ISSN (online): 2319-6599
ISSN (Print): 2319-6580

Diversity of 2, 4 Dichlorobiphenyl degrading consortium of *Pseudomonas* isolates GSa and GSb for degradation of Poly Chlorinated Biphenyl congeners

Degradation of PCB congener by 2, 4 CB degrading *Pseudomonas* consortium

[1]Shobha K Jayanna, [2]Devaraja Gayathri*, [3]Somaraja Palegar Krishnappa

Department of Microbiology, Davangere University, Shivagangothri, Davangere-577002, India
*gayathridevaraja@gmail.com, [1]shobha_jayanna@rediffmail.com, [3]somarajas@gmail.com

Abstract

Objective: Polychlorinated biphenyls (PCB) are persistent organic pollutants that are widely distributed in the environment. PCBs are aromatic compounds have more than 210 congeners, nonvolatile, chemically inert and do not undergo oxidation, reduction or addition reactions, elimination or electrophilic substitution reactions except under extreme conditions. Their improper disposal in storage and disposal area has negative impact on the ecosystem. Although Chemical methods are available for the degradation, they tend to emit more toxic chemicals. Alternative the biological methods are safer and cost effective. In this context, 2, 4 Dichlorobiphenyl, (a PCB congener) degrading bacterial isolates which have been evaluated for substrate affinity using PCB congener mix.
Methods: In the present study, 2, 3, 5, 6 tetrachlorobiphenyls and 3, 5', 3, 5 tetrachlorobiphenyl in PCB congener mix degradation by previously characterised *Pseudomonas* isolates GSa and GSb was studied using GC-MS.
Findings: Two constantly overlapping bacterial isolates identified as *Pseudomonas* sp., capable of degrading 2, 4 Chlorobiphenyl degrading, showed its diversity of degrading other PCB congener mix. On GC-MS analysis of the cell free extract showed 60 and 70% degradation as per the ECD values.
Applications: Therefore, the present paper is first of its kind, as 2, 4 CB degrading Pseudomonads in tern degrade other PCB congeners perhaps showing wide application on *in situ* bioremediation since the contaminated site contains variety of congeners.

Key words: Bioremediation, 2, 4 CB, PCB congener, *Pseudomonas* sp, Diversity

1. Introduction

Among organic pollutants, chlorinated aromatic organic compounds especially cause great concern because of their toxicity, persistence and bioaccumulation. Aromatic compounds are the most prevalent and persistent pollutants in the environment. Petroleum, oil contaminated soil commonly contain a mixture of polycyclic aromatic hydrocarbon and heterocyclic aromatics. Large number of pollutants particularly Polychlorinated biphenyl (PCB) compounds pose great threat to environment, therefore there is a need to decrease the PCB concentration using indigenous bacteria. United Nation Environmental Programme (UNEP) has established a list of 12 classes of Persistent Organic Pollutants (POP) such as Polychlorinated Dibenzo-*p*-Dioxins (PCDDs) and Dibenzo Furans (PCDFs), Dichloro Diphenyl Trichloroethane (DDT), toxaphene, and dieldrin, including Polychlorinated biphenyls (PCB) [1]. Soil contaminated with PCB can be found worldwide as a result of industrial activity [2]. POP, particularly Polychlorinated biphenyls (PCB) accumulate in different niches of biosphere significantly affecting ecological balance [3].

PCB were first manufactured commercially in 1929 by the Swann Chemical Company in Anniston, AL. Theodore Swann had developed a commercially viable process to manufacture biphenyl from benzene by bubbling benzene through lead [4]. The degree of chlorination varies depending on the reaction conditions, and ranges from 21% to 68% (w/w). Commercial PCB mixtures were sold based on the percentage of chlorine by weight, with each manufacturer utilizing their own system for identifying their products. In the Arochlor series, a 4-digit code is used; biphenyls are generally indicated by 12 in the first 2 positions, while the last 2 numbers indicate the percentage of chlorine in the mixture *viz* Arochlor 1260, is a PCB mixture containing 60% chlorine [5].

