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Alterations in Lipid Composition of Membranes from *Rhizobium meliloti* Exposed to Parathion

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Rhizobia are exposed to pesticides either present in the soil or applied to inoculated seeds of legume crops. From an ecotoxicological point of view, a population of Rhizobium was considered a highly sensible test parameter for the assessment of side-effects of agrochemicals on soil microorganisms (Domsch et al 1983). Both adverse and innocuous effects of pesticides on essential functions of Rhizobium have been reported (Alexander 1977, Mallik and Tesfai 1983). However, little is known about the effects of insecticides on the chemical composition of bacterial membranes. We had observed (Rosas et al 1985) that membranes from Escherichia coli growing in a medium with parathion accumulated phospholipids with similar fatty acids but different base profiles from that which existed in controls. The aim of the present study was to determine if Rhizobium meliloti exposed to parathion presented membranes with modified chemical composition.

MATERIALS AND METHODS

Rhizobium meliloti 300hl3 was kindly provided by the Centro de Recursos Microbiologicos from Porto Alegre (Brasil). Cells were grown at 28 ± 2°C in a defined saline medium dispensed in Erlenmeyer flasks shaken at a constant rate. The medium contained, in grams per liter: Mannitol 10; Yeast Extract 1; K_2HPO_4 0.5; $MgSO_4 \cdot 7H_2O$ 0.2; NaCl 0.1 (pH 7.0). Stock solution of parathion was prepared and added as described elsewhere (Rosas et al 1985).
Cells were harvested at the exponential phase of growth concentrated and washed. Membranes were isolated using the method detailed by Evans (1969). Dry weight was determined following lyophilization. Proteins were evaluated by the method of Lowry et al (1959). Lipids were extracted and subjected to thin-layer chromatography using previously reported procedures (Rosas

et al 1980). Total lipid phosphorus as well as individual phospholipids were determined by the method of Dodge and Phillips (1967). The methyl-esters of the fatty acids were prepared with the boron trifluoride-methanol reagent, examined in a Varian 2100 gas chromatograph, identified and evaluated as described previously (Rosas et al 1980).

RESULTS AND DISCUSSION

As an initial step of our investigation, we have exposed R.meliloti 300h13 to a parathion concentration which approximated pest control application rates (McRae and Celo 1974) and also pesticide pollution in soil water (Cook et al 1983).

Table 1. Phospholipids and proteins of membranes isolated from R.meliloti 300h13 at the exponential phase of growth with parathion

mg/g dry membrane	Control	Parathion
Lipid phosphorus (L)	1.484 ± 0.038	1.831 ± 0.058 P < 0.05
Total proteins (P)	273.17 ± 6.19	378.25 ± 13.60 P < 0.001
L/P ratio x 10^3	5.4	4.8

Values reported are the means of four experiments ± standard errors.

Table 2. Phospholipidic composition of membranes isolated from R.meliloti 300h13 at the exponential phase of growth with parathion

Phospholipid	Control	Parathion
Phosphatidylcholine (PC)	42.9(*)	32.5
Phosphatidylethanolamine (PE)	16.4	23.6
Phosphatidylglycerol (PG)	12.9	20.1
Phosphatidylserine (PS)	10.2	8.9
Cardiolipin (CL)	3.7	3.1
Phosphatidylinositol (PI)	1.4	1.3
Phosphatidic Acid (PA)	6.0	5.2
Unidentified phospholipid	6.5	5.3
$\frac{PS + PE + PC}{PG + CL}$	4.2	2.8

(*) Results are expressed as percentage of total lipid phosphorus. They are the means of four experiments.

We found that membranes isolated from parathion treated cells had a modified chemical composition. Table 1 shows that membranes had significant increases in both

protein and lipid phosphorus contents and the ratio between phospholipids and proteins was weakly decreased. The data of Table 2 establish some differences in the phospholipidic composition of membranes from control and cells exposed to the insecticide. Although phosphatidylcholine remained the dominant phospholipid species, its percentage was decreased, while phosphatidylethanolamine and phosphatidylglycerol were increased. Consequently, the ratio among phospholipids (PS + PE + PC / PG + CL) synthetized from the lipo-nucleotide cytidine-5'diphosphate-diglyceride (CDP-DG) by separate branchs was diminished. We had reported a similar altered ratio in membranes isolated from Escherichia coli exposed to parathion (Rosas et al 1985).

Table 3. Fatty acids of membranes isolated from R.meliloti 300hl3 at the exponential phase of growth with parathion.

Fatty acid	Control	Parathion
Myristic	trace	trace
Palmitic	8.72 ± 1.13	9.50 ± 1.81
Palmitoleic	trace	trace
9,10-Methylene-hexadecanoic	trace	trace
Stearic	1.05 ± 0.21	0.95 ± 0.31
Cis-vaccenic	72.06 ± 2.25	88.20 ± 1.96
Unidentified	1.51 ± 0.53	1.73 ± 1.25
Lactobacillic	12.77 ± 0.53	trace

Results are reported as percentage of total fatty acid. Each figure represents the mean of four experiments ± standard error. If the fatty acid was present as less than 0.9% of the total, it is expressed as trace.

Fatty acid profiles (Table 3) of membranes of parathion treated cells showed that lactobacillic acid almost disappeared and its metabolic precursor, cis-vaccenic acid, was slightly increased.
The chemical composition of membranes isolated from R.meliloti 300hl3 exposed to parathion had some similarities with that of cytoplasmic membranes isolated from R.meliloti 102F70 bacteroids by Miller and Tremblay (1984). They also found differences in fluidity, thermotropic behaviour, and respiratory proteins of the membranes of bacteroids and free-living Rhizobia of the same strain. Our results leave open the possibility of variations in membrane physical chemistry as well as in membrane associated functions. The persistence of the observed changes is currently being studied in our laboratory, and complemented by evaluations conducted directly with soil.

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REFERENCES

Alexander M (1977) Introduction to Soil Microbiology, 2nd Edn, Wiley, New York, pp 438-456

Cook AM, Grossenbacher H, Hutter R (1983) Isolation and cultivation of microbes with degradative potential. Experientia 39:1191-1198

Dodge JT, Phillips GB (1967) Composition of phospholipids and of phospholipid fatty acids and aldehydes in human red cells. J Lip Res 8:667-674

Domsch KH, Jangnow G, Anderson TH (1983) An ecological concept for the assessment of side-effects of agrochemicals on soil microorganisms. Residue Rev 86:65-105

Evans TJ (1969) Membrane adenosine-triphosphatase of Escherichia coli. Activation by monovalent cations. J Bacteriol 100:914-922

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275

Mallik MAB, Tesfai K (1983) Compatibility of Rhizobium japonicum with commercial pesticides in vitro. Bull Environ Contam Toxicol 31:432-437

Miller RW, Tremblay PA (1984) Cytoplasmic membrane of Rhizobium meliloti bacteroids. I Alterations in lipid composition, physical propierties, and respiratory proteins. Can J Biochem Cell Biol 61:1334-1340

Rosas SB, Secco MC, Ghittoni NE (1980) Effects of pesticides on the fatty acid and phospholipid composition of Escherichia coli. Appl Environ Microbiol 40:231-234

Rosas SB, Storani MMC de, Ghittoni NE (1985) Fatty acids and phospholipids of membranes isolated from Escherichia coli growing in a medium with parathion. Bull Environ Contam Toxicol 34:265-270

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