

Metabolism of 3-Methylindole by a Methanogenic Consortium

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A methanogenic 3-methylindole (3-MI)-degrading consortium, enriched from wetland soil, completely mineralized 3-MI. Degradation proceeded through an initial hydroxylation reaction forming 3-methyloxindole. The consortium was unable to degrade oxindole or isatin, suggesting a new pathway for 3-MI fermentation.

Aromatic N-heterocyclic compounds, including indoles, are produced by the chemical industry for a variety of applications, including pharmaceuticals, pesticides, and dye-stuffs. Widespread use of these chemicals contributes to the pollution of soil, groundwater, and surface waters (6, 10, 12). In many cases, the habitats that these chemicals enter are anaerobic. Some members of this chemical class are toxic (13). 3-Methylindole (3-MI) has been shown to induce acute bovine pulmonary edema and emphysema (4).

Indole degradation in sewage sludge (2, 8, 9) and in soil and sediments (9) under both methanogenic and denitrifying conditions has been investigated. Recently, Gu and Berry (7) examined indole metabolism by an indole-degrading methanogenic consortium enriched from sewage sludge. Their studies established that indole degradation proceeded through a two-step hydroxylation pathway yielding oxindole and isatin subsequent to cleavage between the C-2 and C-3 atoms on the pyrrole ring of indole. The biodegradability of indolic compounds, including 3-MI, under sulfate-reducing conditions was examined by Bak and Widdel (1). They isolated from marine sediment a sulfate-reducing bacterium, *Desulfobacterium indolicum*, that used indole as the sole electron donor and carbon source. Although a 3-MI degrader was not isolated, their study demonstrated that 3-MI can serve as a carbon source for anaerobes.

In this study, we used a 3-MI-degrading consortium enriched from wetland soil classified as belonging to a fine-loamy, mixed, nonacid, thermic family of Typic Sulfaquents (5) and collected in Surry County, Virginia. Surface soil (5 to 10 cm; pH 6.8) was collected in a 2-liter, narrow-neck glass bottle, which was filled to the neck and capped for transport to the laboratory. Indolic compounds, 3-MI, indole, oxindole, and isatin (purity, 97 to 99%), purchased from Aldrich Chemical Co., Inc. (Milwaukee, Wis.), were used without further purification. 3-Methyloxindole, the identity of which was confirmed by mass spectrometry, UV spectroscopy, and proton nuclear magnetic resonance spectrometry, was obtained from a 3-MI-degrading enrichment culture as described by Gu and Berry (7). A mineral salts medium, adjusted to pH 7.1, was prepared as described by Boyd et al. (3). Wetland soil cultures were prepared by adding 3-MI (10 μmol dissolved in 0.1 ml of methanol) to 160-ml serum bottles. Methanol was evaporated by use of O_2 -free N_2 . Deoxygenated mineral salts medium (60 ml; total solids content, 51 mg ml^{-1}) and wetland soil slurry (40 ml) were added to the serum bottles, which were then closed with thick butyl rubber stoppers and aluminum crimp seals.

Cultures were monitored, and transfers were made every 25 days (approximately), on the basis of substrate depletion, by adding 20 ml of the 3-MI-depleted culture to serum bottles containing 80 ml of freshly prepared mineral salts medium (plus 3-MI). Degradation experiments, conducted in triplicate, were initiated by transferring 20 ml of 3-MI-depleted culture medium from the enrichment cultures to 160-ml serum bottles containing 80 ml of mineral salts medium with either 100 or 150 μmol of the selected indolic compound. Indole-amended sterile control cultures were prepared by adding 20 ml of inoculum and 80 ml of medium to serum bottles as described above and autoclaving the bottles for 30 min. Following sterilization, indoles (dissolved in 0.1 ml of methanol) were injected into the controls with a syringe (fitted with a 0.2- μm -pore-size membrane filter and needle). Unamended control cultures were prepared in the same manner but without the addition of indolic substrates. All cultures were incubated under static conditions in the dark at 25°C. Additional serum bottle cultures were initiated for sacrifice in the event that a metabolite was detected during the fermentation trial.

