motility and chemotaxis processes are bacterial abilities that reduce the distance between cells and solid substrates, and thus may enable biphenyl-utilizing bacteria to actively seek new substrates once they are depleted in a given contaminated area.

In this communication, we also review the stress response induced in microorganism grown in these pollutants with particular focus on the versatile possible role of inorganic polyphosphate as a protective agent against stress and as an essential factor in microbial mobility and biofilm formation.

2. Motility and chemotaxis

Mobility (swimming, swarming and twitching) serves the planktonic organism in seeking nutrients, avoiding toxins and finding a suitable surface for aggregation. Bacteria swim using flagella and move on surfaces by a gliding movement. They may respond directly to ambient conditions or, more frequently, to temporal changes in stimulus intensity. Bacterial chemotaxis, a movement under the influence of a chemical

gradient, either toward (positive chemotaxis) or away (negative chemotaxis) from the chemical gradient, helps bacteria to locate most advantageous conditions for their growth and survival. Although most of the chemotaxis studies have focused on hydrophilic substances that are not pollutants (Stock and Surette, 1996), in the last decade, chemotaxis toward different environmental pollutants has received more attention and many microorganisms with the chemotactic abilities toward different xenobiotic compounds have been isolated and characterized (review in Pandey and Jain, 2002; Parales and Harwood, 2002). It is clear that chemotaxis is a selective advantage to the bacteria for guiding them to sense and locate pollutants that are present in the environment and, in many cases, the chemoattractant is a compound that serves as carbon and energy source, whereas a chemorepellent is toxic for the bacteria (Fig. 1).

Recently, it was reported that two motile byphenyl-degrading bacteria (*Pseudomonas putida* P106 and *Rhodococcus erythropolis* NY05) showed significant positive chemotactic response toward biphenyl (Wu et al., 2003). By using swarming motility and capillary assays, we found the same result toward biphenyl in *Pseudomonas* sp. B4, another highly motile biphenyl-utilizing bacterium (Gordillo et al., unpublished results). We also tested different chlorobiphenyls (CBs) and PCBs, and found these compounds to be

