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A Dynamic Analytic Approach to Evaluate Metabolism of Imidaclothiz following Application by Biotransformation in *Stenotrophomonas maltophilia*

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To mimic the action of imidaclothiz in environment more easily, pure strain were used to biotransform it. Hydroxyl imidaclothiz and olefin imidaclothiz are formed as the two main metabolites of imidaclothiz during its metabolism by *Stenotrophomonas maltophilia*. A new method has been developed for the simultaneous analysis of these metabolites by HPLC at 250 nm as a unimodal elution peak, even though wavelengths of 269, 250 and 314 nm are ideal for the individual analysis of imidaclothiz, hydroxyl imidaclothiz and olefin imidaclothiz, respectively. The solubilities of imidaclothiz, hydroxyl imidaclothiz and olefin imidaclothiz were determined to be 0.25, 0.9 and 1.15 g/l, respectively, by HPLC using a standard curve equation. Although hydroxyl imidaclothiz could be converted to olefin imidaclothiz under acidic conditions (e.g., HCl or H_2SO_4) it could not be transformed to olefin imidaclothiz by *S. maltophilia.* Consequently, we have proposed a partial metabolic pathway for imidaclothiz, which involves the simultaneous formation of olefin imidaclothiz and hydroxyl imidaclothiz. These results of pure biotransformation made it easier to understand the dynamic activities of insecticides in environment and will hopefully be referenced and support furthering studies on the metabolism of imidaclothiz in soil in future following application.

Key words: Imidaclothiz, metabolic pathway, stability, Transformation, *Stenotrophomona smaltophilia.*

Neonicotinoids are one of the most rapidly expanding classes of insecticides in the pesticide market (Buchholz *et al*. 2002; Elbert *et al*. 2008; Tomizawa *et al*. 2005). Although they have been reported to be non-toxic to mammals, residual levels of these pesticides can be readily detected in the environment and several food stuffs, which could impact the health of consumers (Ming Wu *et al*. 2010; anetaBargañska *et al*. 2014). Furthermore, the metabolites of insecticides can sometimes show higher activity than the insecticides themselves. As suggested by Nauen, this eventuality could provide an explanation as to why some insecticides, such as imidacloprid, exert their activity over longer periods of time and with better control when they are applied as a soil drench rather than a foliar application (Nauen *et al*. 1998, 2001). To develop a deeper understanding of the activity of insecticides, it is therefore necessary to analyze their metabolites and dynamics following their application, especially when they are applied as a soil drench.

Imidaclothiz, or 1-(2-chloros-5-thiazolylmethyl)-N-nitroimida-zolin-2-

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ylideneamine, is a new compound of the neonicotinoid family (Gupta *et al*. 2008). We previously demonstrated that imidaclothiz could be converted to hydroxy limidaclothiz and olefin imidaclothiz by *S.maltophilia* (Dai *et al*. 2010). We also demonstrated that the activities of hydroxyl and olefin imidaclothiz were similar to that of imidaclothiz against the horsebean aphid *Aphis craccivora* and the mosquito larva *Culexpipiens*, which suggested that soil fertilization and seed soaking would be better methods for the application of imidaclothiz than foliage spray (Dai *et al*. 2010). These results suggested that the metabolites should be considered to the same extent as the parent pesticide during any analysis designed to evaluate the residues of imidaclothiz. Herein, we have developed a new analytical method for the simultaneous evaluation of the metabolites of imidaclothiz as well as their stability in different solutions. The metabolic pathway of imidaclothiz has been studied using pure microbio transformation to develop a deeper understanding of their fate both in vivo and the environment. The results of this study should therefore provide a better understanding of the metabolism of imidaclothiz in soil.

MATERIALS AND METHODS

Chemicals and bacteria

Imidaclothiz (>97% purity) were provided by Nantong Jiangsu Agrochemical& Chemical Limited Corporation, China. All of the solvents used for the HPLC analysis were purchased as the HPLC grade from Agilent Technologies (USA). All of the other solvents and inorganic reagents used in this study were purchased as the analytical grade from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Piperonylbutoxide (PBO) was purchased from Sigma–Aldrich Corporation (USA).

The bacterium *S. maltophilia* CGMCC 1.1788 was obtained from China General Microbiological Culture Collection Center (Beijing, China).

Bacterial cultivation and biotransformation

The method used in the current study has been described previously (Dai *et al*. 2010).

