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# Interaction of Higher Marine Fungi with the Herbicide Atrazine. II. Sorption of Atrazine to Four Species of Marine Fungi

Mark J. Schocken and Marilyn K. Speedie

*Department of Medicinal Chemistry and Pharmacognosy, University of Maryland at Baltimore, 636 West Lombard St., Baltimore, MD 21201*

The marine fungi contribute much to the formation of vascular plant detritus in estuaries due to their abundance and degradative potential as evidenced by distribution studies (KOHLMEYER and KOHLMEYER 1979) and biochemical analysis (GESSNER 1980; MORRISON and WHITE 1980; WHITE et al. 1980). In addition to its role in the estuarine food web, detritus also concentrates pollutants through adsorption to the organic matrix and uptake by the attached microbial community (ODUM and DRIFMEYER 1978). The higher marine fungi, therefore, are well positioned to modify pollutants, including pesticides, through adsorption, uptake and biodegradation. Conversely, pollutants toxic to these fungi may perturb the marine detrital food web. Reports of the interaction of marine fungi with pesticides are quite limited (HODKINSON 1976; SGUROS and QUEVEDO 1978).

The interaction of the marine fungi with the herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) is of special importance, as the widespread use of this herbicide on agricultural lands has resulted in its accumulation in the surface microlayer, water column, suspended particulates and bottom sediments of the productive Chesapeake Bay (CORRELL et al. 1978; WU et al. 1980). Atrazine has been identified as one of several persistent pesticides which may potentially cause problems in aquatic environments (MORLEY 1977).

This report describes the extent of atrazine bioaccumulation by four species of marine fungi previously shown to mediate the loss of atrazine from an artificial seawater medium (ASWM) under cometabolic conditions (SCHOCKEN, SPEEDIE, AND KIRK, Mycologia, in press). Studies of pollutant bioaccumulation by microorganisms rarely differentiate between adsorption and absorption (BAUGHMAN and PARIS 1981). However, this study does distinguish between loosely associated (desorbable) atrazine and that which is either very strongly adsorbed to the cell surface or internalized. The sum of the adsorption and absorption processes which result from atrazine removal from the surrounding medium has been termed "sorption" and has been the focus of this study.

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## MATERIALS AND METHODS

### Selection of fungi

Four species of higher marine fungi (*Dendryphiella salina* (Suth.) Pugh et Nicot, *Leptosphaeria oraemaris* Linder, *Periconia prolifica* Anastasiou, and *Trichocladium achrasporum* (Meyers et Moore) Dixon in Shearer et Crane were chosen from 50 isolates based upon the extent of their interaction with atrazine (SCHOCKEN, HARTLEY, and SPEEDIE, 1981, Abstr. Annu. Meet. Am. Soc. Microbiol., Q87, p. 215; SCHOCKEN et al., Mycologia, in press) and their distribution patterns within Chesapeake Bay (SHEARER 1972; KIRK and BRANDT 1980).

### Materials

Analytical standards of atrazine (99.24%) were provided by the United States Environmental Protection Agency, Beltsville, MD. Radiolabeled ($^{14}$C-ring) atrazine (specific activity: 44 µCi/mg) as well as unlabeled dealkylated and dechlorinated *s*-triazine derivatives were gifts of the Ciba Geigy Corp. (Greensboro, NC). Solvents used for extraction and thin-layer chromatography (TLC) were reagent grade or better. All other chemicals used were of the highest purity available from suppliers. TLC was carried out on silica gel precoated analytical aluminium backed strips with fluorescent indicator (EM Laboratories, Elmsford, NY).

### Methods

The four species of marine fungi were grown on maintenance medium and inoculated into an artificial seawater medium. Both media have been previously described (SCHOCKEN et al., Mycologia, in press). Inocula consisted of either filtered spore/mycelial suspensions (*D. salina*, *L. oraemaris*, and *T. achrasporum*) or mycelial plugs (*P. prolifica*).

Fungi were inoculated (in triplicate) into ASWM containing glucose (0.1%), ammonium nitrate (0.02%), $^{14}$C-atrazine (1 µCi) and unlabeled atrazine (10 ppm). Cultures were incubated at 27-28 C on a New Brunswick rotary shaker at 150 rpm and harvested between 17 and 25 days. Mycelia were filtered over Whatman no. 4 paper using gentle suction. Filtrates were saved and mycelia transferred to 25 ml receiving flasks and washed with three portions of 5.0 ml ASWM. Radioactivity present in both filtrates was determined by liquid scintillation counting (LSC). Mycelia were then transferred to 10.0 ml methanol and ultrasonicated in an ice bath until microscopic examination of portions of cellular material revealed extensive cellular disruption. The resulting material was centrifuged at 30,000g for 20 min at 4 C. The supernatant was decanted and its radioactivity determined by LSC. The pellet of cellular fragments was solubilized by either alkali (Protosol, New England Nuclear, Boston, MA) or the perchloric acid procedure of MAHIN and LOFBERG (1966). Radioactivity released upon solubilization was determined by LSC.

