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BIODEGRADATION OF METHYL-PARATHION BY BACTERIA ISOLATED OF AGRICULTURAL SOIL

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Key words: pesticides, methyl-parathion, bacteria, biodegradation, phosphotriesterase, parathion-hidrolase

ABSTRACT

A methyl-parathion degrading consortium of bacteria was isolated from agricultural soils in central Mexico (Morelos state), using methyl-parathion as the only carbon source. The ability of the consortium to degrade methyl-parathion, was assessed with a mineral medium containing 15 mg/L of pesticide; remnants concentration of methyl-parathion were measured by gas chromatography. Different colonies were chosen from the consortium obtained, depending on their color, growth shape, morphology, consistency, borders and surface. As much as eleven different genera of bacteria were found in the consortium, which were tested for enzymatic activity by measuring change in absorbance at 410 nm, when a 15 mg/L solution of methyl-parathion was exposed to an extract containing the enzyme (a phosphotriesterase), to produce *dimethylthiophosphoric acid* and *p-nitrophenol*. From the eleven tested species, plus *Flavobacterium* sp. ATCC 27551 strain, only five of them showed phosphotriesterase activity on the methyl-parathion. Because most of the isolated bacteria are pathogens it becomes difficult to establish recommendations towards the extensive use of one of these strains in natural environments. However, these bacteria could be considered as a potential source of enzymes to reduce environmental contamination by methyl-parathion and its residues.

Palabras clave: plaguicidas, paratión metílico, bacterias, biodegradación, fosfotriesterasa, paratión hidrolasa

RESUMEN

A partir de suelos agrícolas del estado de Morelos, México, se aisló un consorcio bacteriano con capacidad de degradar paratión metílico. El aislamiento se llevó a cabo utilizando al plaguicida como única fuente de carbono. La habilidad del consorcio aislado para degradar al paratión metílico se determinó en medio mineral con 15 mg/L del plaguicida y la concentración remanente fue cuantificada por cromatografía de gases. Del consorcio obtenido se aislaron varias colonias con base en su color, forma de crecimiento, morfología, consistencia, bordes y superficie. Se lograron identificar once géneros, a los cuales se les determinó actividad enzimática al medir el cambio de absorbencia a 410 nm. Para ello, se hizo reaccionar una solución de paratión metílico a concentración de 15 mg/L con un extracto enzimático con actividad de fosfotriesterasa, para producir *ácido dimetiltiofosfórico* y *p-nitrofenol*. De las doce especies probadas, donde se incluyó a *Flavobacterium* sp. ATCC 27551, sólo cinco especies mostraron actividad de fosfotriesterasa sobre el paratión metílico. Las bacterias aisladas son principalmente patógenas, por lo que es muy difícil recomendar su uso en am-

bientes naturales. Sin embargo, son una fuente potencial de enzimas que pueden reducir la contaminación ambiental por paratión metílico así como algunos residuos del mismo.

INTRODUCTION

Pesticides are organic compounds manufactured and used for pest control. The use of these chemicals has had ecological effects that have not been repaired. Pesticides are dispersed in the environment and become pollutants. Environmental pollution caused by both, excessive and continuous use of pesticides, begins when these compounds enter the environment by diverse causes (accidental spills, direct applications, residuals due to facility cleaning of containers, state of equipment used and methods used to apply the products). Quality of soils, ground water, continental and coastal waters as well as the air, are all affected (Ortiz *et al.* 1997).

An important way to avoid ecological damages and human health problems caused by the presence of pesticides, is to reduce their concentrations in the environment, precluding either lixiviation to groundwater or possible incorporation to natural food chains. Options to lowering environmental pollution include chemical treatment, incineration and physical removal of the contaminated soil. However, these methods are expensive and inefficient. On the other hand, biological methods are potentially more efficient, inexpensive and can achieve complete mineralization of the pollutants. However, success of biological methods depends on the microorganism's efficiency and the stability of the enzymes in contaminated environments. For this reason, different technologies that might be applied for bioremediation of contaminated soils and groundwater have been developed. These methods offer various advantages, such as safety, economy and speed of process, among others (Chapalamadugu and Chaudry 1992).