Samples (1 ml) of culture medium were collected, stored, and analyzed by high-performance liquid chromatography (HPLC) as described by Gu and Berry (7). Methane production in serum bottle cultures was measured by gas chromatography as outlined by Gu and Berry (7). In preparation for metabolite identification, contents of replicate serum bottle cultures were filtered (no. 1 filter; Whatman, Inc., Clifton, N.J.), acidified, and extracted three times with 25 ml of CH_2Cl_2 . The extract was evaporated to dryness, the residue was dissolved in CH_2Cl_2 and dried by passage through Na_2SO_4 , and the volume was reduced to 0.2 ml under N_2 . Thin-layer chromatography with 0.25-mm-thick precoated silica gel plates (no. 06-600C, Fisherbrand; Fisher Scientific, Springfield, N.J.) was used to separate components. The mobile phase used to separate the 3-MI metabolite ($R_f = 0.62$) was CH_2Cl_2 -methanol (100:1 [vol/vol]) and that used for separation of the indole metabolite ($R_f = 0.46$) was hexane- CH_2Cl_2 -ethyl acetate (6:1:3 [vol/vol/vol]). Metabolites were extracted from the silica gel with methanol. A portion of the methanol extract was evaporated under N_2 in preparation for mass spectrometry. The remainder was used to determine the UV spectrum. Mass spectral data, confirming the identification of 3-MI and indole metabolites, were obtained with a 7070E-HF high-resolution mass spectrometer (VG Analytical, Manchester, United Kingdom) with a direct insertion probe at an electron energy of 70 eV.

The same pattern of substrate (3-MI) disappearance and metabolite appearance was observed for all transfer cultures, including the ninth one (Fig. 1). On the basis of an

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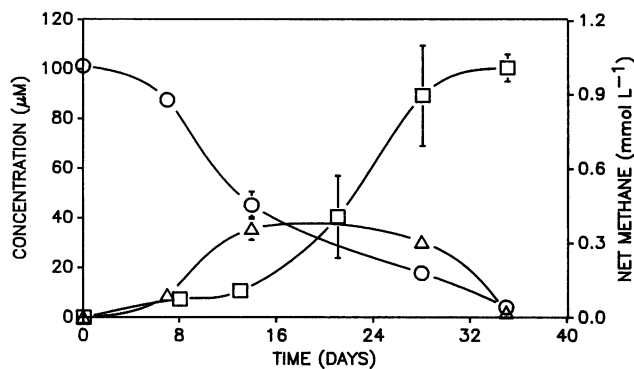


FIG. 1. Disappearance of 3-MI (○) and appearance of 3-methyl-oxindole (△) and methane (□) in transfer cultures (ninth transfer).

earlier investigation, the metabolite was expected to be 3-methyloxindole (1,3-dihydro-3-methyl-2H-indol-2-one) (7). No appreciable losses of 3-MI were observed in sterile controls. Net methane production in 3-MI-degrading enrichment cultures proceeded according to the predicted stoichiometric relationship $C_9H_9N + 8.5H_2O \longrightarrow 2.75CO_2 + 5.25CH_4 + NH_4HCO_3$ (11), providing evidence that 3-MI was mineralized. The identity of the metabolite was confirmed by comparison with an authentic 3-methyloxindole standard on the basis of the HPLC retention time (4.10 min), the UV absorbance (λ_{max} , 249 nm), and mass spectral analysis (molecular ion peak at m/z 147).

The 3-MI-degrading enrichment cultures were able to transform indole. The accumulation of a metabolite in the culture medium correlated stoichiometrically with the disappearance of indole (Fig. 2). Net differences in methane production between indole-amended and unamended control cultures were negligible, indicating that indole was not mineralized by this consortium. Autoclaved sterile controls showed a negligible disappearance of indole. On the basis of results from previous investigations (2, 7), oxindole (1,3-dihydro-2H-indol-2-one) was suspected to be the metabolite. The identity of the metabolite was confirmed by comparison with an authentic oxindole standard on the basis of the HPLC retention time (3.23 min), the UV absorbance (λ_{max} , 247 nm), and mass spectral analysis (m/z 133).

Since indole did not serve as a carbon and energy source for the 3-MI-degrading methanogenic consortium, oxindole

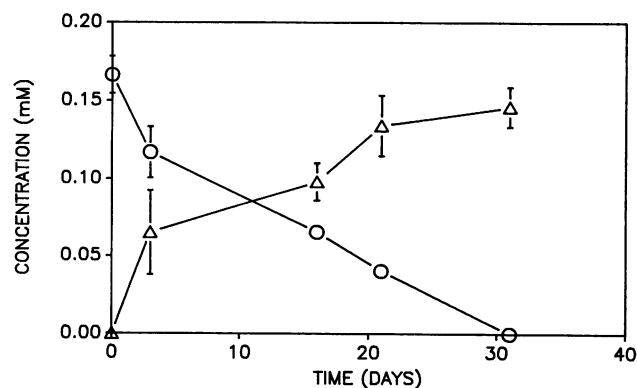


FIG. 2. Disappearance of indole (○) and appearance of oxindole (△) in transfer cultures (ninth transfer).

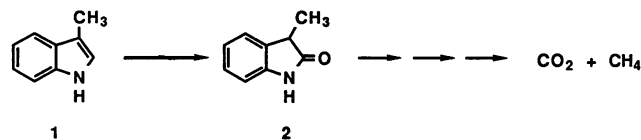


FIG. 3. Proposed pathway for 3-MI (compound 1) fermentation by a 3-MI-degrading methanogenic consortium enriched from wetland soil. 3-MI is first converted to 3-methyloxindole (compound 2).

production probably occurred as a result of 3-MI hydroxylase(s) activity in the 3-MI-grown cell inoculum. Neither oxindole nor isatin was degraded by the 3-MI-degrading consortium.

