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Chemosphere 86 (2012) 1127–1132

Contents lists available at SciVerse ScienceDirect

Chemosphere

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Technical Note

Isolation of mesotrione-degrading bacteria from aquatic environments in Brazil

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ARTICLE INFO

Article history:
Received 13 May 2011
Received in revised form 15 December 2011
Accepted 15 December 2011
Available online 13 January 2012

Keywords:
Xenobiotic
Callisto
Pantoea ananatis
Biodegradation
Bioremediation

ABSTRACT

Mesotrione is a benzoylcyclohexane-1,3-dione herbicide that inhibits 4-hydroxyphenyl pyruvate dioxygenase in target plants. Although it has been used since 2000, only a limited number of degrading microorganisms have been reported. Mesotrione-degrading bacteria were selected among strains isolated from Brazilian aquatic environments, located near corn fields treated with this herbicide. *Pantoea ananatis* was found to rapidly and completely degrade mesotrione. Mesotrione did not serve as a sole C, N, or S source for growth of *P. ananatis*, and mesotrione catabolism required glucose supplementation to minimal media. LC–MS/MS analyses indicated that mesotrione degradation produced intermediates other than 2-amino-4-methylsulfonyl benzoic acid or 4-methylsulfonyl-2-nitrobenzoic acid, two metabolites previously identified in a mesotrione-degrading *Bacillus* strain. Since *P. ananatis* rapidly degraded mesotrione, this strain might be useful for bioremediation purposes.

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1. Introduction

The tremendous increase in crop yields associated with the "green" revolution has been possible, in a large part, due to the discovery and use of herbicides that selectively control weed growth in production fields (Dayan et al., 2009). Although, a key research objective has been to discover new herbicides that control the widest variety of weed species at low application rates and in an environmentally-friendly manner (Alister and Kogan, 2006; Beaudegnies et al., 2009), the use of agricultural chemicals does impact the environment. For instance, these chemicals can change the microbial community structure, by the selection of potential strains responsible for herbicide degradation (Ding et al., 2009), and oxidative stress response to herbicide (Martins et al., 2011). Further, it has been estimated that nearly 80% of world's human population is exposed to pesticides in water (Vörösmarty et al., 2010). The potential transfer of pesticides to water bodies depends on the pesticide used and environmental characteristics (Arias-Estévez et al., 2008). To minimize possible threats, information on the environmental fate of new herbicides is needed.

Mesotrione [2-(4-methylsulfonyl-2-nitrobenzoyl)1,3-cyclohexanedione] (Fig. 1) was first registered for use as a pre- and post-emergent herbicide in 1993 for controlling the growth of broadleaf weeds. Mesotrione has been used in Europe, the USA, and Brazil since 2000, 2001, and 2003, respectively, chiefly under the brand name Callisto (Alferness and Wiebe, 2002). This herbicide functions by inhibiting 4-hydroxyphenylpyruvate dioxygenase (HPPD) activity in target plants, which converts tyrosine to plastoquinone and α-tocopherol, producing bleaching symptoms on leaves (Boydston and Al-Khatib, 2008; Aliferis et al., 2009). The benzoyl at the C2 and C4 positions has been shown to be essential for herbicide function, and the strong electron-withdrawing cyclohexanedione moiety is responsible for slowing the rate of detoxification by plants (Mitchell et al., 2001).

Despite its widespread use, however, few microorganisms have been reported to catabolize mesotrione. Mesotrione has been shown to lower bacterial diversity in enrichment experiments at concentrations ranging from 0.07 to 0.15 mM (Batisson et al., 2009). Durand et al. (2006), studying fungal and bacterial strains in rain water isolated *Bacillus* sp., strain capable to completely biotransforming 5 mM mesotrione. They identified AMBA (2-amino-4-methylsulfonyl benzoic acid) as one of the metabolites, but was not the major one. *In situ* NMR and HPLC was used to demonstrate a rapid transformation of mesotrione. Alferness and Wiebe (2002) suggested that 4-methylsulfonyl-2-nitrobenzoic acid (MNBA) and AMBA were the two major parent metabolites from mesotrione degradation in soil and in water.

Mesotrione, in high concentrations, was shown to have an impact on bacterial community structure on soil in Limagne, France, producing mesotrione-sensitive and mesotrione-adaptative strains.

