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Phospholipids and Proteins from *Rhizobium meliloti* Exposed to Parathion at Different Incubation Times

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A number of rather stable, lipid soluble organic chemicals are widely spread in our environment. The persistence of these chemicals or their metabolites inevitably renders them amenable to uptake by soil microorganisms. The structure and composition of bacterial membranes are profoundly influenced by the environment. Alterations in membrane composition may lead to deleterious effects on fundamental biological processes in which microbial membranes are involved. Rosas et al.(1980) have reported changes in phospholipids and fatty acids from Escherichia coli grown in the presence of four insecticides. More recently, Carranza et al.(1985, 1986) observed alterations in the chemical composition of Rhizobium meliloti exposed to parathion. Antunes Madeira and Madeira (1984) suggested that parathion would perturb normal lipid-protein interactions involved in modulation of enzyme activity from membranes. The aim of the present work was to study the effects of parathion on phospholipids and proteins from R.meliloti at the stationary phase of growth, when the insecticide was added at different times during bacterial growth.

MATERIALS AND METHODS

Rhizobium meliloti 3D0 h13 was kindly provided by Dr. Lilian Frioni (Universidad Nacional de Río Cuarto, Argentina). Cells were grown at 28 ± 2°C in a defined saline medium containing, in grams per liter : Mannitol 10; Yeast Extract 1; K_2HPO_4 0.5; $MgSO_4.7H_2O$ 0.2; NaCl 0.1 (pH 7.0). Stock solution of parathion was prepared as described by Rosas et al.(1985). Parathion was added to 8.6 µM final concentration in the growth medium at 0, 12, 15, 18, 21, 24, 36, and 48 h of incubation. Controls received the equivalent volume of solvent (ethanol). Growth was determined by measuring the optical density at 420 nm.

Cells were harvested at the stationary phase of growth

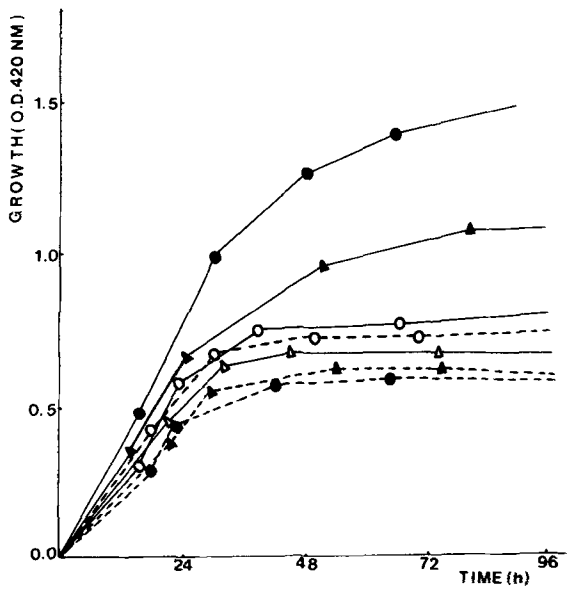

Figure 1. Effect of parathion on the growth of R. meliloti 3D0 h13. Parathion was added to give a 8.6 µM final concentration at different times (from bottom to top: 0, 12, 15, 18, 21, 24 h, and control).

concentrated by centrifugation and washed. Dry weight was determined by lyophilization. Lipids were extracted and subjected to thin-layer chromatography using previously reported procedures (Rosas et al. 1980, 1985). Total lipid phosphorus as well as individual phospholipids were evaluated according to Dodge and Phillips (1967). Proteins were determined by the method of Lowry et al.(1951).

RESULTS AND DISCUSSION

Fig. 1 shows the effects of 8.6 µM parathion on R. meliloti growth. The microorganisms arrived earlier than controls to stationary phase when the insecticide was added from 0 to 21 h of incubation. On the contrary, bacterial growth was similar to that of controls when parathion was added at 24 h of incubation. R.meliloti appeared susceptible to the insecticide during the early logarithmic phase of growth. This effect was not observed when E.coli was treated with parathion (Rosas et al. 1980).

On a dry weight basis, cellular lipid phosphorus

Table 1. Total phospholipids of R.meliloti 3D0 h 13 at the stationary phase of growth with 8.6 µM parathion.

	Total phospholipids mg/g dry weight	% Control
Control	0.989 ± 0.140	
Parathion added at h 0	0.625 ± 0.070(')	63
12	0.765 ± 0.100	77
18	0.787 ± 0.031	80
21	0.865 ± 0.033	87
24	0.840 ± 0.077	85
36	0.876 ± 0.053	89
48	0.905 ± 0.028	92

Data are means ± standard errors from five experiments
(') $p < 0.05$

diminished in microorganisms which were treated with parathion from the start of incubation (Table 1). However, statistically significant differences in lipid phosphorus content between control and treated cells were not observed when the insecticide was added from 12 to 48 h of bacterial growth. The phospholipid composition was maintained in all tested experimental conditions (Table 2).

Because of no statistically significant variations in cellular proteins, the relationships between phospholipids and proteins from parathion treated R.meliloti were slightly lower than those from controls (Table 3).

Although the changes presented in cellular growth, R.meliloti cells which were exposed to 8.6 µM parathion from 12 to 21 h of incubation did not have significant alterations either in phospholipids or in protein contents. When the insecticide was added to the growth medium at the start of incubation, R. meliloti decreased its lipid phosphorus content without changes in the phospholipid composition at the stationary phase of growth. Carranza et al.(1985) had observed that R.meliloti cells which were exposed to parathion had a significant increase in lipid phosphorus content with modifications in the phospholipid composition and a diminution in protein concentration at the logarithmic phase of growth. The difference in cellular composition on parathion treated R.meliloti from logarithmic to stationary phases would be indicative of transitory effects of the insecticide.

Antunes Madeira and Madeira (1984) reported that

Table 2. Phospholipid composition of R.meliloti 3D0 hl3 at the stationary phase of growth with 8.6 µM parathion.

	PI	PS	PA	PC	PE	PG
Control	14	14	11	22	19	20
Parathion						
at h 0	14	15	10	22	18	19
12	15	15	15	18	17	18
18	11	14	11	21	20	21
21	13	15	12	20	23	16
24	13	14	14	20	21	17
36	13	14	14	21	17	20

Results are expressed as % of total lipid phosphorus, means from five experiments. Phospholipids : PI phosphatidyl inositol, PS phosphatidyl serine, PA phosphatidic acid, PC phosphatidyl choline, PE phosphatidyl ethanolamine, PG phosphatidyl glycerol.

Table 3. Protein content of R.meliloti 3D0 hl3 at the stationary phase of growth with 8.6 µM parathion.

	Proteins mg/g dry weight	% Control	Phospholipid/Protein ratio x 10^3
Control	253.7 ± 12.2		4.20
Parathion			
at h 0	215.8 ± 19.4	92	2.90
12	217.7 ± 5.1	92	3.51
18	255.6 ± 7.5	108	3.08
21	243.8 ± 4.1	103	3.51
24	283.0 ± 26.8	120	2.97
36	217.5 ± 13.0	92	3.23

Data are means ± standard errors from five experiments

parathion incorporated better in bilayers with low cholesterol content, and suggested that the insecticide would preferentially accumulate in highly functional membranes, such as bacterial membranes. It is well known that cholesterol is not a bacterial component.

On the other hand, Lohman and Hagedorn (1985) observed a marked parathion adsorption on the lipid containing trilaminar sheath of the green algae Scenedesmus quadricauda. However, little is known about parathion adsorption on biological molecules, especially on microbial compounds.

Further research is needed in order to characterize the nature of parathion interaction with R.meliloti.

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