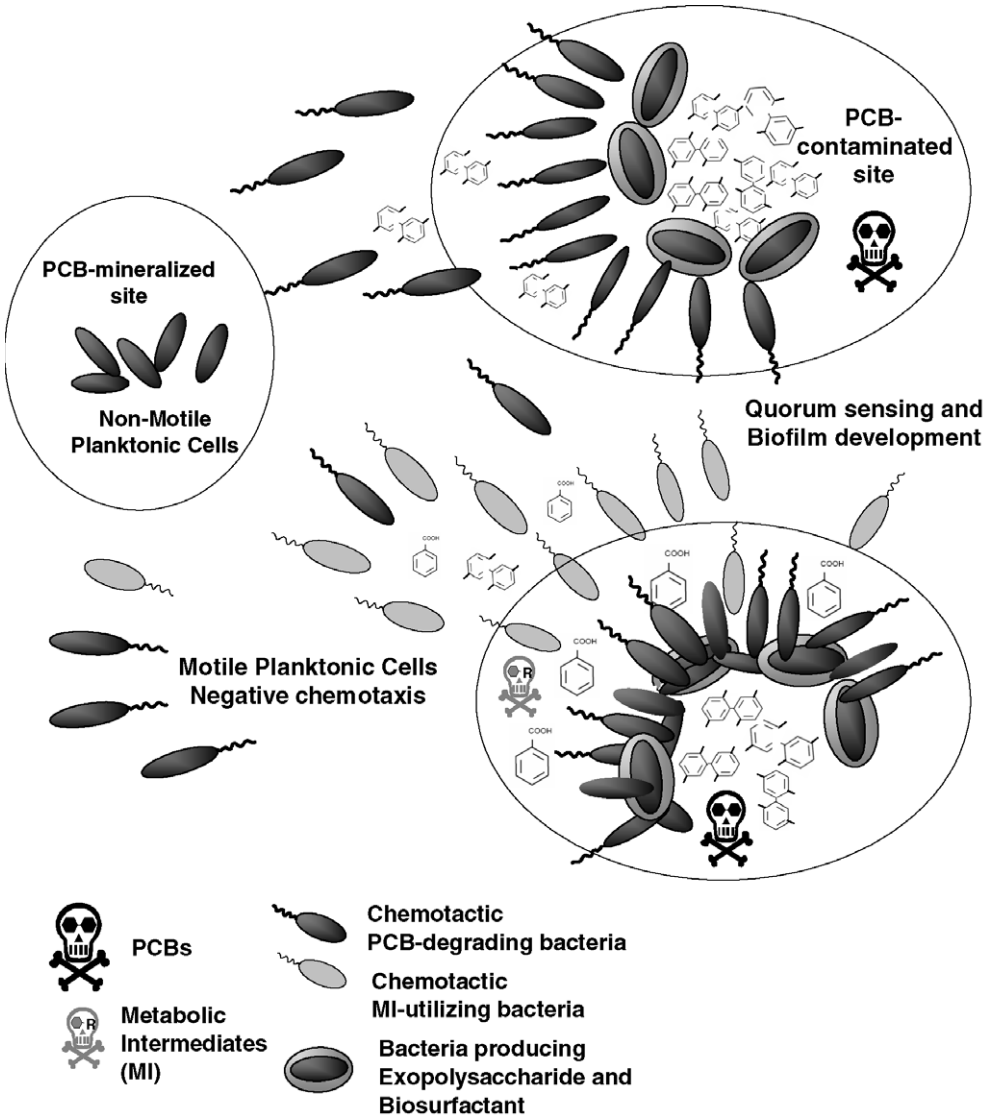

Fig. 1. Role of motility, chemotaxis and biofilm development in PCB-degradation by bacteria.

chemoattractants for *Pseudomonas* sp. B4 cells. The previous growth of cells in biphenyl did not induce chemoattraction toward biphenyl, suggesting that, unlike what has been described in the two naphthalene-degrading bacteria *Pseudomonas* sp. strain NCIB 9816-4 and *P. putida* G7 (Grimm and Harwood, 1997, 1999), chemotaxis of *Pseudomonas* sp. B4 toward biphenyl or CBs is not induced by previous growth of the cells in the substrate to be degraded.

Although *B. xenovorans* LB400 was described as a motile strain (Goris et al., 2004), recently, we and others have found this microorganism to be non-motile (Nielsen et al., 2000; Gordillo et al., unpublished results). Due to its lack of motility, chemotactic assays could not be performed with this bacterium. The loss of preexisting genes or gene activities during evolution is a major mechanism of ecological specialization and it is possible that laboratory domestication may have led to the loss of some functions required for chemotaxis in *B. xenovorans* LB400. Repeated passage of bacterial isolates in liquid culture can select for the loss of many "social" behaviours, including motility function (Velicer, 1999; Velicer et al., 1998, 2000, 2002). Also, exopolysaccharide and aerial structures were robust only in biofilm communities of recent natural isolates but not in laboratory strains, indicating that multicellularity has been lost during laboratory domestication (Zinser et al., 2003). In the future, it will be important to work with both genetically modified laboratory strains and recently isolated microbes, observing them in the context of structured communities to fully appreciate their developmental and degradation potentials.

Chlorobenzoates are intermediate metabolites that are formed and, in some bacteria accumulated, during degradation of PCBs and other chlorinated aromatic compounds (Abramowicz, 1990; Abraham et al., 2002). It was reported that 3- and 4-chlorobenzoate are attractants to *P. putida* PRS2000 previously grown on benzoate or 4-hydroxybenzoate (Harwood, 1989; Harwood et al., 1990). This fortuitous inducible chemoattraction to compounds that are not growth-supporting substrates for this particular strain is frequently found on self-transmissible catabolic plasmids and is possible that this chemotactic response facilitates the transfer of catabolic plasmids by bringing strains missing the appropriate catabolic genes to environments contaminated with chlorobenzoates (Harwood and Ornston, 1984).

