Detection of New Polychlorinated Biphenyl (PCB) Degraders Express *Meta*-cleavage Enzymes in PCB Contaminated Site

Hamdy A. Hassan

Environmental Biotechnology Department, Genetic Engineering and Biotechnology Research Institute, Menoufia University, Sadat city, Egypt
hamdyhassan1973@yahoo.com

Abstract: Oxidation ponds of Sadat city in Egypt had been polluted with polychlorinated Biphenyls (PCBs) compounds. The PCBs content in these ponds were detected with range from 212 to 320 mg PCBs kg^{-1}. 27 bacterial isolates were isolated based on its capability to express *meta*-cleavage enzymes 2,3-dihydroxybiphenyl dioxygenase (DHBD) and catechol 2,3 dioxygenase (C23O) as a key enzyme of aerobic PCBs degradation. A partial 16S rRNA gene sequence revealed that the isolates belonged to the genus *Pseudomonas, Bacillus, Staphylococcus, Rhodococcus*, *Burkholderia*, and *Pandoraea*. The 27 bacterial isolates had the capability to use monochlorinated biphenyl as the sole carbon source. Ten isolates were belonged to genus *Pseudomonas, Burkholderia, Bacillus and Rhodococcus* had also the capability to use dichlorinated biphenyl as the sole carbon source. Only two strains *Burkholderia* sp. HA-OP24 and *Rhodococcus* sp. HA-30 were capable of metabolizing the highly recalcitrant congener 2,4,5,2′,4′,5′-chlorobiphenyl. The new PCBs degraders harbor DHBD and C23O. These genes may act as functional genes in the metabolic pathway of the PCBs, which can be used as a suitable molecular marker for the *in situ* detection of microbial communities in PCBs contaminated sites. A good understanding of these novel strains, may aid not only in answering questions concerning the microbial degradation of polychlorinated biphenyls (PCBs) in natural systems but also to enhance the potential use of bioremediation by facilitating the detection and monitoring of the PCBs degrader with DHBD and C2,3O in the contaminated sites.
[Hamdy A. Hassan. **Detection of New Polychlorinated Biphenyl (PCB) Degraders Express *Meta*-cleavage Enzymes in PCB Contaminated Site.** *Life Sci J* 2013;10(1):1123-1132] (ISSN:1097-8135). http://www.lifesciencesite.com. 173

Keywords: Bacterial community / *meta*-cleavage enzymes / Polychlorinated Biphenyls (PCBs)

1. Introduction

PCBs are man-made chemicals and synthesized by substituting variable number of chlorine atoms (from 1 to 10 chlorine atoms) onto the biphenyl aromatic molecular structure to produce 209 congeners. These congeners were used widely as coolant in power transformers, insulators in capacitors, heat transfer fluids, fire resistance, and plasticizers as well as in consumer products such as ink, paper and paints. PCBs are a main category of persistent organic pollutants (POPs), which present as contaminants in the environment (IPCS, 1993, 1997). The PCBs have impacted our environment through accidents, spills in industrial facilities and mismanagement of storage or disposal areas (Nagayama, 1976; Chen *et al*., 1980).

Exposures to elevated levels of PCBs have caused birth defects and cancer in laboratory animals, and they are a suspected cause of cancer and adverse skin and liver effects in humans (Safe, 1984; Kimbrough, 1987; Piper, 2005; USEPA, 2008). PCBs contaminations in drinking water, sediments, wastewater, foods, and aquatic organisms have been documented (Boon e*t al*., 1985). As a result of these effects from PCBs, the Environmental Protection Agency (EPA) regulates the disposal of PCBs through the Toxic Substance Control Act (TSCA) which requires that the PCBs be disposed in accordance with the methods regulated by the EPA. Incineration is the standard method of destruction for PCBs and the only one approved for the removal of PCBs from the soil and sediment (Blake, 1994). Regulation provides scope to alternate methods like biodegradation/bioremediation as an effective tool for cleanup of PCB-contaminated soils that could demonstrate the destruction of PCBs equivalent to incineration.

One of the problems related to the persistence of PCBs can be explained by the lack of microorganisms capable of degrading this compound (Middeldorp *et al.*, 1990). This problem could be solved by the introduction of specific PCB degrading microorganisms into the environment. Cultivation of microorganisms isolated from PCBs contaminated sites or any variety of ecosystem samples by any chosen cultivation approach will inevitably favor the growth of some community members while others are inhibited or not cultured and allows only around 0.1 to 1% of the total viable bacterial cells present in a variety of ecosystems can be cultured (Amann *et al*., 1995). It is likely to generate more cultivation

methods allow more detections of the PCBs degraders in the contaminated sites.

Some aerobic bacteria capable of oxidizing PCBs have been reported (Pieper and Seeger, 2008). Bacterial strains of *Pseudomonas*, *Burkholderia*, *Comamonas*, *Cupriavidus,* *Sphingomonas*, *Acidovorax*, *Rhodococcus,* *Corneybacterium* and *Bacillus* genera have been characterized (Furukawa and Fujihara, 2008; Seeger and Pieper, 2009), as well as some fungi, such as *Phanerochaete chrysosporium* (Beaudette *et al*., 1998). The degradation of PCBs by microorganisms isolated thus far is started with transforming PCBs into vicinal diols by initial dioxygenase enzymes belong to a large family of Rieske non-heme iron oxygenases (Iwasaki *et.al.,* 2007) by introducing two oxygen atoms into PCBs which dehydrogenated by a dehydrogenase to give chloro, 2,3' dihydroxybiphenyl which subjected to extradiol dioxygenase, which can easily be identified due to their yellow pigmentation of the product (Pieper, 2005).

In this study identification and characterization new PCBs degraders were performed based on culture-dependent bacterial community in PCBs contaminated site (Oxidation ponds-Sadat city in Egypt). The new isolated strains had the capability to grow on PCBs and harboring *meta*-cleavage genes 2,3-dihydroxybiphenyl dioxygenases (DHBD) and catechol 2,3 dioxygenase (C23O) for the upper and lower of PCBs catabolic pathways .

