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Effects of Atrazine on *Ochrobactrum anthropi* Membrane Fatty Acids

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***Ochrobactrum anthropi* is a gram-negative bacillus recognized as a human opportunist pathogen isolated in clinical specimens and not of clinical significance. We report a new aspect of this bacterium, that it has been isolated from activated sludge. In fact, it is able to grow on atrazine (2-chloro-4-ethylamino-6-isopropyl-amine-*s*-triazine) by utilizing it as the only source of carbon. Our results show that atrazine (0.03 g/liter) causes a dramatical increase in the degree of saturation of membrane fatty acids. Analysis and identification of bacterial fatty acids were performed by gas chromatography and gas chromatography-mass spectrometry techniques.**

There is little information about microorganisms capable of degrading environmental contaminants, particularly about the mechanisms by which they react and adapt to new substrates. It has been seen that xenobiotics partition preferentially in membranes, causing an increase in membrane fluidity (4, 12) and that the main target of chemicals with the potential to pollute bacterial and eukaryotic cells is the cell membrane (5).

Many studies have shown that fluidity is strongly regulated to offset physical and chemical changes imposed by the environment by the process of homeoviscous adaptation, which probably enables vital membrane functions to continue (9, 11, 13). The most important mechanism for regulating membrane fluidity against membrane-active substances consists in a change in the fatty acid composition of the cell membrane and, in particular, in a modification in the degree of saturation (2, 3, 5, 7). This phenomenon has been described for organic solvents, but the fact that a pesticide can stimulate change in fatty acid composition has not, to our knowledge, been reported so far.

Ochrobactrum anthropi, classified as group Vd according to the scheme of the Centers for Disease Control and Prevention, is closely related to gram-negative, aerobic, peritrichously flagellated, nonfermentative, nonfastidious bacilli that are positive for oxidase and urease production (6). This organism has occasionally been isolated from clinical material and is considered not of clinical significance (1). *O. anthropi* appears to be recognized as a human opportunist pathogen associated with intravascular catheters (6).

In this study, we have investigated the effects of atrazine on membrane lipids of *O. anthropi*, which is able to grow with pesticide as the only source of carbon and to adapt in such a way as to modify the degree of saturation of membrane fatty acids.

Microorganism and culture condition. The microorganism was isolated from an activated sludge contaminated with atrazine. Culture morphology and biochemical reactions in the API ID 32 E and ID 32 GN (bioMérieux) systems were consistent with the identification of *O. anthropi*. The percent identification and T indices were as follows: 99.4% and 1, respectively, by ID 32 E and 99.9% and 0.74, respectively, by ID 32 GN. The strain was cultivated on agar plates with minimal medium M9 (10) containing succinate (1 g/liter), succinate (1 g/liter) plus atrazine (0.03 g/liter), and atrazine (0.03 g/liter) alone as the growth substrate. Growing cells were harvested at late exponential phase, which was determined as the period at which growth took place at the maximal rate before reaching the stationary phase, taking into account the increase in colony diameter observed with a microscope.

Lipid extraction and transesterification. Fatty acid methyl esters were prepared according to the method previously described by Orgambide et al. (8), with slight modifications. Bacterial cells were carefully removed and transferred to a vial containing 2 ml of distilled water and were centrifuged at 6,000 $\times$ *g* for 10 min. The supernatant fluids were discarded, and the cell pellets were lyophilized. The dry cell residue was extracted twice with 1:1 (vol/vol) $CHCl_3$-MeOH for 4 h at 60°C. The supernatant obtained after centrifugation at 3,000 $\times$ *g* for 5 min was transferred into another vial, and 1/2 volume of $CHCl_3$ and 1/2 volume of H_2O were added. After shaking (mechanical shaker, 2 min) and centrifugation at 3,000 $\times$ *g* for 5 min, two phases were separated. The upper phase was removed and discarded; the lower phase was dried under nitrogen and derivatized with 3 ml of 1 M methanolic hydrochloric acid for 2 h at 80°C. The sample was cooled at room temperature, and fatty acid methyl esters were extracted with 5 ml of hexane for 5 min; the volume of the extract was reduced to 0.01 ml, and then the extract was injected into the gas chromatograph.