How bacterial chemotaxis has the potential to influence the rate of degradation of the chemoattractants was recently demonstrated in the case of naphthalene. Chemotaxis by *P. putida* strain G7 was shown to enhance the degradation of naphthalene diffusing not only from a naphthalene-saturated aqueous buffer contained in a capillary (Marx and Aitken, 2000) but also from a non-aqueous-phase liquid (NAPL) containing naphthalene (Law and Aitken, 2003). NAPLs are organic materials extracted from polluted sites that cause great challenges in the remediation of contaminated soils and sediments. Also, chemotaxis by wild-type *P. putida* G7 increased the rates of naphthalene desorption and degradation relative to rates observed with non-chemotactic and non-motile mutant strains (Law and Aitken, 2003).

Coordinated regulation of bacterial chemotaxis to many xenobiotic compounds and their respective mineralization and/or transformation indicates that this phenomenon might be an integral feature of degradation (Fig. 1). Understanding how microorganisms move and adjust themselves using environmental cues are integral to the complex structure and function of microbial communities. Although motility in response to biphenyl and CBs has been described, understanding the role of all components involved in PCBs chemotaxis is still lacking.

3. Biofilms and bacterial communities

In natural settings, microorganisms most commonly exist as multicellular communities exhibiting a high degree of structure (O'Toole et al., 2000). Microbes often construct and live within surface-associated multicellular communities known as biofilms. The exact structure and physiology of the biofilm all vary with the nature of its resident microbes and local environment (for recent reviews: Stanley and Lazazzera, 2004; Branda et al., 2005). Clay minerals performed an essential role in biofilm formation, possibly acting as a nutrient transfer from PCBs to a pleasant form to the bacteria, and it was suggested that clay minerals might perform a similar function in soil. De novo development of PCB-degrading microbial communities and the formation of combined biofilms consisting of bacteria and clay minerals were observed, in which the clay minerals were arranged around the bacteria to form hutch-like structures (Lunsdorf et al., 2000a,b).

The role of commensal relationships on the spatial structure of a surface-attached microbial consortium with the potential to interact metabolically in the biodegradation of PCB was studied (Nielsen et al., 2000). Highly motile *Pseudomonas* sp. B13(FR1) can metabolize the chlorobenzoate produced by *B. xenovorans* LB400 when grown on chlorobiphenyl and its spatial location close to the *B. xenovorans* LB400 microcolonies occurs only when the consortia is grown in chlor-

obiphenyl. Another important aspect in the spatial relationships in this microbial community is the motility of the secondary user involved in the structure development.

PCBs are poorly bioavailable because of its superhydrophobic and very low solubility in water. Microorganisms that use hydrophobic substrates often produce biosurfactants that enlarge the surface area of the hydrophobic substrates and also their bioavailability by increasing the solubility or desorbing them from surfaces and coordinating attachment and detachment of microorganisms to and from the solid surfaces (Sim et al., 1997; Ron and Rosenberg, 2001). The influences in the PCBs biodegradation efficiency of different biosurfactants such as lipopeptides (Golyshin et al., 1999) or maltotriose esters (Ferrer et al., 2003) and chemically produced surfactants (Singer et al., 2000) have been studied in detail with contradictory results. Whereas in some cases, the addition of surfactants increases the degradation rates, in others the degradation rates decrease. Taking into account that addition of surfactants can produce changes in the community composition resulting in a decrease in the degrader population (Colores et al., 2000) and also reduce bacterial adhesion to surfaces (Stelmack et al., 1999), the net effect of a surfactant on biodegradation will depend on the right balance between the benefits that result from enhanced solubility of solid pollutants versus the reduction in direct adhesion of bacteria to those compounds (Billingsley et al., 1999).

