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ORIGINAL ARTICLE

Aerobic degradation of fenvalerate by a Gram-positive bacterium, *Bacillus flexus* strain XJU-4

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Abstract Synthetic pyrethroid—fenvalerate—is one of the most widespread toxic pollutants and has adverse effect on living systems. However, little is known about its biotransformation mechanism in different microorganisms. To elucidate the pathway that might be involved in the catabolism of fenvalerate, we used *Bacillus flexus* strain XJU-4 (3-nitrobenzoate degrading organism) as an ideal fenvalerate degrading bacterium. Thin layer chromatography, high performance liquid chromatography and gas chromatography–mass spectrometry analysis results revealed that 3-phenoxybenzoate, protocatechuate, and catechol are the three main by-products of fenvalerate metabolism. Additionally, the bacterial cell-free enzymes showed the activities of fenvalerate hydrolyzing esterase, 3-phenoxybenzaldehyde dehydrogenase, 3-phenoxybenzoate dioxygenase, phenol hydroxylase, protocatechuate 2,3-dioxygenase and catechol-2,3-dioxygenase. Thus, in strain XJU-4, protocatechuate and catechol were further metabolized through *meta*-cleavage pathway. Moreover, laboratory-scale soil experiments results suggest that *B. flexus* strain XJU-4 is a suitable contender for bioremediation of pyrethroid fenvalerate-contaminated sites.

Keywords Biodegradation · *Bacillus flexus* strain XJU-4 · Microcosms · Fenvalerate · 3-Phenoxybenzoate

Introduction

For the past few decades, pesticides are continuously being used for both agricultural and industrial purposes (Eqani et al. 2012; Tallur et al. 2015; Talwar et al. 2014). Besides their effectiveness, these pesticides posed several potential health threats to the ecosystem including microorganisms present in the soil (Pandey and Singh 2004) and other wildlife (Eqani et al. 2012). Fenvalerate (a synthetic pyrethroid), is also known as a chiral pesticide and reported to be used nearly 1 kiloton per annum worldwide (Chen et al. 2011a).

Even though, fenvalerate has higher toxicity against pests, but, it was observed that it has lower toxic effect

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toward mammals, birds, and plants (Garey and Wolff 1998). Nevertheless, several studies shown that fenvalerate has endocrine toxicity, genotoxic effects, neurotoxicological effects and as a tumour promoter (Fei et al. 2010; Gu et al. 2010; Hemming et al. 1993; Qu et al. 2008; Wang et al. 2017; Wolansky and Harrill 2008; Xia et al. 2004). This synthetic pesticide has been mainly used in agricultural sector, as well as in the home for sanitation purposes and also on cattle to control pests. Given consideration to its widespread use, several studies revealed that fenvalerate has been often detected into the soil, sediment and water (Ismail and Maznah 2005; McKinlay et al. 2008; Xue and Xu 2006). Fenvalerate half-life in soil ranged between 360 and 1440 h; however, it depends on microorganisms, moisture, temperature, pH, soil properties (Ismail and Maznah 2005). Typically, transformation of fenvalerate proceeds through several ways, including volatilization, photolysis, hydrolysis and microorganisms in the eco-geological system (Chen et al. 2011a). Generally, in the environment, 3-phenoxybenzoate has been identified as a common intermediate of pyrethroids including fenvalerate and has higher toxic effects than parent (pyrethroids) compounds (Xia et al. 2004; Yuan et al. 2010; Zhu et al. 2016).

It has been widely reported in the literature that the microorganisms played an essential role in the degradation and detoxification of fenvalerate and other pyrethroid residues in the environment (Chen et al. 2011a; Yu et al. 2013). There are several reports on the degradation of fenvalerate by various microorganisms like *Achromobacter* sp., *Acinetobacter* sp. strain JN8, *Bacillus cereus*, *Cladosporium* strain HU, *Owenweeksia hongkongensis,* genus of *Pseudomonas, Sphingomonas* sp. F-7 and *Stenotrophomonas* sp. strain ZS-S-01(Boricha and Fulekar, 2010; Chen et al. 2011a, b; Deborah et al. 2013; Fulekar 2009; Jin et al. 2014; Maloney et al. 1988; Yu et al. 2013). However, it is necessary to understand the mechanism of fenvalerate metabolism in different bacteria, which is a critical step for enhancing existing bioremediation techniques for fenvalerate removal in the eco-geological system. In this paper, we proposed a pathway for the degradation of fenvalerate by *Bacillus flexus* strain XJU-4 under aerobic condition. Furthermore, we have also investigated the bioremediation of fenvalerate in the soil using bacterium; *B. flexus* strain XJU-4.