The hydroxylation reaction initiating the metabolism of 3-MI by the 3-MI-degrading methanogenic consortium (Fig. 3) was identical to that carried out by an indole-degrading methanogenic consortium enriched from sewage sludge (7). In both cases, degradation was initiated by the addition of oxygen to position 2 of the pyrrole ring. In indole fermentation, the initial hydroxylation reaction (forming oxindole) was followed by a second hydroxylation reaction (forming isatin) prior to ring cleavage between the C-2 and C-3 atoms on the pyrrole ring of indole.

In the present study, indole was converted to oxindole by the 3-MI-degrading methanogenic consortium. This consortium was unable, however, to hydroxylate position 3 of oxindole (forming isatin) and was also unable to mineralize indole. These results, together with the fact that this consortium failed to degrade oxindole or isatin, provide strong evidence that the fermentation of 3-MI did not proceed through the oxindole-isatin pathway. To verify this contention, it will be necessary to isolate and identify the metabolite produced immediately after the formation of 3-methyloxindole. Fermentation of 3-MI through the oxindole-isatin pathway would require that the methyl group be removed.

Fermentation of indole proceeds through the oxindole-isatin pathway, as demonstrated in a recent study conducted by Gu and Berry (7). Those researchers also reported on an indole-degrading methanogenic consortium that hydroxylated 3-MI, forming 3-methyloxindole, but was unable to use this indolic compound as a carbon and energy source. These results, together with the results from the current study, suggest there may be two different fermentation pathways for indole degradation.

REFERENCES

- Bak, F., and F. Widdel. 1986. Anaerobic degradation of indolic compounds by sulfate-reducing enrichment cultures, and description of *Desulfobacterium indolicum* gen. nov., sp. nov. *Arch. Microbiol.* **146**:170-176.
- Berry, D. F., E. L. Madsen, and J.-M. Bollag. 1987. Conversion of indole to oxindole under methanogenic conditions. *Appl. Environ. Microbiol.* **53**:180-182.
- Boyd, S. A., D. R. Shelton, D. Berry, and J. M. Tiedje. 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. *Appl. Environ. Microbiol.* **46**:50-54.
- Carlson, J. R., E. O. Dickinson, M. T. Yokoyama, and B. Bradley. 1975. Pulmonary edema and emphysema in cattle after intraruminal and intravenous administration of 3-methylindole. *Am. J. Vet. Res.* **36**:1341-1347.
- Edmonds, W. J., R. L. Hodges, and C. D. Peacock, Jr. 1990. Tidewater Virginia tidal wetland soils: a reconnaissance characterization. VAES bulletin 90-2. Virginia Agricultural Experiment Station, Virginia Polytechnic Institute and State University, Blacksburg.
- Gloffely, D. E., A. W. Taylor, A. R. Isensee, J. Jersey, and S. Glenn. 1984. Atrazine and simazine movement to Wye River

- Estuary. *J. Environ. Qual.* **13**:115–121.
7. **Gu, J.-D., and D. F. Berry.** 1991. Degradation of substituted indoles by an indole-degrading methanogenic consortium. *Appl. Environ. Microbiol.* **57**:2622–2627.
 8. **Madsen, E. L., and J.-M. Bollag.** 1989. Pathway of indole metabolism by a denitrifying microbial community. *Arch. Microbiol.* **151**:71–76.
 9. **Madsen, E. L., A. J. Francis, and J.-M. Bollag.** 1988. Environmental factors affecting indole metabolism under anaerobic conditions. *Appl. Environ. Microbiol.* **54**:74–78.
 10. **Nelson, C. R., and R. A. Hites.** 1980. Aromatic amines in and near the Buffalo River. *Environ. Sci. Technol.* **14**:1147–1149.
 11. **Symons, G. E., and A. M. Buswell.** 1933. The methane fermentation of carbohydrates. *J. Am. Chem. Soc.* **55**:2028–2036.
 12. **Wehtje, G., L. N. Mielke, J. R. C. Leavitt, and J. S. Schepers.** 1984. Leaching of atrazine in the root zone of an alluvial soil in Nebraska. *J. Environ. Qual.* **13**:507–513.
 13. **Wilkes, D. J.** 1981. Animals: bioenvironmental effects, p. 1–163. *In* H. M. Braunstein, E. D. Copenhaver, and H. A. Pfuderer (ed.), *Environmental, health, and control aspects of coal conversion—an information overview*, vol. 2. Ann Arbor Science Publishers, Inc., Ann Arbor, Mich.