Among pesticides, methyl-parathion (*O,O-dimethyl O-4 nitrophenil phosphorotioate*) is a contact and ingestion organophosphate insecticide, having P=S bond. Due to its low persistence in the environment it is used mainly in agriculture, although it is used widely in home dwellings and industries too. According to the CICOPLA-FEST (1997), it is extremely dangerous for warm-blooded animals (mammals and birds). In Mexico it is recommended for about 70 crops, including vegetables, fruit-bearing plants and cereals (SARH 1994). Furthermore, this kind of pesticides are used for tick control in cattle dippings and this residual wastewaters are disposed without previous treatment.

The route to degrade ethyl-parathion has been studied with bacteria isolated from soils, and specific strains have been characterized as *Pseudomonas diminuta* and *Flavobacterium* sp. ATCC 27551, which contain a phosphotriesterase enzyme, inicially called *parathion hydrolase* (Chapalamadugu and Chaudry 1992, Scanlon and Reid 1995). The enzyme is encoded by a *opd* (organophosphate degrading) gene of *P. diminuta* MG. The gene was first identified in the pCMS1 plasmid and an identical gene was also found in a plasmid of *Flavobacterium* sp. ATCC 27551 (Grimsley *et al.* 1998). Such enzyme causes hydrolysis of ethyl-parathion to yield *p-nitrophenol* and *diethyl thiophosphoric acid* (**Fig. 1**) (Munnecke and Hsieh 1976, Serdar *et al.* 1982), although it does not show a high enzymatic activity for methyl-parathion, suggesting that the enzyme is alkyl-components-specific. Therefore, since this pesticide is broadly used in Mexico, it would be important to have bacterial strains able to degrade it so as to supply strains that allow degradation of this pesticide in biological treatment of wastes and contaminated soils.

The goal of this work was to increase the microbial degradation process of methyl-parathion, using indigenous soils microorganisms that have been exposed to these kind of compounds during long periods. Also, to assess soil bacteria's ability to degrade the organophosphate pesticide methyl-parathion.

MATERIAL AND METHODS

Reagents

Tripticasein soy agar (TS) was obtained from Bioxon; potassium monobasic phosphate, potassium dibasic phosphate, ammonium sulphate, magnesium sulphate, sodium chloride, calcium chloride, iron sulphate, sodium molibdate

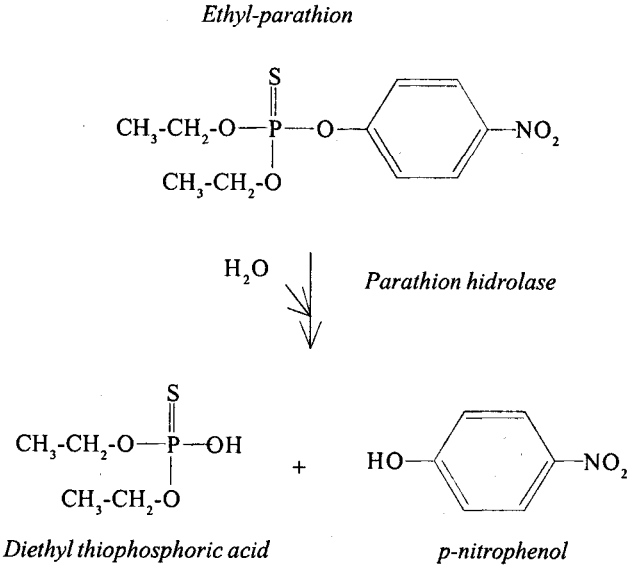

Fig. 1. Hidrolysis of ethyl-parathion by a phosphotriesterase enzyme (parathion hidrolase)

and manganese sulphate and Tris buffer were purchased from J. T. Baker. p-Nitrofenol and bovine serum albumine were obtained from Sigma Chemical Co. Analytical grade methyl-parathion (98% purity) was purchased from Ultra Scientific (Analytical Standards).

Mineral medium composition

The mineral medium had the following composition (per liter): KH_2PO_4, 0.2 g; K_2HPO_4, 0.5 g; $(NH_4)_2SO_4$, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; $FeSO_4 \cdot 7H_2O$, 0.025 g; Na_2MoO_4, 0.005 g; $MnSO_4$, 0.0005 g (pH 7.0 ± 0.3). The phosphoric salts were sterilized separately (125° C for 25 min) to prevent precipitation and later aseptically added to the rest of salts.