PCB have been widely used in industry as heat transfer fluids, hydraulic fluids, solvent extenders, flame retardants, organic diluents, dielectric fluids [4], and in electromagnets, liquid filled cables, gasketing and dampening belts, voltage regulators, vacuum pumps, microwave ovens and transformer oils [6].

ISSN (online): 2319-6599
ISSN (Print): 2319-6580

Toxicity of PCB has been recognized since 1930 [7]. According to the Department of Health and Human Services (DHHS), U.S. Environment Protection Agency (EPA) and International Agency for research on Cancer (IARC), PCB are suspected to be carcinogenic in animals and humans [7, 8]. PCB are known to cause reproductive defect in humans [9, 10, 11]and animals [12]. PCB also cause neurological [13], endocrinal [14, 15]and other defects. These also adversely affect fetal and infant development [16] in addition to higher mutagenic and carcinogenic ability [17].

Possible methods for PCB degradation include physical, chemical and biological. Conventional technologies are typically expensive and destructive. Incineration is the only physical method approved for the removal of PCB. This method would produce toxic byproducts such as PCDDs (commonly known as dioxins) and PCDFs which are found to be more dangerous than PCB compounds [18].

Microorganisms that reductively dechlorinate PCB are widespread in contaminated sediments and involve species related to *Dehalococcoides* [19] and phenol by Enterobacteriaceae bacterium strain DRY7 [20]. PCB dechlorination has been mostly attributed to complex bacterial consortia and little is known about metabolic pathways, molecular basis, and the enzymes implicated in the process.

Further, diverse aerobic bacteria capable of oxidizing PCB have been reported [21]. *Burkholderia xenovorans* LB400 was able to degrade a broad range of PCB [22, 23, 24] and was found to be a model bacterium for PCB degradation. *Rhodococcus jostii* RHA1 was another potent PCB degrading soil bacterium [25, 21]. Biphenyl grown cells of *Paenibacillus* sp. KBC101 efficiently degraded di-to nonachlorobiphenyls [26].

Although, 2, 4 Dichlorobiphenyl (2, 4 CB) was degraded by *Pseudomonas* isolates GSa and GSb [27], their consortium showed 90% degradation on GC MS [28]. However, data on consortium usage for degradation of PCB congeners are scarce. Therefore, in the present work the diversity of the consortium in degrading PCB compounds such as 2, 3, 5, 6 tetrachlorobiphenyls and 3, 5', 3, 5 tetrachlorobiphenyl in PCB congener mix have been reported.

2. Material and methods

2.1 *Chemical*

PCB congener mixture was obtained from Sigma Aldrich (USA). All solvents used in the present study are of analytical grade.

2.2 *Bacteria*

Previously isolated and characterized the consortium of *Pseudomonas*isolates GSa and GSb [27-29] was used for the present study.

2.3 Inoculum preparation and Growth of the consortium on PCB compounds

About 100µl (0.1ml) of growth medium from log phase of the consortium was inoculated to the SMS medium with PCB congener (4µg/ml). The consortium was incubated with PCB congener as a sole source of carbon and energy. After 48h of incubation, the bacterial isolates were centrifuged 5000rpm/15min) and the extract was subjected to GC-MS analysis.

2.4 *Sample preparation for GC-MS analysis*

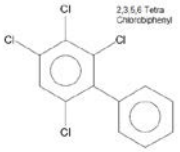
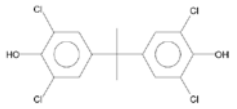
The spent medium was acidified with dilute HCl (0.1M) and extracted with hexane (1:3 v/v) 3 times. Later, the resulting extract was dried over anhydrous sodium sulphate and evaporated to dryness. The residue obtained was dissolved in methanol and characterized by GC-MS analysis [27].