Materials and methods

Chemicals and media

Fenvalerate, phenol, 4-hydroxy-3-phenoxybenzoic acid, 3-phenoxybenzoic acid, protocatechuic acid, gentisic acid, 4-chlorocatechol, catechol, 4-nitrocatechol and 3-methylcatechol with more than 97% purity were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemical compounds were of highest (analytical) grade obtained by commercial sources. The stock solutions of substrates like 3-Phenoxybenzoate and fenvalerate were prepared at 100 mM concentration by dissolving in methanol and acetone, respectively. The individual stock solutions were sterilized by membrane filtration and added into autoclaved enrichment medium under sterile condition to get the required concentrations. The enrichment medium (mineral-salts medium, MSM 1) that contained K_2HPO_4, 6.30; KH_2PO_4, 1.82; NH_4NO_3, 1.00; $MgSO_4{\cdot}7H_2O$, 0.20; $CaCl_2{\cdot}2H_2O$, 0.10; $Na_2MoO_4{\cdot}2H_2O$, 0.006; $MnSO_4{\cdot}H_2O$, 0.06, and $FeSO_4{\cdot}7H_2O$, 0.10 gl^{-1}. The bacterial cell suspension was measured by plate-count technique (Mulla et al. 2012).

Microorganism and growth condition

The 3-nitrobenzoate degrading *B. flexus* strain XJU-4 used in the current study was formerly isolated and identified in our laboratory (Mulla et al. 2011a, b). For growth study, 5 ml ($OD_{600\ nm}$ of 0.7) of organism was initially inoculated into 95 ml of MSM 1 (in 500 ml Erlenmeyer flask) supplemented with fenvalerate (2 mM) as well as 3-phenoxybenzoate (3 mM), respectively, and kept on a rotary shaker (150 rpm) under dark condition at 30 °C. *B. flexus* strain XJU-4 growth at different intervals was quantified at 600 nm by spectrophotometer (Hitachi U-2800, Tokyo, Japan). The bacterial culture was preserved on fenvalerate-mineral salts agar slants as well as 3-phenoxybenzoate-mineral salts agar slants, respectively.

Utilization of fenvalerate and other aromatic compounds

The *B. flexus* strain XJU-4 ability to consume individual substrates (supplemented at initial concentration of 2 mM) such as fenvalerate, 3-phenoxybenoate, 4-hydroxy-3-phenoxybenzoate, phenol, protocatechuate, gentisate, catechol, 3-methylcatechol, 4-chlorocatechol, 3-chlorocatechol and 4-nitrocatechol as a sole source of carbon was studied by quantifying *B. flexus* strain XJU-4 growth under liquid MSM 1 as described above. Degradation of fenvalerate and 3-phenoxybenzoate during growth of strain XJU-4 was determined at different incubation periods by high performance liquid chromatography (HPLC) analysis. Uninoculated controls were used to measure any conversion of fenvalerate (2 mM) as well as 3-phenoxybenzoate (3 mM) affected by abiotic factors. The effect of various concentration of fenvalerate (1–4 mM) as well as 3-phenoxybenzoate (1–4 mM) on the growth of *B. flexus* strain XJU-4 was observed after 6 days of incubation.