Reference strain

Flavobacterium sp. ATCC 27551 strain, reported as bearing the *opd* gene (Mulbry and Karns 1989) was used as reference strain.

Sampling sites selection

Sampling sites were selected according to data from SAGAR (1996), regarding agricultural soils where methyl-parathion and other organophosphates had been constantly applied in large amounts. The soils are located in the north-eastern zone of Morelos state (central Mexico).

Soils sampling and analytical procedure

Five hundred gram samples were manually taken at approximately 20 cm depth the soil surface (within the arable layer) in the chosen sites, and were air dried at ambient conditions during a minimum time span of 48 hr. Once driend, the soil was manually ground and passed through a No. 10 sieve (2 mm) to eliminate large particles before being analysed (Ortiz *et al.* 1993a). Chemical analysis of soil samples was performed according to Ortiz *et al.* (1993a y b). Additional sampling was done to isolated degrading microorganisms (bacteria) at 10 cm below the soil surface at the chosen sites. Sterile Petri dishes were used to store and transport samples at 4° C until isolation was performed (Van-Elsas and Smalla 1997). Soil characteristics were the following: moisture, 35%; pH 7.52; electric conductivity, 730 µS/cm; organic matter, 2.88%; total nitrogen, 0.12%; available phosphorus, 35.49 mg/kg and cation exchange capacity, 18.85 cmol/kg.

Isolation of bacteria

One gram of soil sample was suspended in 5 mL of sterile mineral medium and this suspension was considered as the inoculum. Afterwards, 0.2 mL of inoculum were transferred to 20 mL of mineral medium in 125-mL flasks and supplemented with methyl-parathion as the only carbon source, at 5, 10 and 15 µg/mL (acclimation period). Methyl-parathion was added initially as a filter-sterilized methanol solution (Millipore membrane, 0.25 µm pore size) such was evaporated to dryness before addition of mineral medium in the flask. The culture was incubated at room temperature and constant shaking (120 rpm , Lab Line shaker), during 24 hours. Bacterial count was done by Agar Plate Method (Clark 1965).

Degradation of methyl-parathion by consortium

Ability of the consortium bacteria isolated to degrade methyl-parathion, was assessed with mineral medium (MM) containing 15 mg/L of pesticide. Bacterial cells scraped three times from stock slants were suspended in 5 mL of sterile water. In Erlenmeyer flask with 20 mL of MM, were inoculated 200 µL each suspension, by triplicate. The flasks were incubated on a orbital shaker at 120 rpm. Every 24 hours, a sub-sampled (1 mL) was taken and residual methyl-parathion was extracted three times with ethyl acetate. The extract was dried with anhydrous sodium sulfate followed by filtration through glass-fiber paper (Whatman GF/B). The filtrate was evaporated to dryness under nitrogen and redisolved in 50 µL of dichlorometane. The amount of residual pesticide was determinated on a Hewlett Packard 6890 Gas Chromatograph equipped with a nitrogen and phosphorus detector (NPD) and a HP-5 5% phenylmethylsilicone capillary column (30 m by 0.2 mm, inner diameter). The operating conditions were as follows: injector temperature, 240°C; detector temperature 280°C; oven temperature, 100 to 250°C at 12°C / min; carrier flow rate 2 mL/min. Growth of bacteria was monitored by the Agar Plate Method. The following systems were run as controls: mineral medium and methyl-parathion solution without bacteria, and mineral medium with inoculum without methyl-parathion.

Process to select and obtain pure colonies

Different colonies were chosen from the isolated consortium considering color, shape of growth, morphology, consistency, borders, and surface. They were streaked separately in trypticasein soy agar (TS agar) plates containing 15 mg/L of the pesticide (final concentration). Those colonies considered as different were plated on TS agar with pesticide. They were incubated as stated above. This procedure was repeated several times to ensure purity of isolated colonies.