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doi:10.1016/j.chemosphere.2011.12.041

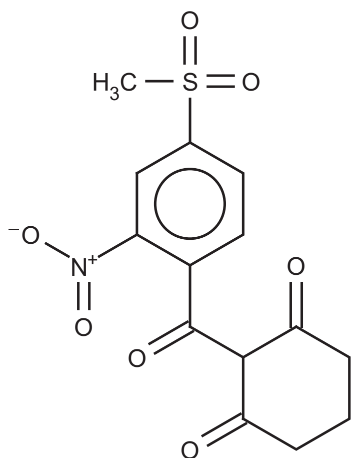

Fig. 1. Mesotrione structure.

Bacillus sp. and *Arthrobacter* sp. were isolated as adapted strains, but only the former was able to completely and rapidly biotransform mesotrione (Batisson et al., 2009). Crouzet et al. (2010) studied the responses of microbial communities to pure or formulated (Callisto) mesotrione, applied at three different doses, and found MNBA and AMBA as biodegradation products in high applied rates.

In this study, we report on the isolation of a *Pantoea ananatis* strain that completely degraded mesotrione in a pathway that does not produce AMBA and MNBA intermediate products. The bacterium was isolated from water environments surrounded by mesotrione treated fields. To our knowledge, this is the first report of *P. ananatis* being involved in pesticide degradation although *Pantoea* sp. strains have previously been reported to be associated with animal and plant diseases, can readily be found in the environment (Brady et al., 2008; Baldwin et al., 2009; Smits et al., 2011). Results of this study may lead to a better understanding about the fate of mesotrione in water.

2. Material and methods

2.1. Chemicals

A commercial formulation of Callisto, containing 48% of active ingredient mesotrione (Fig. 1), was used in this study. In culture media pure chemical mesotrione was used and was kindly provided by Syngenta Crop Protection, Greensboro, NC (USA). For HPLC and LC–MS/MS experiments, we used analytical standard of Pestanal (Sigma–Aldrich). All other chemicals used in HPLC and LC–MS/MS were ACS grade and were from Fischer Scientific, Pittsburg, PA.

2.2. Samples collection and strains isolation

Samples (100 mL) of water were collected from a lake and streams surrounded by corn fields treated with mesotrione (Callisto), at Capão da Onça School Farm – Ponta Grossa State University, Ponta Grossa-PR, Brazil (longitude: 50°03' W, latitude: 25°05' S and an average altitude of 1000 m). Two samples were collected in each location, and plated in triplicate in mesotrione minimal medium (MMM), containing 10 mM potassium phosphate buffer, pH 7.0; and following compounds in g L^{-1}: 3 $NaNO_3$; 0.5 $MgSO_4$; 0.5 KCl; 0.01 $FeSO_4$; 0.04 $CaCl_2$; 0.001 $MnSO_4$; 0.4 glucose; 15 agar, and 0.04 mM (1 × FR, Filed Rate) mesotrione (Callisto). Plates were incubated at 18–37 °C. Strains with high tolerance to mesotrione were isolated and stored in 50% glycerol at −86 °C until used.

2.3. Bacterial Identification by sequencing 16S rDNA

The bacterial strains were cultivated on LB Agar in g L^{-1} (10 bacto peptone, 5 yeast extract, 10 NaCl, 15 Agar) and after incubation a single colony was collected for strain identification. The 16S rRNA genes were amplified using primers 8F 5′-AGAGTTTGATCCTG GCTCAG-3′ and 1492R 5′-GGTTACCTTGTTACGACTT-3′ (Lane, 1991) and sequenced at the University of Minnesota, BioMedical Genomics Center in St. Paul, MN USA as previously described (Munoz et al.,