Chemical surfactants have the advantage of low price but its toxicity to the cells reduces the rate of PCB biodegradation. The contrasting effects of the surfactant functions are a result of the poorly understood complexity of interactions between soil/sediment, pollutant, surfactant and microorganisms in different environments. Interestingly, genetically modified microorganism (GEM) with the capacity for degrading both, chemical surfactants and PCBs resulted in the simultaneous increase in PCB-degrader populations and contaminant bioavailability (Lajoie et al., 1992, 1997).

Because it is well known that the rates of degradation of the individual congeners can vary significantly depending on their initial concentrations and on the composition and complexity of the PCB mixtures (Bedard and Quensen, 1995), the aerobic PCB-degradation products formed by numerous bacterial strains (Massé et al., 1989; Bedard and Haberl, 1990; Maltseva et al., 1999; Drenzek et al., 2004), have established the basis for designing novel bacterial communities in effective PCB-biofilm reactors (Kastanek et al., 2004; Fava et al., 1996, 2000).

The importance to study PCB-biofilm communities in the degradation of these contaminants could be illustrated in the single-stage coupled anaerobic/aerobic reactor system for the degradation of Aroclor 1242. The close contact of aerobes and anaerobes within the granular biofilm facilitated the exchange of metabolites, thus improving the stability and system performance for the near-complete mineralization of Aroclor, and the sequential biodegradation process accomplished in the single reactor system (Tartakovsky et al., 2001). Another example was a stable PCB-dechlorinating consortium (2,3,4,5-CB enrichment culture) developed from the source of sediment that attacks only meta- and para-dechlorination of 2,3,4,5-CB and freshly added Aroclor 1260. The culture attacks only doubly flanked chlorines of PCBs, chlorines that are flanked on each side by another chlorine bound to a carbon atom, indicating a high degree of specificity for PCB dechlorination (Wu et al., 2000).

Microbial diversity changes in PCB-dechlorinating community in response to different culture conditions have been monitored in situ by molecular screening techniques based on 16S rRNA analysis (i.e. ARDRA: amplified ribosomal DNA restriction analysis, DGGE: denaturing gradient gel electrophoresis and TRFLP: terminal restriction fragment length polymorphism). All these procedures have provided an alternative approach to identify new species within a community and study the prevalence and survival of the PCB-degrader in the environment (Nogales et al., 2001; Watts et al., 2001). However, the exact modes in which PCBs are degraded by microbial communities under environmental conditions require further investigations.

4. PCBs toxicity and bacterial stress responses

Many studies discuss in more detail the extent of PCBs influence in different human diseases such as cancer, neurobehavioural effects, abnormal thyroid and immune function in children and low birth weight (Kimbrough, 1995; Kimbrough and Krouskas, 2003), and the overall toxicity of some PCB congeners in many soil and aquatic animals is well documented as a serious environmental problem (Seegal, 1996). Exposure to PCBs and related halogenated aromatic compounds induces significant behavioral dysfunctions in laboratory and contaminated resident animals, particularly following exposure during gestation and lactation.

Although many genetic, enzymological and biochemical analysis of PCB-degradative pathways have provided the basis for the bioremediation of PCBs, little is known about the adaptive responses of PCB-degrading bacteria during their growth in this kind of organochlorine compounds.

In some bacteria, it has been reported that stress proteins related with one stimulus can be induced during exposure to other stresses. For example, various heat shock proteins were synthesized when the cells were exposed to hydrogen peroxide (VanBogelen et al., 1987; Dukan and Touati, 1996), UV light (VanBogelen et al., 1987) or chemical agents including aromatic compounds (Ramos et al., 1995; Vercellone-Smith and Herson, 1997). Also, *Escherichia coli* cells in a nutrient-limited environment evolved into a state of enhanced resistance to various stress conditions, such as high osmotic pressure, high temperatures and oxidative stress (Matin, 1991).

