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GROWTH OF PCB-DEGRADING BACTERIA ON COMPOUNDS FROM PHOTOSYNTHETIC PLANTS

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ABSTRACT

Compounds produced by photosynthetic plants were shown to support the growth of PCB-degrading bacteria, and the organisms retained their ability to metabolize PCBs. These results indicate that the rhizosphere zone surrounding the roots of some plant species may selectively foster the growth of PCB-degrading microbes. Thus, introduction of a carefully selected plant species at PCB-contaminated sites has promise as a new means of enhancing and maintaining microbial degradation of PCBs.

INTRODUCTION

Discovery in the late 1960s that polychlorinated biphenyls persist in the environment much longer than other man-made chemicals suggested that biological organisms (specifically bacteria) were incapable of degrading this class of synthetic compounds. However during the last 25 years several research groups have successfully isolated different strains of PCB-degrading bacteria from environmental samples and characterized their enzymatic properties under laboratory conditions. For example, Bedard et al. (1986) published a list of 25 different bacterial strains which were capable of degrading PCBs under aerobic conditions. Furthermore, it has been shown in the laboratory that combinations of these various strains will effectively degrade the PCBs in aroclor mixtures (Bedard et al. 1987). Thus, PCB-degrading bacteria do exist in the wild, but the persistence of PCBs in contaminated soil indicates that the indigenous bacteria that are genetically capable of metabolizing PCBs are ineffective under most environmental conditions. Thus the primary challenge for successful bioremediation of PCB-contaminated soil is to devise ways to encourage the growth and PCB-metabolism of a select group of microbes which are either indigenous to PCB-sites, or are introduced to the sites.

The only published reports of efforts to test PCB-bioremediation in the field are studies conducted by General Electric. In 1987, a 20-week study was conducted at a race drag strip in New York (McDermott et al. 1989) to evaluate PCB degradation in the top 3-6 cm of soil when inoculated 3 times weekly with a bacterium (Pseudomonas putida LB400) known to metabolize PCBs in the laboratory (Bedard et al. 1987).

Although PCB degradation was observed, the results were disappointing because only 10-20% of the available PCBs were metabolized, and the rate of degradation was only 50% of that observed in the laboratory. More encouraging are recent results reported by GE from field studies conducted during the fall of 1991 on sediments in the Hudson River (Harkness et al. 1992). In this work, it was shown that addition of oxygen, inorganic nutrients, and biphenyl enhanced PCB biodegradation by amounts ranging from 37 to 55% in comparison to controls. Provision of oxygen and inorganic nutrients are conducive to increased microbial activity in general, and have consistently been demonstrated to enhance the biodegradation of soil pollutants. In contrast, the addition of biphenyl was a specific, direct effort in the GE study to selectively stimulate PCB-degradating bacteria.

Although laboratory (Focht and Brunner 1985) and limited field studies (Harkness et al. 1992) have shown the merit of adding biphenyl to PCB-contaminated soil, the low water solubility of biphenyl would make it almost impossible to uniformly mix or distribute this compound into large volumes of soil which exist at many PCB-contaminated sites. Thus, there is a need to find other compounds which support the growth of PCB-degraders, and ways to mix them into PCB-contaminated soil. One possibility which we have investigated is naturally occurring compounds produced and potentially released into the soil by plants. We compared the yield and PCB-degrading properties of 3 different bacterial strains grown on 1 of 15 different plant compounds (Table 1) with the yield and metabolic properties of the same bacteria grown on biphenyl.

Table 1. Plant compounds examined in the growth study.

Chemical class	Chemical	Molecular weight	Sugar moiety
Catechins	Catechin	290.30	-[a]
(flavan-3-ols)			
C-glycoflavones	Vitexin	432.36	Glucose
Cinnamic acids	p-coumaric acid	164.20	-
Coumarins	Coumarin	146.14	-
	Scopoletin	192.16	-
Dihydrochalcones	Phloridzin	436.40	Glucose
Flavanones	Naringenin	272.30	-
(dihydroflavones)	Naringin	580.53	Rhamno-glucose
Flavanonols	Dihydrofisetin	288.26	-
(dihydroflavonols)	(fustin)		
Flavones	Apigenin	270.20	-
	Chrysin	254.20	-
Flavonols	Morin	302.20	-
	Myricetin	318.20	-
	Quercetin	302.20	-
Miscellaneous	Maclurin	262.21	-
	Biphenyl[b]	154.20	-

[a]No sugar moiety is associated with this compound.
[b]Reported to be present in a few plant species, but is not considered to be widely distributed or abundant in the plant kingdom as is the case for the other compounds in this list.

