Utilization of Methylthio-s-Triazine for Growth of Soil Fungi

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Aspergillus niger van Tieghem, Aspergillus tamarii Kita, and Aspergillus flavus Link ex Fries utilized the methylthio moiety of 2,4-bis(isopropylamino)-6-methylmercapto-s-triazine (prometryne) as a sulfur nutrient source. Other soil fungal isolates not affected by prometryne concentrations to 1 mg/ml culture included: Aspergillus oryzae (Ahlburg) Cohn, Curvularia lunata (Wakker) Boedijn, Trichoderma viride Persoon ex Fries, Alternaria tenuis Nees ex Corda, Penicillium funiculosum Thom, and Paecilomyces variotii Bainier.

The methylthio analogue of propazine, [2-chloro-4,6-bis(isopropylamino)-s-triazine], termed prometryne [2,4-bis(isopropylamino)-6-(methylthio)-s-triazine], has reduced soil residual herbicide activity with an increased usage spectrum as compared to the corresponding chlorinated s-triazine.

Kearney et al. (5) found that s-triazine metabolites of Aspergillus fumigatus Fresenius differed from the 2-chloro-4,6-bis(ethylamino)-s-triazine (simazine) metabolites occurring within green plants and proposed a degradation pathway that does not involve the hydroxy analogue reported to occur in higher plants. Gysin (2) proposed that some soil microorganisms appear to degrade prometryne by oxidation of the methylthio group to a sulfoxide or sulfone. Other soil reactions and known degradation pathways of s-triazine herbicides were recently reviewed by Harris et al. (3).

Objectives of this study were to screen prominent soil fungal isolates for their ability to utilize the methylthio moiety and to determine interaction growth responses in factorial prometryne and sulfate-sulfur level combinations.

METHODS AND MATERIALS

Isolates of common soil-inhabiting fungi including Alternaria tenuis Nees ex Corda, Curvularia lunata (Wakker) Boedijn, Trichoderma viride Persoon ex Fries, Penicillium funiculosum Thom, Paecilomyces variotii Bainier, Aspergillus flavus Link ex Fries, Aspergillus niger van Tieghem, Aspergillus tamarii Kita, and Aspergillus oryzae (Ahlburg) Cohn were cultured on broth media containing levels of 0, 0.01, 0.1, 0.5, and 1.0 mg/ml of prometryne. An 80% emulsifiable prometryne formulation was used having 13.28% methylthio sulfur. The growth medium and culture techniques were essentially the same as were used in the substituted urea degradation studies reported previously (8) with cultures incubated at 30 C for 96 hr.

Prometryne degradation with differential sulfate-sulfur media levels was determined for A. niger, A. tamarii, and A. flavus cultured on nutrient broth containing 0, 0.01, 0.1, 0.5, and 1.0 mg/ml of prometryne in factorials including 0, 2.5, 12.5, and 25.0 μg of sulfur per ml of culture as sodium sulfate with culture techniques as reported previously (4).

Triazine extraction of residual media and purification for thin-layer chromatography detection were essentially the procedures of Mattson (6) for hydroxy-s-triazine metabolites, and results were confirmed with fluorescence quenching of prometryne spots by using Whitenberg's tert-butyl hypochlorite method (9). Quantitation of plate readings was accomplished with adaptation of the Polaroid recording procedure (1).

RESULTS AND DISCUSSION

All soil fungal isolates used in these culture studies indicated high tolerance for the methylthio-s-triazine, prometryne, at concentrations up to 0.1% in broth media without presynthesized growth factors. However, differences in growth rates were apparent although not statistically significant. Fungi that continued a uniform growth to a prometryne culture level of 10 mg/ml with low coefficients of variation were A. niger 5.45%, A. flavus 6.38%, A. tamarii 10.05%, and A. oryzae 8.80%. These isolates are soil epipedian microfloral dominants of the acid, siliceous, thermic, Psammemic paleustalf, Eufaula sand, used in this study.

