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Mutagenic Deactivation of 7, 12-Dimethylbenz(a)anthracene in Nonacclimated Soil

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불순응된 토양에서 이메틸벤조안트라센 돌연변이 유발성의 불화성화

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ABSTRACT

Mutagenic characteristics deactivation of 7, 12-dimethylbenz(a)anthracene was studied in a nonacclimated sandy loam soil at low and neutral pH soil conditions. Soil extracts containing transformation products were separated into three fractions based on HPLC retention time(polarity). Highly polar transformation products of 7, 12-dimethylbenz(a)anthracene demonstrated a negative mutagenic response with the Ames mutagenicity assay, strain TA-100, for both low and neutral pH soils. Moderate and low polar fractions, however, induced mutagenicity for both soil samples with mutagenic ratios similar to those of the parent compound.

INTRODUCTION

Many PAH constituents in hazardous waste have been identified as having mutagenic and carcinogenic characteristics. The application of hazardous waste to soil is restricted to include those wastes that will be rendered less hazardous or nonhazardous by chemical or biochemical reactions in the soil. Biochemical detoxication potential of PAH compounds in soil systems, thus, is important in the design and management of hazardous waste / soil systems containing these PAH compounds for treating and disposing of the wastes without effecting environmental damage and public health hazards(1).

Soil pH may be an important engineering variable for the control of PAH metabolism in soil systems(2). In low pH soil habitats, fungi play a major role in microbial transformations. When soil pH increass to neutrality, the microbial community is dominated by bacterium. Fungi, during the metabolism of PAH compounds, produce epoxide intermediates which are known to be tumor initiators in carcinogenesis(3), while bacteria do not form these intermediates(4). Thus differences in the PAH metabolism between acid and neutral soil may provide an engineering management technique for the detoxification and assimilation of PAH constitutents in soil systems without mutagen formation.

One important PAH compound having high carcinogenic potency is 7, 12-dimethylbenz(a)anthracene. Intragastric or intravenous administration of a single dose of 7, 12-dimethylbenz(a)anthracene induces skin tumors(5), and lung tumors(6).

The objectives of this study were to investigate the mutagenic characteristics deactivation of 7, 12-dimethybenz(a)anthracene in a nonacclimated sandy loam soil. The effect of soil pH on mutagenic characteristics of transformation intermediates was also investigated.

MATERIALS AND METHODS

Mutagenic deactivation studies were performed with the McLaurin sandy loam soil at low pH and the same

oil adjusted to neutral pH soil condition. The McLaurin soil was selected on the basis of its low pH(pH = 4.8). For the neutral pH incubation, the soil pH was adjusted to 7.5 by adding 70mg of $CaCO_3$ to the McLaurin soil.

Soil samples of each pH value were incubated and extracted as described previously(7) except 1000g(dry weight) of 7, 12-dimethylbenz(a)anthracene treated soils(1mg/g) were placed in a 3 l glass beaker. A large amount of soil (100g) was used in order to obtain sufficient amounts of 7, 12-dimethylbenz(a)anthracene metabolites for a mutagenicity assay. Soil extracts were separated into three metabolite fractions based on HPLC retention time(polarity) (0-15 min, 15-33 min, and 35-45 min) and isolated by preparative HPLC on a 21.2mm I.D. x 25cm 15-μ octadecylsilane column(Supelco Inc., Bellefonte, PA) using a water/acetontrile gradient(35%-100%) at a flow rate of 8ml. The HPLC fractions were evaporated to dryness under an aerated hood and reconstituted with dimethylsulfoxide(DMSO).

Mutagenic activities of 7, 12-dimethylbensz(a)anthracene and metabolite fractions were measured with the Ames test(8) using the Salmonella typhimurium TA-100. TA-100, which detects mutagens causing base-pair substitutions, was supplied by Dr. Bruce N. Ames(University of California, Berkeley, CA). Samples were tested on triplicate plates in the standard plate incorporation assay at four dose levels with enzyme activation(S9). 2-Aminofluorene(10 μg/plate) was used as a positive control. Mutagenic potential of test samples was expressed as the mutagenic ratio, i.e., ratio of number of colonies in the presence of a test sample to the number of colonies on a control growth place in the absence of the test sample.