Analytical methods

The degradation by-products were isolated from culture supernatants of strain XJU-4 grown on fenvalerate by extraction using diethyl ether and verified by thin layer chromatography (TLC) on glass plates coated with silica gel G using two different solvent systems: (a) chloroform–acetic acid (95:5, vol/vol) and (b) benzene–methanol–acetic acid (45:8:4, vol/vol). The by products were observed at 254 nm under ultraviolet (UV) chamber and/or by exposing iodine vapours on TLC glass plates in TLC chamber. Phenolic compounds were confirmed by their characteristic colour on spraying with 2% solution of 2,6-dichloroquinone-4-chlorimide in methanol (Gibbs reagent) or using diazotized *p*-nitroaniline (Mulla et al. 2011b). ortho-Dihydroxy compounds were confirmed by spraying with Arnow's reagent (1937). GC–MS analysis was performed using a Shimadzu QP2010 Plus (Tokyo, Japan) as described previously (Mulla et al. 2011b). The by-products were analyzed by HPLC (Shimadzu, Kyoto, Japan) equipped with SPD-10AVP UV-Detector. The separation was executed on Silica gel-packed C_{18} column (4.6 × 250 mm) of particle size (5 µm) (Phenomenex) by isocratic condition using 50 mM phosphate buffer (pH 7.0) plus acetonitrile (3:7) for isolated metabolite I and 3-phenoxybenzoate whereas 40 mM acetic acid plus methanol (1:1) for fenvalerate, isolated metabolite II as well as isolated metabolite III. The flow rate of both mobile phase were kept at 1 ml min^{-1}. UV–Visible spectra were obtained using spectrophotometer (Hitachi U-2800, Tokyo, Japan).

Enzyme assays

The bacterial culture was grown on MSM 1 supplemented with appropriate concentration of individual substrate like fenvalerate and glucose were harvested by centrifugation at 6500×*g* for 12 min at 4 °C. The bacterial cell pellets were washed and resuspended in two volume of 50 mM phosphate buffer (pH 7.0). The bacterial culture cell-free extracts were prepared by sonication (Ultrasonic processor, model XL 2010) for 5 min and centrifugation (at 11,000×*g*) was carried out at 4 °C up to 42 min. The supernatant free from cell debris was used for further enzymatic studies.

Fenvalerate hydrolyzing esterase activity was assessed spectrophotometrically by determining with decrease in absorbance at 239 nm due to disappearance of substrate, fenvalerate. 3-Phenoxybenzaldehyde dehydrogenase activity was assessed spectrophotometrically by determining with increase in absorbance at 340 nm, due to the formation of NADPH. 3-Phenoxybenzoate dioxygenase activity was assessed spectrophotometrically by determining with increase in absorbance at 295 nm (Tallur et al. 2008). 4-Hydroxy-3-phenoxybenzoate hydroxylase was assessed spectrophotometrically at 30 °C by determining with decrease in absorbance at 340 nm, due to the substrate dependence oxidation of NADPH (Tallur et al. 2008). Phenol hydroxylase activity was quantified according to Neujahr and Gaal (1973). Protocatechuate 2,3-dioxygenase, protocatechuate 3,4-dioxygenase and protocatechuate 4,5-dioxygenase activities were quantified according to Mulla et al. (2011b). Catechol-1,2-dioxygenase activity was quantified according to Hayaishi et al. (1957). Catechol-2,3-dioxygenase activity was quantified according to Kim et al. (1992). Protein was quantified by the method of Lowry et al. (1951).

Microcosm experiments

Soil used in microcosm experiment was collected from agriculture field around Dharwad, India. The soil contained 23.0% clay, 21.0% silt, 37.0% sand, 0.67% organic carbon, 0.068% total nitrogen and had a pH of 7.40. Microcosm experiments were carried out as described previously (Chen et al. 2011a). 100 g of soil were placed in 500 ml beakers. Water content of each beaker was adjusted into 40% (maximum water holding capacity) and was maintained whole experimental study by adding double distilled water whenever necessary. Further, fenvalerate was added into each beaker at a final concentration of 2.5 mM in acetone solution, thoroughly mixed and kept for a while to evaporate acetone. Four different types of microcosms experimental set up were made (a) examination microcosm with non-sterile soil, (b) examination microcosms with sterile soil, (c) control microcosms with sterile soil, and (d) control microcosms with non-sterile soil. Sterilization was performed for the collected soil samples by autoclaving at 121.5 °C for 40 min. Examination microcosms with sterile and non-sterile soils were inoculated with microbial suspension (*B. flexus* strain XJU-4) by drip irrigation method to give a final concentration of 2 × 10^7 cells colony-forming units (CFUs) g^{-1} soil, whereas the control microcosms (without strain XJU-4) with non-sterile and sterile soils were left non-bioaugmented. All the microcosms were concealed using perforated aluminium foil and finally kept at 30 °C for incubation up to 10 days. Soils samples (5 g) were collected at regular intervals and extracted for fenvalerate residual analysis by HPLC (as described above method).

