# Biodegradation of PCBs by Plant-Bacteria And Plant-Fungi Systems

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## OBJECTIVE

PCB-degrading bacteria exist in the wild, but the persistence of PCBs in the environment indicates that under conditions prevailing at PCB-sites the indigenous bacteria are ineffective in degrading PCBs, even though some of them are genetically capable of metabolizing PCBs. 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.

Both laboratory studies[1] and field studies[2] have demonstrated that the addition of biphenyl as a microbial substrate favors the degradation of PCBs. Large scale provision of biphenyl at contaminated terrestrial sites poses problems. The low water solubility of biphenyl would make it difficult to mix evenly into soil, and retention of the chemical in the soil for extended time periods is questionable because of volatilization.

We hypothesize that the roots of some plant species secrete compounds similar to biphenyl which promote the growth of PCB-degrading bacteria and/or fungi but inhibit the growth of other competing microbes. Thus, plants have the potential to selectively foster the growth of PCB-degrading bacteria and/or fungi. It must be emphasized that all species should not be presumed to have this influence. An effective plant species must have the genetic capacity to synthesize a selective microbial substrate and release it into the soil in sufficient amounts to influence the microbial community in the rhizosphere in favor of PCB-degrading bacteria and/or fungi.

## METHODS

The suitability of plant flavonoids to support the growth of PCB-degrading bacteria was examined by comparing the growth of three different PCB-degrading bacterial strains on biphenyl versus 14 different compounds (apigenin, catechin, chrysin, coumarin, dihydrofistin, maclurin, morin, myricetin, naringenin, naringin, phloridzin, quercetin, scopoletin, and vitexin. The compounds tested served as the sole carbon

source for pure cultures grown in liquid media. The three strains of bacteria tested were provided by the General Electric Company who had demonstrated the ability of each strain to degrade PCBs[3]. The PCB-degrading properties of bacteria grown on flavonoids were examined after 3 transfers in each of the compounds studied. The ability of each organism to metabolize PCBs was measured with the assay described by Bedard.[3]

A screening study was conducted to identify plant species which release large amounts of phenols from their roots. Twelve different plant species were grown in sand culture in the greenhouse for 3 to 5 months at which time the pots were eluted with three liters of $H_20$, and the phenol content of the eluates was determined according to the method of Amorim et al.[4] HPLC chromatography was used to analyze root exudates for their flavonoid content. The ability of exudate from each plant species to support the growth of a PCB-degrading bacterium was tested by growing H850 in pure culture with the exudate serving as the sole carbon source.

The degradation of PCBs by pure cultures of ectomycorrhizal fungi was examined by incubating selected fungi with mixtures of PCB congeners for 5 days and measuring the disappearance of individual congeners by GC analysis as described by Bedard.[3]

RESULTS

Microbial growth on plant flavonoids: Three PCB-degrading bacterial strains obtained from General Electric (MB1, H850, and LB400)[3] have been tested for their ability to grow on 14 different plant flavonoids as the sole carbon source. All three bacterial strains grew on at least 4 of the 14 compounds, and the H850 strain grew better on 6 of the compounds than it did on biphenyl. The PCB degrading properties of each of these strains was examined after 3 successive passes in media with one of the flavonoids serving as the sole carbon source. The bacterial strains examined retained their capacities to degrade PCBs after growth on plant flavonoids. These results prove that some of the natural products produced by plants will support the growth of PCB-degrading bacteria and that the bacteria grown on these compounds retain their ability to metabolize PCBs. The substrate specificity which was observed emphasizes the necessity to identify specific plant species which produce and release those compounds which are instrumental in fostering the growth of the PCB-degrading bacteria.

Release of flavonoids from plant roots: Two criteria were used to select the species examined for flavonoid release: 1) all test plants had been reported to accumulate flavonoids in some tissue (usually leaves) and 2) the plants were suitable for growth under conditions prevailing at contaminated sites. Concerning the latter criterion, species were selected which are perennial, easy to grow, have large root systems, widely distributed in the U.S., and have a high tolerance for stress conditions such as poor soil and drought. Analysis of the exudates collected from 12 different plants

showed that seven of the twelve species examined released substantial amounts of phenolics into the soil solution. Further analysis of some of these exudates with HPLC chromatography has shown the presence of numerous, yet unidentified, flavonoids. Further examination has shown that exudates collected from two of the plant species supports the growth of H850, a PCB-degrader. These results support the hypothesis that the rhizosphere of certain plant species will favor the growth and survival of PCB-degrading bacteria.

Ectomycorrhizal fungi: It is well established that the growth in the soil of certain species of ectomycorrhizal fungi is dependent on the presence of specific plant species[5]. In addition to bacteria, fungi associated with plant roots may also have desirable PCB-degrading properties as shown previously for other xenobiotics.[6] Eighteen different pure cultures of ectomycorrhizal fungi have been tested for their ability to metabolize a mixture of 19 different PCB congeners with chlorine contents ranging from 2 to 6. Six of the cultures tested have been demonstrated to metabolize PCBs. In 5 days, Gautieria crispa 4936, the most active fungus tested to date, metabolized 5 different congeners by amounts ranging from 20 to 50% of the starting material. No endomycorrhiza have been tested, because they can not be propagated as pure cultures, and they do not form extensive fungal mats under field conditions as do ectomycorrhizal fungi. Three tree species (cottonwood, Ponderosa pine and willow) which are known to be colonized by some species of mycorrhizal fungi are being tested for their ability to support the growth of the fungi demonstrated in our laboratory to metabolize PCBs. To our knowledge this is the first effort made to examine the influence of mycorrhizal fungi on PCBs.

CONCLUSIONS

Flavonoid compounds produced by plants have been shown in our laboratory to support the growth of PCB-degrading bacteria. Organisms grown on plant flavonoids retained their ability to metabolize PCBs. Ectomycorrhizal fungi were also demonstrated for the first time 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. Introduction of a carefully selected plant species at PCB-contaminated sites is a new and promising means of giving a survival advantage to PCB-degrading microbes over other competing soil organisms. Increasing the population size of a desired microbe, and extending its longevity at a contaminated site are both conducive to enhanced degradation of soil pollutants such as polychlorinated biphenyls.

# TRANS

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