Characterization of the bacteria selected from the soil

A BBL Crystal system was used along with other biochemical tests to characterize and identify bacteria, as well as motility, carbohydrates fermentation, citrate, indole, methyl red and oxydase. The procedure to identify was performed in accordance with *Bergey's manual of Systematic Bacteriology* (Krieg and Holt 1984).

Enzymatic activity determination

Enzymatic activity was determined by measuring change in absorbance at 410 nm, when a 15 mg/L solution of methyl-parathion was exposed to an enzymatic extract containing the enzyme (a hydrolase) to produce *dimethylthiophosphoric acid* and *p-nitrophenol*. This was carried out in a Tris buffer pH 9 (Masaphy *et al.* 1996). These measurements were carried out in an Espectronic 601 Milton Roy spectrophotometer, bearing deuterium and tungsten lamps. An activity unit is defined as the 1.0 µmol hydrolysis of methyl-parathion/min.

Each genera was cultivated in 125 mL Erlenmeyer flasks with 50 mL of mineral medium. However, pure colonies did not grow in this medium, and therefore we proceeded to cultivate them in TS broth. 15 mg/L of methyl-parathion were added to each culture and were incubated during a period of 48-60 hours at 28°C. To obtain the enzymatic extract, the procedure reported by Masaphy *et al.* (1996) was used taking as reference the location of the enzyme reported for *Flavobacterium* sp. ATCC 27551, which is intracellular and not excreted to the medium. Therefore, it was necessary to extract the proteins from the cell's interior by breaking the cellular membranes. First, the culture was centrifuged for five minutes at 5,000 rpm at 4°C. Supernatant was discarded, since it was considered as the extracellular enzymatic extract. The pellet cells were washed twice by adding 10 mL of phosphate buffer (Na_2HPO_4 10 mM), pH 7.2. The supernatant was also discarded, since this step was carried out to wash the cells.

After washing the cells, the pellet was resuspended in phosphate buffer. Bacterial cells were broken with a sonifier (BWR Scientific, Branson Sonifier 450), during two minutes. The resulting suspension was centrifuged 10 min at 10,000 rpm and the supernatant was considered as the intracellular enzymatic extract. The pellet was suspended with phosphate buffer and was centrifuged as mentioned earlier. The supernatants were combined in a test tube with threaded cap and were kept frozen until their analysis.

The total protein concentration in the extract was measured using the Lowry method (García and Vázquez 1998), with bovine serum albumin as standard. To measure the enzymatic activity, we considered the occurrence of *p-nitrophenol* (PNP). The enzyme breaks the methyl-parathion to produce this metabolite and *dimethylthiophosphoric acid*, producing one PNP mole per each mole of hydrolyzed methyl-parathion. For this purpose, a mixture of 100 to 800 µL of enzymatic extract (depending on the genus they come from, which was determined with different tests), methyl-parathion at a final concentration of 15 mg/L and Tris buffer at pH 9.00 was prepared. The mixing was prepared directly in quartz cuvets and then Tris buffer, methyl-parathion and the enzymatic extract were added to start the reaction. The increase in absorbance was measured by the appearance of PNP.

Absorbance of the reaction mixtures was compared to a PNP standard curve, which covered a concentration range from 1.5 to 6 mg/L. Additionally, absorbance changes were measured in blank samples which consisted of Tris buffer and methyl-parathion as well as Tris buffer and enzymatic extract for each genus, under the same conditions mentioned above.

Strain conservation

Each isolated bacterial strain was preserved in glycerol and conserved at -70° C in a REVCO freezer.

RESULTS

Acclimation

Before putting soil microorganisms to acclimation process to methyl-parathion, a viable count was performed ($1X10^6$ CFU/mL). The Petri dishes used as blank, did not showed any growth after the incubation period. Later, a viable count was performed for each concentration of the pesticide and the results were $7.5 X10^5$, $3 X 10^7$ and $4.9 X 10^7$ CFU/mL, when mineral medium was supplemented with 5, 10 and 15 mg/L of methyl-parathion, respectively. It can be noted that bacterial growth was greater as the concentration of the methyl-parathion increased.

Growth kinetics and degradation by isolated soil consortium

Figure 2 shows the results of kinetic accomplished with one consortium. As it can be observed, while growth increases, methyl-parathion concentration decreases. Under these experimental conditions, the amount of methyl-parathion lost in culture after eigth days of incubation was 80.27% (15 mg/L initial concentration and 2.96 mg/L at the end of the experiment).