MATERIALS AND METHODS

Materials: Apigenin, catechin, chrysin, p-coumaric acid, morin, myricetin, naringin, and scopoletin were obtained from Sigma Chemical Company (St. Louis, MO). Coumarin was obtained from Merck & Co. Inc. (Rahway, NJ), and naringenin was obtained from General Biochemicals (Chagarin Falls, OH). Dihydrofisetin, and maclurin were purchased from ICN Biomedicals, Inc. (Cleveland, OH). Quercetin was purchased from California Biochemical Research (Los Angeles, CA), and vitexin was obtained from Atomergic Chemetals Corp. (Plainview, NY).

Three known PCB-degrading bacteria were obtained from General Electric (Alcaligenes eutrophus H850, Corynebacterium sp. MB1, and Pseudomonas putida LB400). These cultures were maintained on PAS medium with biphenyl as the sole carbon source (Bedard et al. 1986). The plant compounds tested (Table 1) were dissolved in 95% ethanol and filter-sterilized through a 0.2 μm filter (Gelman). The initial stock solutions were diluted with sterile distilled H_20 to secure 4 stock solutions for each chemical tested. Two ml of each solution were added to individual test tubes containing 8.0 ml of PAS-salts medium. The final concentrations of the test-substrates were 2.0, 1.0, 0.5, and 0.2 g/L. The plant compounds were the sole carbon source in each medium. Individual culture tubes were inoculated with 100 μl of a bacterial suspension grown in PAS-biphenyl medium until an OD of 1.0 was reached at 615 nM (Bedard et al. 1986). The test cultures were incubated at 23°C on a rotary shaker. Cultures were run in triplicate for each compound at each concentration.

One ml aliquots were aseptically removed from each culture tube on days 0,3,6,9,12,15,21, and 27. Individual aliquots were diluted using 3.0 ml of distilled H_20 and the OD was measured on a Bausch-Lomb spectronic 20. Three sets of controls were included: 1) medium incubated without an inoculum, 2) medium minus organic substrate with inoculum, and 3) medium minus organic substrate without an inoculum. The OD values reported in Table 2 were corrected by subtracting the OD values for control-1, described above.

Plant compounds which served as the best growth substrates for each of the bacterial strains were investigated further to determine if bacteria grown on these compounds retained their ability to metabolize PCBs. Each bacterial strain was grown for three successive 27-day periods on biphenyl and each of three different plant compounds provided at the optimal growth concentration, as reported in Table 2. Cells grown on PAS + biphenyl medium were used as the original inoculum. The PCB-degradation properties of the inoculum cells was determined and shown to be essentially the same as that observed for cells maintained on biphenyl during the plant compound study (Table 3,4,5).

The ability of cells grown on different carbon sources to metabolize PCBs was determined with an assay patterned after that of Bedard et al. (1986). A two ml suspension of bacteria with an approximate OD reading of 1.0 was placed in a 20 ml vial containing a mixture of either 10 or 11 different PCB-congeners each at a 5 mM concentration. The congeners and mixtures examined were the same as those used by Bedard et al. (1986). Each incubation vial was shaken for 24 h after which time unreacted PCBs were recovered by subjecting the contents of each vial to a Triton-X-100 plus hexane extraction (Bedard

et al. 1986). The amount of each congener recovered was quantified by GC analysis, and the extent of metabolism was determined by comparing the amount of each PCB congener recovered from living cells with that recovered from dead-cell controls.

