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Effects of Aldicarb on the Biochemical Composition of *Rhizobium meliloti*

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Aldicarb is a systemic carbamate pesticide which is widely used for protection of commercial crops from attack by insects, nematodes and other pests. As for organophosphorus insecticides, the essential basis of carbamate insecticidal activity is the inhibition of acetylcholinesterase. However, the two classes behave differently on their mechanism of inhibition (Corbett et al 1984). Aerobic and anaerobic degradation of aldicarb were investigated in vitro (Bank and Tyrrell 1984, 1985; Given and Dierberg 1985) in soils (Ou et al 1985a, 1985b) and in groundwater (Miles and Delfino 1985). Although microbial degradation appears to be the main factor contributing to the disappearance of aldicarb in soils, effects of the insecticide on soil microorganisms have not been reported. Little is known about the action of organophosphorus and carbamate pesticides on bacterial biochemistry. We observed that Escherichia coli (Rosas et al 1980, 1985) and Rhizobium meliloti (Carranza et al 1985, 1986; Thüar et al 1987) modified their cellular concentration of proteins, phospholipids, fatty acids, and carbohydrates, when parathion was added to the growth medium. The purpose of initiating the present study was to determine the result of aldicarb addition to pure culture of R.meliloti.

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 which was described elsewhere (Carranza et al 1985, 1986). Analytical grade aldicarb [2methyl-2(methylthio)propionaldehyde-O(methylcarbamoyl)oxime] was a gift from Union Carbide Argentina. Stock solution of aldicarb was prepared in absolute ethanol and diluted 1000-fold by volume in the medium to give a final concentration of 5 mg/mL. Controls had the equivalent volume of absolute ethanol.

Bacterial growth was recorded as optical density at 420 nm against a medium blank. Cells were harvested at the logarithmic and at the stationary phases of growth, 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). Total cellular carbohydrates and excreted polysaccharides were analyzed as glucose by using the anthrone-sulphuric method of Trevelyan and Harrison (1952).

RESULTS AND DISCUSSION

R.meliloti cultivated in a broth medium which has received 5 mg aldicarb/L at the start of incubation did not change its growth pattern. In similar experimental conditions, we had previously observed (Carranza et al 1985, Thuar et al 1987) that 2.5-5 mg parathion/L decreased the growth rate of R.meliloti.

Chemical composition of microbial cells was determined at logarithmic and stationary phases of growth. Tables 1 and 2 show that the content of proteins and phospholipids, as well as phospholipid composition of aldicarb treated microorganisms were the same as controls. On the other hand, Table 1 indicates that both total cellular carbohydrates (TCC) and polysaccharides excreted to the medium (EPS) were diminished. The results are suggestive of a delayed carbohydrate synthesis.

Fast-growing rhizobia are known for their capacity to produce a variety of cellular polysaccharides such as metabolizable glycogen and cell surface polysaccharides (β-glucans, lipopolysaccharides, capsular polysaccharides). In addition, rhizobia excrete copious amounts of soluble exopolysaccharides (EPS) when grown in synthetic culture media. Zevenhuizen (1984) corroborated that EPS excretion and TCC accumulation began in the logarithmic phase and continued in the stationary phase of growth; glucans gradually leaked out of the cells and were found in the EPS fraction of the medium. We calculated the relative increases of TCC and of EPS from logarithmic to stationary phases of R.meliloti growth, as percentages of logarithmic phase values. Table 3 shows that aldicarb treated cells decreased TCC accumulation and increased EPS excretion. These findings leave open the possibility of changes in carbohydrate metabolism when R.meliloti was exposed to the insecticide.

Table 1. Gross cellular composition of *R.meliloti* 3D0 h13 and excreted polysaccharides (EPS) when 5 mg aldicarb/L broth medium was added at the start of incubation.

Phase of growth	Condition	Proteins mg/g	Phospholipids,a dry weight of	Polysaccharides,b cells	EPS,b mg/mL medium
Logarithmic	Control	173.7 ± 17.3	0.877 ± 0.129	0.474 ± 0.025	0.676 ± 0.060
	Aldicarb	170.2 ± 14.7	0.816 ± 0.165	0.294 ± 0.022	0.316 ± 0.031
Stationary	Control	183.1 ± 17.8	0.905 ± 0.140	1.156 ± 0.045	0.982 ± 0.089
	Aldicarb	181.3 ± 16.2	0.968 ± 0.230	0.469 ± 0.031	0.687 ± 0.058

Results are means ± standard errors of five experiments.
a: lipid phosphorus equivalents
b: glucose equivalents

Table 2. Effects of aldicarb on the phospholipid composition of R.meliloti 3D0 h13

	PI	PS	PA	PC	PE	PG
Control cells	12	11	20	19	20	18
Aldicarb treated	10	14	19	21	21	15

Results are expressed as % of total lipid phosphorus (Table 1) means from five experiments. Phospholipids: PI: phosphatidylinositol, PS: phosphatidylserine, PA: phosphatidic acid, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PG: phosphatidylglycerol

Table 3. Carbohydrates increases from logarithmic to stationary phases of R.meliloti 3D0 h13 growth calculated as % of logarithmic phase values.

	Cellular Carbohydrates	Excreted Polysaccharides
Control cells	144	45
Aldicarb treated	59	117

Although variations in cellular composition of aldicarb treated R.meliloti were less impressive than those we had previously observed in parathion treated bacteria (Carranza et al 1985) the presence of aldicarb and parathion in the growth medium led to alterations in rhizobial carbohydrates. A possible role for rhizobial polysaccharides in the nodulation process has been a subject of great interest for a number of years, but has not been demonstrated conclusively. The current hypothesis receiving the most support (Halverson and Stacey 1986) is that rhizobial cell surface polysaccharides function as signal molecules which induce host responses for infection and nodulation. Hence, changes in the metabolism of rhizobial carbohydrates might affect the development of the plant-microbe interaction.

Several studies have shown that the major metabolic pathway of aldicarb in soils is rapid oxidation to aldicarb sulfoxide followed by slower oxidation to aldicarb sulfone or hydrolysis to aldicarb sulfoxide oxime (Ou et al 1985a, 1985b). Further research is needed in order to know the degradation pathway of aldicarb and to characterize the actual toxic substance in our experimental conditions.

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