The increase in the level of the stress protein GroEL observed when *Pseudomonas* sp. B4 was grown in the presence of biphenyl and different CBs (Chávez et al., 2004) is in agreement with the behaviour of *Pseudomonas* sp. DJ-12 where several stress-shock proteins, including DnaK and GroEL, were induced when subjected to stress conditions such as the presence of biphenyl and 4-chlorobiphenyl (Park et al., 2001), indicating that the pollutant compounds served both, as carbon sources and as chemical stressors. It was found that chlorinated derivatives were more toxic than the non-chlorinated compounds. In addition, the stress-shock proteins DnaK and GroEL, which contribute to the resistance of the cytotoxic effect of the phenoxy-herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), were induced at different 2,4-D concentrations in exponentially growing cultures of *Burkholderia* sp. YK-2 (Cho et al., 2000). In some bacteria, PCBs possibly exert their toxic effects by accumulating in the cytoplasmic membrane and disrupting its function. In this case, the degradation ability of biphenyl is important not only for energy acquisition but also for detoxification of biphenyl (Delawary et al., 2003).

Organochlorine compounds including PCBs are also well known as increasing oxidative stress in several biological systems (Voie and Fonnum, 2000; Coteur et al., 2001; Mariussen et al., 2002; Ruiz-Leal and George, 2004). Recent studies have implicated oxidative stress as a possible causative mechanism for the non-target toxicity of organochlorinate compounds. In living microorganisms that have been subjected to environmental stresses, oxidative stress is caused by both overproduction of reactive oxygen species (ROS) and depletion of antioxidants. Overproduction of ROS was found in *Pseudomonas* sp. B4 cells exponentially grown under different conditions by using the oxidative stress-sensitive probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (Chávez et al., 2004). The exact source of this oxidative stress should be further investigated. However, it is possible that ROS might be generated by the oxidation reactions catalyzed by the biphenyl dioxygenases, which play a critical role in the bacterial aerobic degradation of aromatic compounds and contain mononuclear iron and Rieske-type (2Fe–2S) clusters. These enzymes catalyze the enantiospecific addition of oxygen (O_2) to substrates with pi-electron systems to form *cis*-dihydrodiols (Gibson and Subramanian, 1984; Furukawa et al., 2004). In the presence of benzene, by an uncoupling reaction, 40% to 50% of the O_2 consumed by NDO benzene was reduced to hydrogen peroxide, which spontaneously may decompose by forming strong oxidizing agent such as hydroxyl radicals via Fenton-type reaction (Lee, 1999).

Moreover, not only biphenyl and its chloroderivates, but also its metabolic intermediates such as dihydrodiols, dihydroxybiphenyls and catechols, were found to be highly toxic for bacteria. By using recombinant *E. coli* strains expressing different subsets of *B. xenovorans* LB400 *bph* genes as a model system, the concomitant accumulation of the two firsts CBs metabolic intermediates affected the cell viability much more than CBs themselves. Cell viability of *B. xenovorans* LB400 and of *E. coli* when exposed directly to 2,3-dihydroxybiphenyl also decreased considerably (Cámara et al., 2004). Also it has been reported that catechols, like dihydroxybiphenyls, in the presence of molecular oxygen and combined with heavy metals (e.g. Cu^{2+}, Fe^{3+}) can also cause oxidative DNA damage (Schweigert et al., 2001). From microorganisms to mammals, the wide chemical reactions and modes of action of catechols may be responsible for their toxicity.

Recently, Raman confocal microscopy was used to discriminate between cultures of *B. xenovorans* LB400 exposed to different pollutants (Singer et al., 2005). This novel bioassay could be used as a tool to study bioavailability and toxicity in PCB contaminated environments.

The increase in the levels of the general stress proteins and oxidative stress observed in many biphenyl-utilizing bacteria indicate that these bacteria adjust their physiology with a stress response when confronted with compounds that supply both as carbon and energy sources and, at the same time, as chemical stressors. This cross-protection against various

stresses is thought to be an adaptive response by bacteria in surviving increasingly complex natural environments.