Stability of hydroxyl imidaclothiz in different solutions

Hydroxyl imidaclothiz was heated for 30 min at 30 °C in 0.1 M solutions of aqueous HCl, H_2SO_4, AcOH, H_2O and NaOH. HPLC analysis was used to determine the hydroxyl imidaclothiz content before and after the incubation process.

Inhibition of the hydroxylation and dehydrogenation of imidaclothiz by PBO

The inhibitory activity of PBO towards the biotransformation of imidaclothiz was examined in whole cells according to the method described by Matsuzaki and Wariishi (2004) using the procedures described in our previous study (Dai *et al*. 2007).

HPLC analysis

HPLC analysis was conducted using an Agilent 1100 HPLC system (Agilent, USA) with a Zorbaxoctadecylsilyl silica gel (ODS) column(250 × 4.6 mm, 5 mm). The column was eluted at a flow rate of 1 ml/min with a mobile phase of consisting of 0.01% (v/v) acetic acid in water and acetonitrile (65:35 " v/v) for imidaclothiz. The HPLC system was equipped with an Agilent G1314A UV detector to monitor the elution of the different materials.

RESULTS AND DISCUSSION

Determination of the most suitable wavelengths for detecting the residues by HPLC

It is clear from the chromatograms shown in Figure 1 that there were two products being tested by HPLC at 269 nm. A detection wavelength of 269 nm was selected for the current study because it represents the most suitable wavelength for the detection of imidaclothiz. It is noteworthy, however, that this wavelength may not have been the most suitable wavelength for the detection of metabolites. Products 1 and 2 were previously obtained by preparative HPLC and identified as olefin imidaclothiz and hydroxyl imidaclothiz, respectively (Dai *et al*. 2010). The best wavelengths for the detection of these compounds were determined to be 314 and 250 nm for olefin imidaclothiz and hydroxyl imidaclothiz, respectively. Previous work with these compounds showed that they exhibited the same activity towards insects as the parent compound (Dai *et al*. 2010). This result therefore demonstrated that the activity of the metabolites should be considered to the same extent as the parent insecticide following its application. This result also suggested that the levels of specific

metabolites in plants should be monitored following the application of the parent insecticide. With this in mind, it is important to select a suitable wavelength that allows for the simultaneous detection of imidaclothiz and its metabolites. A wavelength of 250 nm was selected in the current study for the simultaneous detection of hydroxyl imidaclothiz and olefin imidaclothizin the residues by HPLC using a unimodal elution peak. The solubilities of imidaclothiz, hydroxyl imidaclothiz and olefin imidaclothiz were also determined by HPLC to be 0.25, 0.9 and 1.15 g/l, respectively, by HPLC using the standard curve equation. It is well-known that metabolites are more water-soluble than their parent compound, which enables them to be readily transported to other tissues when they are

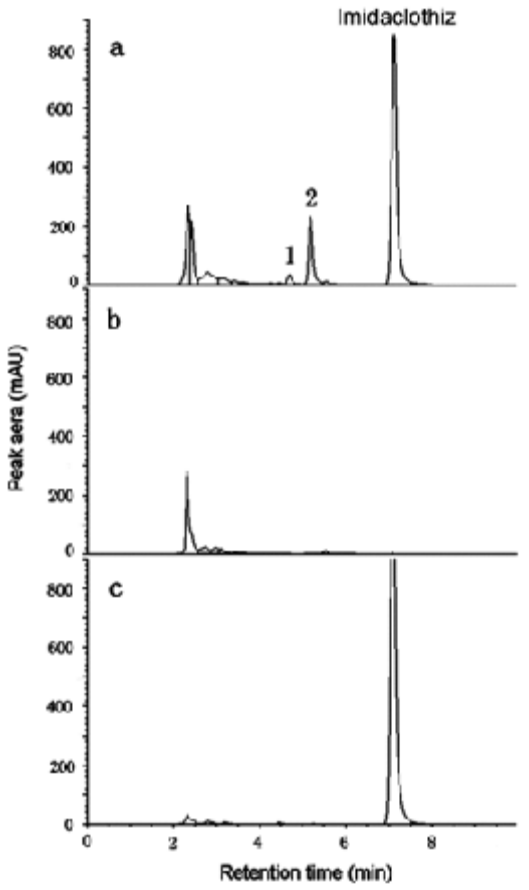

Fig. 1. HPLC spectra for imidaclothizfollowing its transformation by *S.maltophilia* (a: experimental groups with imidaclothiz and *S.maltophilia*, b: bacterial cells without imidaclothiz, c: control groups with imidaclothiz only)