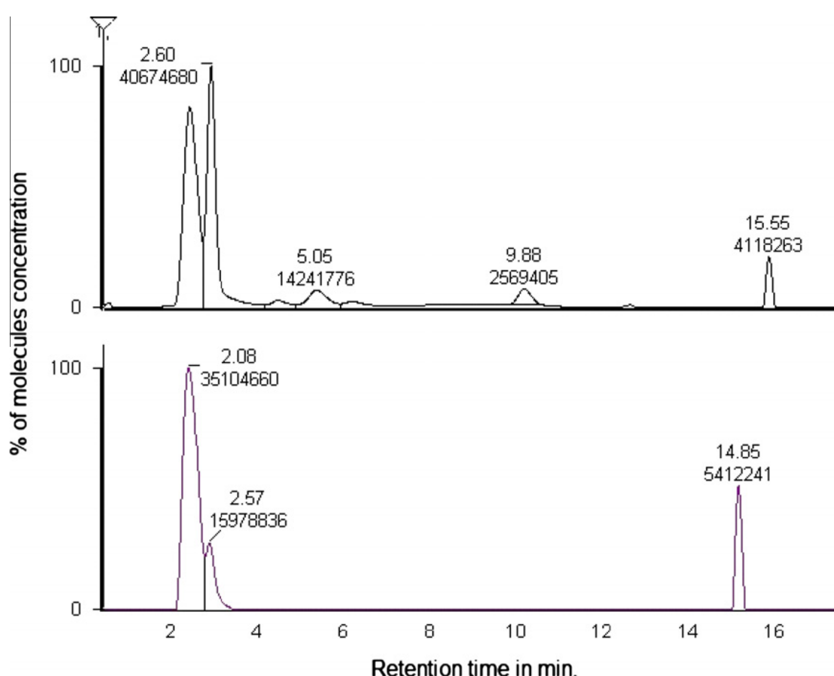

Fig. 2. HPLC chromatograms of mesotrione degradation by *P. ananatis*. Upper panel: mesotrione degradation peaks of *P. ananatis* after 24 h of incubation with MMM + herbicide. *Bottom panel*: mesotrione peak in 0 h of incubation in BMM + mesotrione (mesotrione RT = 14.85 min).

2011). Results were analyzed by blast analysis using the Ribosomal Database Project website (http://rdp.cme.msu.edu).

2.4. Mesotrione degradation analysis

To determine mesotrione degradation capability, strains were grown in 100 mL LB broth containing 0.04 mM mesotrione (Syngenta) in 500 mL flasks and incubated at 30 °C, with shaking at 200 rpm. Cells were harvested after 24 h and centrifuged at 8000 g for 15 min at 4 °C. The bacterial pellet was washed, twice, in MM (MMM without mesotrione) and the cells were resuspended in 10 mL 0.04 mM mesotrione MMM, and incubated at 30 °C on a rotary shaker (200 rpm). Samples (1 mL) were directly taken from the incubation medium (0 and 24 h), and centrifuged at 10000g for 5 min. The supernatant (0.9 mL) was frozen until analysis by HPLC. Prior to HPLC analyses, samples were syringe filtered (0.22 μm). HPLC analyses were accomplished with Waters Alliance 2695 high performance liquid chromatography, coupled with photo diode array. A Zorbax, RX-C8 column (2.1 mm ID × 150 mm long × 5 μm film thickness), was used for separation. The column temperature was maintained at 35 °C and the mobile phase, at 0.2 mL min^{-1}, was a gradient starting with 90% water (0.1% formic acid) (A): 10% Acetonitrile (B); 90% A at 0 min; 90% A at 3 min; 50% A at 10 min; 10% A at 15 min; 10% A at 20 min; 90% A at 25 min; 90% A at 30 min. The sample injection volume was 50 μL and samples were maintained at 20 °C in the autosampler to minimize potential analyte decomposition, while the column was maintained at 35 °C to improve the peak resolution.

2.5. Nutrient uptake and mesotrione degradation

To determine if mesotrione was used as carbon, nitrogen or sulfur source, and to correlate these capacities with herbicide degradation, bacterial cultures were incubated in different media, in triplicate, for 24 h at 30 °C, with shaking at 200 rpm. The following media were investigated: (A) MMM; (B) MMM with no carbon (no glucose); (C) MMM with no nitrogen (replacing $NaNO_3$ salt with 3 g L^{-1} NaCl); and (D) MMM with no sulfur (replacing SO_4 salts with 0.5 g L^{-1} $MgCl_2$; 0.01 g L^{-1} $FeCl_3$; and 0.001 g L^{-1} $MnCl_2$). Samples (100 μL) were taken after 24 h for bacterial growth measurements using a spectrophotometer at 660 nm. After 5 d, 3 mL samples were collected for HPLC degradation analyses. HPLC samples were centrifuged at 10000g for 5 min. The supernatant (0.9 mL) was frozen until analysis by HPLC. Prior to HPLC analyses, samples were syringe filtered.

