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Biotransformation of naphthalene and diaryl ethers by green microalgae

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Abstract

The role of green microalgae in the biotransformation of naphthalene (a polycyclic aromatic hydrocarbon) and diaryl ethers was investigated using axenic cultures of *Chlorella vulgaris* and two environmental isolates, *Scenedesmus* SI1 and Ankistrodesmus SI2. Biotransformation experiments with dense cell cultures showed that these three green algae transformed toxic xenobiotics to more polar metabolites. *Chlorella vulgaris* metabolized naphthalene to 1-naphthol (0.36–0.65%). *Ankistrodesmus* SI2 biotransformed dibenzofuran to six metabolites (total over 7%), three of which (possibly four) were identified as monohydroxylated dibenzofurans, the remaining two may be dihydroxylated derivatives. *Scenedesmus* SI1 biotransformed dibenzo-*p*-dioxin to three metabolites, one of which was tentatively identified as 2-hydroxydibenzo-*p*-dioxin (approximately 3.8%), the remainder may be dihydroxylated derivatives. This is the first time that the biotransformation of diaryl ethers by green microalgae has been investigated.

Introduction

Polycyclic aromatic hydrocarbons (PAHs), such as naphthalene (NPH), are released into the environment during the combustion of materials such as fossil fuels and hydrocarbons. Diaryl ethers such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) arise, for example, as by-products of manufacturing of chlorophenols and their derivatives (Hay 1982).

The microbial degradation of NPH is well documented and the biochemical pathways have been elucidated (Cerniglia 1984, 1992; Gibson & Subramanian 1984). Diaryl ethers and their halogenated derivatives show a high resistance to biodegradation due to their limited bioavailability and toxicity and tend to persist in the environment. A few bacteria have recently, however, been reported to be capable of catabolizing and, in some cases, even mineralizing unsubstituted and halogenated diaryl ethers. These bacteria include *Sphingomonas* (Wittich et al. 1992; Schmidt et al. 1992, 1993; Harms et al. 1995; Keim et al. 1999), *Brevibacterium* (Engesser et al. 1989) and *Staphylococcus* (Monna et al. 1993). Additionally, the yeast *Trichosporon beigelii* was shown to biotransform diphenyl ether (DE) to hydroxylated derivatives (Schauer et al. 1995).

While the roles of bacteria, fungi and yeasts in the biodegradation or biotransformation of xenobiotics are well-established in many genera, the incidence of reports involving biotransformations of xenobiotics by algae is scarcer. Prokaryotic and eukaryotic marine and freshwater algae, including cyanobacteria, green algae and diatoms (red and brown) are known to metabolize NPH to a series of metabolites, (Cerniglia et al. 1979, 1980a, b, 1982).

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There are no reports of purely algal degradation or biotransformation of diaryl ethers, hence the objectives of the present study were to identify algal strains with the ability to biotransform these xenobiotics to more polar metabolites.

Materials and methods

Algal cultures

Chlorella vulgaris [CCAP 211/11B, Beijerinck 1890] was obtained from the Culture Collection for Algae and Protozoa (CCAP) at the Institute of Freshwater Ecology, Ambleside, Cumbria, UK. *Scenedesmus* SI1 was isolated from wet soil from the river banks at the confluence of the industrially polluted River Derwent and River Tyne, Gateshead, County Durham, UK, whereas *Ankistrodesmus* SI2 was isolated from pond water draining from Forestry Commission conifer plantations in Harwood Forest, central Northumberland; the water was thus acidic and dark brown with peat solubles. These algae were successfully cleaned from other microbial contaminants and have since been deposited with CCAP as *Scenedesmus* sp. CCAP 276/123 and *Ankistrodesmus* sp. CCAP 202/27.

Growth media

C. vulgaris was maintained in Bristol's Medium (BM) (Bold 1949) supplemented with trace elements (2ml per litre of a stock solution containing, per litre, 0.25 μg $CuSO_4$.5H2O; 0.27 μg $(NH_4)_6Mo_7O_{24}.4H_2O$; 0.6 μg $CoCl_2.6H_2O$; 4.5 μg $MnCl_2.4H_2O$; 1.2 μg $ZnSO_4.7H_2O$; 0.12 μg $FeCl_3.6H_2O$ and 1.9 μg Na_2.EDTA). Following sterilization of this pH 6.6 solution, a filter-sterilized vitamin supplement containing equal amounts of cyanocobalamin, thiamine.HCl and biotin was added to final concentrations of 0.04 $\mu g.l^{-1}$.

Scenedesmus SI1 and *Ankistrodesmus* SI2 were cultivated in Woods Hole MBL Medium (MBL) (Nichols 1973). This pH 7.2 solution was also supplemented with the same vitamins as BM.