2. Materials and Methods

Sadat City and Oxidation ponds descriptions

Sadat City is one of the largest industrial city in Egypt, located in the west of Menoufia Province, at 93 km from Cairo. Area: 500 km^2, Population: 900,200. It is a desert area which includes more than 109 factories with several industrial activites such as iron-steel industry, paints, ceramic, chemicals, foods, fertilizers, biocides, organic products, textile, paper, batteries, dyes, printing materials and recycled plastics, as a result the Oxidation ponds were highly contaminated with PCBs. The treatment of such emissions is carried out depending on the oxidation pond system (Elaxada ponds). However, the sweeping overflow of this discharge moves downward to the lower land forming large pools (2.6-4 hectares area, and 2-4 m depth).

Chemical and physical analysis of the soil samples from the oxidation ponds

Four different soil samples were collected from the shores of the ponds in different treatments stages of the oxidation ponds. The soil samples were then stored at 4°C until use within 7 days. From each sample homogenized, freeze-dried soil samples (1 mg). Chemical and physical analyses were performed according to standard techniques (Sparks, 1996). Soil organic C was determined by the method of dichromate oxidation. pH was also measured by glass electrode in 1:2 H_2O suspensions, total N was measured by the standard Kjeldahl method. PCBs were analysed by extracting the soil samples and concentrated under a gentle stream of N_2 to about 200 µl and completed to 1000 µl with hexane. One µl aliquots were analysed by capillary gas chromatography performed on a Hewlett Packard 5890 Series II gas chromatograph equipped with a HP Ultra 2 capillary column (50 m by 0.2 mm; film thickness 0.11 mm) and FID detector. Hydrogen served as the carrier gas with flow rate 1ml/min. Injector temperature was set to 250°C and detector temperature was 300°C. The oven program was: 80°C for 3 min, 90°C to 288°C at 6°C min^{-1} followed by an isothermal period of 20 min

DNA Extraction from the four soil samples

Total DNA from the PCBs-contaminated soil samples (10 g wet weight) were extracted according to the protocol for DNA extraction with the Fast prep DNA kit for soil (Bio 101). DNA was visualized on 1% agarose gels. Yield of genomic DNA was determined spectrophotometrically by measuring the absorbance at 260 nm. Purity was determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm (a pure DNA has an A_{260}/A_{280} ~ 2.0) The DNA extracts from PCBs- contaminated samples containing approximately 200 ng ml^{-1} DNA were 50- or 100-fold diluted in Tris-HCl buffer (10 mM, pH 8.0) and used as template DNA in PCR.

Isolation and identification of PCBs degraders harboring 2,3-dihydroxybiphenyl dioxygenase

Chlorobiphenyl-degrading bacteria were initially isolated by traditional enrichment culture method from the soil samples prepared using 1 g of soil in 1 L Erlenmeyer flasks containing 100 ml of mineral medium with 2-Chlorobiphenyl (2-CB) and 4-Chloobiphenyl (4-CB) separately with concentration 2 mM as the sole source of carbon and energy. Due to limited aqueous solubility, stock solutions of each 2-Chlorobiphenyl (2-CB) and 4-Chloobiphenyl (4-CB) were prepared in 2,2,4,4,6,8,8-heptamethylnonane (HMN), a non-degradable carrier, to provide an initial concentration of 2 mM. For PCBs compounds mentioned below, which have limited aqueous solubility, the concentrations given represent the total mass in both the aqueous and HMN phases, divided by the aqueous volume. After one month of cultivation at 30°C, 10% of the culture was transferred to fresh medium and cultured for another month. Organisms in CB enriched cultures were obtained by a spray plate technique. Desired dilutions (0.1 ml) were spread on mineral medium agar, immediately after an ethereal solution of 2-CB or 4-

CB was uniformly sprayed onto the surface of the agar; the plates were sealed with paraffin film and incubated for 4–6 weeks. CB-degrading microorganisms were identified by compound-cleared zones surrounding individual colony. Such colonies were purified on mineral medium agar containing 2 mM 2-CB or 4-CB were sprayed with filter sterilized 2,3-dihydroxybiphenyl (DHB) (10 mM). Colonies turning yellow due to extradiol cleavage of DHB were purified by repeated subculturing and streaking on minimal medium agar plates supplemented with 2-CB and 4-CB as sole carbon source. The new PCBs degraders were identified partially using two primer sets targeting the 16S rRNA, the first primer set (16F8 5`-AGAGTTTGATCCTGGCTCAG 3`; 16R518 5`- CGTATTACCGCGGCTGCTGG-3`), and the second primer set (16F945 5`-GGGCCCGCACAAGCGGTGG-3` 16R1492 5`-TACGGYTACCTTGTTACGACTT-3`), the condition as described by Lane (Lane, 1991) corresponding to position 1-518 and 945- 1492 respectively according to the *E. coli* numbering system were directly determined from PCR fragments after purification with GeneJET™ PCR Purification Kit (Fermentas).

Growth on different PCBs compounds

Pure cultures were tested for their ability to grow on a variety of defined carbon sources. The tests were performed in minimal media supplemented with the tested compounds as a sole carbon source. Substrates were added to 6 ml MM in Balch tubes at a concentration of 100 ppm and inoculated with 10^5–10^6 cells/ml of phosphate buffer (pH 7.2), tubes were crimp sealed with Teflon-coated stoppers and incubated on shaker at 100 rpm. Tested substrates included monochlorobiphenyls (2-CB and 4-CB), dichlorobiphenyls (2,3CB, and 2,4CB), and polychlorinated biphenyls (2,4,5,2′,4′,5′-Chlorobiphenyl) Stock solutions of all substrates were autoclaved prior to use. Growth was evaluated by microscopy and visual monitoring of turbidity in conjunction with periodic HPLC analyses to measure test compound disappearance or appearance of products. In these substrate-screening studies, growth was scored as positive if turbidity was notably greater than in controls lacking the test compound, microscopic examination revealed an increase in cell numbers, and HPLC analysis showed loss of the test compound. Growth tests were conducted in triplicate for each substrate. In time course experiments, three replicate tubes were sacrificed at each time point.