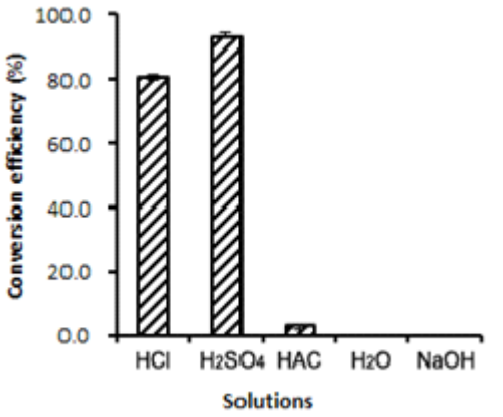

Fig.2 Stability of hydroxyl imidaclothiz in 0.1M solutions of HCl, H_2SO_4, HAC, NaOH and H_2O. Hydroxyl imidaclothiz was heated for 30min at 30°C. The transformation efficiency was determined as the ratio provided by the difference in the content of hydroxyl imidaclothiz before and after incubation compared with the original content. The data have been presented as the mean ± SD (n = 6) from three independent experiments,with two replicates for each experiment

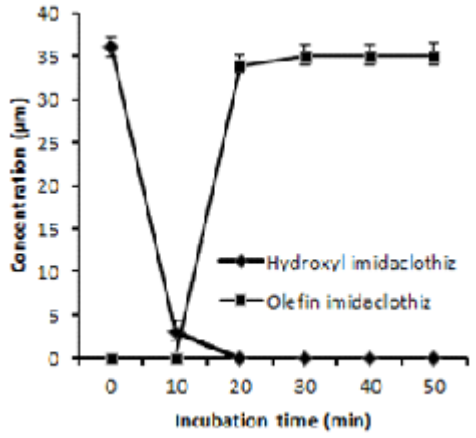

Fig. 3 Time-course experiments for the reaction of hydroxyl imidaclothiz in 0.1M HCl at 30 °C over a period of 70min. Hydroxyl imidaclothiz(0.36 mM) was incubated at 30 °C and a sample was collected every 10min for HPLC analysis. The concentrations of hydroxyl imidaclothiz and olefin imidaclothiz were calculated using the standard curve equation. The data have been presented as the mean ± SD (n = 6) from three independent experiments,with two replicates for each experiment

transformed in plants or animals. It should therefore be considered that the level of contamination resulting from metabolites in water systems will increase considerably because of the enhanced solubility of metabolites.

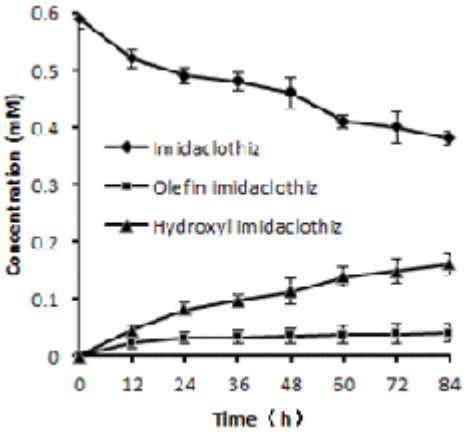

Fig. 4. Time-course experiments for the biotransformation of imidaclothiz by *S. maltophilia*. All of the transformations were carried out in 100-ml Erlenmeyer flasks containing10 ml of a cell suspension with 500 mg of sucrose and 2 mg of imidaclothiz.The data have been presented as the mean ± SD (n = 9) from three independent experiments,with three replicates for each experiment

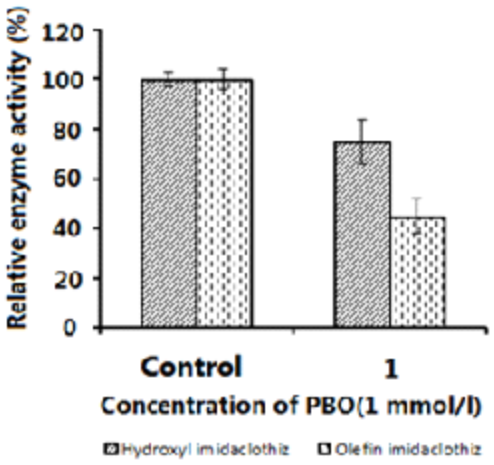

Fig. 5 Effect of PBO on the hydroxylation and dehydrogenation activities of resting cells. The transformation broth consisted of 10 ml of phosphate buffer containing 30 ml of fermentation broth (OD_{600} = 6), which had been harvested by centrifugation. The reactions were conducted in a 100-ml flask containing 10 ml of the transformation broth with an additional 2 mg of imidaclothiz. The experiments were conducted in duplicate, and the data have been expressed as the mean ± SD (n = 6)