Biotransformation experiments

Large volumes of exponential-phase cultures of *C. vulgaris* grown in BM plus 10 mM glucose in the light, or *Scenedesmus* SI1 and *Ankistrodesmus* SI2 cultures grown in MBL plus 10 mM acetate in the light, were harvested by centrifugation (22,100 $\times$ g for 30 min [*C. vulgaris*] or 45 min [*Scenedesmus* SI1 and *Ankistrodesmus* SI2] in a Beckman J2-21 centrifuge at 4 °C). The cells were washed twice in basal liquid medium by the same method of centrifugation and were resuspended in the same medium to a density of 1.3 to 6.3 $\times$ 10^7 cells.ml^{-1} (judged by haemacytometer counts). These dense cultures were divided (15-25 ml) among sterile 100ml Erlenmeyer flasks with Teflon-sealed screw caps containing 0.1 mM xenobiotic; boiled cell controls with 0.1 mM xenobiotic; cell suspensions without the xenobiotic and a no-cell control (plain medium) with 0.1 mM xenobiotic. The xenobiotics naphthalene (NPH) (diaryl), dibenzo-*p*-dioxin (DD) and dibenzofuran (DF) (diaryl ethers) were added from 100mM stock solutions in dimethylformamide (DMF). The resulting volume of DMF in the cultures exhibited no toxic effects on the algae (Todd et al. 2001; Todd 1999).

Algae were incubated under a light-dark cycle of 12–12 h under illumination provided by white fluorescent light (7.52 μmol photons $m^{-2}.s^{-1}$) at 18 °C and shaken at a speed of 100 rev.min^{-1}. The flasks were sampled daily for 5 days; cells were removed from samples by centrifugation (10,000 $\times$ g; 4 °C; 15 min in a microfuge) and the supernatants were frozen at -20 °C for later analysis by HPLC. Cells were pelleted from cultures remaining after the incubation period and to extract possible metabolites, supernatants were extracted 4 times with ethyl acetate (neutral extract), after which the resulting aqueous layer was acidified with 85% (w/v) orthophosphoric acid to pH 2.5 and the process was repeated to obtain the acidic extract. Both extracts were then dried over anhydrous sodium sulphate and filtered; the solvent was removed and the residues were dissolved in methanol to provide the respective extracts. Axenity of cultures was routinely examined throughout experimental work by streaking onto nutrient agar and potato dextrose agar plates and inoculation into nutrient broth. Cultures were also examined microscopically by phase contrast for adhering bacteria.

Chemical analysis and identification of metabolites

HPLC

HPLC was performed on a Gilson 805 Manometric Module comprising 305 and 306 pumps with a Gilson 234 automatic sampling injector, a Milton Roy® Spectromonitor® 3100 set at 210 nm. The integration system comprised Lab Systems VG chromatography servers linked to Lab Systems XChrom

software. The separation was achieved on an Anachem S5 OD52 column (250 × 46mm) run isocratically with a 60 : 40 (by vol.) methanol : water mobile phase containing 1 ml.l^{-1} orthophosphoric acid (H_3PO_4) at 1 ml.min^{-1}. Integration was started at 4 min after injection of samples to eliminate the strongly absorbing DMF solvent peak derived from the presentation of the xenobiotics to algal cultures in this water-miscible solvent. In order to detect both metabolites and the initial substrate, a solvent gradient of methanol:water from 60 : 40 to 95 : 5 (by vol.) containing 1 g.l^{-1} H_3PO_4 was required. Metabolites from a representative set of duplicate flasks were quantified by constructing calibration curves on the basis of known concentrations of each compound (not shown). Several repetitions of these biotransformation experiments showed that metabolites were always formed from a particular xenobiotic to the same proportion.

Colorimetric tests for phenolic compounds

Biotransformation experiments were set up with the algae as above and after incubation for 5 days, metabolites were extracted into ethyl acetate from neutral supernatants and concentrated. A series of reactions was set up with 1ml Fast Red GG Salt (*p*-nitrobenzenediazonium tetrafluoroborate) or Fast Violet B Salt (4-benzoylamino-2-methoxy-5-methylbenzenediazonium chloride hemi [zinc chloride] salt) (0.1% in 0.1 N HCl) to which a few drops of appropriate xenobiotic (1 mg.ml^{-1} in EtOAc); or an authentic standard of known possible hydroxylated metabolites (1 mg.ml^{-1} in EtOAc), or 0.5 ml of the appropriate neutral ethyl acetate incubation extract were added. After mixing and allowing the tube contents to stand for 30 min, the solution was neutralized to pH 7.0 with K_2CO_3 and the coupled dye extracted into 0.5 ml EtOAc and the colour reactions were observed. An identical series of samples was separately treated with 0.2ml of Folin-Ciocalteau's Phenol Reagent (1 : 4 in distilled water), followed after 10 min by the addition of a few drops of sodium bicarbonate (5% w/v) until the mixture was distinctly alkaline. If phenols were present in high concentration, a blue colour developed immediately. At much lower concentrations, the colour developed and intensified over 5-10 h.