2.6. Metabolite identification

Analysis for loss of the mesotrione parent compound was accomplished with Waters Alliance 2695 HPLC, Milford, MA, coupled to an Applied Biosystems, Carlsbad, CA, API 3200 LC–MS/MS. A Zorbax, RX-C8 column (2.1 mm ID × 150 mm long × 5 μm film thickness, Agilent Technology, Santa Clara, CA), was used for separation as described above. All compounds were detected using LC–DAD (Waters 2996 Photo diode array detector) with DAD monitoring at 210–400 nm, and LC–MS/MS negative ionization or thermo spray – full range scan mode, from 80 to 400 amu, with the following mass spectrometer conditions: Curtain Gas interface: 207 kPa, IS voltage: −4000 V, Gas 1: 207 kPa, Gas 2: 207 kPa, Ions source temperature: 400 °C, dwell time: 200 ms.

Sample extracts were acidified with formic acid and passed through a conditioned C18 solid phase extraction cartridge (SPE). The SPE was eluted with MeOH, the solution was evaporated to dryness with N_2, and then brought to 1 mL with 50:50 Acetonitrile:H_2O.

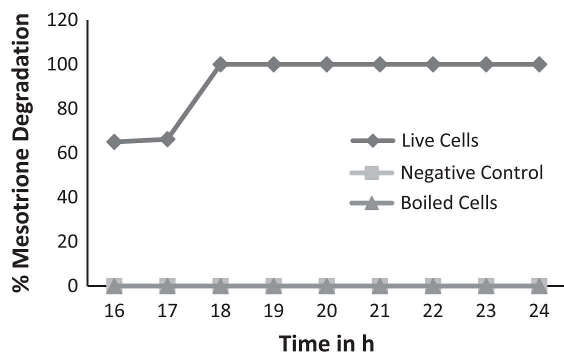

Fig. 3. Percentages of mesotrione degradation based on HPLC data.

3. Results and discussion

3.1. Strain isolation

A mesotrione degrading bacterium, *P. ananatis* (Genbank Accession AF264684), was found among 359 microorganisms that grew on minimal medium with 1 × FR mesotrione (Callisto). In contrast, Crouzet et al. (2010) did not find mesotrione degradation activity in 1 × FR. One of the reasons for this may be due to the fact that

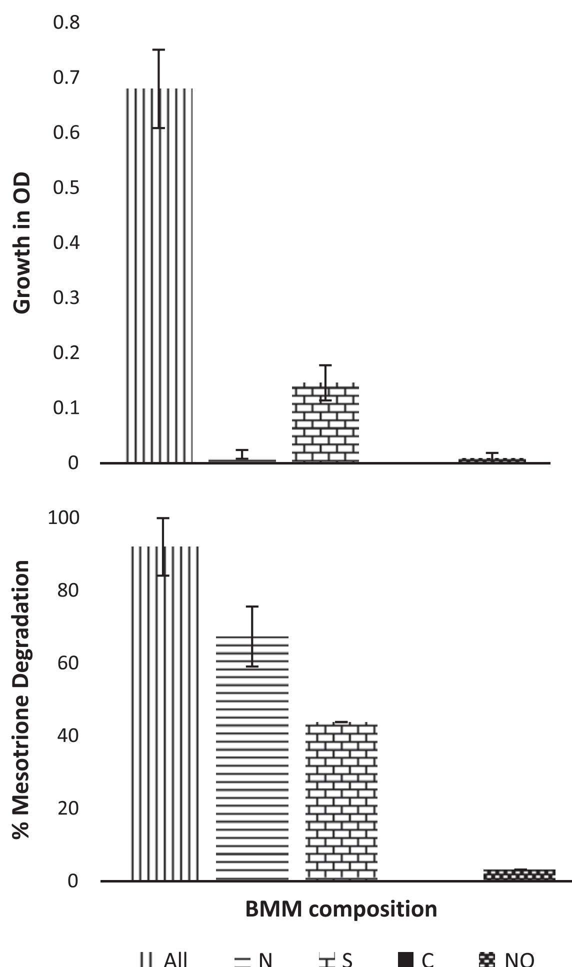

Fig. 4. *P. ananatis* growth data and mesotrione degradation data at same selective culture conditions. *Upper graphic*: growth data of *P. ananatis* in 25 mL of MMM media, in carbon, nitrogen and sulfur uptake tests, in 125 mL flasks, and 5 d of incubation at 30 °C. All = MMM; N = MMM − nitrate; S = MMM − sulfur; C = MMM − glucose; NO = MMM − mesotrione − glucose. *Bottom graphic*: HPLC data from mesotrione degradation by *P. ananatis* after 5 d of incubation at 30 °C, in the same experimental conditions of upper graphic.

