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Metabolic Pathway of Fenpropathrin By *Rhodopseudomonas palustris* PSB07-19

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The intermediates in fenpropathrin biodegradation process by *Rhodopseudomonas palustris* PSB07-19 were detected by gas chromatograph/mass spectrometer (GC/MS). Three intermediates, n-isopropyl-3-phenylpropanamide, 2,4-Dimethylamphetamin and 2-amino-1-[o-methoxyphenyl] propane were detected, and the retention times of these intermediates were 7.983 min, 8.764 min, and 9.236 min, respectively. Based on these intermediates, the deduced fenpropathrin biodegradation protocol was descriptive. This study hints exsiting different biodegradation pathway of pyrethroids residue, and would enrich our knowledge about removing and cleaning up pyrethroids residue in environments.

Key words: Biodegradation, Fenpropathrin, *Rhodopseudomonas palustris* PSB07-19, Biodegradation pathway.

Fenpropathrin, is one of most utilizing and indispensability of pyrethroids for protecting crops, vegetables and fruit trees. However, the adverse effects of its residue to agricultural environments and humanity itself are increasingly concern, for example, it is extremely toxic to the aquatic invertebrates[1,2]. For some of high toxic of organophosphorous peisticides progressive forbidding and being instead by pyrethroid pesticides, the risk of pyrethroid pesticides residues (of course, including fenpropathrin) was more and more critical.

As a practical solution for detoxifying pyrethriod pesticides residues, biodegradation is received attention in recent studies, for its merits of high efficiency, economical and without secondary pollution. Lots of organisms with excellent biodegrading capacity to parts of typical pyrethroid pesticides residues, such as fenvalerate, cypermethrin bifenthrin, deltamethrin and cyfluthrin, was isolated and well characterized[3-7]. Compared to these pyrethroid pesticides residues, little attempts for fenpropathrin biodegradation were carried out. In our previous study, *Rhodopseudomonas palustris* strain PSB07-19 was successful isolated for biodegrading fenpropathrin, nevertheless, it is not touching on biodegradation mechanism of fenpropathrin residue[8].

The objective of the present study was focused on detecting of intermediates in the process of biodegradation of fenpropathrin residue by *R. palustris* PSB07-19, and deducing the biodegradation protocol pathway.

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MATERIALS AND METHODS

Chemicals and media

Sample of fenpropathrin (95.0% pure analytical grade) were kindlyproviding by Dr Xingan Liu (Institute of Plant Protection of Chinese Academy of Agricultural Science). The standard samples of fenpropathrin (99.0% pure analytical grade) were purchased from the Tianjing orient green technology Co., Ltd (Jiangshu province, China). Fenpropathrin was dissolved in acetone as a stock solution (100 g/l).

The photosynthetic bacteria medium (PSB medium) used according to previous published reference [9], consists of (per 1 liter deionized water): K_2HPO_4, 0.2g; KH_2PO_4, 0.8g; $MgSO_4$, 0.2g; $CaSO_4 \cdot 2H_2O$, 0.1g; $NaMoO_4 \cdot 2H_2O$, 0.0033g; $FeSO_4 \cdot 7H_2O$, 0.005g; yeast extract 1.5g, taken to pH 7.2 with 10 mol/l NaOH. Technical agar (15g) was added to 1 liter PSB medium for a solid plate medium. After autoclaving, the fenpropathrin was added at the concentrations stated below.

Preparation of inoculation of *R. palustris* PSB07-19

The R. palustris PSB07-19, which stored in -80!, was activated by spread plate method in solid PSB medium, cultured in approximate 2000 lx illumination, 30±1 ! until appearing clear single colonies[9]. Then one of single colony was inoculated into PSB medium and cultured in same conditions. The starting number of cells was adjusted to approximately 10^9 cfu/ml as inoculants.