Chemical analyses

Neutral ethyl acetate extracts from several experiments were pooled and further concentrated under N_2; metabolites were purified by collecting effluent fractions (Pharmacia FRAC-100 collector) during HPLC resolution. Appropriate fractions were pooled and evaporated to dryness. For UV spectra determination, residues were redissolved in a solution of 0.1 M 3-(N-morpholino)propane sulphonic acid (MOPS) buffer, pH 7.0 : methanol (1 : 1, by vol). The spectrum of the sample (0.2 ml) was measured between 220 nm and 350 nm in a Cary Model 4E recording double-beam spectrophotometer against a blank of the buffer-methanol mixture. To determine if the metabolite produced was identical to a known compound, a 0.2 ml aliquot of a 0.05 mM solution of the appropriate authentic compound in the same solvent mixture, was examined in the same way and the respective spectra obtained were compared.

For electron impact (EI, 55 eV, 300 μA) mass spectrometry, residues were redissolved in chloroform and mass spectra were obtained using a Micromass Autospec Mass Spectrometer operating at 55e.v. and 300 μA trap current. The mass ion and fragmentation patterns of the mass spectrum were compared with that of known metabolites from the NIST chemical library.

Chemicals

Naphthalene (NPH) and dibenzofuran (DF) were purchased from Sigma and Aldrich, respectively. Dibenzo-*p*-dioxin (DD); 2-, 3- and 4-hydroxydibenzofuran (2-OH-DF, 3-OH-DF, 4-OH-DF), and 2-hydroxydibenzo-*p*-dioxin (2-OH-DD) were a kind gift from T. Keim and W. Francke, Institut für Organische Chemie, Universität Hamburg, Germany. All chemicals and solvents were of the highest purity available.

Results

Initial trials showed that each alga was biotransforming only one of the three test substrates to more polar metabolites – NPH by *C. vulgaris*, DD by *Scenedesmus* SI1 and DF by *Ankistrodesmus* SI2. Detailed studies were then performed using these three substrates to characterize the products.

Transformation of NPH by C. vulgaris

Analysis by HPLC of cell-free supernatants and neutral extracts of dense glucose-grown suspensions of *C. vulgaris* incubated with 0.1 mM NPH indicated the formation of Metabolite I (M-I), with retention time 8.94 min (Figure 1); all other peaks observed were accounted for in the control incubations (not shown). No

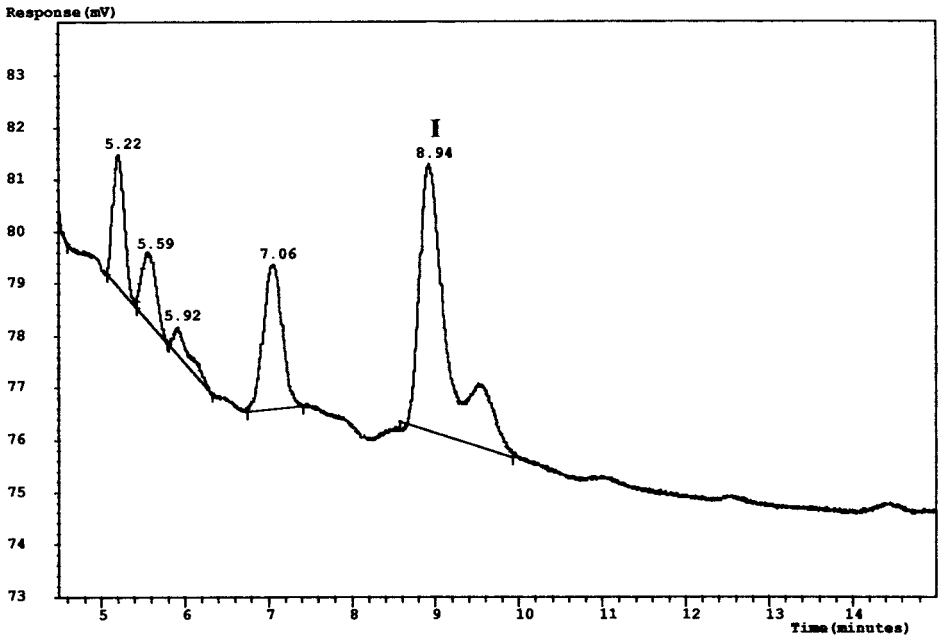

Figure 1. HPLC elution profile of a neutral ethyl acetate extract obtained after incubation of *C. vulgaris* (grown photoheterotrophically on glucose) with naphthalene (0.1 mM) for 5 days. Cultures comprised 48.5 μg protein ml^{-1}.

metabolites were observed in the acidic extracts. M-I appeared in the culture flasks by the second day of incubation and gradually increased in amount to day 5 (Figure 2). On comparing the HPLC retention time of M-I with those of known metabolites of NPH, it was shown that M-I had a retention time similar to that of 1-naphthol (8.6 min) but separated clearly from 2-naphthol (7.6 min). Small quantities (0.5 μM) of either 1-naphthol or 2-naphthol were then added to separate aliquots of the incubation mixture supernatant and the mixtures re-analysed by HPLC. M-I and 1-naphthol co-eluted at 8.6 min resulting in an increase of the metabolite peak area, whereas M-I (8.6 min) and 2-naphthol (7.7 min) were clearly distinct compounds (Todd 1999). M-I was thus confirmed as 1-naphthol; the amount of conversion of NPH to 1-naphthol in the 0.1mM NPH incubation mixtures was 0.36–0.65% (from calibration curve).