As it can be observed (**Fig. 2**), the greater percentage of degradation was obtained during the first four days, although the growth was registered until the day seven. Concentration of methyl-parathion in our blank, kept constant which suggests that decrease should be exclusively due to activity of the microorganisms. In this experiment the amount of pesticide adsorbed to cellular material, was not quantified; therefore, this percentage includes transformation and adsorption caused by growth of bacteria.

Characterization of the consortium obtained

From the isolated consortium we obtained a total of eleven different genera. **Figure 3** shows the number of colonies that were isolated from agricultural soils, and grouped by species, after being identified. It can be observed that most of the isolated colonies belong to *Pseudomonas aeruginosa*, followed by *Xanthomonas maltophilia* with five colonies, *Proteus vulgaris* with

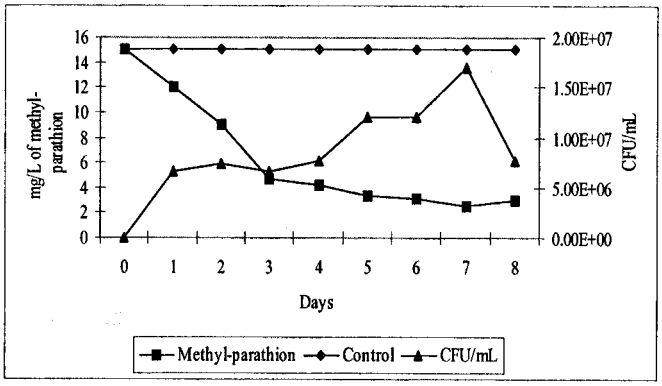

Fig. 2. Degradation of methyl-parathion by an isolated consortium of soil bacteria. Cells were incubated in mineral medium and 15 mg/L of pesticide

three colonies and *Chromobacterium violaceum* with two; the rest of the species had only one colony.

Enzymatic activity of each one of the isolated species

Table I shows the values of the enzymatic activity, which is defined as the time the hydrolase parathion needs to produce one *p-nitrophenol* mol. This table also shows the values of the specific activity that is defined as the quantity of protein that is required to produce a *p-*

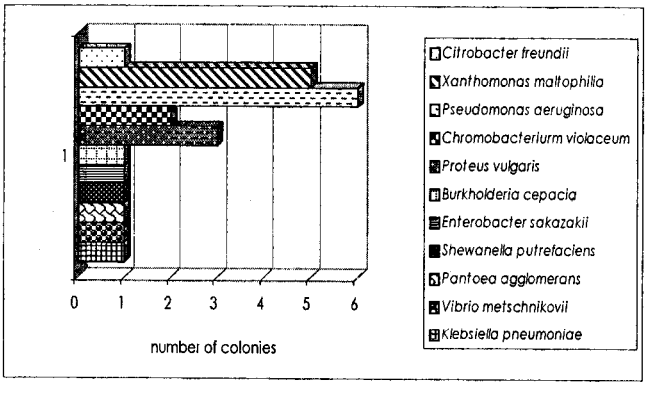

Fig. 3. Results of total number of isolate colonies from agricultural soils that were sampled

nitrophenol mol per minute. As mentioned before, the enzymatic activity was measured through the occurrence of PNP in a certain period of time. **Figure 4** shows the results of this measurement for each one of the species.

From the twelve tested species, including *Flavobacterium* sp. ATCC 27551 strain, reported as bearing the *opd* gene, only five species showed hydrolase activity on the methyl-parathion. Nevertheless, the five species showed different behaviors concerning this property. In general, the enzymatic activity of the different isolated species can be summarized as follows:

Pseudomonas aeruginosa> Klebsiella pneumoniae> Burkholderia cepacia> Enterobacter sakazakii> Citrobacter freundii

The species that did not show both enzymatic activity and specific activity were not able to hydrolyze methyl-parathion, since no occurrence of *p-nitrophenol* was observed. However, these species showed a high concentration of total protein, such as the *Chromobacterium violaceum*, where parathion hydrolase was not expressed. Therefore, the hydrolase parathion activity was not detected. One possible explanation is that they lost its capacity to express this activity after being exposed to preservation.