Transformation of 2,3-Dihydroxybiphenyl and catechol by resting cells from PCBs degraders

The resting cells of the 2CB or 4CB degrader isolated strains from the oxidation ponds were prepared after growth in minimal medium supplemented with 2CB or 4CB as the sole carbon sources (2mM). The cells were harvested during late exponential growth, washed twice with 50 mM sodium phosphate buffer (pH 7.4), and resuspended in 5 ml of the same buffer to give an OD_{600nm} of 4.0. Resting cells of the CBs degrader isolated strains were incubated at 30°C with 100 µM of catechol, or DHB in 2 ml eppendorf tubes containing with 1.5 ml cell suspension. For quantification of substrate depletion, samples (200 µl) were taken at appropriate time intervals, centrifuged and analyzed by HPLC.

Data deposition

The sequences reported in this study has been deposited in the GenBank database for the first primer with accession numbers (HQ918231-HQ918254) and for the second primer set with accession numbers (JF264732- JF264755).

3. Results

The chemical and physical properties of the four soil samples

The chemical and physical properties of the soil samples as well as the evaluation of its pollution degree may help to estimate the impact of pollutants on the quality of soil under investigation, if they are complemented with measurement of biological properties (Margesin *et al*., 2000). The soil samples consisted mainly of sand with some clay, were acidic with pH 5.47-5.90, generally all the four samples had low TOC and TN, sample 3 (OP-3) showed less organic carbon content 4.8 g TOC kg^{-1} soil while sample 4 (OP- 4) showed the slightly higher organic carbon content 8.8 g TOC kg^{-1} soil, the PCBs contents in the four soil samples were very high amounts with rang 212-320 mg PCBs kg^{-1} soil sample (Table 1).

Isolation and characterization of strains exhibiting 2,3-dihydroxybiphenyl dioxygenase activity.

Dilutions of soil samples were spread on minimal media plates supplemented with monochlorinated biphenyl (2-CB and 4-CB). After incubation of plates from 4-7 days, Whereas there was no significant difference in the number of colony forming units from the four differently contaminated soil samples tested (approximately 3 x 10^6 CFU/g of soil), there were a correlation between presence of extradiol dioxygenase activity and the capability to grow on 2-CB and 4-CB contaminants, all colonies were analysed for the dioxygenase activity by spraying with 10mM 2,3-dihydroxybiphenyl or catechol solutions. A subset of colonies with different colony morphotypes and exhibiting yellow coloration upon spraying, which exhibited dioxygenase activity could be isolated. 27 bacterial isolates were isolated; the isolation was achieved by selective enrichment and subsequent plating on minimal medium

containing the PCBs substrate (2-CB and 4-CB) as the sole source of carbon. 27 isolates were characterized by sequencing 16S rRNA partially with two primer sets. One primer set (16F8, 16R518) corresponding to position 1-518 according to the *E. coli* numbering system and the other primer set (16F945, 16R1492) corresponding to position 945-1492 according to the *E. coli* numbering system. Analysis using the RDPII database revealed that 18 isolates HA-OP1, HA-OP2, HA-OP3, HA-OP4, HA-OP5, HA-OP6, HA-OP7, HA-OP8, HA-OP9, HA-OP10, HA-OP13, HA-OP14, HA-OP15, HA-OP18, HA-OP19, HA-OP20, HA-OP21 and HA-OP22, were belonged to the genus *Pseudomonas*; two isolates HA-OP25 and HA-OP30 belonged to genus *Rhodococcus;* two isolates HA-OP29 and HA-OP31 belonged to *Bacillus,* another isolates HA-OP17, HA-OP24, HA-OP26 and HA-OP28 were belonged to genus *Staphylococcus, Burkholederia*, *Brevibacillus and Pandoraea* respectively. Two phylogenetic relationships between isolated strains with related aromatic compounds degraders taxa were determined by a neighbor-joining distance analysis of their 16S rRNA gene sequences for the first primer set (Figure 1) and for the second primer set (Figure 2) *Pseuudomonas* sp., Strains HA-OP3, HA-OP4, HA-OP5, HA-OP6, HA-OP7, HA-OP9, and HA-OP10 showed highest similarity with each other and grouped into one cluster either for the first primer set (Figure 1), or for the second primer set (Figure.2). Some *Pseuudomonas* strains as *Pseuudomonas* sp. Strains HA-OP13, HA-OP14, HA-OP15, HA-OP18, HA-OP19, HA-OP20, HA-OP21 and HA-OP22 were clustered together and have high similarity in the first primer set with *Pseudomonas* sp. C16w, *Pseudomonas* sp. ARDRA PS2, *Pseudomonas* sp. CT-1, and *Pseudomonas* sp. DK2009-3a which have previously been reported to be able to grow on hydrocarbon, BTEX, naphthalene and fenamiphos & oxamyl respectively (Figure 1) (Junca and Pieper, 2004; Viñas *et al.,* 2005). In case of the second primer with *Pseudomonas veronii* UFZ-B54 and *Pseudomonas* sp. BZ27 which have previously been reported to be able to grow on Chlorobenzene and hydrocarbons respectively. The remained *Pseudomonas* isolates *Pseudomonas* sp. HA-OP1 showed high similarity in case of the first and second primer set with *Pseudomonas aeruginosa* W3, which has been reported as anthracene degrader (Figures1, 2). *Brevibacillus* sp. HA-OP26 showed high similarity with the polyaromatic hydrocarbon degrader *Brevibacillus* brevis BEA. *Rhodococcus* sp. HA-OP25 showed high similarity with *Rhodococcus* sp. CH9 as phenol degrader and *Rhodococcus* sp. HA-OP30 with high PCBs substrate diveristy (Table 2) has high similarity with *Rhodococcus* erythropolis, which has been reported as dibenzofurane degrader. *Burkholderia* sp. HA-OP24 showed high PCBs substrate diveristy (Table 2) has high similarity with *Burkholderia* sp. isolate N2P5 (Mueller, 1997) for the first primer set and *Burkholderia fungorum* DBT1 for the second primer set as a polyaromatic hydrocarbon and dibenzothiophene degrader, whereas *Bacillus* sp. Strains HA-OP29, and HA-OP31 and *Staphylococcus* sp. Strain HA-OP17 showed highest similarity with the type strain of *Staphylococcus* sp. PN/Y which has previously been reported as phenanthrene degrader (Somnath *et al.*, 2007).