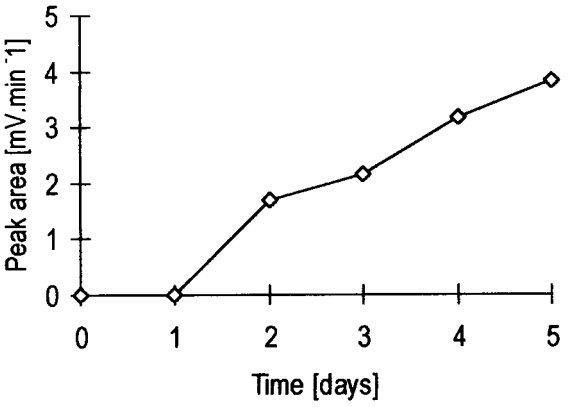

Figure 2. Time course of formation of Metabolite I from naphthalene by *C. vulgaris*. Aliquots of 0.5ml were taken daily from incubations for 5 days; cells were removed by centrifugation before analysis by HPLC. Mean peak areas are shown for Metabolite I (retention time 8.94 min) [◊].

Transformation of DF by Ankistrodesmus *SI2*

Acetate-grown cells formed six metabolites (designated M-II to M-VII) from DF with retention times of 5.62 min (M-II), 6.39 min (M-III) and 13.8 to 19.2 min (M-IV to M-VII) (Figure 3). None of these metabolites were formed in control flasks or in acidic extracts.

M-II to M-VI were formed at varying concentrations over the 5-day incubation period (Figure 4); M-VII was only detected in the neutral ethyl acetate extract of the culture remaining on the final day of the incubation. M-II was always produced to the highest concentration but may have started autooxidation after day 3.

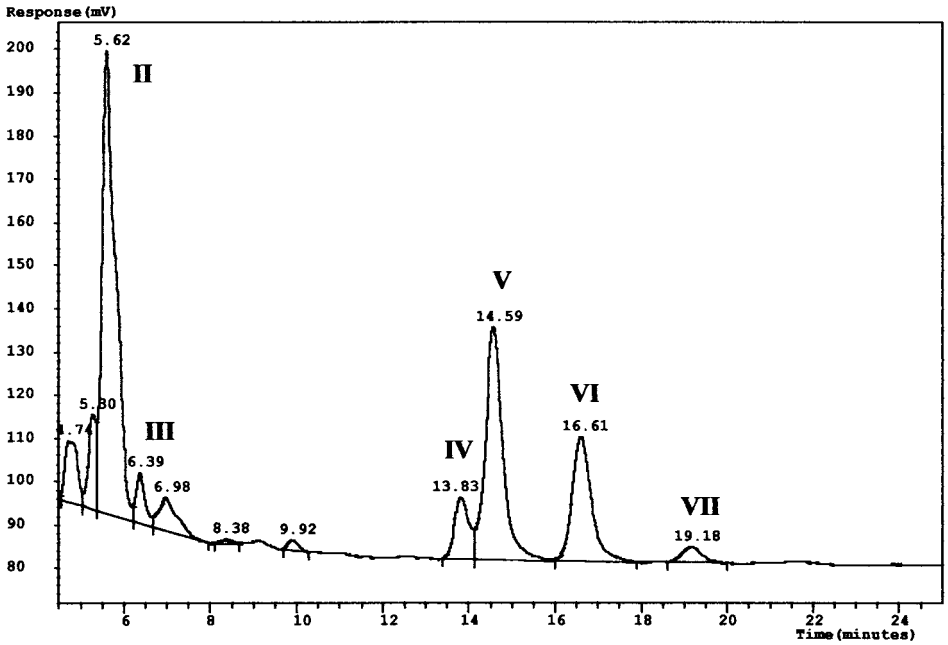

Figure 3. HPLC elution profile of a neutral ethyl acetate extract obtained after incubation of *Ankistrodesmus* SI2 (grown photoheterotrophically on acetate) with dibenzofuran (0.1 mM) for 5 days. Cultures comprised 11.0 μg protein ml^{-1}.