Determination of fatty acid composition. Fatty acid analysis was performed with a Dani 8520 gas chromatograph, equipped with a flame ionization detector, and a CP Sil5 (25 m; inside diameter, 0.32; film thickness, 0.2 μm) nonpolar capillary column or a Supelco SP-2330 polar column. The temperature program ranged from 80 to 230°C (4°C/min). The inlet system, which involved the use of a split-splitless injection technique, was equipped with a programmed temperature vaporization introduction system, with helium as the carrier gas. The bacterial fatty acids were identified by comparison of the retention

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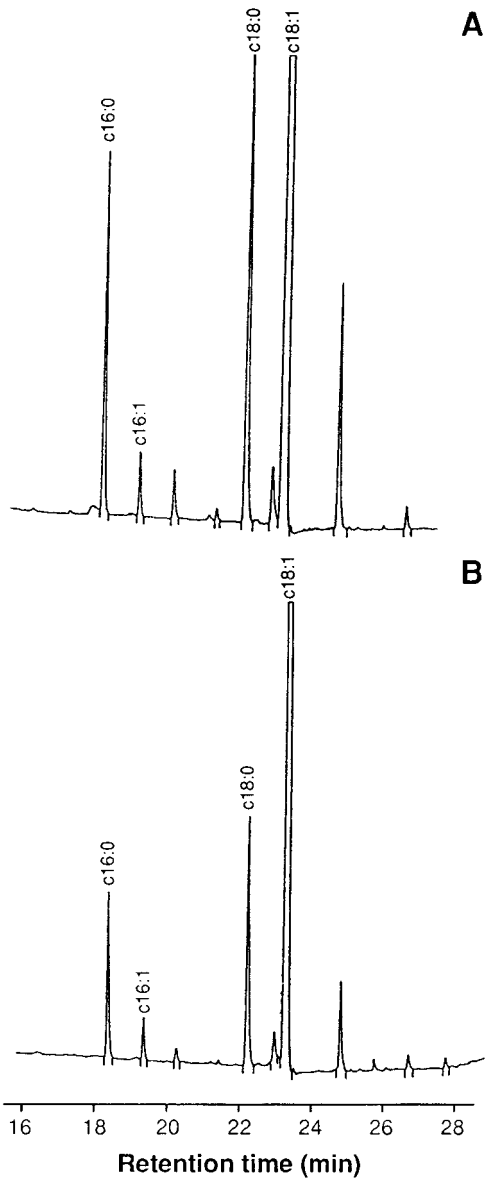

FIG. 1. Parts of capillary gas chromatogram (column, SP 2330) with the major membrane fatty acids of *O. anthropi* indicated. Cells were grown in the presence of atrazine (0.03 g/liter) (A) or succinate (1 g/liter) (B).

times of the gas chromatographic peaks with those of authentic standards, and identities were subsequently confirmed by mass spectrometry (VG TS-2500 mass spectrometer; Fisons Inc.) in electron impact mode (70eV) (data not shown). The relative amounts of the fatty acids were determined from the peak areas of the methyl esters with a Chromatopac C-R6A integrator (Shimadsu, Kyoto, Japan). Replicate determinations indicated that the relative errors [(standard deviation/mean) × 100] of the values were 2 to 5%.

In view of the increasing importance of *O. anthropi* in clinical microbiology and its possible use in environmental bioremediation processes, the present study was performed to identify the membrane fatty acid profile and to evaluate the effects of atrazine in the degree of saturation of membrane fatty acids.