3. Results and discussion

Two bacterial isolates (GSa and GSb) showing overlapping growth pattern was identified previously as *Pseudomonas* sp was chosen for the present study in the consortium. In various natural and manmade environments, many species of microorganisms stably coexist by interacting with each other and effectively exerts various function and mixed microbial cultures were efficient in biodegradation of recalcitrant compounds [30]. Upon utilization of 2, 4 CB an increase in turbidity and production of yellow colour indicated the production of 2-hydroxy-6-oxo-6phenyl-hexa-2, 4-dienoic acid (HOPDA), a *meta* cleavage product [31]. Although, *Pseudomonas* consortium GSa and GSb capable of degrading 2, 4 CB, their ability to degrade PCB Congener was analysed [27, 28, 29].

ISSN (online): 2319-6599
ISSN (Print): 2319-6580

Table 1. List of PCB compounds degraded by the consortium of Pseudomonas spp GSa and GSb.

Sl. No	Highest peak	*m/z* values and intensities	Library Match	Structure
1	18.05	Not detected	-	
2	21.57	176 (20), 221(100), 244 (20.5), 258 (40), 277 (90), 294(80.5), 295 (80.5)	2, 3, 5, 6 tetrachlorobi phenyl	
3	24.90	281(30.8), 291(40.5), 292.73 (40.1), 295 (10), 342 (80), 349 (80.5), 361.99 (100), 364.06 (90.3), 366.31 (50)	3, 5', 3, 5 tetra chloro biphenyl	
4	26.65	Not detected	-	

On GC-MS analysis, the consortium GSa and GSb with PCB congener mixture resulted in reduction of peak of four PCB compounds (18.05min, 21.57min, 24.90 min and 26.65min). Although four PCB compounds degraded by the consortium, only two were detected by computer library of NIST-MS Type Version-2, 2005 (Table 1). The peaks detected were 2, 3, 5, 6 tetrachlorobiphenyl at 21.57min (60% degradation by ECD values) (Figure 1) and 3, 5', 3, 5 tetrachlorobiphenyl at 24.65 min (Figure2a and b) (70% degradation by ECD values).

Figure 1. GC-MS of 2, 3, 5, 6 tetra chlorobiphenyl in PCB congener mix showing reduction in peak at 21.57min by the consortium (GSa and GSb) and Mass Ionization spectrum of 2, 3, 5, 6 eluting at 21.57min.

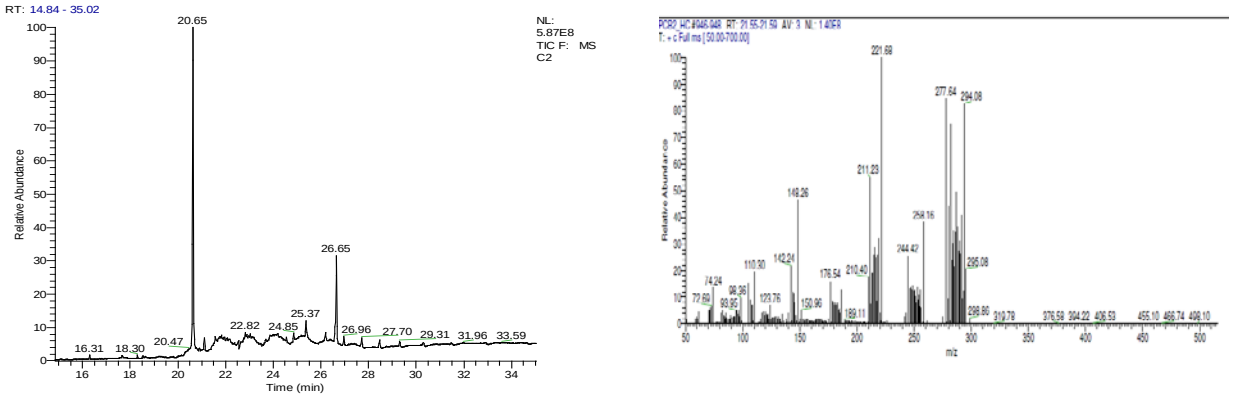
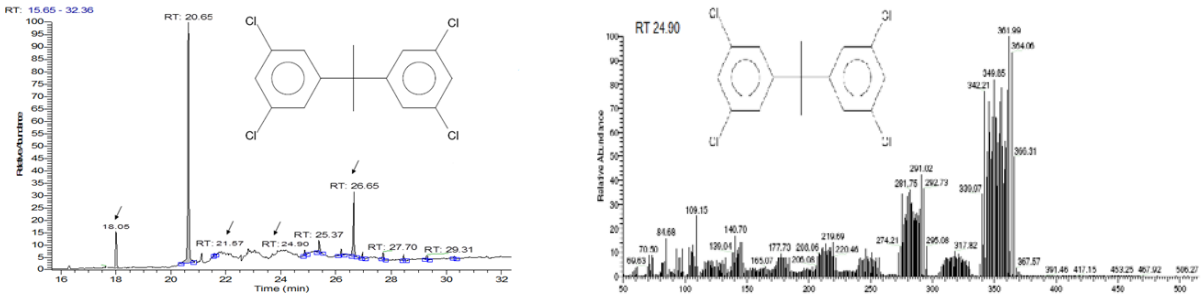