Statistical analysis

In this study all experiments were carried out by triplicate and their results are provided as mean ± standard deviation (SD). Experimental data obtained were evaluated

statistically using one-way ANOVA (SPSS. 7.5). A $P \leq 0.05$ was recorded as statistically significant (Mulla et al. 2011b).

Results and discussion

Utilization of fenvalerate and other aromatic compounds by bacterial culture

The strain XJU-4 utilized fenvalerate, 3-phenoxybenzoate, phenol, catechol, protocatechuate as a sole source of carbon and energy but not 4-hydroxy-3-phenoxybenzoate, gentisate, 3-chlorocatechol, 4-chlorocatechol, 4-nitrocatechol and 3-methylcatechol. The bacterium, *B. flexus* strain XJU-4 growth on fenvalerate (2 mM) as a sole source of carbon is shown in Fig. 1. The bacterial culture was completely degraded 2 mM concentration of fenvalerate within 6 days of incubation (Fig. 1). The accumulation of 3-phenoxybenzoate in the culture medium during growth of *B. flexus* strain XJU-4 on fenvalerate is shown in Fig. 1. Similarly, the fenvalerate degrading bacterium was also utilized 3-phenoxybenzoate (3 mM) as a sole source of carbon and energy (Fig. 2). The accumulation of protocatechuate and catechol in the culture medium during growth of *B. flexus* strain XJU-4 on 3-phenoxybenzoate is shown in Fig. 2. The effect of various concentrations of fenvalerate and 3-phenoxybenzoate on strain XJU-4 showed maximum growth at 2 and 3 mM, respectively (data was not shown).

Identification of metabolites

The analysis of culture supernatants of *B. flexus* strain XJU-4 grown on fenvalerate by UV, TLC and HPLC revealed that the presence of three metabolites (I–III). Their R_f values and λ_{max} were found to be identical to that of authentic 3-phenoxybenzoate, protocatechuate and catechol (Table S1, Supplementary Information, SI). The mass spectrum of isolated metabolite I (Fig. S1A, SI) showed molecular peak M^+ at *m/z* 214, is in good agreement with empirical formula $C_{13}H_{10}O_3$. The spectral data were identical with that of 3-phenoxybenzoate (Fig. S1B, SI). The mass spectrum of isolated metabolite II (Fig. S1C, SI) showed molecular peak M^+ at *m/z* 154, is in good agreement with empirical formula $C_7H_6O_4$. The spectral data corresponded well with that of authentic protocatechuate (Fig. S1D, SI). The mass spectrum of isolated metabolite III (Fig. S1E, SI) showed molecular peak M^+ at *m/z* 110, is in good agreement with empirical formula $C_6H_6O_2$. The spectral data corresponded well with that of authentic catechol (Fig. S1F, SI). On the basis of these results, the presences of three by-products of fenvalerate biodegradation were identified as 3-phenoxybenzoate (first metabolite), protocatechuate (second metabolite) and catechol (third metabolite) with their authentic compounds (Fig. S1, SI).

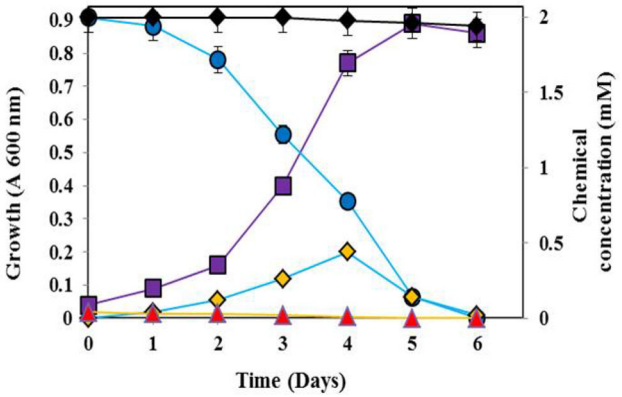

Fig. 1 Utilization of fenvalerate (2 mM) (-●-) during growth (-■-) of *Bacillus flexus* strain XJU-4 with the accumulation of 3-phenoxybenzoate in the culture filtrate (-◆-). Uninoculated (with 2 mM fenvalerate) (-◆-) and inoculated (without 2 mM fenvalerate) controls (-▲-) in the mineral-salts medium. Data values represent the averages of triplicate determinations