5. Inorganic polyphosphate and stress responses

Inorganic polyphosphate (polyP) is a polymer of tens or hundreds of orthophosphate (Pi) residues linked by high-energy phosphoanhydride bonds. It has been detected in many bacteria and fungi and in smaller amounts in every microbe, plant and animal so far examined (Kornberg et al., 1999; Kulaev and Kulakovskaya, 2000). Since polyP contains phosphorus, one of the most important vital elements present in of all major subunits of life: amino acids, nucleotides, sugars and phospholipids recently has been quite obviously shown to be a "molecule for many reasons" of living cells (Kornberg, 1999).

The principal enzyme that synthesizes polyP enzymes involved in the metabolism of polyP in bacteria are the polyphosphate kinase 1 (PPK1) that catalyzes the reversible conversion of the terminal phosphate of ATP into polyP and the exopolyphosphatase (PPX) that processively hydrolyzes the terminal residues of polyP to liberate Pi (Kornberg et al., 1999). Both PPK1 and PPX are highly conserved in many bacterial species, including some of the major pathogens (Tzeng and Kornberg, 1998; Kornberg et al., 1999; Cardona et al., 2002).

The accumulation of polyP in bacteria is a natural phenomenon and finely regulated process that depends on phosphate and energy sources availability as well as in the presence of K and Mg ions (Nesmeyanova, 2000). In essence, polyP accumulation responds to different external factors (e.g. nitrogen or sulfur deficiency or unfavorable pH conditions) whether they are components of the nutrient medium, or its physical and chemical conditions (Harold, 1966; Rao and Kornberg, 1999; Nesmeyanova, 2000).

The accumulation of polyP in bacteria is a tightly regulated process and functions as a phosphate donor, energy source and chelator for divalent cations. In addition, polyP can be involved in the functioning of global regulatory systems. The involvement of polyP in the regulation of both, enzyme activities and expression of large groups of genes is the basis of survival for different bacteria, including pathogens, under stress conditions and adaptation to the stationary-growth phase (Kornberg et al., 1999; Kulaev and Kulakovskaya, 2000). Mutant bacterial cells lacking polyP survive poorly during growth in the stationary phase and are less resistant to heat, oxidants, osmotic challenge, antibiotics and UV (Crooke et al., 1994; Kim et al., 2002; Rao and Kornberg, 1996; Rao et al., 1998; Tsutsumi et al., 2000).

PolyP accumulation in response to nutrient deprivation has also been reported in the genus *Pseudomonas* and the PCB-degrading bacteria *Pseudomonas* sp. B4 and *B. xenovorans* LB400 accumulated a great amount of large electron-dense granules when grown in biphenyl and CBs in all stages of growth and in glucose only when the cells entered the stationary phase (Chávez et al., 2004). By means of energy dispersive X-ray (EDAX) analysis and electron energy loss spectroscopy (EELS) with an integrated energy-filtered transmission electron microscope (EFTEM), it was demonstrated that these granules were mainly composed by phosphate and most likely polyP (Fig. 2C–D; Chávez et al., 2004). Also, when exponentially grown cells of *Pseudomonas* sp. B4 were shifted from a medium with glucose to the same medium but containing biphenyls and different CBs, a great increase in polyP accumulation in the form of electron-dense granules was observed (Fig. 2A–B; Chávez et al., 2004).

Interestingly, PPK is essential in *P. aeruginosa* not only for various forms of motility (Rashid and Kornberg, 2000; Rashid et al., 2000a) but also for biofilm development, quorum sensing, production of virulence factors and for virulence in the burned-mouse pathogenesis model (Rashid et al., 2000b).