Figure 2 a). GC-MS of 3, 5', 3, 5 tetra chlorobiphenyl in PCB congener mix showing reduction in peak at 24.90min.b). Mass Ionization spectrum of peak 24.90min showing 3, 5', 3, 5 tetra chlorobiphenyl.



a. b.

There was a colour change from colourless to yellow was observed in culture media of the consortium. The yellow colour observed was an indication of meta cleavage product 2-hydroxyl-6-oxo-6-penta 2, 4-dienoic acid (HOPDA)[32].

Further, *Burkholderia xenovorans* LB400T, *Rhodococcus* sp. strain RHA1, and *Pseudomonas pseudoalcaligenes* KF707 hardly degraded the coplanar PCB (3, 4, 3', 4'-CB), and strain KBC101 degraded highly chlorinated PCB (11%)

and the coplanar PCB (56%) [21]. Thus the ability of the consortium (GSa and GSb) to degrade PCB appears to be higher than that of previously reported strong PCB degraders, such as *Rhodococcus* sp. RHA1. Compared with the substrate utilization of the other bacterial species, the consortium GSa and GSb perhaps contain specific enzymes that catalyze the oxidation of chlorinated compounds, having broad substrate specificity. According to the degradation profile of several PCB congeners, the dioxygenase of the consortium seems to have different substrate specificity than the dioxygenase of Gram-negative bacteria (*Pseudomonas pseudoalcaligenes* KF70 and *Burkholderia xenovorans* LB400T) and Gram-positive bacteria (*Rhodococcus* sp. strain RHA1) [25,22]. Therefore, the present result perhaps useful in reducing xenobiotic level in contaminated soil if applied for *in situ* bioremediation.