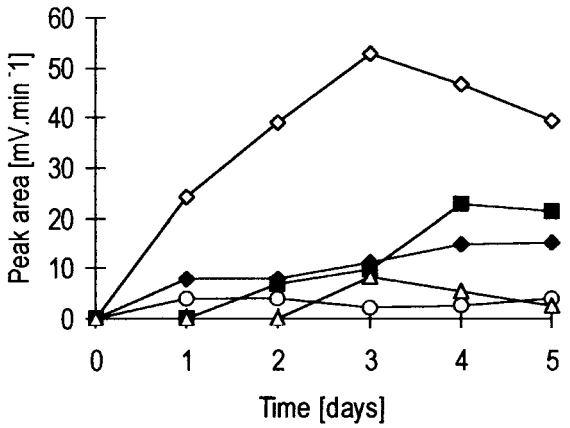

Figure 4. Time course of formation of metabolites from dibenzofuran by *Ankistrodesmus* SI2. Aliquots of 0.5ml were taken daily from incubations for 5 days; cells were removed by centrifugation before analysis by HPLC. Mean peak areas are shown for Metabolite II (retention time 5.62 min) [◊], Metabolite III (6.39 min) [■], Metabolite IV (13.83 min) [○], Metabolite V (14.59 min) [♦] and Metabolite VI (16.61 min) [△].

To identify metabolites, the same extracts were re-analysed by HPLC when spiked separately with solutions of three isomeric monohydroxylated dibenzofurans; other known metabolites of DF, such as dihydroxylated derivatives, were not available for comparison. M-IV, -V and -VI co-chromatographed (with a resulting increase in peak area at the appropriate retention times) with 4-, 2- and 3-hydroxydibenzofuran (4-, 2- and 3-OH-DF), respectively (Todd 1999). It is possible that M-VII was 1-OH-DF because it eluted closely after M-IV, -V and -VI, and that M-II and -III were different dihydroxylated isomers of DF because they eluted close together but were more polar than M-IV to M-VII. From calibration curves (not shown), the conversion of DF to 2-, 3- and 4-OH-DF in incubations (Fig. 3) was 1.6% (M-V), 0.4% (M-VI) and 0.5% (M-IV), respectively, a total of 2.5% conversion into these three derivatives. As M-II was produced to concentrations at least twice as much as the sum of M-IV, -V and -VI, there was an overall conversion of DF of about 7%.

The phenolic nature of the metabolites from DF was further confirmed by the reactions of 5-day incubation mixtures with Folin-Ciocalteau's Phenol Reagent and with acidic Fast Red GG salt. The original DF substrate gave no reaction with these reagents but the 5-day incubation mixtures and 2-OH-DF gave almost identical colours (Todd 1999, not shown). The other OH-DF isomers and 1-naphthol, which was used as a phenolic standard, also showed bright colours with these reagents.

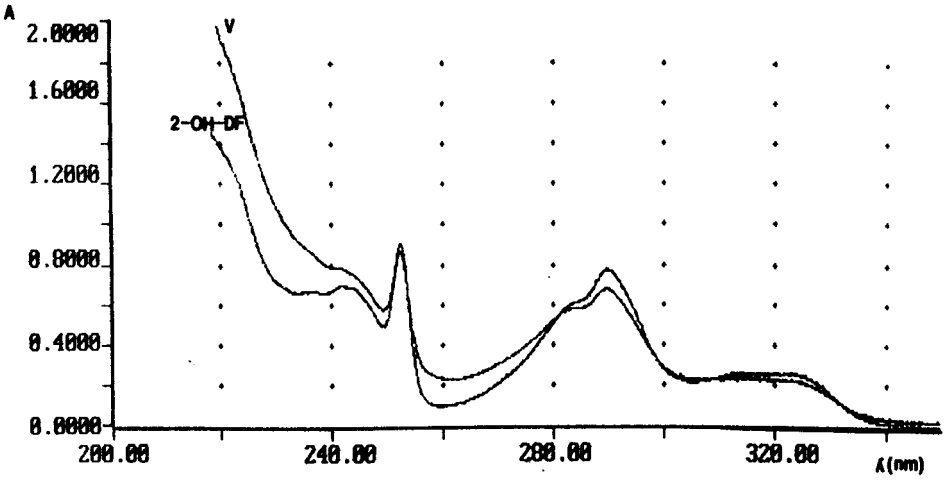

Figure 5. UV Spectra of Metabolite V and 2-hydroxydibenzofuran (2-OH-DF). A full spectral scan (220-350 nm) of absorbance (A) was taken for samples of both compounds, light path 10 mm.