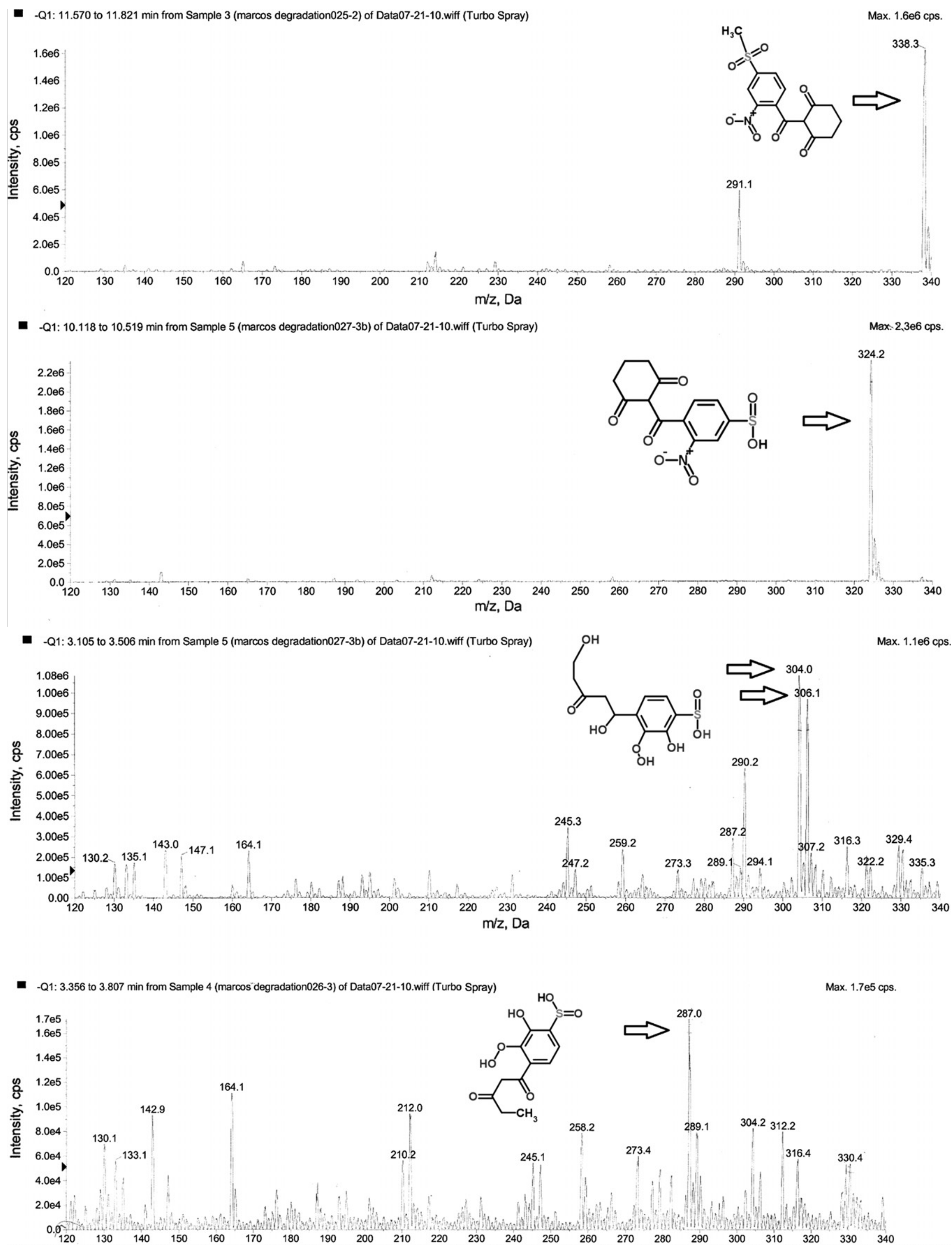

Fig. 5. Mass spectra of metabolites and possible structures. ESI mass spectra generated metabolites molecular weight, which was used to predict the more stable molecular structure. After the H in negative mode is lost, then the resultant structure would have the mass indicated at spectrogram.

we used commercial Callisto, containing 48% mesotrione as active ingredient, while the Callisto used by Crouzet et al. (2010) contained 10% active ingredient.