It is important to point out that in the case of the collection strain (under the experimental conditions of this work), the parathion hydrolase was not expressed either, even though it also showed a high concentration of total protein. The culture conditions of all the species were homogeneous, and were taken from previous reports for *Flavobacterium* sp. ATCC 27551. The optimal conditions for the enzyme activity in this species is: pH within a range of 7-10; temperature of 40 °C and presence of bivalent cations, among others (Brown 1980, Chaudry *et al.* 1988, Dumas *et al.* 1989, Mulbry and Karns 1989). Therefore, with the results obtained and under these experimental conditions, the most efficient strain for the first hydrolysis reaction of methyl-parathion was *Pseudomonas aeruginosa.*

DISCUSSION AND CONCLUSIONS

Soil and the aquatic environments are characterized by the presence of multiple xenobiotic organic substances. Final destination of these substances in the environment is strongly affected by the nature and concentration of other components in aqueous and solid phases, especially colloids and macromolecules (organic and minerals), which often form complexes with organic contaminants (Masaphy *et al.* 1996). In this work the hydrolysis efficiency of methyl-parathion was studied in an aqueous medium (mineral medium) by a bacteria consortium isolated from agricultural soils.

Concerning the microorganisms adaptation process to laboratory conditions and to different concentrations of methyl-parathion, some preliminary studies demonstrate that when bacteria are exposed to a selection pressure, as a single organic substrate, very high population densities can be obtained (Alexander 1994). According to the results obtained, population density in-

TABLE I. RESULTS OF ENZYMATIC ACTIVITY BY SPECIES

SPECIE	Total protein (μg/mL)	Enzymatic activity (μmol/min)	Specific activity (μmol/min/mg prot)
Burkholderia cepacia	**236.50**	**0.06**	**1.25**
Chromobacterium violaceum	610.00	ND	ND
Citrobacter freundii	**41.67**	**0.02**	**0.67**
Enterobacter sakazakii	**330.00**	**0.04**	**0.23**
Flavobacterium sp. ATCC 27551	155.00	ND	ND
Klebsiella pneumoniae	**178.00**	**0.08**	**2.22**
Pantoea aglomerans	178.00	ND	ND
Proteus vulgaris	96.50	ND	ND
Pseudomonas aeruginosa	**27.50**	**0.10**	**4.54**
Shewanella putrefaciens	173.24	ND	ND
Vibrio metschnnikovii	224.50	ND	ND
Xanthomonas maltophilia	61.50	ND	ND

ND: not detected

creased proportionally to the quantity of pesticide added.

Under the experimental conditions of this work, a decrease of methyl-parathion was observed in the medium, which suggests an hydrolysis caused by the enzymatic action of the bacteria group. From the results of this work, we cannot conclude if this hydrolysis takes later on to a complete mineralization (breakdown of the *p-nitrophenol* ring) or if this metabolite remains in the medium. However, Spain and Gibson (1991) point out that *Moxarella* species use *p-nitrophenol* as a carbon and nitrogen source. According to various studies (Chapalamadugu and Chaudhry 1992), the advantage of a consortium isolated with the pesticide as the only carbon source, allows a bacterial species to carry out first the hydrolysis and allows other species to use the compounds resulting from this hydrolysis (*dimethylthiophosphoric acid* and *p-nitrophenol*), as nitrogen, phosphorus and carbon source.

On the other hand, the isolated bacteria have been in the presence of some organophosphosphate pesticide in the soil, that probably has forced them to generate new enzymes (such as *parathion hydrolase* for which no natural substrate is known), and also new metabolic routes for the degradation of this type of organic compound. The environmental conditions, the pH of the soil, the agricultural practices and the quantity of pesticide added in each region, among other causes, can be the decisive factor to force bacteria to use xenobiotic compounds (such as the pesticides), as substrate for their growth.