It has been shown that *E. coli* cells overproducing the yeast exopolyphosphatase (PPX1; Wurst et al., 1995) have the same behaviour of a mutant whose polyphosphate kinase gene is disrupted (Shiba et al., 2000). By using broad-range vectors (Lefebre and Valvano, 2002), the recombinant yeast exopolyphosphatase (PPX1) was overproduced in *Pseudomonas* sp. B4 to remove as much of cellular polyP content as possible (Chávez et al., unpublished results). Examining the polyP-deficient phenotype by using this approach has some advantages over the PPK knockout strategy because it can be used in several strains with good reproducibility depending on the broad-range vector employed. Also it is possible to remove short-chain polyP that remain unaffected in a *ppk* mutant and avoid the appearance of the small-colony-size variant by using a fresh transformant carrying the PPX1 gene on the plasmids (Shiba et al., 2000).

Among other morphological and physiological modifications, it was found that polyP-minus *Pseudomonas* sp. B4 cells were non-motile in swarming motility plates and by examination under light microscopy (Fig. 2E–H; Chávez et al., unpublished results). Al-

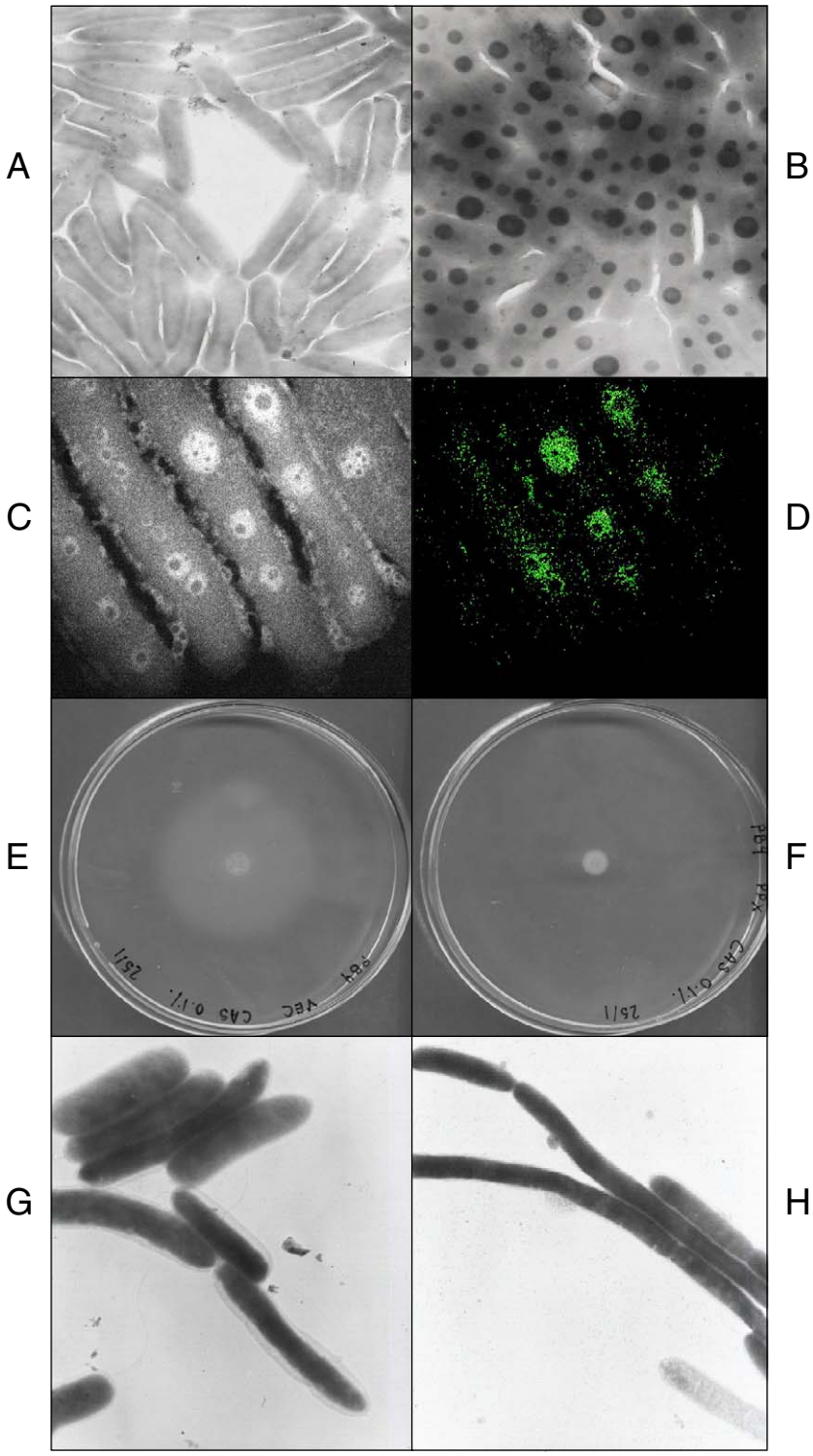

Fig. 2. PolyP accumulation in PCB-degrading bacteria. Presence of polyP granules in *Pseudomonas* sp. B4 grown in glucose (A) and 4Cl-biphenyl (B) as the sole carbon sources. Electron micrograph negative image of *Pseudomonas* sp. B4 cells with electron dense granules (C) and phosphate distribution image (green, D), obtained by electron spectroscopy imaging (ESI). Swarming motility assay plates of wt *Pseudomonas* sp. B4 cells (E; electron microscopy morphology detail in G) and *Pseudomonas* sp. B4 cells overexpressing yeast exopolyphosphatase (F; electron microscopy morphology in H). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

though the exact roles of polyP in the adaptive response of biphenyl-utilizing bacteria to PCBs remain to be elucidated, understanding polyP metabolism might be important to improve the interaction of the microorganism with their toxic substrates and therefore the efficiency of bioremediation processes.