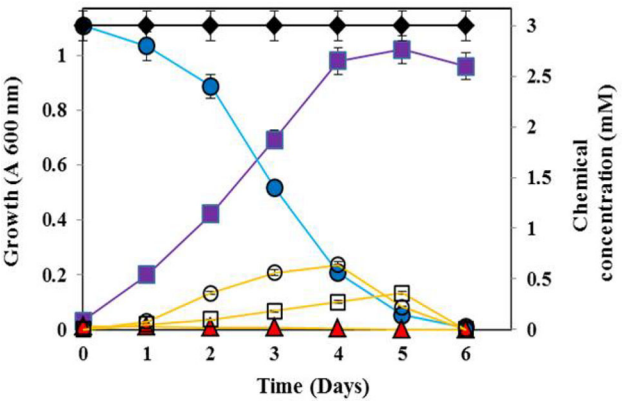

Fig. 2 Utilization of 3-phenoxybenzoate (3 mM) (-●-) during growth (-■-) of *Bacillus flexus* strain XJU-4 with the accumulation of protocatechuate (-○-) as well as catechol (-□-) in the culture filtrate. Uninoculated (with 3 mM 3-phenoxybenzoate) (-◆-) and inoculated (without 3 mM 3-phenoxybenzoate) controls (-▲-) in the mineral salts medium. Data values represent the averages of triplicate determinations

Bacterial cell-free enzymes involved in fenvalerate catabolism

The activities of various enzymes involved in the degradation of fenvalerate are given in Table 1. The cell-free extract of the *B. flexus* strain XJU-4 grown on fenvalerate contained the activities of fenvalerate hydrolyzing esterase, 3-phenoxybenzaldehyde dehydrogenase, 3-phenoxybenzoate dioxygenase, phenol hydroxylase, protocatechuate

Table 1 Specific activities of enzymes in the cell-free extract of *Bacillus flexus* strain XJU-4 grown on fenvalerate

Enzyme	Specific activity[a] (units/mg of protein)
Fenvalerate hydrolyzing esterase	0.164 ± 0.002
3-Phenoxybenzaldehyde dehydrogenase	0.242 ± 0.002
3-Phenoxybenzoate dioxygenase	0.354 ± 0.004
4-Hydroxy-3-phenoxybenzoate hydroxylase	0.000 ± 0.00
Phenol hydroxylase	0.398 ± 0.002
Protocatechuate 2,3-dioxygenase	0.476 ± 0.003
Protocatechuate 3,4-dioxygenase	0.000 ± 0.00
Protocatechuate 4,5-dioxygenase	0.000 ± 0.00
Gentisate-1,2-dioxygenase	0.000 ± 0.00
Catechol-1,2-dioxygenase	0.000 ± 0.00
Catechol-2,3-dioxygenas	0.487 ± 0.004

Glucose-grown cells did not contain these activities

Values are the mean ± standard deviation (SD) of triplicates

[a] One unit is defined as the formation or consumption of 1 μmol of the product or substrate, respectively, per min

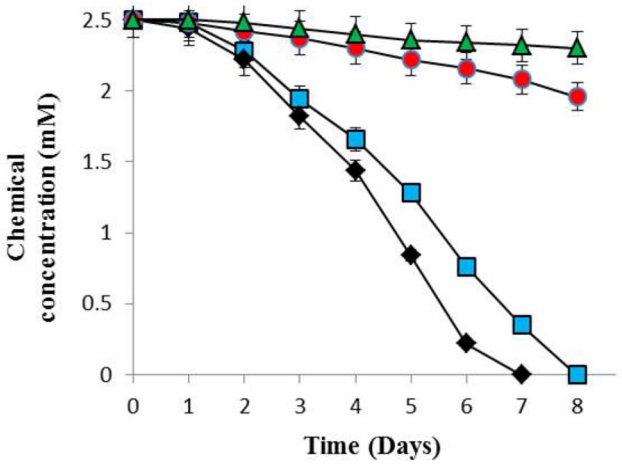

Fig. 3 Dynamics of degradation of fenvalerate (2.5 mM) by strain XJU-4 during microcosm studies [**a** microcosm (inoculated) with sterile soil (-■-), **b** microcosm (inoculated) with non-sterile soil (-◆-), **c** control (uninoculated) with sterile soil (-▲-) and **d** control (uninoculated) with non-sterile soil (-●-)]. Data values represent the averages of triplicate determinations