Full-scan spectra of intermediates

Metabolites were isolated from the culture filtrates by extraction with ethyl acetate, which centrifuged and removed of the cells of *R. palustris* PSB07-19 grown in fenpropathrin (100 mg/l), the residue was dissolved in methanol. The intermediates of residues were analyzed by GC/MS. GC/MS analyses were performed according to previous publication[10]. The parameters of GC/MS (Agilent 6890N/5975 series GC-MSD, Agilent) were as following: equipping for electron ionization (EI) (70eV) with a trap current of 100mA and a source temperature as 200 °C. The setting of mass scanning was full-scan spectra over the ranges of *m/z* of 45-500 at 2 s per scan. Data collection and processing were performed using Agilent MSD chemstation software containing the Agilent chemical library.

RESULTS AND DISCUSSION

Intermediates in biodegrading process of fenpropathrin by *R. palustris* PSB07-19

Analysis of intermediates in the culture extracts in the processing of fenpropathrin biodegradation by *R. palustris* PSB07-19 using GC/MS, appearing several compounds in mass spectrum figure. Based on mass spectrum chemicals library of Agilent MSD chemstation, three intermediates were identified, compound I was in line with n-isopropyl-3-phenylpropanamide (Fig 1), compound II corresponded well with 2,4-Dimethylamphetamin (Fig 2), and compound III was identified as 2-amino-1-[o-methoxyphenyl] propane (Fig 3). The retention times of these compounds were 7.983 min, 8.764 min, and 9.236 min, respectively.

Bioremediation is an increasing concern on removing or cleaning up of agricultural contaminants. The first and foremost for potential application of some an organism are explicating