The stability of metabolites in different solutions

The results of our previous study revealed that 5-hydroxyl imidacloprid could be converted to olefin imidacloprid under acidic conditions in a high molar yield (Dai *et al*. 2006). With this in mind, it was envisaged that hydroxyl imidaclothiz could also be converted to olefin imidaclothizin a similar manner. Based on the results shown in Figure 2, it is clear that hydroxyl imidaclothiz can be converted to the corresponding olefin under acidic conditions but not under alkaline conditions, even in water. Furthermore, these data show that hydroxyl imidaclothiz is stable under neutral and alkaline conditions but not stable under acidic conditions, which suggested that imidaclothiz could behave differently depending on the conditions provided by the soil. Furthermore, the level of conversion could be related to the concentration of free [H^+] because very little conversion was observed in the presence of AcOH, which provides a weakly acidic environment. It is noteworthy that the level of hydroxyl imidaclothiz decreased quickly over time, whereas the level of olefin imidaclothiz remained the same to begin with and then increased. These data suggested that an intermediate was being formed during the course of the metabolism process (Fig. 3).

Proposed mechanism for the metabolism of imidaclothiz in *S.maltophilia*

A time-course study for the biotransformation of imidaclothiz by *S. maltophilia* revealed that hydroxylimidaclothiz and olefin imidaclothiz could be simultaneously detected (Fig.

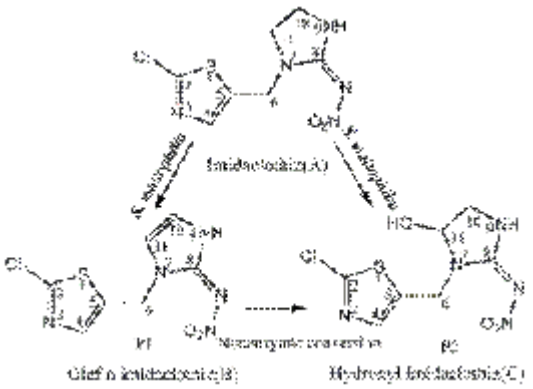

Fig. 6 Proposed partial metabolic pathway forimidaclothiz by *S.maltophilia*

4). Furthermore, the detection process was unaffected by the gradual increase in the amount of hydroxyl imidaclothiz and the simultaneous accumulation of a small amount of olefin imidaclothiz. However, it remained unclear at this stage whether the olefin imidaclothiz was derived from hydroxyl imidaclothiz by a biotransformation process or a chemical reaction. It was also unclear whether the olefin imidaclothiz was a substrate that could be converted to hydroxyl imidaclothiz. To develop a deeper understanding of the nature of these processes, we used pure olefin imidaclothiz and hydroxyl imidaclothiz as substrates and transformed them separately using *S. maltophilia* (Data not show). The results of these experiments showed that these substrates could not be interconverted, which suggested that olefin imidaclothiz was formed as a direct result of the dehydrogenation of imidaclothiz. These results also indicated that the hydroxylation of imidaclothiz to hydroxy limidaclothiz occurred simultaneously rather than the dehydration of the hydroxy limidaclothiz produced from imidaclothiz. Cytochrome P450 monooxygenase enzymes are generally involved in hydroxylation reactions (Schulz-Jander *et al*. 2002). The results of a PBO inhibition assay revealed that PBO inhibited the hydroxylation and dehydrogenation activities of resting cells by 70 and 45%, respectively, at a concentration of 1 mmol/l PBO (Fig. 5). These results further suggested that olefin imidaclothiz and hydroxyl imidaclothiz were reacting independently with different enzymes. Based on these results, we have proposed a metabolic pathway for imidaclothiz, which is shown in Fig. 6.

CONCLUSIONS

These primary results led us to understand deeply of imidaclothiz's fate in environment. So we can choose the proper wavelength as 250 nm for monitoring residues of imidaclothiz and acidic condition is encouraged to be an application environment because hydroxyl imidaclothiz with less biological activity can be easily converted to olefin imidaclothiz with good activity so that longer time and better control are achieved. The solubility of the metabolites showed us that imidaclothiz will be more water soluble following application. Biotransformation of metabolites helped us to know the metabolism pathway of imidaclothiz in vivo and vitro. This data will support further studies on the metabolism of imidaclothiz in soil in future. It demonstrated that pure biotransformation was an easy way to understand the dynamic activities of pesticides following application.

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