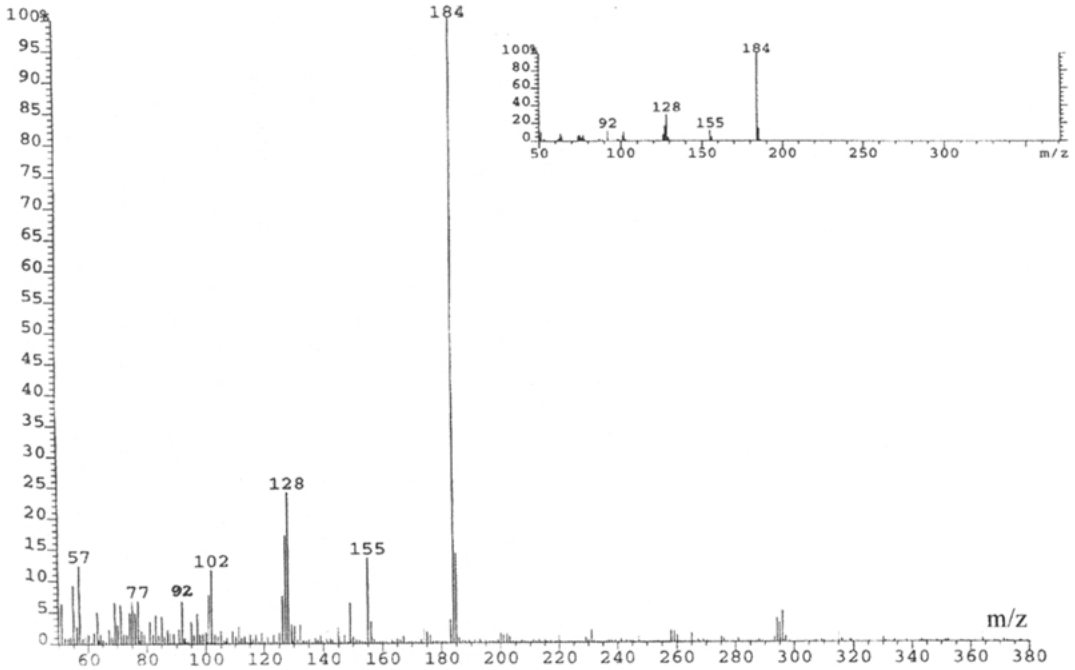

Figure 6. Mass Spectrum of Metabolite V. The mass spectrum (EI) was taken at 55 eV and 300 μA trap current. The inset shows the MS of authentic 2-hydroxydibenzofuran (#18917), retrieved from the NIST chemical library of spectra, and is included for comparison.

M-V was purified (see methods) and further evidence for this being 2-OH-DF came from a comparison of the UV spectrum of the two compounds between 220nm and 350 nm (Figure 5); the two spectra and the absorption maxima/minima showed close overall similarity.

Final confirmation for the tentative identity of M-V with 2-OH-DF was obtained from EI mass spectrometry of solutions of these two compounds in chloroform (Figure 6). The EI mass spectrum of M-V showed prominent mass ions at m/z 184 (M^+) and at 185 ($M^+ + 1$) and major fragments at 155 (M-29) due to loss of –CHO; 128 (M-56) from loss of $C_4H_8^+$ from splitting of the furan ring; 102 (M-82) from the loss of furylmethylene $C_4H_4O\text{-}CH_2^+$ from the mass ion; 92 (M-92) due to the loss from the mass ion of

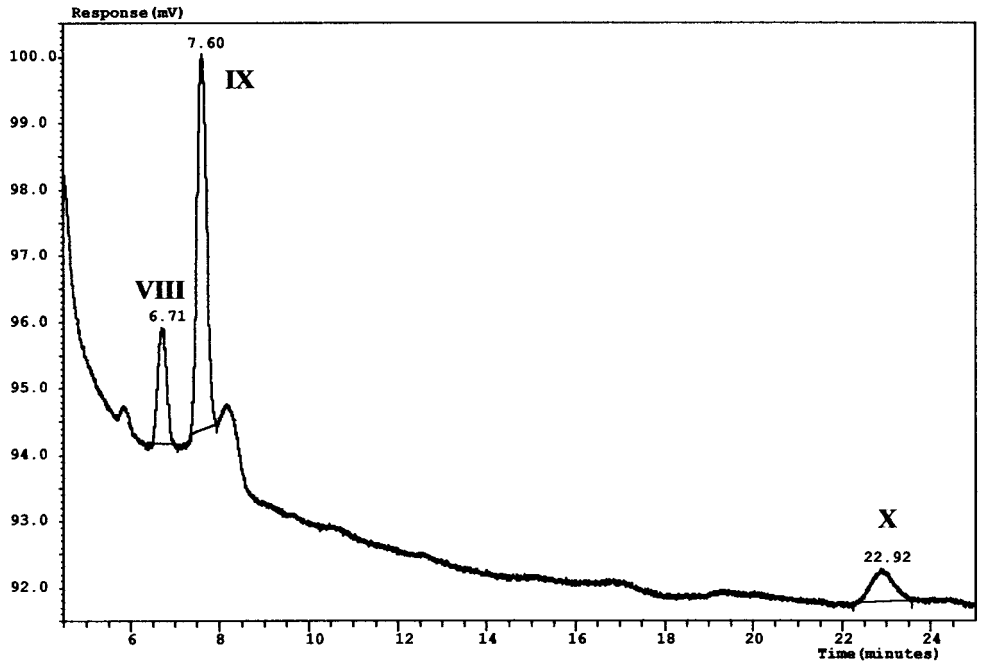

Figure 7. HPLC elution profile of a direct culture sample from the incubation of *Scenedesmus* SI1 (grown photoheterotrophically on acetate) with dibenzo-*p*-dioxin (0.1 mM) for 5 days. Cultures comprised 12.7 μg protein ml^{-1}.