Substrate diversity of bacterial strains

The growth of the isolates on 2CB; 4CB; 2,3CB; 2,4CB and 2,4,5,2′,4′,5′-Chlorobiphenyl as sole carbon sources is summarized in Table 2. All the strains were able to utilize 2CB; 4CB quite rapidly, though its growth patterns varied which for different isolates. Interestingly, growth was observed on some diCBs including 2,3-, 2,4-DCB for ten strains, seven strains were belonged to genus *Pseudomonas*; one strain was belonged to genus *Burkholderia* (*Burkholderia* sp. HA-OP24); one strain was belonged to genus *Rhodococcus* (*Rhodococcus* sp. HA-OP30)*;* and one strain was belonged to genus *Bacillus* (*Bacillus* sp. HA-OP31). Furthermore, growth were observed on 2,4,5,2′,4′,5′-Chlorobiphenyl only with two strains *Burkholderia* sp. HA-OP24 and *Rhodococcus* sp. HA-OP30. There was a colour change from colourless to yellow observed in culture media of *Pseudomonas* sp. HA-OP2, *Pseudomonas* sp. HA-OP4, and *Pseudomonas* sp. HA-OP20. It is noteworthy that this *meta*-cleavage product persisted throughout the incubation period.

Transformation of 2,3-Dihydroxybiphenyl and catechol by resting cells from PCBs degraders

To analyze for the differences in extradiol dioxygenase activities (2,3 dihydroxybiphenyl dioxygenase or catechol 2,3 dioxygenase) in the 27 strains using the resting cells grown on 2-CB and 4-CB were incubated with 2,3DHB and Catechol for detection of the *meta*- cleavage activity in both the upper and lower pathway of PCBs degredation, the activities were detected colorimetric by the production of yellow metabolites. The yellow color observed could be considered as an indication of meta-cleavage product—hydroxyl 6-oxo-6-penta 2,4-dienoic acid (HOPDA) in the case of 2,3DHB and and 2-hydroxymuconic semialdehyde for catechol. A rapid coloration due to *meta*-cleavage of DHB and catechol were observed with high activities in both 2-CB and 4-CB cells. Quantification of transformation rates for the all 27 strains by HPLC revealed, higher activities with 2,3 DHB in both 2-CB and 4-CB grown cells, than with catechol (Table 3). The strains

Burkholderia sp. HA-OP24 and *Rhodococcus* sp. HA-OP30 showed highest activities with 2,3 DHB in 2-CB grown cells with 60 and 55 µM/min in g protein respectively, while the highest activities with 2,3 DHB in 4-CB were also observed with the both two strains *Burkholderia* sp. HA-OP24 and *Rhodococcus* sp. HA-OP30 54 and 49 55 µM/min in g protein respectively (Table 3).

Table 1. Soil characteristics of soil samples used for isolation of PCBs bacterial degrader.

Soil sample	pH	PCBs content [mg PCBs kg^{-1}]	Total organic carbon [g TOC kg^{-1}]	Total nitrogen [g TN kg^{-1}]	C:N ratio
OP- 1	**5.90**	**280± 20**	**6.4 ± 0.4**	**0.4 ± 0.03**	**16.00**
OP- 2	**5.47**	**212 ± 18**	**7.8 ± 0.9**	**0.4 ± 0.04**	**19.50**
OP- 3	**5.64**	**313 ± 24**	**4.8 ± 0.7**	**0.2 ± 0.03**	**24.00**
OP- 4	**5.54**	**320 ± 26**	**8.8 ± 1.0**	**0.5 ± 0.10**	**17.60**

Table 2. PCB-degraders and its Substrate utilization spectrum.

Isolated strains	MCB		DCB		PCB
	2CB	4CB	2,3CB	2,4CB	2,4,5,2′,4′,5′-Chlorobiphenyl
***Pseudomonas* sp. HA-OP1**	+	+	-	-	-
***Pseudomonas* sp. HA-OP2**	+[1]	+[2]	+	+	-
***Pseudomonas* sp. HA-OP3**	+	+	-	-	-
***Pseudomonas* sp. HA-OP4**	+[2]	+[1]	+[2]	+	-
***Pseudomonas* sp. HA-OP5**	+	+	+	+	-
***Pseudomonas* sp. HA-OP6**	+	+	-	-	-
***Pseudomonas* sp. HA-OP7**	+	+	-	-	-
***Pseudomonas* sp. HA-OP8**	+	+	-	-	-
***Pseudomonas* sp. HA-OP9**	+	+	+	+	-
***Pseudomonas* sp. HA-OP10**	+	+	-	-	-
***Pseudomonas* sp. HA-OP13**	+	+	-	-	-
***Pseudomonas* sp. HA-OP14**	+	+	+	+	-
***Pseudomonas* sp. HA-OP1 5**	+	+	+	+	-
***Pseudomonas* sp. HA-OP18**	+	+	-	-	-
***Pseudomonas* sp. HA-OP19**	+	+	-	-	-
***Pseudomonas* sp. HA-OP20**	+	+	+[3]	+[3]	-
***Pseudomonas* sp. HA-OP21**	+	+	-	-	-
***Pseudomonas* sp. HA-OP22**	+	+	-	-	-
***Staphylococcus* sp. HA-OP17**	+	+	-	-	-
***Burkholderia* sp. HA-OP24**	+	+	+	+	+
***Rhodococcus* sp. HA-OP25**	+	+	-	-	-
***Rhodococcus* sp. HA-OP30**	+	+	+	+	+
***Bacillus* sp. HA-OP27**	+	+	-	-	-
***Pandoraea* sp.HA-OP28**	+	+	-	-	-
***Bacillus* sp. HA-OP29**	+	+	-	-	-
***Bacillus* sp. HA-OP31**	+	+	+	+	-
***Brevibacillus* sp. HA-OP26**	+	+	-	-	-

+, Growth; –, no growth. Culture supernatant fluid turned a yellow color that was; [1]permanent; [2]disappeared with time and; [3]occasional

Table 3. Transformation of 2,3 dihydroxybiphenyl (DHB) and Catechol by the resting cells of the different isolated strainsfrom the oxidation ponds as a PCBs contaminated site on 2chlorobiphenyl (2CB) and 4 chlorobiphenyl (4CB). Transformation was Quantified by determination of substrate depletion by HPLC.

