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Metabolism of 2-chlorobiphenyl by Suspension Cultures of Paul's Scarlet Rose

John S. Fletcher,[1] Alan W. Groeger,[1] and James C. McFarlane[2]

[1]Department of Botany and Microbiology, University of Oklahoma, Norman, Oklahoma 83019 and [2]U.S. Environmental Protection Agency, 200 S.W. 35th Street, Corvallis, Oregon 97333

Several studies have shown that plant-soil systems were capable of degrading chlorinated biphenyls, with the resulting accumulation of breakdown products in both the plants and the soil (Moza et al., 1974, 1976, 1979). However, since these studies were performed under field conditions, it is not certain what portion of the degradation was due to plant metabolism and what portion to that of soil microbes which have been demonstrated in pure culture to degrade chlorinated biphenyls (Pal et al., 1980). This question is addressed in the present research by determining the metabolism of 2-chlorobiphenyl provided aseptically to axenic cultures of Paul's Scarlet rose.

MATERIALS AND METHODS

Suspension cultures of Paul's Scarlet rose were grown in 250 ml Erlenmeyer flasks containing 80 ml of MPR medium (Nesius et al., 1972). Transfer techniques and culture conditions used in this study were the same as those described in our previous reports (Danks et al., 1975; Mohanty and Fletcher, 1978). The nonradioactive 2-chlorobiphenyl used in this study was purchased from Foxboro Company, New Haven, CT, and the radioactive material (2-chlorobiphenyl-chlorophenyl-ring UL-^{14}C, specific activity = 11.0 mCi/mmol) was purchased from Pathfinder Laboratories, Inc., St. Louis, Missouri.[1] Thin layer chromatography in our laboratory of the ^{14}C-2-chlorobiphenyl showed that the compound was 97% pure. Stock solutions of each of the purchased compounds were prepared in methanol and stored at -20°C until they were used.

The influence of 2-chlorobiphenyl on the growth of rose cultures during late logarithmic growth, day 11 to 14 (Mohanty and Fletcher, 1978), was examined by comparing the growth of control cultures with that of test cultures whose medium was augmented on day 11 with 2-chlorobiphenyl to give a final concentration of 0.28 ppm. Harvesting of the cultures and dry weight determinations were made as previously described (Mohanty and Fletcher, 1978).

Send reprint requests to John S. Fletcher at the above mailing address.

[1] Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

The kinetics of ^{14}C exchange between living cells and medium containing ^{14}C-2-chlorobiphenyl was determined by removing aliquots of media from cultures at selected times during a 72-hour incubation period, and assaying the aliquots for ^{14}C. Control flasks containing heat-killed cells (autoclaved for 25 minutes) were also examined. Radioactive 2-chlorobiphenyl was added to 11-day-old cultures in the same manner and amount as described below for the metabolism investigation. Analysis was conducted on triplicate flasks of each treatment.

Metabolism of ^{14}C-2-chlorobiphenyl was examined by aseptically adding 200 ul of stock solution containing 1 μCurie of ^{14}C (9.1 umoles of substrate) to four 11-day-old cultures. The final concentration of the chlorobiphenyl in the cuture medium was 0.24 ppm. Two of the chemically augmented cultures were alive and 2 had been heat killed by autoclaving them for 25 minutes prior to adding chlorobiphenyl. The cultures augmented with 2-chlorobiphenyl were maintained under normal growth conditions (Danks et al., 1975) for 76 hours. At the conclusion of the incubation period, the sterility of the cultures was established by standard microbial plating techiques, and the cells were then filtered from their medium and washed with deionized water as previously described (Danks et al., 1975). The cells and medium collected from each culture flask were then extracted according to the procedures of Bligh and Dyer (1959) as modified by Scheel and Sandermann (1977). Cells from a single culture (approximately 15 g fresh weight) were placed in a 30 ml mixture of chloroform and methanol (1:2, v/v) and ground for 5 minutes with a Virtis grinder set at medium speed. Individual homogenates were then filtered through a Whatman 934-AH fiberglass filter. The residue retained on the filter was washed with three 15-ml rinses of chloroform and methanol (1:2, v/v) and an 8-ml rinse of H_2O. The combined filtrates were placed in a separatory funnel with an additional 25 ml of chloroform and 29 ml of H_2O. When the solvents had partitioned into discrete phases, they were separated from each other and stored at 5°C. Ten ml of the medium collected from each culture was extracted according to the procedures described above for the cells.

Duplicate 1-ml samples of the chloroform and methanol-H_2O extracts from both cells and medium were assayed for ^{14}C. One-ml samples were placed in 15 ml of Opti-Fluor and the radioactivity was determined with a Packard model 2000CA liquid scintillation analyzer. The insoluble residues recovered from both the cells and the medium were oxidized with a Packard Tri-Carb-306 sample oxidizer, and the released $^{14}CO_2$ from each sample was trapped in an 20-ml mixture of Carbo-Sorb and Permafluor V (9:11 v/v).

Thin layer chromatograms of the methanol-H_2O and chloroform extracts were prepared by applying appropriate aliquots onto silica gel plates and developing them in toluene-ethanol-methanol (4:1:1), and heptane, respectively. The location of radioactive material on the chromatograms was determined by autoradiography. The appearance of new metabolites was determined by comparing the autoradiograms of the extracted material with that of the parent compound.

RESULTS AND DISCUSSION

The average dry weight of rose cultures grown under standard conditions was 285 mg on day 11 (Table 1). A comparison of the growth from day 11 to 14 of control and 2-chlorobiphenyl treated cultures showed no significant difference at the 95% confidence level (t-test). Therefore, it can be concluded that a 0.28 ppm concentration of 2-chloribiphenyl, which is well within the water solubility of this compound (Hutzinger et al., 1974) had no significant influence on the growth of Paul's Scarlet rose cultures during their late log-phase period of growth.