either $-^{+}C_6H_3OH$ (hydroxyaryl) or $-C_6H_4O^{+}$ (aryl ether), and at 77 (phenyl, $C_6H_5^{+}$). The same fragmentation pattern is evident in the NIST library spectrum of 2-OH-DF. A high resolution determination of the molecular mass ion of M-V gave m/e 184.051598. The calculated molecular mass of 2-OH-DF, $C_{12}H_8O_2$, is 184.052430. The identity of M-V with 2-OH-DF was thus strongly indicated.

Transformation of DD by Scenedesmus *SI1*

Three metabolites (M-VIII, -IX and -X) were observed by HPLC in the acetate-grown *Scenedesmus* SI1 incubations with 0.1mM DD; retention times were approximately 6.7 min (M-VIII), 7.6 min (M-IX) and 22.9 min (M-X) (Figure 7). None of these metabolites formed in the control flasks. When neutral ethyl acetate extracts of the final day incubations were analyzed by HPLC, areas were recorded in controls with approximately the same retention times for M-VIII and -IX, but only in trace amounts less than 0.1% of the experimental value; M-VIII and -IX were therefore abundantly produced metabolites of DD. The concentrations of metabolites formed from DD increased daily (Figure 8) but M-IX (7.6 min) was always formed to higher concentrations than M-VIII (6.7 min) and M-X (22.9 min).

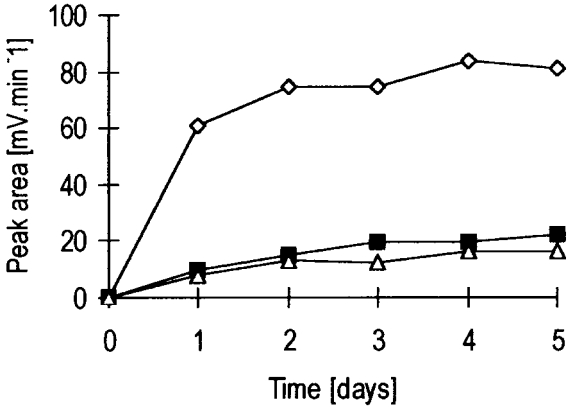

Figure 8. Time course of formation of metabolites from dibenzo-*p*-dioxin by *Scenedesmus* SI1. Aliquots of 0.5 ml were taken daily for 5 days from which cells were removed by centrifugation before analysis by HPLC. Mean peak areas are shown for Metabolite VIII (retention time 6.7 min) [■], Metabolite IX (retention time 7.6 min) [◇] and Metabolite X (retention time around 22.9 min) [△].

With the aim of identifying any of these metabolites, the neutral ethyl acetate extract from experimental incubations was re-analysed by HPLC with

the inclusion of a small amount of 2-hydroxydibenzo-*p*-dioxin (2-OH-DD); our only authentic monohydroxylated DD available. M-X co-chromatographed with 2-OH-DD (Todd 1999); by the end of the 5-day incubation period, approximately 3.8% of the DD could be accounted for as the tentative metabolite 2-OH-DD.

The phenolic nature of the metabolites of DD was further confirmed by reacting the neutral ethyl acetate extracts of 5-day incubation mixtures with Folin-Ciocalteau's Phenol Reagent and with Fast Violet B salt in 0.1 N HCl. The experimental extract showed a similar blue colour to that of 2-OH-DD in the reaction with Folin-Ciocalteau's Phenol Reagent after addition of Na_2CO_3 which deepened on standing, whilst the substrate DD, in contrast, remained colourless (Todd 1999, not shown). In reactions with the Fast Violet B salt, the incubation mixture with the metabolites showed the same dark red colour as that of 2-OH-DD in this reaction, whereas the colour observed with DD was pale yellow (not shown). The identical colours of the 5-day incubation mixtures and 2-OH-DD in these reactions further confirm the phenolic nature of the metabolite.

Attempts to purify the metabolites for identification by UV absorption spectra and mass spectrometry were unsuccessful, but due to M-VIII and M-IX being more polar than M-X, it is possible that these were indeed dihydroxylated derivatives of DD.