It was possible to isolate 11 different species that came from a consortium that was able to grow in methyl-parathion as the only carbon source. The consortium itself was able to grow and to reduce in 86% the methyl-parathion concentration in the medium. However, when the culture was put in a mineral medium, with each one of the strains separately, it was not able to grow, and therefore it did not use the pesticide. This suggests that the isolated bacteria can use the pesticide in a co-metabolism process. The physiological basis for the co-metabolism is not well known, but the more accepted hypothesis is related with the specificity of the enzymes. Many enzymes present in microbial cells catalyze reactions that include different but chemically related substrates. If the product of the activity of some of these enzymes is not an appropriate substrate for any other enzyme, such compound will be accumulated, although the initial enzyme converts its natural substrate into products that provide energy and a carbon source to the active species.

In previous studies, similar observations were already made to prove that the complete degradation of methyl-parathion is carried out in a more efficient way in mixed cultures of bacteria than in pure cultures (Munnecke 1976, Chaudry *et al.* 1988). However, Pahm and Alexander (1993), mentioned that the bacteria cultivated in pure media are useful to determine and to evaluate the degradation capacity shown separately by each species.

The reason why a specific species cannot use a xenobiotic compound is that it is possible that in natural environments such species takes part in the metabolism in a very specific function. So, a cooperation process is established between microorganisms to allow the existence of a great quantity of reactions in order to establish a single metabolic route. On the other hand, since many metabolic routes depend on enzymes that are coded in a plasmid, the ability of the microorganisms to degrade organic compounds depends on the stability of the plasmids or of their genes.