Isolated Strains	2-CB cells		4-CB cells	
	DHB µM/min *g* protein	Catechol µM/min g protein	DHB µM/min g protein	Catechol µM/min g protein

***Pseudomonas* sp. HA-OP1**	**30**	**10**	**34**	**11**
***Pseudomonas* sp.HA-OP2**	**25**	**11**	**33**	**13**
***Pseudomonas* sp.HA-OP3**	**33**	**12**	**37**	**16**
***Pseudomonas* sp.HA-OP4**	**35**	**10**	**41**	**17**
***Pseudomonas* sp.HA-OP5**	**29**	**15**	**34**	**11**
***Pseudomonas* sp.HA-OP6**	**34**	**13**	**35**	**12**
***Pseudomonas* sp.HA-OP7**	**36**	**12**	**33**	**14**
***Pseudomonas* sp.HA-OP8**	**30**	**14**	**36**	**11**
***Pseudomonas* sp.HA-OP9**	**36**	**13**	**39**	**16**
***Pseudomonas* sp.HA-OP10**	**37**	**14**	**38**	**11**
***Pseudomonas* sp.HA-OP13**	**39**	**18**	**33**	**18**
***Pseudomonas* sp.HA-OP14**	**30**	**15**	**39**	**19**
***Pseudomonas* sp.HA-OP15**	**34**	**11**	**41**	**11**
***Pseudomonas* sp.HA-OP18**	**30**	**11**	**33**	**19**
***Pseudomonas* sp.HA-OP19**	**30**	**11**	**32**	**11**
***Pseudomonas* sp.HA-OP20**	**33**	**12**	**31**	**16**
***Pseudomonas* sp.HA-OP21**	**41**	**15**	**39**	**15**
***Pseudomonas* sp.HA-OP22**	**30**	**10**	**29**	**11**
***Staphylococcus* sp. HA-OP17**	**35**	**18**	**38**	**10**
***Burkholderia*sp. HA-OP24**	**60**	**22**	**54**	**19**
***Rhodococcus*sp. HA-OP25**	**36**	**11**	**39**	**10**
***Brevibacillus*sp. HA-OP26**	**33**	**10**	**31**	**11**
***Bacillus* sp. HA-OP27**	**30**	**13**	**34**	**17**
***Pandoraea*sp.HA-OP28**	**30**	**10**	**37**	**11**
***Bacillus* sp. HA-OP29**	**36**	**12**	**38**	**14**
***Rhodococcus*sp. HA-OP30**	**55**	**23**	**49**	**18**
***Bacillus* sp. HA-OP31**	**29**	**11**	**30**	**13**

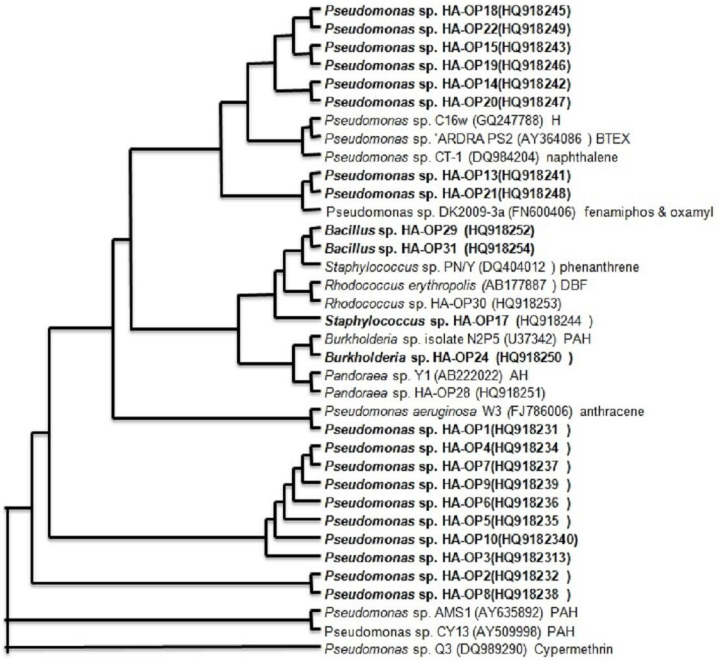

Figure 1. Phylogenetic tree based on the comparison of partial 16S rRNA gene fragments, corresponding to position 1-518 according to the E.coli numbering system, showing the relationship of the new PCBs degraders strains (with bold line and the accession numbers after the strain name) with the nearest strain for each, that have already reported before as aromatic degraders. "H" hydrocarbon, "DBF" dibenzofurane, "PAH" Polyaromatic hydrocarbon, "AH" aromatic hydrocarbon.