Discussion

We investigated the ability of three green algae from different sources to biotransform the environmental pollutants NPH (a PAH), DD and DF (diaryl ethers). *C. vulgaris*, *Scenedesmus* SI1 and *Ankistrodesmus* SI2 biotransformed NPH, DD and DF, respectively, to more polar metabolites consisting of aromatic structures. Previous work in our laboratory using phenol as a simple aromatic substrate showed that these three algae do not degrade the aromatic ring to acquire intermediates, reducing power or ATP, therefore, the majority of NPH, DD and DF remained untransformed. Green algal formation of 1-naphthol from NPH has been previously observed with an *Oscillatoria* species (a blue-green alga), a *Chlamydomonas* species and other *Chlorella* species (Cerniglia et al. 1980a, b), in which the total extent of NPH metabolism ranged from 0.1–2.4%. The amount observed here of 0.65% conversion of NPH into 1-naphthol is thus comparable with these other algal species. Contrastingly, there is no information available on the biotransformation of diaryl ethers by green algae, although the yeast *Trichosporon mucoides* biotransformed DF to five major metabolites (without ring cleavage), which were identified as 1-, 2-, 3-, 4-OH-DF and 2,3-dihydroxy-DF (Hammer et al. 1998). *T. mucoides* transformed more than 50% of the DF within 8 hours, thus the rate of transformation observed by this yeast was much higher than that observed here by the alga *Ankistrodesmus* SI2. Strains of the bacteria *Sphingomonas* actually mineralise DF and DD for growth via hydroxylated metabolites (Wittich et al. 1992), thus the transformation of 3.8% of DD to a monohydroxlyated DD by the alga *Scenedesmus* SI1 is low in comparison.

The formation of monohydroxylated derivatives such as 1-naphthol (M-I); 2-, 3-, 4- OH-DF (M-V, -VI, -IV) and 2-OH-DD (M-X) suggests the use of a cytochrome P-450 monooxygenase system in these algae similar to that in yeast and fungi, with which aerobic conditions would be obligatory. Supplementary anaerobic biotransformation experiments (not presented) showed that in the absence of oxygen, no metabolites were formed thus indicating that the biotransformations normally taking place required the incorporation of molecular oxygen into the aromatic ring by oxygenases, rather than by incorporation of –OH derived from water (Todd 1999).

Monohydroxylation could occur by direct hydroxylation of the aromatic ring, through cytochrome P-450, with the reduction of the remaining oxygen molecule to water (Cerniglia et al. 1980a; Cerniglia 1984). Another possibility is that the monohydroxylated compounds are formed by spontaneous isomerization, via the NIH shift, of the relative arene oxides formed by monooxygenation, again through cytochrome P-450, as described by Cerniglia and Gibson (1977) for the oxidation of NPH and anthracene by the fungus *Cunninghamella elegans*, and by Schauer et al. (1995) for the oxidation of diphenyl ether by the yeast *Trichosporon beigelii*.

If the unidentified M-II and M-III from DF, and M-VIII and M-IX from DD are indeed dihydroxylated derivatives, then there are also several possible mechanisms for their formation. The pathway via *trans*-dihydrodiols involves two separate steps of monooxygenation, as shown by *T. beigelii* and *T. mucoides* which biotransformed monohydroxylated derivatives of DE (Schauer et al. 1995) and DF (Hammer et al. 1998). In contrast, the route via *cis*-dihydrodiols

would implicate a dioxygenase mechanism usually seen in prokaryotic microorganisms (Dunn & Gunsalus 1973; Ensley et al. 1982; Yen & Serdar 1988; Gibson et al. 1990). Although *cis*-dihydrodiols were not detected in the present study, this mechanism of formation cannot be ruled out because 1-naphthol (M-I) can also be formed by the dehydration of naphthalene *cis*-dihydrodiol (Cerniglia et al. 1980a). These studies showed evidence for the functioning of both mono- and di-oxygenase systems in prokaryotic and eukaryotic microalgae, but the mechanisms for the formation of 1-naphthol as a major metabolite were not elucidated.

Additionally, the almost complete transformation of the PAH benzo[*a*]pyrene to *cis*-dihydrodiols was shown by a *Selenastrum*, *Scenedesmus* and *Ankistrodesmus* species (Lindquist & Warshawsky 1985a, b; Schoeny et al. 1988; Warshawsky et al. 1995). As no *trans*-dihydrodiols were observed in these experiments with benzo[*a*]pyrene, a metabolic route similar to that observed in bacteria, rather than that observed in fungal and mammalian systems, seemed likely in these algae.

In the present study, it cannot be said whether the algae biotransformed the xenobiotics by a monooxygenase or dioxygenase pathway. This is, however, the first report of a purely algal biotransformation of diaryl ethers. Further studies would be required to determine the mechanism of hydroxylation used by *C. vulgaris*, *Ankistrodesmus* SI2 and *Scenedesmus* SI1 to biotransform NPH to 1-naphthol (M-I); DF to hydroxylated derivatives (M-IV, -V, -VI), and DD to the tentatively identified metabolite 2-hydroxydibenzo-*p*-dioxin (M-X), respectively.

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