The analysis showed that lipids of this bacterial strain contain three different types of fatty acids: saturated ($C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, and $C_{18:0}$), *cis*-monounsaturated ($C_{16:1\ 9c}$ and $C_{18:1\ 11c}$), and cyclopropane ($C_{19\ cyc}$) fatty acids.

Figure 1 shows parts of a capillary gas chromatogram from cells grown on succinate and on atrazine as the sole sources of energy and carbon, respectively. In this figure the major saturated and unsaturated fatty acids are represented: palmitate ($C_{16:0}$), stearate ($C_{18:0}$), palmitoleate ($C_{16:1}$ 9c), and *cis*-vaccenate ($C_{18:1\ 11c}$).

The fatty acid profile of the bacterial strain grown on solid culture media with succinate and atrazine, at the concentrations indicated in Table 1, points out that the presence of the herbicide increases the content of the total saturated fatty acids (64%) with respect to the control in succinate (31%). The relative amounts of the fatty acids were determined from the peak areas of the methyl esters as described above. Moreover, atrazine decreases the percentage of the total unsaturated fatty acids with respect to the control, 8.01 and 35.1%, respectively. The ratio of saturated to unsaturated fatty acids was defined as either $C_{18:0}/C_{18:1\ 11c}$ or $C_{16:0}/C_{16:1\ 9c}$. When bacteria were grown in the presence of atrazine, at a concentration of 0.03 g/liter, as its sole source of carbon, there was a dramatic increase in the ratio for both C_{18} and C_{16} fatty acids with respect to the bacteria grown in the optimal condition, that is, succinate at 1 g/liter. In fact, the ratios for C_{18} fatty acids were 0.28 in cells grown on succinate and 2.8 in those grown on atrazine (a 10-fold increase). For C_{16} fatty acids, the ratio was 7.6 on succinate and 18.2 on atrazine alone (2.5-fold increase). When atrazine (0.03 g/liter) was added to succinate (1 g/liter), no significant differences in fatty acid compositions were observed. To verify if the fatty acid profile was modified because of the low concentration of the carbon source, experiments with succinate at a concentration of 0.05 g/liter were performed. At this concentration an increase of saturated fatty acid was observed, but it was not as notable as that obtained with atrazine at 0.03 g/liter. On the other hand, when atrazine and succinate were both used at a concentration of 0.5 g/liter, only atrazine was able to dramatically increase the ratio of saturated to unsaturated fatty acids. The same results were obtained when atrazine was used at a concentration of 0.03 g/liter, thus demonstrating that the presence of atrazine was responsible for the phenomenon.

A change in fatty acid composition leading to an increase in the degree of saturation of the membrane lipids is a well-known reaction of bacteria against membrane-active substances, such as organic solvents or aromatic compounds (2, 3,

TABLE 1. Capillary gas chromatographic analysis of membrane fatty acid composition

Substance and concn	Fatty acid (% of total fatty acids)[a]							
	$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	$C_{16:1}$	$C_{16:0}$	$C_{18:1}$	$C_{18:0}$	$C_{19:0cyc}$
Succinate, 1 g/liter	0.89 ± 0.1	1.62 ± 0.07	3.5 ± 0.12	2.1 ± 0.05	15.9 ± 0.73	33.0 ± 0.35	9.3 ± 0.51	33.6 ± 0.78
Atrazine, 0.03 g/liter	3.42 ± 0.2	8.0 ± 0.35	1.3 ± 0.04	1.9 ± 0.06	34.72 ± 2.01	6.11 ± 0.3	17.06 ± 0.51	28.64 ± 1.3

[a] Results were determined from the peak areas of methyl esters. Cells were grown on solid media with succinate or atrazine and incubated at 30°C.

5, 7). Data presented in this paper show that the contact of *O. anthropi* with a pesticide structurally unrelated to the compounds known to induce changes in membrane fatty acids produces an increase in the content of saturated fatty acids. Studies to understand which mechanism(s) underlies this phenomenon are in progress.

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