3.2. Mesotrione degradation analysis

HLPC data analyses showed that *P. ananatis* could degrade all the added mesotrione (Fig. 2). After 17–18 h of incubation (Fig. 3).

Only one mesotrione peak could be seen at 0 h of bacterial incubation. However, in 24 h, three different degradation peaks could be observed (Fig. 2). Batisson et al. (2009) also reported that a *Bacillus* sp. from soil of Limagne (Puy-de-Dôme, France) could rapidly and completely degrade mesotrione in 24 h. In our studies, however, we report for the first time that Gram negative bacteria can also degrade this herbicide.

Results in Fig. 3 show that live cells are required for degradation, there was no degradation by heat killed *P. ananatis*. This also shows that mesotrione was not lost from the culture medium by cell adsorption.

3.3. Nutrient uptake and mesotrione degradation

Fig. 4 shows growth data of *P. ananatis* inoculated in different media, and corresponding analyses for mesotrione degradation by HPLC. These data indicate the bacteria did not use mesotrione as sole carbon source for growth, in contrast to what was found with the *Bacillus* sp. strain (Batisson et al., 2009). Thus glucose was necessary for *P. ananatis* to start growth and degradation (Kao et al., 2004).

Mesotrione inhibits the enzyme HPPD, which, in plants, is a component of the biosynthetic pathway that converts tyrosine to plastoquinone and α-tocopherol. Plastoquinone is a critical cofactor for phytoene desaturase, a component of the carotenoid biosynthetic pathway. Depletion of plastoquinone levels by inhibition of HPPD results in depletion of carotenoids (Mitchell et al., 2001). In *P. ananatis* the *crtE* gene codify for diphosphate (GGPP) synthase, which produces a condensation of three molecules of isopentenyl diphosphate with one of dimethylallyl diphosphate to produce GGPP. The condensation of two molecules of GGPP into phytoene by phytoene synthase is encoded by *crtB* bacteria gene. Phytoene desaturase, encoded by *crtI*, converts phytoene to lycopene and, after some more metabolic steps, reach carotene production (Farré et al., 2010). We suggest that *P. ananatis* strain degrades mesotrione not for using it as carbon source, but as a strategy to avoid the inhibition of carotene synthesis.

3.4. Metabolite identification

LC-MS/MS analysis of mesotrione has been done in both positive (Gervais et al., 2008) and negative mode (Gomides Freitas et al., 2008). Analysis in negative mode of culture medium following mesotrione degradation by *P. ananatis* is shown in Fig. 5, and several degradation peaks were observed; no degradation peaks were observed in positive mode. We could predict three steps in the mesotrione degradation pathway, represented by the higher degradation peaks in the spectrograms. The masses associated to the peaks in spectrograms are consistent with the calculated masses and proposed structures.

It was previously shown that MNBA and AMBA were the major products of mesotrione degradation found in soil (Alferness and Wiebe, 2002; Bonnet et al., 2008; Batisson et al., 2009). These compounds, mainly AMBA (Bonnet et al., 2008), have been considered more toxic than mesotrione itself (Crouzet et al., 2010). Results of LC/MS/MS analysis for the water-borne *P. ananatis* strain suggest a possible new mesotrione degradation pathway that does not go through MNBA and AMBA intermediates. Durand et al. (2010), in further studies with *Bacillus* sp. 3 B6, considered a new degradation pathway, which leads to intermediary hydroxylamino derivatives, some of which are toxic and mutagenic. Mesotrione was considered a toxic molecule due to the strong withdrawing of 4-nitro and sulfur groups (Mitchell et al., 2000) and is more stable in water and more resistant to photolysis than sulcotrione, a triketone herbicide (Chaabane et al., 2007). The authors indicated that the major difference between these structures, the NO_2 group, was responsible the stability of mesotrione.

Interestingly, *E. coli* K strains TOP10 and DH5-α, and *E. coli* B strains CGSC 5713 (Lemonnier et al., 2008) and CGSC 5365 (Khankal et al., 2008) were also found in this study to degrade 100% of the added mesotrione (0.04 mM) in 24 h in mesotrione degradation analysis by HPLC (data not shown). This suggests that mesotrione degradation by bacteria may not be as unique as previously thought, especially if carried out via cometabolic processes. We believe this is the reason for the difficulties in finding specific mesotrione degradation genes (Durand et al., 2006).