RESULTS

Several of the plant compounds tested were able to support the growth of the three bacterial strains tested, but equivalent amounts of growth by each bacterium on each compound was not observed. Alcaligenes eutrophus H850 (Table 2) grew on 11 of the 13 compounds tested. The best growth was on naringin, but 5 other compounds (phloridzin, apigenin, catechin, morin, and dihydrofisetin) supported growth better than biphenyl. Pseudomonas putida LB400 grew on catechin, chrysin, maclurin, and myricetin; however, biphenyl was the best substrate. Corynebacterium sp. MB1 also grew best on biphenyl but naringin, catechin, coumarin, and myricetin also served as excellent sole carbon sources. Five other compound (p-coumaric acid, naringenin, maclurin, chrysin, and morin) supported MB1 growth. The compounds which did not support growth were tested to determine if they were inhibitory to the test strains of bacteria. Normal growth occurred when each test compound was provided at 2.0 g/L in combination with nutrient agar. Therefore, it was concluded that the lack of growth noted for some bacteria-substrate combinations (Table 2) was due to the inability of the organism to use the plant compound as a sole carbon source, and not chemical inhibition by the plant compounds.

Table 2. The relative growth of PCB-degrading bacteria after incubation in PAS-salts medium with a single carbon source provided at its optimal concentration.

H850		LB400		MB1	
Naringin	1.600[ae]	Biphenyl	1.120[a]	Biphenyl	1.320[a]
Phloridzin	1.480[de]	Maclurin	0.530[c]	Naringin	1.300[b]
Apigenin	1.270[d]	Myricetin	0.450[b]	Catechin	1.130[b]
Catechin	1.180[d]	Catechin	0.150[b]	Coumarin	1.100[b]
Morin	1.080[d]	Chrysin	0.110[b]	Myricetin	1.040[c]
Dihydrofisetin	1.030[d]	----------		p-Coumaric	0.700[b]
Biphenyl	1.010	Naringin	0.090[e]	Naringenin	0.560[b]
Myricetin	1.010[c]	Naringenin	0.080[d]	Maclurin	0.460[b]
Vitexin	0.950[c]	Morin	0.040[d]	Chrysin	0.240[d]
Maclurin	0.880[b]	Coumarin	0.000[bcde]	Morin	0.230[d]
Coumarin	0.410[b]	p-Coumaric Acid	0.000[bcde]	----------	
Chrysin	0.200[e]	Quercetin	0.000[bcde]	Quercetin	0.050[e]
----------		Scopoletin	0.000[bcde]	Scopoletin	0.000[bcde]
Quercetin	0.050[e]				
Scopoletin	0.040[e]				

[a]Absorbance following 27 days of growth measured between 510 to 615 depending on absorbance properties of substrate.
[b]Concentration of 0.2 g/L.
[c]Concentration of 0.5 g/L.
[d]Concentration of 1.0 g/L.
[e]Concentration of 2.0 g/L.

When each of the strains was grown for three transfers on those compounds which gave the best growth support, the ability of the strains to metabolize PCBs was very similar to that of bacteria grown on biphenyl (Table 3,4, and 5). Naringin, the plant compound, providing the best growth support for H850 (Table 2) also fostered the greatest metabolic activity of this organism towards PCBs (Table 3). LB400 had the greatest capacity to metabolize PCBs when grown on myricetin (Table 4), where it was observed that 16 of the 19 congeners supplied were altered. MB1 cultures grown on coumarin (Table 5) metabolized 13 different congeners which exceeded the number used by the biphenyl controls.

DISCUSSION

Several naturally occurring plant phenolics were demonstrated to support the growth of three strains of PCB-degrading bacteria, and the organisms retained their ability to metabolize PCBs. These results suggest that phenolic compounds produced and released into the soil by plant roots could serve as a natural carbon source for PCB-degrading bacteria and thereby stimulate PCB degradation in the same

Table 3. Metabolism of PCBs by Alcaligenes eutrophus H850 following growth for 3 transfers on different sole carbon sources.