Polyphosphate accumulating organism also play a critical role in several environmental and biotechnological problems such as heavy metals detoxification (Alvarez and Jerez, 2004; Renninger et al., 2004) and biological phosphorus removal, a treatment process that is widely used to remove excess phosphate in wastewater (Keasling et al., 2000; McGrath and Quinn, 2003; Seviour et al., 2003). Taking into account that *Pseudomonas* sp. B4 and *B. xenovorans* LB400 accumulated high levels of polyP (Chávez et al., 2004) and that PCBs and heavy-metals were found simultaneously in contaminated environments (Shin et al., 2004; Gillan et al., 2005), it should be interesting to find out if these PAOs could be used not only in bioremediation of PCBs but also in heavy metals and phosphorus removal in polluted sites.

6. Concluding remarks

Recently, a draft genome sequence of the model PCB-degrader *B. xenovorans* LB400 was generated by The Joint Genome Institute (JGI/ORNL annotation December 2003), suggesting a total of 9851 open reading frames (ORFs) in approximately 9.7 Mbp of genome size. By using microarray analysis, all the cellular processes and physiology adjustments relevant for PCBs degradation were studied in a genomic context (Denef et al., 2004). The use of this functional genomic approach as well as other proteomic and metagenomic studies will help to elucidate all the cellular processes relevant for PCB-degradation not only of bacterial strains in laboratory conditions but also of bacterial populations in their natural environments. These results may allow the development of strategies to remove the bottlenecks that limit efficient PCB-degradation and facilitate the construction of microorganism or bacterial communities with ideal PCB-degrading abilities.

Now is generally accepted that the ideal PCB-degrader should be a highly tolerant microorganism that not only constitutively expresses the degradation enzymes with wide substrate specificities without accumulating toxic intermediates, but also should be a microbe producing surfactants that solubilize PCBs, and that survives and propagates in the pollutant environment until the end of the clean-up process. Here we also reviewed other cellular characteristics that may favor PCB bioavailability such as motility, attachment to surfaces and biofilm formation capabilities. These abilities may enable cells to actively seek new substrates once they are depleted in the contaminated area. Therefore, knowledge of all these cellular processes is essential to predict the survival and activity of bacteria exposed to such adverse conditions and to select for PCB-degraders with superior tolerances to these pollutants.

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