Crouzet et al. (2010) studied the response of microbial communities to pure and formulated mesotrione (Callisto). They reported that MNBA and AMBA were major biodegradation products from cells receiving high concentrations of mesotrione. They concluded that $100 \times$ FR doses with pure mesotrione seemed to induce stronger microbial response than Callisto, because this contains adjuvants with potential side-effects in some microbial populations. Callisto caused impact on soil microbial activity sooner than mesotrione. Our results show that, in agreement with Crouzet work, *P. ananatis* was isolated from an aquatic environment, probably with low herbicide concentration, and could not grow at $100 \times$ FR Callisto, due the adjuvants side-effects, but was able to degrade mesotrione 0.04 mM ($1 \times$ FR active ingredient).

4. Conclusions

A new mesotrione degradation pathway for *P. ananatis* is suggested in this work, resulting in intermediate products other than MNBA or AMBA, which are more toxic than the original herbicide molecule. Mesotrione is not a carbon source to *P. ananatis,* and its degradation is probably due to a cometabolic process with the glucose as an energy starter. *P. ananatis* could not grow in high Callisto concentrations in media culture, probably due to adjuvants' side-effects. Fast metabolism and no toxic degradation products are desirable characteristics for herbicide bioremediation process.

Acknowledgments

The authors thank CAPES for financial support to M. Pileggi and to CNPq for travel grants to the USA. We also thank Syngenta Crop Protection for providing pure mesotrione.

References

Alferness, P., Wiebe, L., 2002. Determination of mesotrione residues and metabolites in crops, soil, and water by Liquid Chromatography with Fluorescence Detection. J. Agric. Food Chem. 50, 3926–3934.

Aliferis, K.A., Materzok, S., Paziotou, G.N., Chrysayi-Tokousbalides, M., 2009. *Lemna minor* L. as a model organism for ecotoxicological studies performing ^{1}H NMR fingerprinting. Chemosphere 76, 967–973.

Alister, C., Kogan, M., 2006. ERI: environmental risk index. A simple proposal to select agrochemicals for agricultural use. Crop. Prot. 25, 202–211.

Arias-Estévez, M., López-Periago, E., Martínez-Carballo, E., Simal-Gándara, J., Mejuto, J.-C., García-Río, L., 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agric. Ecosyst. Environ. 123, 247–260.

Baldwin, C.D., Howe, G.B., Sampath, R., Blyn, L.B., Matthews, H., Harpin, V., Hall, T.A., Drader, J.J., Hofstadler, S.A., Eshoo, M.W., Rudnick, K., Studarus, K., Moore, D., Abbott, S., Janda, J.M., Whitehouse, C.A., 2009. Usefulness of multilocus polymerase chain reaction followed by electrospray ionization mass spectrometry to identify a diverse panel of bacterial isolates. Diagn. Microbiol. Infect. Dis. 63, 403–408.

Batisson, I., Crouzet, O., Besse-Hoggan, P., Sancelme, M., Mangot, J.F., Mallet, C., Bohatier, J., 2009. Isolation and characterization of mesotrione-degrading *Bacillus* sp. from soil. Environ. Pollut. 157, 1195–1201.

Beaudegnies, R., Edmunds, A.J.F., Fraser, T.E.M., Hall, R.G., Hawkes, T.R., Mitchell, G., Schaetzer, J., Wendeborn, S., Wibley, J., 2009. Herbicidal 4-hydroxyphenylpyruvate dioxygenase inhibitors. A review of the triketone chemistry story from a Syngenta perspective. Bioorg. Med. Chem. 17, 4134–4152.

Bonnet, J.L., Bonnemoy, F., Dusser, M., Bohatier, J., 2008. Toxicity assessment of the herbicides sulcotrione and mesotrione toward two reference environmental microorganisms: *Tetrahymena pyriformis* and *Vibrio fischeri*. Arch. Environ. Contam. Toxicol. 55, 576–583.

Boydston, R.A., Al-Khatib, K., 2008. Exudation of mesotrione from potato roots injures neighboring plants. Weed Sci. 56, 852–855.

Brady, C., Cleenwerck, I., Venter, S., Vancanneyt, M., Swings, J., Coutinho, T., 2008. Phylogeny and identification of *Pantoea* species associated with plants, humans and the natural environment based on multilocus sequence analysis (MLSA). Syst. Appl. Microbiol. 31, 447–460.

Chaabane, H., Vulliet, E., Joux, F., Lantoine, F., Conan, P., Cooper, J.-F., Coste, C.-M., 2007. Photodegradation of sulcotrione in various aquatic environments and toxicity of its photoproducts for some marine micro-organisms. Water Res. 41, 1781–1789.