RESULTS AND DISCUSSION

Results for Ames assay testing with strain TA-100 for 7, 12-dimethylbenz(a)anthracene metabolites at low and neutral pH soil are presented in Tables 1, and 2, respectively. The most polar metabolic fraction(Fraction 1) demonstrated a negative mutagenic response(mutagenic ratio less than 2 as described in USEPA(1983)(9) for both soil pH conditions. Metabolite Fractions 2 and 3, however, induced a positive response for both soils. The mutagenic ratios of these metabolite fractions were similar to those of the parent PAH compound. (Table 3)

Table 1. Results for Ames assay testing for metabolites formed ffom 7,12-dimethylbenz (a)anthracene by McLaurin sandy loam soil (low pH).

Metabolite Fraction[a]	Sample Amount (μg/plate)	Revertant Colonies/Plate		Mutagenic Ratio	
		14d	28d	14d	28d
1	0	84	86		
	10	88	86	1.0	1.0
	50	104	96	1.2	1.1
	100	110	102	1.3	1.2
2	0	84	86		
	10	147	149	1.8	1.7
	50	215	184	2.6	2.1
	100	263	219	3.1	2.5
3	0	84	86		
	10	181	190	2.2	2.2
	50	234	195	2.8	2.3
	100	262	268	3.1	3.1

aFraction 1 = HPLC retention time < 15 min, 2 = 15-33 min, and 3 = 35-45 min.

Table 2. Results for Ames assay testing for metabolites formed from 7,12-dimethylbenz (a)anthracene by McLaurin sandy loam soil (neutral pH).

Metabolite Fraction[a]	Sample Amount (μg/plate)	Revertant Colonies/Plate		Mutagenic Ratio	
		14d	28d	14d	28d
1	0	88	87		
	10	83	86	0.9	1.0
	50	96	101	1.1	1.2
	100	100	108	1.1	1.2
2	0	88	87		
	10	154	88	1.8	1.0
	50	192	160	2.2	1.8
	100	248	207	2.8	2.4
3	0	88	87		
	10	213	209	2.4	2.4
	50	254	251	2.9	2.9
	100	272	234	3.1	2.7

[a]Fraction 1 = HPLC retention time < 15 min, 2 = 15-33 min, and 3 = 35-45 min.

Table 3. Results for Ames assay testing for 7,12-dimethylbenz(a)anthracene.

Sample Amount (μg/plate)	Revertant Colonies/Plate	Mutagenic Ratio
Nonsoil extract		
0	87	
10	189	2.2
50	218	2.5
100	263	3.0
Soil Extract		
Low pH		
0	87	
10	190	2.2
50	214	2.5
100	260	3.0
Neutral pH		
0	87	
10	181	2.1
50	227	2.6
100	272	3.1

The mutagenic potential of 7, 12-dimethylbenz(a)anthracene in the soil systems was greater at 14 days than at 28 days after PAH incorporation into soils. The detoxication potential of 7, 12-dimethylbenz(a)anthracene may be important for engineering management and control of hazardous wastes containing this PAH compound since toxicity reduction as a function of incubation time in the soil can be used to assess the success of treatment. The phenomenon of detoxication was also reported by other investigator(10) who found that the mutagenicity of polar transformation products of benzo(a)pyrene increased and then decreased with incubation time, or treatment time, in the soil.

Mutagenic responses for the metabolites formed from low and neutral pH soil were not different (Tables 1 and 2). Soil pH adjustment changed the microbial populations by one order of magnitude, but significant amounts of bacteria and fungi still existed in both soils(Table 4). Thus, differences in the metabolic products between low and neutral pH soil are not likely and similar mutagenic responses between the two soil treatments may result.

Table 4. Effect of soil pH on microbial populations in McLaurin sandy loam soil.

Soil pH	cfu/g soil	
	Bacteria	Fungi
4.8	1.1×10^5	5.3×10^4
7.5	2.5×10^6	4.0×10^3

요 약

불순응된 사질토에서 7,12－이메틸벤조안트라센 돌연변이 유발성의 불활성화에 대한 연구가 행해졌다. 토양에서 형성된 이메틸벤조안트라센의 극성이 강한 대사산물은 Ames분석에서 돌연변이성을 나타내지 않았다. 극성이 중간 또는 약한 대사산물은 돌연변이성을 보였으며, 그 변이비는 모체화합물의 변이비와 유사하였다.

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