Table 1. Influence of 2-chlorobiphenyl on the dry weight increase of suspension cultures of Paul's Scarlet rose during late logarithmic growth, day 11 to 14.

Treatment	Day 11 mg	Day 14	Increase %
Control	285.0[a] (42.3)[b]	640.1 (31.6)	225
2-chlorobiphenyl added	285.0 (42.3)	701.6 (21.8)	246[c]

[a] Each value is the average of 6 replicates.
[b] Values in parentheses are the standard error.
[c] Calculation was based on 11-day-old control values.

Monitoring of the ^{14}C content in the medium of the living culture supplemented with ^{14}C-chlorobiphenyl showed two distinct phases of change (Figure 1). Initially, there was a decline in soluble radioactivity in the medium (0 to 1 h), but thereafter the radioactivity in the medium increased. The rapid loss of radioactivity from the medium between 0 and 1 h also occurred for the heat-killed culture, but the reappearance of radioactivity in the medium did not occur. The rapid disappearance of ^{14}C from the flasks containing either living or dead cells suggested that the monochlorinated biphenyl adhered to cell material without the involvement of metabolism. In contrast to this, the reappearance of ^{14}C in the medium of only the living cultures indicated that chlorobiphenyl was transformed into water soluble compound(s) which was released from the cells. The living rose cells may have formed a glycosylated derivative of the ^{14}C-chlorobiphenyl in a manner similar to the metabolism of pentachlorophenol by soybean tissue cultures (Langebartels and Harms, 1984).

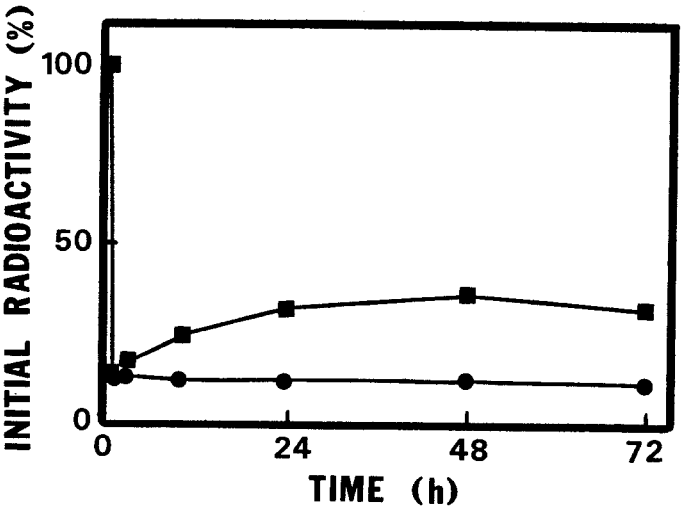

Figure 1. Changes in the ^{14}C content of the medium containing ^{14}C-chlorobiphenyl and living (■) or dead (●) cells. Standard errors fell within the dimensions of the symbols (n = 3).

Major differences in the distribution of ^{14}C among the fractions recovered from the living as compared to nonliving cultures (Table 2) clearly showed that living plant cells maintained under axenic conditions were capable of metabolizing 2-chlorobiphenyl. In the living cultures, the three fractions recovered, methanol-H_2O, chloroform, and insoluble residue, contained 43, 17, and 40%, respectively, of the radioactivity. In contrast to this, the radioactivity recovered from the nonliving cultures was primarily in the chloroform fraction (96%), and all of this radioactivity was demonstrated by chromatographic means to be the parent compound, 2-chlorobiphenyl.

The ability of plant cultures to metabolize 2-chlorobiphenyl was further substantiated by showing through chromatographic analysis that none of the 759,000 DPM in the methanol-H_2O fraction of the living cells, or the 364,000 DPM in the medium of these cells was present as 2-chlorobiphenyl. However, the autoradiograms did show 6 distinct bands of radioactivity present on chromatograms of the cell extract and 5 on chromatograms of the medium. Three of the bands on both the medium and the cell extract chromatograms had equivalent RF values. Thus, if each band observed on the autoradiograms represented a single compound, the methanol-H_2O extract of the cells contained 6 radioactive compounds, none of which were 2-chlorobiphenyl, and the medium contained 5 such compounds. This investigation has shown for the first time that plant cells maintained under axenic conditions are capable of metabolizing a chlorinated biphenyl.

Table 2. Distribution of ^{14}C in extracts prepared from the cells and medium following a 76 hour incubation period of 11-day-old living and nonliving rose cultures in medium containing ^{14}C-2-chlorobiphenyl.

Fraction	Living						Dead[a]					
	Cells		Medium (DPM x 10^3)		Total		Cells		Medium (DPM x 10^3)		Total	
Methanol-H_2O	759[b]	(29)[c]	364	(14)	1,123	(43)	12	(0.6)	62	(3)	74	(3.6)
Chloroform	173	(7)	266	(10)	439	(17)	1,805	(88)	170	(8)	1,975	(96)
Insoluble Residue	300	(11)	750	(29)	1,050	(40)	8	(0.4)	3	(0.1)	11	(0.5)
Total	1,232	(47)	1,380	(53)	2,612	(100)	1,825	(89)	235	(11.1)	2,060	(100.1)

[a] Autoclaved for 25 minutes at 121°C.

[b] The DPM values are the averages obtained from 2 replicates.

[c] Percent of the total counts recovered, 2.61 x 10^6 for living and 2.06 x 10^6 for dead cells.

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