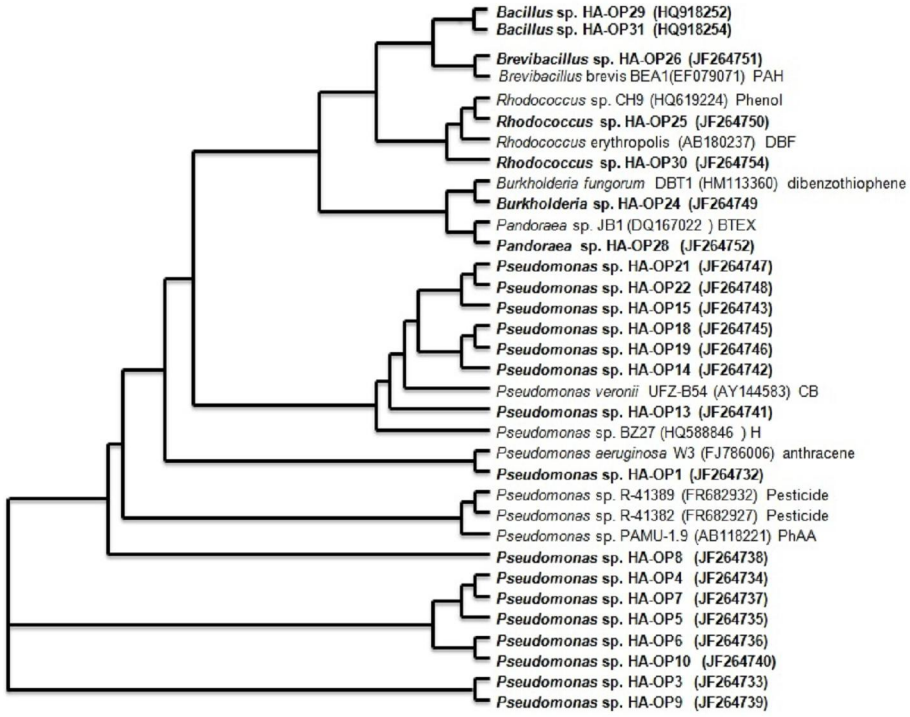

Figure 2. Phylogenetic tree based on the comparison of partial 16S rRNA gene fragments, corresponding to position 945-1492 according to the E.coli numbering system, showing the relationship of the new PCBs degraders strains (with bold line and the accession numbers after the strain name) with the nearest strain for each, that have already reported before as aromatic degraders. "H" hydrocarbon, "DBF" dibenzofurane, "PAH" Polyaromatic hydrocarbon, "AH" aromatic hydrocarbon, "DBT" dibenzothiophene .

4. Discussion

Soil microorganisms play important roles in maintaining soil quality and ecosystem health. Development of effective methods to analyze bacterial community structures and encourages the growth of selected microorganisms that are capable of transforming PCBs are challenges for successful bioremediation of PCBs- Contaminated soils. The microbial degradation of PCBs has been extensively studied in recent years. The genetic organization of biphenyl catabolic genes has been elucidated in various groups of microorganisms, and also their structures have been analyzed with respect to their evolutionary relationships, and new information on mobile elements has become available. In the same context key enzymes, specifically DHBD and C23O have been intensively characterized, structure/sequence relationships have been determined and enzymes were optimized for PCB transformation. However, due to the complex metabolic network responsible for PCBs degradation, optimizing degradation by single bacterial species is necessarily limited (Pieper, 2005).

A wide number of microorganisms are able to aerobically transform some of the 209 possible PCB congeners via the biphenyl pathway, which degrades biphenyl to benzoate and 2-hydroxypenta-2,4-dienoate (Abramowicz, 1990; Furukawa, 2000). In fact, the range of PCBs that is transformed by the biphenyl pathway is highly dependent upon the bacterial strain, 27 isolates were capable to transform the monochlorinated biphenyl and only 10 isolates can transform dichlorinated biphenyl, whereas the rest of strains unable to transform higher chlorinated biphenyl, It is generally believed that biodegradation of PCBs decreases with the increase in chlorine substitution (Furukawa *et al*., 1979). Based on the analysis of various biphenyl degrading isolates it could be deduced that lower chlorinated congeners are more easily transformed compared to higher chlorinated congeners and PCB congeners with chlorines on one aromatic ring only are more easily degraded than those bearing chlorine substituents on both aromatic rings (Pieper, 2005). From the 27 strains only two strains that have the ability to transform the higher chlorinated biphenyls *Burkholderia* sp. HA-OP24 and *Rhodococcus* sp. HA-OP30 (Table 2), behave like *Burkholderia* sp. strain LB400 and *Rhodococcus jostii* RHA1, which transform up to hexachlorinated biphenyls (Bopp, 1986; Kohler *et al*., 1988; Bedard and Haberl, 1990; Warren *et al*., 2004; Mary e*t al*., 2006; McLeod *et al*.,

2006), this may be explained by the highest activities, which were observed with the two strains against 2,3DHB and catechol due to the high induction of *meta*-cleavage enzymes 2,3 dihydroxybiphenyl dioxygenase or catechol 2,3 dioxygenase in both upper and lower pathways respectively, this indication may be a good marker for isolation of PCBs degraders with broad spectrum based on the highest activity of *meta*-cleavage in both the upper and lower pathway. However, each isolate exhibited a particular activity spectrum with regard to the type and extent of PCB congeners metabolized, with some strains having a narrow spectrum and other bacteria like *Burkolderia xenovorans* LB400 is able to degrade a broad range of PCBs (Haddock *et al*., 1995; Seeger *et al*., 1995a; 1995b; 1997; 1999; 2001) and is a model bacterium for PCB degradation. *Rhodococcus jostii* RHA1 is another potent PCB-degrading soil bacterium (Seto *et al*., 1995; Warren *et al*., 2004; McLeod *et al*., 2006).

A critical step in improving the microbial catabolic activities for the degradation of PCBs is understanding the reactivities of the catabolic biphenyl pathway for PCB metabolites. 2,3-Dihydroxybiphenyl 1,2-dioxygenase (DHBD; EC 1.13.11 .39) is the fourth enzyme of the biphenyl pathway and has also been identified as an important determinant of PCB degradation, as it is competitively inhibited by some chlorinated (Cl) metabolites (Seah *et al*, 2000, 2001). It utilizes a mononuclear nonheme iron (II) center to cleave 2,3-dihydroxybiphenyl (DHB) in an extradiol fashion. Most biphenyl-degrading bacteria included all the strains in this study can metabolize biphenyl by *meta*-cleavage reaction product (Table 3), which is yellow in color providing an easy colorimetric test for a rapid screening of bacterial colonies correlating with the contamination levels and carrying 2,3-dihydroxybiphenyl 1,2-dioxygenase activity, and or catechol 2,3 dioxygenase (Happe *et al.*, 1993; Hamdy *et al*., 2008) as a result of the transformation of 2,3-dihydroxybiphenyl to the yellow *meta*-cleavage product 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid in the upper pathway and in case of using catechol as indicator for the lower pathway, it transformed into yellow color 2-hydroxymuconic semialdehyde, we can summeraize that the environmental conditions and, probably, the contamination level were selective for strains possessing 2,3-dihydroxybiphenyl 1,2-dioxygenase and catechol 2,3 dioxygenase activity.

Overall, our study has demonstrated for the first time the ability of micro-organisms enriched from Oxidation ponds of Sadat city to degrade PCBs, suggesting the natural attenuation potential of PCBs degraders at Oxidation ponds. Also, the study identifies that PCBs are degraded primarily via the *meta*-pathway using the 2,3 dihydroxybiphenyl dioxygenase or catechol 2,3 dioxygenase at PCBs contaminated site. These observations are important because those functional genes have been reported that play an important role in the degradation of PCBs compounds (Pieper, 2005; Pieper and Seeger 2008).