4. References

1. UNEP, Guidelines for the identification of PCBs and materials containing PCBs. http://www.pops.int/documents/guidance/NIPsFinal/pcbid1.pdf . Date accessed: 1999.
2. C. Backe, I. T. Cousins, P. Larrson. PCB in soils and estimated soil air exchange fluxes of selected PCB congeners in the south of Sweden. *Environmental Pollution,* 2004; 128(1-2), 59-72.
3. C, Graham, J. J. Ramsden, *Introduction to global warming*, *Complexity and security.* IOS Press, 147-184. 2008.
4. M. D. Erickson, R. G. Kaley, Applications of Polychlorinated biphenyls, *Environmental Science Pollution Research*. 2011; 18, 135-151.
5. WHO, Environmental Health Criteria 140: Polychlorinated biphenyls and terphenyls. 2nd (Edn), Geneva. 1993.
6. C. Obinna, Nwinyi, Degradation of Askarel (PCB Blend) by indigenous aerobic bacteria isolates from dump sites in ore, Ondo State. Nigeria. *Australian Journal of Basic and Applied Sciences.* 2010;4,3938-3948.
7. D. H. Pieper, M, Seeger Bacterial metabolism of Polychlorinated biphenyls, *Journal of Molecular Microbiology and Biotechnology*. 2008; 15**,** 121-138.
8. D. H. Pieper, Aerobic degradation of Polychlorinated biphenyls. *Applied Journal of Microbiology and Biotechnology.* 2005; 67**,** 170-191.
9. R. Loch-Caruso. Uterine muscle as a potential target of Polychlorinated biphenyls during pregnancy. *International Journal of Hygiene and Environmental Health.* 2002; 205, 21-130.
10. S. Safe, K. Connor, K. Ramamoorthy, K. Gaido, S. Maness. Human exposure to endocrine active chemicals: Hazard assessment problems. *Regulatory Toxicology and Pharmacology*. 1997; 26**;** 52-58.
11. S. T. Vater, S. F. Valazquez, V. J. Cogliano. A case study of cancer data set combinations for PCBs. *Regulatory Toxicology and Pharmacology.* 1995; 22(1), 2-10.
12. De S. Flora, M. Bagnasco, P. Zanacchi. Genotoxic, carcinogenic and teratogenic hazards inthe marine environment with special references to the Mediterranean Sea. *Mutation Research.* 1991; 258**,** 285-320.
13. S. L. Schantz, J. J. Widholm, D. C. Rice. Effects of Polychlorinated biphenyls exposure on neuro psychological function in children. *Environmental Health Perspectives*. 2003; 111**,** 357-376.
14. G. Ross. The public health implications of Polychlorinated biphenyls (PCBs) in the environment, *Ecotoxicology and Environmental Safety,* 2004; 59, 275-291.
15. C. L. Portigal, S. P. Cowell, M. N. Fedorek, C. M. Butler, P. S. Renni, C. C. Nelson. Polychlorinated biphenyls interference with androgen–induced transcriptional activation and hormone binding. *Toxicology and Applied Pharmacology*. 2002; 179(3), 185-194.
16. G. Winneke, J. Walkowiak, H. Lilienthal. Polychlorinated biphenyl induced neuro developmental toxicity in human infants and its potential mediation by endocrine dysfunction. *Toxicology.* 2002; 181-182, 161-165.
17. O. M. Faroon, S. Keith, D. Jones, D. C. Rosa. Carcinogenic effects of Polychlorinated Biphenyls. *Toxicology and Industrial Health.* 2001; 17**,** 41-62.
18. Laura J. Amend, Peter B. Lederman. Critical evaluation of PCB remediation technologies. *Environmental Progress and Sustainable energy*. 1992; 11**,** 173-177.
19. W. R. Abraham, B. Nogales, P. N. Golyshin, D. H. Pieper, K. N. Timmis. Polychlorinated Biphenyl degrading microbial communities in soils and sediments. *Current Opinion in Microbiology*. 2002; 5, 246-253.
20. M. H. Hamdan , M. A. Othaman, Z. Suhaili, M. S. Shukor , M. I. E. Halmi , S. R. S. Abdullah, N. A. Shamaan, M. Y. Shukor. Isolation and characterization of a heavy metal reducing Enterobacteriaceae Bacterium Strain DRY 7 with the ability to assimilate phenol and diesel. *Indian Journal of Science and Technology.* 2016;9(23),1-10.
21. M. Seto, E. Masai, M. Ida, T. Hatta, M. Fukuda, K. Yano. Multi polychlorinated biphenyl transformation systems in the gram-positive bacterium *Rhodococcus* sp. strain RHA1. *Applies and Environmental Microbiology.* 1995; 61, 4510-4513.