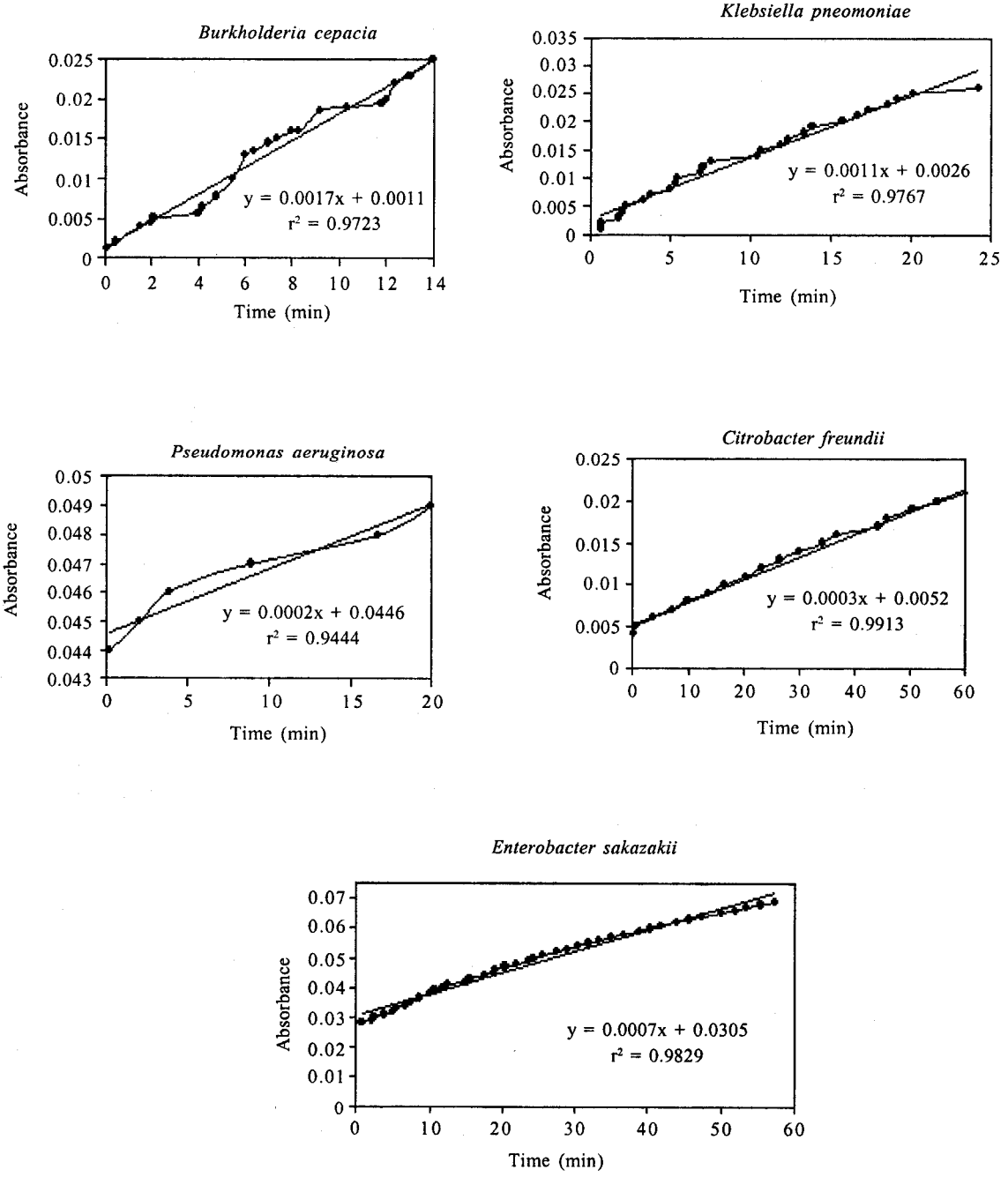

Fig. 4. Results of measurements of *p-nitrophenol* for to obtain enzimatic activity

Pseudomonas diminuta contains the gene that is located in the plasmid pCMS1 and that codes for the enzyme called parathion hydrolase (Serdar *et al.* 1982, Serdar and Gibson 1985). It is an esterase that hydrolyses the organophosphate pesticides, particularly ethyl-parathion, and whose hydrolysis products are *diethylthiophosphoric acid* and *p-nitrophenol*. *Flavobacterium* ATCC 27551 produces a constitutive enzyme that hydrolyze phosphotriester-type bonds. There are also differences between the enzymes expressed by *Flavobacterium* ATCC 27551 and those of other microorganisms concerning the affinity to the substrate.

In this study we observed that *Flavobacterium* ATCC 27551 did not degrade the methyl-parathion; i.e., the occurrence of *p-nitrophenol* was not observed. Therefore, it is deduced that these strains enzymes were not expressed, or the affinity to this substrate was very low or null. This could be due to several reasons. On one hand, temperature was not controlled in this work's experiments. On the other hand the activity of the enzymes tends to get lost

through several routes, such as the interaction with minerals or other components in the reaction mixture, proteolytic agents or due to the adsorption to the surface of the experimental apparatus (Masaphy *et al.* 1996).

In other studies, different species of *Pseudomonas* sp., *Flavobacterium* sp., *Bacillus* spp., and *Arthrobacter* spp. have been isolated from the soil. These species can hydrolyze several organophosphate pesticides and use them as their only carbon source (Shelton and Somich 1988).

With regard to the enzymatic activity, from the eleven isolated and identified bacteria, only five species showed parathion hydrolase activity, since the tested enzymatic extract reacted on the methyl-parathion to produce *p-nitrophenol*.

The isolated bacteria and their enzymes have a fundamental role in the detoxification of polluted environments with methyl-parathion and possibly with other organophosphate pesticides. Previous works have reported that an enzyme that acts particularly on an organophosphate also hydrolyses other similar compounds. Munnecke and Hsieh (1976) found that a raw enzymatic extract of a bacterial consortium isolated with ethyl-parathion showed enzymatic activity for eight different organophosphate pesticides. Adhya *et al.* (1981) observed that a strain of the *Pseudomonas* genus hydrolyzed the ethyl-parathion, but not to the methyl-parathion, suggesting that the alkyl components, along with the phosphorous, are more important in the degrading ability than in the aromatic portion.

It can be observed that bacteria isolated from agricultural soils are mainly pathogens (Palleroni 1984 and Brenner 1984, mentioned by Krieg and Holt 1984, Govan *et al.* 1996), and therefore it is very difficult to establish a recommendation for the extensive use of one of these strains in natural environments. Even though a parathion hydrolase activity was observed, it is important to consider public health risks that could be present. Nevertheless, it is a potential source of enzymes that can reduce the pollution by these pesticides in the environment, using them directly in field or using specific reactors under controlled conditions. For this purpose, research must continue in order to know the nature of the enzyme, its optimal activity characteristics and its life span in natural environments.

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