Crouzet, O., Batisson, I., Besse-Hoggan, P., Bonnemoy, F., Bardot, C., Poly, F., Bohatier, J., Mallet, C., 2010. Response of soil microbial communities to the herbicide mesotrione: a dose-effect microcosm approach. Soil Biol. Biochem. 42, 193–202.

Dayan, F.E., Cantrell, C.L., Duke, S.O., 2009. Natural products in crop protection. Bioorg. Med. Chem. 17, 4022–4034.

Ding, N., Guo, H., Hayat, T., Wu, Y., Xu, J., 2009. Microbial community structure changes during Aroclor 1242 degradation in the rhizosphere of ryegrass (*Lolium multilorum* L.). FEMS Microbiol. Ecol. 70, 305–314.

Durand, S., Amato, P., Sancelme, M., Delort, A.-M., Combourieu, B., Besse-Hoggan, P., 2006. First isolation and characterization of a bacterial strain that biotransforms the herbicide mesotrione. Lett. Appl. Microbiol. 43, 222–228.

Durand, S., Sancelme, M., Besse-Hoggan, P., Combourieu, B., 2010. Biodegradation pathway of mesotrione: complementarities of NMR, LC–NMR and LC–MS for qualitative and quantitative metabolic profiling. Chemosphere 81, 372–380.

Farré, G., Sanahuja, G., Naqvi, S., Bai, C., Capell, T., Zhu, C., Christou, P., 2010. Travel advice on the road to carotenoids in plants. Plant Sci. 179, 28–48.

Gervais, G., Brosillon, S., Laplanche, A., Helen, C., 2008. Ultra-pressure liquid chromatography-electrospray tandem mass spectrometry for multiresidue determination of pesticides in water. J. Chromatogr. A 1202, 163–172.

Gomides Freitas, L., Singer, H., Muller, S.R., Schwarzenbach, R.P., Stamm, C., 2008. Source area effects on herbicide losses to surface waters – a case study in the Swiss Plateau. Agric. Ecosyst. Environ. 128, 177–184.

Kao, C.M., Chai, C.T., Liu, J.K., Yeh, T.Y., Chen, K.F., Chen, S.C., 2004. Evaluation of natural and enhanced PCP biodegradation at a former pesticide manufacturing plant. Water Res. 38, 663–672.

Khankal, R., Luziatelli, F., Chin, J.W., Frei, C.S., Cirino, P.C., 2008. Comparison between *Escherichia coli* K-12 strains W3110 and MG1655 and wild-type *E. coli* B as platforms for xylitol production. Biotechnol. Lett. 30, 1645–1653.

Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematics. John Wiley, West Sussex, UK.

Lemonnier, M., Levin, B.R., Romeo, T., Garner, K., Baquero, M.-R., Mercante, J., Lemichez, E., Baquero, F., Blázquez, J., 2008. The evolution of contact-dependent inhibition in non-growing populations of *Escherichia coli*. P. Roy. Soc. B-Biol. Sci. 275, 3–10.

Martins, P.F., Carvalho, G., Gratão, P.L., Dourado, M.N., Pileggi, M., Araújo, W.L., Azevedo, R.A., 2011. Effects of the herbicides acetochlor and metolachlor on antioxidant enzymes in soil bacteria. Process. Biochem. 46, 1186–1195.

Mitchell, G., Bartlett, D.W., Fraser, T.E.M., Hawkes, T.R., Holt, D.C., Townson, J.K., Wichert, R.A., 2001. Mesotrione: a new selective herbicide for use in maize. Pest. Manage. Sci. 57, 120–128.

Munoz, A., Koskinen, W.C., Cox, L., Sadowsky, M.J., 2011. Biodegradation and mineralization of metolachlor and alachlor by *Candida xestobii*. J. Agric. Food Chem. 59, 619–627.

Smits, T.H.M., Rezzonico, F., Duffy, B., 2011. Evolutionary insights from *Erwinia amylovora* genomics J. Biotechnol. doi:10.1016/j.jbiotec.2010.10.075.

Vörösmarty, C.J., McIntyre, P.B., Gessner, M.O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S.E., Sullivan, C.A., Liermann, C.R., Davies, P.M., 2010. Global threats to human water security and river biodiversity. Nature 467, 555–561.