Acknowledgement

The author is grateful to the Science and Technological Development Fund (STDF) Government of Egypt for financial support to carry out this work as a part of project 46.

References

1. **Abramowicz, D. (1990).** Aerobic and Anaerobic Biodegradation of PCBs: *A Review. Crit. Rev. Biotechnol.* **10,** 241–251.
2. **Amann, R.I., Ludwig, W., and Schleifer, K.H. (1995).** Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* **59**, 143-169.
3. **Beaudette, L.A., Davies, S., Fedorak, P.M., Ward, O.P., and Pickard, M.A. (1998).** Comparison of gas chromatography and mineralization experiments for measuring loss of selected polychlorinated biphenyl congeners in cultures of white rot fungi. *Appl. Environ. Microbiol*. **64**, 2020-2025.
4. **Bedard, D.L., and Haberl, M.L. (1990).** Influence of chlorine substitution on the degradation of polychlorinated biphenyls by eight bacterial strains. *Microb. Ecol*. **20,** 87–102.
5. **Blake, J. (1994).** An approach to the regulation of bioremediation of polychlorinated biphenyls. In R.E. Hichee (Ed.), Bioremediation of chlorinated and polycyclic aromatic hydrocarbon compounds, pp. 432-435. Boca Ratón, Fl: Lewis Publishing, Inc.
6. **Boon, J.P., Van, Z. M.B., and Govaert, M.J.M.A. (1985).** Organochlorines in benthic polychaetes (Nephtys spp.) and sediments from the southern North Sea: identification of individual PCB components. *Neth J Sea Res* **19**, 93–105
7. **Bopp, L.H. (1986).** Degradation of highly chlorinated PCBs by *Pseudomonas* strain LB400. *J. Ind. Microbiol.* **1,** 23–29.
8. **Chen, P.H., Wong, C.K., Rappe, C., and Nygren, M. (1980).** Polychlorinated biphenyls, dibenzofurans and quaterbiphenyls in toxic rice bran oil and in the blood and tissue of patients with PCB poisoning (Yu-Cheng) in Taiwan. *Environmental Health Perspectives* **59**, 59-65.

9. **Furukawa, K., Tomizuka, N., and Kamibayashi, A. (1979).** Effect of chlorine substitution on the bacterial metabolism of various polychlorinated biphenyls. *Appl. Environ. Microbiol* **. 38,** 301-310.
10. **Furukawa, K. (2000).** Engineering dioxygenases for efficient degradation of environmental pollutants. *Curr. Opin. Biotechnol*. **11**, 244–249.
11. **Furukawa, K., and Fujihara, H. (2008).** Microbial degradation of polychlorinated biphenyls: biochemical and molecular features. *J. Biosci. Bioeng*. **105**, 433-49.
12. **Haddock, J.D., Horton, J.R., and Gibson, D.T. (1995).** Dihydroxylation and dechlorination of chlorinated biphenyls by purified biphenyl 2,3-dioxygenase from Pseudomonas sp. Strain LB400. *J. Bacteriol*. **177**, 20-26.
13. **Hamdy A.H.A., Nguyen, B., Victor, W., Howard, J., and Dietmar, H.P**. **(2008).** Two angular dioxygenases contribute to the metabolic versatility of dibenzofuran-degrading Rhodococcus sp. Strain HA01. *Appl. Environ. Microbiol*, Vol. **74,** 3812–3822.
14. **Happe, B., Eltis, L. D., Poth, H., Hedderich, R., and Timmis**, **K.N. (1993).** Characterization of 2,2',3-trihydroxybiphenyl dioxygenase, an extradiol dioxygenase from the dibenzofuran- and dibenzo-p-dioxin-degrading bacterium *Sphingomonas* sp. strain RW1. *J. Bacteriol*. **175,** 7313-7320.
15. **IPCS. (1993).** Environmental health criteria 140. Polychlorinated biphenyls and tetraphenyls. WHO, Geneva.
16. **IPCS. (1997).** Environmental health criteria 195. Hexachlorobenzene, WHO, Geneva.
17. **Iwasaki, A., Takagi, K., Yoshioka, Y., Fujii,K., Kojima, Y., and Harada, N. (2007).** Isolation and characterization of a novel simazine-degrading β- proteobacterium and detection of genes encoding s-triazine-degrading enzymes. *Pest. Manag. Sci*.**63,** 261-268.
18. **Junca, H., and Pieper, D.H**. **(2004).** Functional gene diversity analysis in BTEX contaminated soils by means of PCR-SSCP DNA fingerprinting: comparative diversity assessment against bacterial isolates and PCR-DNA clone libraries. *Environ. Microbiol.* **6,** 95-110.
19. **Kimbrough, R.D. (1987).** Human health effects of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs). *Annu. Rev. Pharmacol. Toxicol*. **27**, 87-111.
20. **Kohler, H.P., Kohler-Staub, E.D., and Focht, D.D. (1988).** Cometabolism of polychlorinated biphenyls: enhanced transformation of Aroclor 1254 by growing bacterial cells. *Appl. Environ. Microbiol*. **54,** 1940–1945.
21. **Margesin, R., Zimmerbauer, A., Schinner, F**. **(2000).** Monitoring of bioremediation by soil biological activities. *Chemosphere* **40**, 339-346.
22. **Mary B.L., Petra, P., Martina, M., Tomáš, M., David, P.N., and John, S.F. (2006).** Polychlorinated Biphenyl (PCB)-Degrading Bacteria Associated with Trees in a PCB-Contaminated Site. *Appl. Environ. Microbiol*. **72**, 2331–2342.
23. **McLeod, M.P., Warren, R.L., Hsiao, W.W., Araki, N., Myhre, M., Fernandes, C., Miyazawa, D., Wong, W., Lillquist, A.L., Wang, D., Dosanjh, M., Hara, H., Petrescu, A., Morin, R.D., Yang, G., Stott, J.M., Schein, J.E., Shin, H., Smailus, D., Siddiqui, A.S., Marra, M.A., Jones, S.J., Holt, R., Brinkman, F. S., Miyauchi, K., Fukuda, M., Davies, J.E., Mohn, W.W., Eltis, L.D**. **(2006).** The complete genome of Rhodococcus sp. RHA1 provides insights into a catabolic powerhouse. *Proc. Natl. Acad. Sci. USA* **103,** 15582-15587.
24. **Middeldorp, P. J. M., Briglia, M., and Salkinoja-Salonen**, **M. S. (1990).** Biodegradation of pentachlorophenol in natural soil by inoculated *Rhodococus chlorophenolicus*. *Microb. Ecol*. **20**, 129-139.
25. **Mueller, J.G., Devereux, R., Santavy, D.L., Lantz, S.E., Willis, S.G., and Pritchard, P.H**. **(1997).** Phylogenetic and physiological comparisons of PAH-degrading bacteria from geographically diverse soils. *Antonie Leeuwenhoek* **71**, 329-343.
26. **Nagayama L., Kuratsune M., and Masuda Y. (1976).** Determination of chlorinated dibenzofurans in Kanechlors and "Yosho oil". *Bull. Environ. Contam. Toxicol.* **15**, 9-13.
27. **Pieper, D.H. (2005).** Aerobic degradation of polychlorinated biphenyls. *Appl. Microbiol. Biotechnol*. **67**, 170-191.
28. **Pieper, D.H., Seeger, M. (2008).** Bacterial metabolism of polychlorinated biphenyls. *J. Mol. Microbiol. Biotechnol*. **15**, 121-138.
29. **Safe, S. (1984).** Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): Biochemistry, toxicology, and mechanism of action. *CRC Crit. Rev. Toxicol.* **13,** 319-393.
30. **Seah, S.Y.K., Labbé, G., Nerdinger, S., Johnson, M.R, Snieckus, V, Eltis, L.D**. **(2000).**