The culture filtrates (containing non-sorbed radioactivity) were combined and extracted with 3 x 75 ml ethyl acetate. The organic phase was taken to dryness under reduced pressure; the residue redissolved in a small quantity of ethyl acetate, and spotted along with unlabeled reference standards on silica gel TLC plates. The methanol extracts of the sonicated mycelia were treated similiarly. The plates were developed in several solvent systems (hexane-n-butanol-acetic acid [4:5:0.1]; ethyl acetate-hexane [3:7]; chloroform-ethanol-acetic acid [90:5:5]) in order to separate atrazine from its dealkylated and dechlorinated derivatives. Spots corresponding to the unlabeled standards were located by the quenching of the fluorescent indicator. The location of radioactivity on the developed plates was determined by scraping 1 cm sections of the developed chromatograms into counting vials, adding scintillation fluid and determining radioactivity present by LSC.

## RESULTS

The four species of higher marine fungi used in this study were able to mediate the loss of the herbicide atrazine from a surrounding artificial seawater environment under cometabolic conditions. The nature and extent of removal is shown in Table 1. Atrazine and/or atrazine degradation products, desorbable by successive ASWM washes, were associated with each species. The percentage of atrazine held in this type of "loose" association ranged from 0.8±0.1% (*T. achrasporum*) to 2.2±0.6% (*L. oraemaris*). The percentage of atrazine and/or its methanol soluble degradation products which either were tightly adsorbed to cellular surfaces or internally stored by the fungus was greatest in *T. achrasporum* (4.6±3.3%) and *P. prolifica* (4.0±0.4%). The results presented in Table 1 suggest that radioactivity was also associated with the methanol-extracted particulate fractions (cell fragments). This was particularly evident with *P. prolifica* in which 1.6±0.2% of the initial radioactivity was released only upon solubilization of the particulate fraction. Similarly, 2.5±0.4% of the initial radioactivity added to *P. prolifica* cultures were found to be associated with its abundantly produced asexual spores.

It was determined by extraction and TLC analysis (previously described in MATERIALS AND METHODS) that the majority of radioactivity both in the culture filtrates and associated with the fungi corresponded to $^{14}$C-atrazine. Greater than 95% of the radioactivity in the ethyl acetate extracts of the culture filtrates corresponded to atrazine. The radioactivity released by the methanol extraction of the sonicated mycelia also indicated that the majority corresponded to atrazine (92.1% for *P. prolifica;* 93.9% for *T. achrasporum;* 94.7% for *L. oraemaris; D. salina* was not determined). Radioactivity not corresponding to atrazine (in both the ethyl acetate and methanol extracts) was found to cochromatograph with the more polar dealkylated and dechlorinated *s*-triazine derivatives. Results therefore suggest that although atrazine degradation does occur, it is not extensive under the conditions employed in this study.

TABLE 1. SORPTION OF ATRAZINE TO FOUR SPECIES OF MARINE FUNGI

| Distribution of radiolabel[a] (%) | Organism *D.salina* | *L.orae-maris* | *P. pro-lifica* | *T. achra-sporum* |
|---|---|---|---|---|
| Activity sorbed released by ASWM washings | 1.6±0.2%[b] | 2.2±0.6% | 1.7±0.1% | 0.8±0.1% |
| released by sonication and MeOH extraction | 0.2±0.1% | 1.2±0.3% | 4.0±0.4% | 4.6±3.3% |
| released by solubilization of cell fragments | 0.1±0.0% | 0.2±0.1% | 1.6±0.2% | 0.4±0.1% |
| released by solubilization of spores | n/a[c] | n/a | 2.5±0.4% | n/a |
| Activity non-sorbed | 98.5±4.3% | 93.8±3.8% | 82.0±3.9% | 91.5±2.5% |
| Activity recovered | 100.3±4.3% | 97.4±3.4% | 91.9±3.8% | 96.9±1.0% |

[a]Corrected for background and quench.
[b]Mean ± SD, n=3.
[c]Not applicable.

## DISCUSSION

The $^{14}$C-atrazine which can be removed by successive ASWM washes indicates a relatively loose binding between the herbicide and the fungal cell surface. Results indicated (Table 1) that this type of removal of herbicide from the surrounding environment was most evident for *L. oraemaris* relative to the other three species of fungi. Interestingly, other studies have indicated that an adhesive polysaccharide capsule is produced by members of the genus, *Leptosphaeria* (SZANISZLO et al. 1968; SZANISZLO and MITCHELL 1971). Although binding may be attributable to a variety of mechanisms, one may speculate that the adhesive biopolymer produced by *L. oraemaris* may play a role in the association of atrazine with the marine fungus.

The recovery of radioactivity initially added to the cultures

was more or less complete (within experimental error) for *D. salina* and *L. oraemaris*. However, recoveries were incomplete particularly with respect to *P. prolifica*. This suggests that the fungus may have the ability to mineralize the molecule. Studies are therefore being conducted to determine the extent of $^{14}CO_2$ evolved from *P. prolifica* cultures incubated with $^{14}$C-ring labeled atrazine.

The four species of higher marine fungi used for this study are widely distributed in the estuarine environment (SHEARER 1972; GESSNER and KOHLMEYER 1976; KIRK and BRANDT 1980) and would therefore have the potential to interact with atrazine in nature. The ability to remove soluble atrazine from the surrounding medium through sorption processes suggests an environmental role for the marine fungi. This study has shown that a portion of the sorbed herbicide is loosely bound to the cell surface whereas another portion is more tightly associated and consequently less available to the external environment. The combined processes result in bioaccumulation of atrazine and may contribute to its transport and redistribution through the estuary. This may be especially significant with *P. prolifica* whose abundantly produced spores would greatly increase the organism's surface area.

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