The factorial prometryne X sulfate sulfur studies indicated A. flavus, A. tamarii, and A. niger...
TABLE 1. Fungal growth with prometryne at differential sulfur levels
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| Fungus        | Conc of S (Na2SO4) | Prometryne conc in media | TLC assay of Media ± sp% |
|---------------|--------------------|--------------------------|--------------------------|
|               | 0                  | 0.01                     | 0.10                     | 0.20                     | 0.50                     | 1.00                     |
| Aspergillus niger | 0                  | 0.14e                    | 0.12                     | 0.53                     | 0.94                     | 5 ± 2                    |
|                | 2.5                | 0.66                     | 0.70                     | 0.69                     | 1.08                     | 1.45                     | 6 ± 1                    |
|                | 12.5               | 2.21                     | 2.24                     | 2.28                     | 2.73                     | 3.31                     | 8 ± 2                    |
|                | 25.0               | 3.81                     | 3.98                     | 3.71                     | 4.83                     | 4.91                     | 8 ± 3                    |
| A. tamarii    | 0                  | 0.11                     | 0.13                     | 0.12                     | 0.64                     | 1.16                     | 8 ± 3                    |
|                | 2.5                | 0.54                     | 0.50                     | 0.53                     | 0.80                     | 1.12                     | 8 ± 2                    |
|                | 12.5               | 2.33                     | 2.04                     | 1.81                     | 2.10                     | 2.40                     | 10 ± 2                   |
|                | 25.0               | 3.35                     | 3.50                     | 3.01                     | 3.21                     | 3.78                     | 9 ± 3                    |
| A. flavus     | 0                  | 0.11                     | 0.10                     | 0.27                     | 1.01                     | 1.47                     | 9 ± 3                    |
|                | 2.5                | 0.64                     | 0.63                     | 0.70                     | 1.43                     | 1.86                     | 8 ± 2                    |
|                | 12.5               | 1.95                     | 2.05                     | 2.28                     | 2.60                     | 3.40                     | 10 ± 2                   |
|                | 25.0               | 4.41                     | 4.14                     | 4.71                     | 4.23                     | 4.66                     | 10 ± 3                   |

a F values for all treatment levels, interactions, and per cent degradation were highly significant. Calculated interaction response surface equations in which Y = growth (mg) X1 = prometryne (mg/ml), X2 = S (µg/ml), CV = coefficient of variation. A. niger Y = 37 + 157 X1 + 3.9 X2 − 3.3 X1 X2 − 0.011X1 X2 + 0.138X1 X2 ; R2 = 0.974, CV = 5.45%. A. tamarii Y = 26 + 120 X1 + 3.1 X2 + 2.1 X1 X2 − 0.022X1 X2 + 0.012X1 X2 ; R2 = 0.791, CV = 11.16%. A. flavus Y = 47 + 131 X1 + 5.5 X2 + 0.7 X1 X2 − 0.049 X1 X2 − 0.37 X1 X3 ; R2 = 0.978, CV = 9.34%. e

b Expressed as micrograms per milliliter. j
c Expressed as milligrams per milliliter.
d Expressed as decrease per milligram per milliliter.
e Mycelium dry weight in grams.

capabilities for methythio moiety utilization of prometryne as a sulfur source (Table 1). Interaction in total growth within prometryne and sulfate-sulfur level combinations were highly significant with low coefficients of variation. Sulfur contents of the four prometryne levels as milligrams per milliliter media were 0.001, 0.013, 0.063, and 0.125, respectively. The non-triazine sulfur content (emulsifier) of this prometryne formulation was less than 0.5 µg/mg of prometryne and separately would not support measurable fungus growth in these studies. Efficiency in utilizing the methylthio group increased with growth level. Highest growth levels were attained with sulfate sulfur amendment, and highly significant growth interaction responses were apparent within treatment combinations.

The marked response of ubiquitous soil microfloral associations to sulfur levels has long been utilized in soil fertility manipulations. Results from previous studies (4) established growth responses of A. niger isolates to sulfur-containing compounds and to media sulfate-sulfur levels. Sulfate supplied at 50 µg/ml of media resulted in nearly peak mycelial yields with an estimated 50% response attained with the 25-µg level. Culture vigor induced below optimal sulfate levels applied in combination with prometryne levels may account for an increased utilization of the methylthio moiety.

Calculation of three-dimensional response surfaces characteristic of growth interaction effects with sulfate-sulfur and prometryne levels were obtained with the least-squares method for a solid configuration and are useful in projecting response estimates. Results with A. niger and A. tamarii yielded highly significant convex response curvatures (quadratic) for interaction effects with the intermediate treatment-level combinations with very low coefficient of variation. The A. flavus isolate, a particularly vigorous decomposer of resistant carbonaceous compounds, utilized the methylthio sulfur more effectively than the other fungi selections in this study with a low coefficient of variation, 9.34%. The highly significant concave surface response curvature with intermediate treatment-level interactions indicated that growth would continue to increase beyond the upper treatment limit restraints used in these studies.

Efforts were unsuccessful for inducing these fungi to utilize this triazine compound as an only
source of nitrogen or as an only carbon source. Although Mickovski and Verona (7) reported apparent nitrogen utilization by *A. niger* from prometryne, as indicated by fungal growth on agar media, they did not report chemical measurement of degradation products or determine the nontriazine emulsifier contribution.

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