Identification of a serine hydrolase as a key determinant in the microbial degradation of polychlorinated biphenyls. *J. Biol. Chem.* **275**, 15701-15708.

31. **Seah, S.Y.K., Labbé, G., Kaschabek, S.R., Reifenrath, F., Reineke, W. and Eltis, L.D. (2001).** Comparative specificities of two evolutionarily divergent hydrolases involved in microbial degradation of polychlorinated biphenyls. *J. Bacteriol*. **183**, 1511-1516.
32. **Seeger, M., Timmis, K.N., Hofer, B. (1995a).** Degradation of chlorobiphenyls catalyzed by the bph-encoded biphenyl-2,3-dioxygenase and biphenyl-2,3-dihydrodiol-2,3-dehydrogenase of Pseudomonas sp. LB400. *FEMS Microbiol. Lett.* **133**, 259-264.
33. **Seeger, M., Timmis, K.N., Hofer, B. (1995b).** Conversion of chlorobiphenyls into phenylhexadienoates and benzoates by the enzymes of the upper pathway for polychlorobiphenyl degradation encoded by the *bph* locus of *Pseudomonas* sp. strain LB400. *Appl. Environ. Microbiol*. **61,** 2654-2658.
34. **Seeger, M., Timmis, K.N., Hofer, B. (1997).** Bacterial pathways for the degradation of polychlorinated biphenyls. *Mar. Chem***. 58**, 327-333.
35. **Seeger, M., Zielinski, M., Timmis, K.N., Hofer, B. (1999).** Regiospecificity of dioxygenation of di-to pentachlorobiphenyls and their degradation to chlorobenzoates by the bph-encoded catabolic pathway of Burkholderia sp. strain LB400. *Appl. Environ. Microbiol.* **65**, 3614-3621.
36. **Seeger, M., Cámara, B., Hofer, B**. **(2001)**. Dehalogenation, denitration, dehydroxylation, and angular attack on substituted biphenyls and related compounds by a biphenyl dioxygenase. *J. Bacteriol*. **183**, 3548-3555.
37. **Seeger, M., Pieper, D.H. (2009).** Genetics of biphenyl biodegradation and co-metabolism of PCBs. In: Microbiology of hydrocarbons, oils, lipids, and derived compounds, Vol. 2, pp. 1179-1199, Springer, Heidelberg, Germany.
38. **Seto, M., Kimbara, K., Shimura, M., Hatta, T., Fukuda, M., and Yano, K**. **(1995).** A novel transformation of polychlorinated biphenyls by Rhodococcus sp. strain RHA1. *Appl. Environ.Microbiol*. **61**, 3353-3358.
39. **Somnath M., Subhankar C., and Tapan, K.D**. **(2007)**. A novel degradation pathway in the assimilation of phenanthrene by Staphylococcus sp. strain PN/Y via meta-cleavage of 2-hydroxy-1-naphthoic acid:formation of trans-2,3-dioxo-5-(29-hydroxyphenyl)-pent-4-enoic acid. Microbiology, **153**, 2104–2115.
40. **Sparks, D.L. (1996).** Methods of Soil Analysis, Part 3, Chemical Method, SSSA. Book Series Number 5, Soil Science Society of America, Madison, WI.
41. **U.S. Environmental Protection Agency**. **(2008).** "Polychlorinated Biphenyls (PCBs) U.S. EPA, Toxicity and Exposure Assessment for Children's Health teach database at: http://www.epa.gov/teach
42. **Viñas M, Sabaté J, Solanas AM**. **(2005).** Bacterial community dynamics and PAHs degradation during bioremediation of a heavily creosote-contaminated soil. *Appl. Env. Microbiol* . **71,** 7008–7018.
43. **Warren, R., Hsiao, W.L., Kudo, H., Myhre, M., Dosanjh, M., Petrescu, A., Kobayashi, H., Shimizu, S., Miyauchi, K., Masai, E., Yang, G., Stott, J.M., Schein, J.E., Shin, H., Khattra, J., Smailus, D., Butterfield, Y.S., Siddiqui, A., Holt, R., Marra, M.A., Jones, S.J., Mohn, W.W., Brinkman, F.S., Fukuda, M., Davies, J., Eltis, L.D**. **(2004).** Functional characterization of a catabolic plasmid from polychlorinatedbiphenyl- degrading *Rhodococcus* sp. Strain RHA1. *J. Bacteriol.* **186**, 7783-7795.

1/7/2013