PCB Congener	Biphenyl	Apigenin	Naringin	Phloridzin
		(%)[a]		
Open 2,3 and 3,4 sites				
2,3	●	●	●	●
2,4'	●	●	●	●
2,5,4'	●	•	●	•
2,2'	●	●	●	●
2,3,2',3'	●	•	●	•
2,5,2'	●	●	●	●
2,3,2',5'	●	•	●	•
2,4,5,2',3	•			
2,5,3',4'	●	•	●	•
2,3,4,2',5'				
Open 2,3 sites				
4,4'	•			
2,4,4'	•			
2,4,3',4'				
2,4,2',4'				
3,4,3',4'				
Open 3,4 sites				
2,5,2',5'	●	•	●	●
2,4,5,2',5'	•		•	•
Blocked 2,3 and 3,4 sites				
2,4,6,2',4'				
2,4,5,2',4',5'				

[a]Percent of congener removed following a 24 h incubation of the bacterium in a PCB mixture containing 5 mM of each congener. % Metabolism: • 20-39, ● 40-59, ● 60-79, ● 80-100

manner as previously demonstrated when biphenyl was added to PCB-contaminated soil (Focht and Brunner 1985). The release of phenolics into the soil by the finely distributed network of plant roots may be thought of as a naturally occurring injection system capable of evenly distributing a desired and perhaps necessary substrate for the active proliferation and action of PCB-degrading bacteria. However, it is important to recognize that the roots of all plant species do not produce and release equal amounts and kinds of phenolic compounds (Rao 1990); therefore, the rhizosphere zone of all plants must not be considered a haven for PCB-degrading bacteria. It may be that only a few plant species may have the desired characteristics. Awareness of such species would be extremely valuable, because growing such plants at contaminated sites has the potential of selectively fostering the growth of PCB-degrading bacteria over competing organisms. The outcome could be a sustained population of PCB-degrading bacteria which would degrade PCBs over an extended time period. Thus, plant-microbe systems have the potential of providing inexpensive, ecologically stable bioremediation systems.

Table 4. Metabolism of PCBs by Pseudomonas putida LB400 following growth for 3 transfers on different sole carbon sources.

PCB Congener	Biphenyl	Catechin	Maclurin	Myricetin
		(%)[a]		
Open 2,3 and 3,4 sites				
2,3	●	●	●	●
2,4'	●	●	●	●
2,5,4'	●	●	●	●
2,2'	●	●	●	●
2,3,2',3'	●	●	●	●
2,5,2'	●	●	●	●
2,3,2',5'	●	●	●	●
2,4,5,2',3	●			●
2,5,3',4'	●	●	●	●
2,3,4,2',5'	●			●
Open 2,3, sites				
4,4'				●
2,4,4'	●			●
2,4,3',4'				●
2,4,2',4'	●			●
3,4,3',4'				
Open 3,4, sites				
2,5,2',5'	●	●	●	●
2,4,5,2',5'	●	●	●	●
Blocked 2,3 and 3,4 sites				
2,4,6,2',4'				
2.4,5,2',4',5'				

[a]Percent of congener removed following a 24 h incubation of the bacterium in a PCB mixture containing 5 mM of each congener. % Metabolism: • 20-39, ● 40-59, ● 60-79, ● 80-100

Table 5. Metabolism of PCBs by Corynebacterium sp. MB1 following growth for 3 transfers on different sole carbon sources.

PCB Congener	Biphenyl	Catechin	Coumarin	Naringin
		(%)[a]		
Open 2,3 and 3,4 sites				
2,3	●	●	●	●
2,4'	●	●	●	●
2,5,4'	•	•	●	•
2,2'	●		●	
2,3,2',3'	●	●	●	●
2,5,2'	•		•	•
2,3,2',5'	•		•	•
2,4,5,2',3	●	•	●	●
2,5,3',4'			•	
2,3,4,2',5'				
Open 2,3 sites				
4,4'	●	●	●	●
2,4,4'	●	●	●	●
2,4,3',4'	●		●	●
2,4,2',4'				
3,4,3',4'				
Open 3,4 sites				
2,5,2',5'				
2,4,5,2',5'				
Blocked 2,3 and 3,4 sites				
2,4,6,2',4'				
2,4,5,2',4',5'			•	

[a]Percent of congener removed following a 24 h incubation of the bacterium in a PCB mixture containing 5 mM of each congener. % Metabolism: • 20-39, ● 40-59, ● 60-79, ● 80-100

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