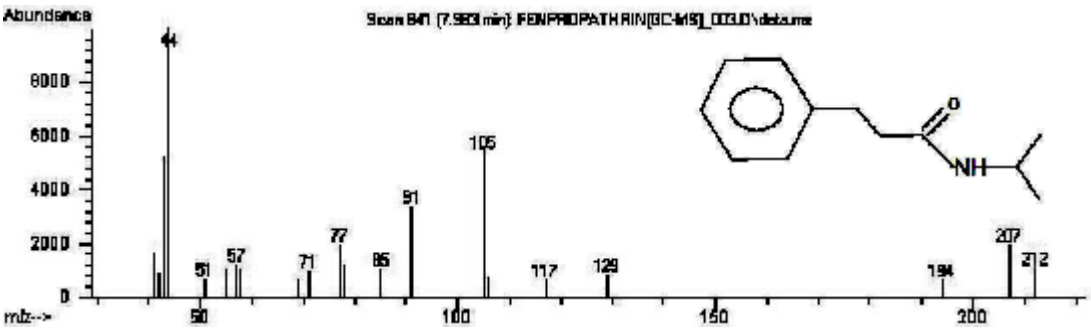

Fig. 1. Mass spectrum of intermediate of N-Isopropyl-3-phenylpropanamide

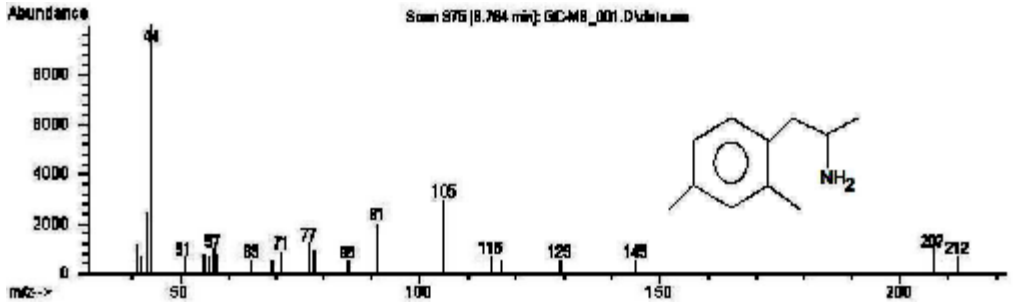

Fig. 2. Mass spectrum of intermediate of 2,4-Dimethylamphetamin

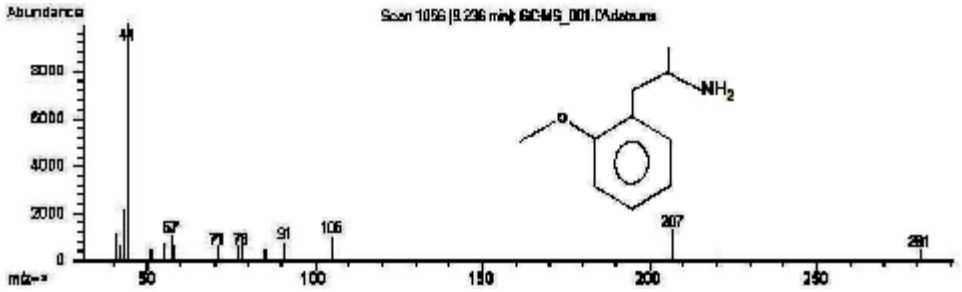

Fig. 3. Mass spectrum of intermediate of 2-Amino-1-[o-methoxyphenyl]propane

the metabolic intermediates, so as to understanding the eco-safety of biodegradation process [11]. One of efficient ways for detecting intermediates is utilizing the GC/MS, and lots of previous studies uncovered the biodegrading pathway of some typical pyrethroids including fenvalerate, permethrin, tetramethrin and deltamethrin [12]. Moreover, Zhang et al [9] detected one esterlysis intermediate in fenpropathrin biodegradation process by *Rhodopseudomonas* sp. strain PSB07-6 using GC/MS. However, to our best knowledge, above three intermediates (Fig 1-Fig3) are never seen in previous studies, hints there is probably different and novel metabolic pathway of fenpropathrin by *R. palustris* PSB07-19.

Proposal pathway

According to the molecular skeleton of fenpropathrin, and detected three intermediates appearing in the biodegradation process, a deduced pathway protocol was descriptive (Fig 4). The biodegradation process of fenpropathrin by *R. palustris* PSB07-19 was including disrupting the single oxygen bridge bond, leading to losing a benzene ring, and then modifying the side chain containing cyano-group.

Formerly studies of biodegradation of cypermethrin demonstrated that there are two

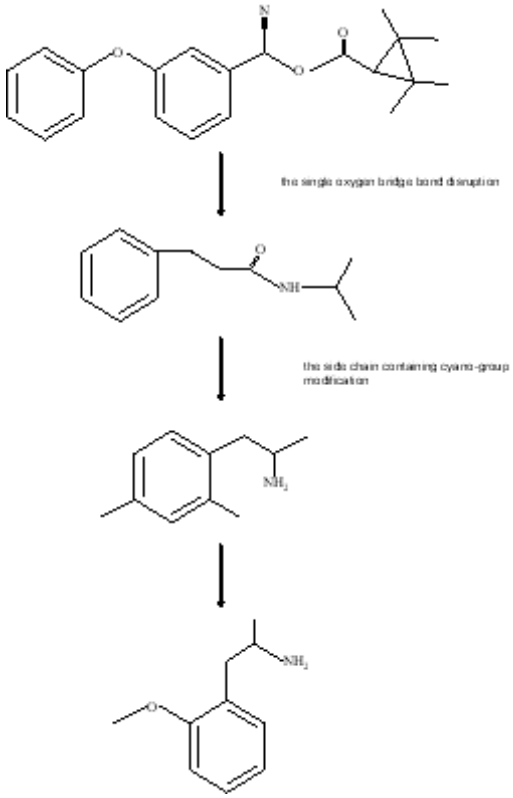

Fig. 4. Deduced pathway protocol of biodegradation of fenpropathrin by *R. palustris* PSB07-19

typical biodegradation pathways. The first one is known as hydroxylase pathway, appearing biodegradation intermediates of DVCA, 3-phenoxy benzoate and 4-hydroxy-3-phenoxybenzoic acid[13-16]. The other one is called as oxygenase pathway, existing biodegradation intermediates of protocatechuate and phenol[17-18]. But the similarity of both pathways is the terminal metabolic products, which are CO_2 and H_2O[17]. The biodegradation pathway of fenpropathrin by *R. palustris* PSB07-19 (Fig 4) is distinct difference with above both biodegradation pathway of cypermethrin, probably because of different biodegrading organism. And further intermediates studies should better understand the fenpropathrin biodegradation pathway.

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