22. M. Seeger, B. Cámara, B. Hofer. Dehalogenation, denitration, dehydroxylation, and angular attack on substituted biphenyls and related compounds by a biphenyl dioxygenase. *Journal of Bacteriology.* 2001; 183**,** 3548-3555.
23. M. Seeger, D. Pieper. *Genetics of biphenyl degradation and co-metabolism of PCBs. In: Microbiology of hydrocarbons, oils, lipids and derived compounds*. Timmis KN Edn., *Springer*, Heidelberg. 2009; 1179-1199.
24. J. D. Haddock, J. R. Horton, D. T. Gibson. Dihydroxylation and dechlorination of chlorinated biphenyls by purified biphenyl 2, 3 dioxygenase from *Pseudomonas* sp. strain LB400. *Journal of Bacteriology.* 1995; 177, 20-26.
25. Michael P. McLeod,René L. Warren, William W. L. Hsiao, Naoto Araki, Matthew Myhre, Clinton Fernandes, Daisuke Miyazawa, Wendy Wong, Anita L. Lillquist, Dennis Wang, Manisha Dosanjh, Hirofumi Hara, Anca Petrescu, Ryan D. Morin, George Yang, Jeff M. Stott, Jacqueline E. Schein, Heesun Shin, Duane Smailus, Asim S. Siddiqui, Marco A. Marra, Steven J. M. Jones, Robert Holt, Fiona S. L. Brinkman, Keisuke Miyauchi, Masao Fukuda, Julian E. Davies, William W. Mohn, Lindsay D. Eltis. The complete genome of *Rhodococcus* sp. RHA1 provides insights into a catabolic powerhouse. *Proceedings of National Academy of Sciences USA* 2006; 103(42), 15582-15587.
26. M. Sakai, S. Ezaki, N. Suzuki, R. Kurane. Isolation and characterization of a novel Polychlorinated-biphenyl degrading bacterium *Paenibacillus* sp. KBC 101. *Journal of Applied Microbiology and Biotechnology*. 2005; 68**,** 111-116.
27. K. J. Shobha, D. Gayathri. Degradation of 2, 4 dichlorobiphenyl via meta cleavage pathway by *Pseudomonas* spp. Consortium. *Current Microbiology.* 2015; 70**,** 871-876.
28. D. Gayathri, K. J. Shobha. 2,4 Dichlorobiphenyl, a congener of polychlorinated biphenyl degradation by *Pseudomonas* sp GSa and GSb. *Indian Journal of Experimental Biology.* 2015*;* 53**,** 536-542.
29. K. J. Shobha, D. Gayathri. Optimization of 2, 4 dichlorobiphenyl (PCB Congener) degradation by *Pseudomonas spp*. *Bulletin of Environment and Scientific Research,* 2014;3, 1-7.
30. J. G. Ibanez, H. Esparza, M. Doria, C. Serrano, F. Intante, M. M. Singh. Introduction to Environmental chemistry, Environmental Chemistry. 2007; XVII, 3-10.
31. P. K. Somaraja, D.Gayathri, N.Ramaiah. Molecular characterization of 2-Chlorobiphenyl degrading *Stenotrophomonas maltophilia* GS-103. *Bulletin of Environmental Contamination and Toxicology,* 2013; 91**,** 148-153.
32. Sunday A. Adebusoye, Flynn W. Picardal, Matthew O, Ilori O Olukayode. Amund Clay Fuqua. Characterization of multiple novel aerobic Polychlorinated biphenyl (PCB) utilizing bacterial strains indigenous to contaminated tropical African soils. *Biodegradation,* 2008; 19, 145-159.

The Publication fee is defrayed by Indian Society for Education and Environment (iSee). www.iseeadyar.org

Cite this article as:

Shobha K Jayanna, Devaraja Gayathri, Somaraja Palegar Krishnappa. Diversity of 2, 4 Dichlorobiphenyl degrading consortium of *Pseudomonas* isolates GSa and GSb for degradation of Poly Chlorinated Biphenyl congeners*. Indian Journal of Bioinformatics and Biotechnology.* Vol 4 (3), August, 2016.