2,3-dioxygenase and catechol 2,3-dioxygenase. The activities of 4-hydroxy-3-phenoxybenzoate, protocatechuate 3,4-dioxygenase, protocatechuate 4,5-dioxygenase and catechol 1,2-dioxygenase were not detected in the cell-free extract. The overall results revealed that a *meta*-cleavage pathway was involved in fenvalerate degradation. On the other hand, the cell-free extract of glucose grown cells (*B. flexus* strain XJU-4) were not contained any of these enzyme activities. Therefore, these results suggest that the enzymes involved in the transformation mechanism were induced by the growth of bacterium on fenvalerate. More studies are needed to further support oxygenase enzymes that are responsible for fenvalerate degradation.

Microcosm experiments

To observe the capacity of *B. flexus* strain XJU-4 to degrade fenvalerate in the soil, we studied microcosm experiments (Fig. 3). The experiment conducted using microcosms with sterile soil; we observed that the complete removal of fenvalerate (2.5 mM) was achieved by strain XJU-4 within 8 days. On the 2nd day, 0.22 mM (8.8%) degradation was detected and degradation was 0.84 mM (33.6%) by 4th day. The degradation was 1.74 mM (69.6%) by 6th day. Almost complete degradation of fenvalerate was observed at 8th day. In other tested microcosms with non-sterile soil, complete fenvalerate degradation by bacterium was observed within 7 days. On the other hand, in control experiments (non-sterile and sterile soil without *B. flexus* strain XJU-4), the degradation of fenvalerate was observed only by 21.6 and 8%, respectively, after 8 days of incubation (Fig. 3).

From the results it is confirmed that the *B. flexus* strain XJU-4 degraded synthetic pyrethroid pesticide (i.e., fenvalerate) by hydrolysis of ester linkage to form 3-phenoxybenzoate via 3-phenoxybenzaldehyde. The 3-phenoxybenzoate was further metabolized into phenol as well as protocatechuate. UV, TLC, HPLC, GC–MS analysis and enzymatic studies have confirmed that 3-phenoxybenzoate, phenol, protocatechuate and catechol were the major by-products of fenvalerate metabolism in the presence of strain XJU-4. Additionally, the presence of higher activities of protocatechuate 2,3-dioxygenase and catechol 2,3-dioxygenase in the fenvalerate-grown cells has suggested that protocatechuate and catechol were further metabolized via *meta*-cleavage pathway under oxidation condition. Looking into Fig. 4, it can be seen the pathway for the degradation of phenoxybenzoate moiety of fenvalerate in *B. flexus* strain XJU-4.

The initial step of fenvalerate degradation into 3-phenoxybenzoate (in *B. flexus* strain XJU-4) was similar to that described in other organisms such as *Bacillus circus*, *Pseudomonas fluorescens*, *Achromobacter* sp., *Sphingomonas* sp. F-7 and *Stenotrophomonas* sp. strain ZS-S-01 (Chen et al. 2011a; Maloney et al. 1988; Yu et al. 2013). However, later pathway of fenvalerate metabolite, 3-phenoxybenzoate proceeds in *B. flexus* strain XJU-4 was vary from those reported for other organisms. For example, *B. flexus* strain XJU-4 catabolized 3-phenoxybenzoate into protocatechuate and phenol, and similar to that reported for the genus of *Pseudomonas* (Halden et al. 1999, 2000) and *Micrococcus* (Tallur et al. 2008). On the other hand, in *Bacillus cercus*, *Pseudomonas fluorescens* and *Achromobacter* sp., 3-phenoxybenzoate is biotransformed into

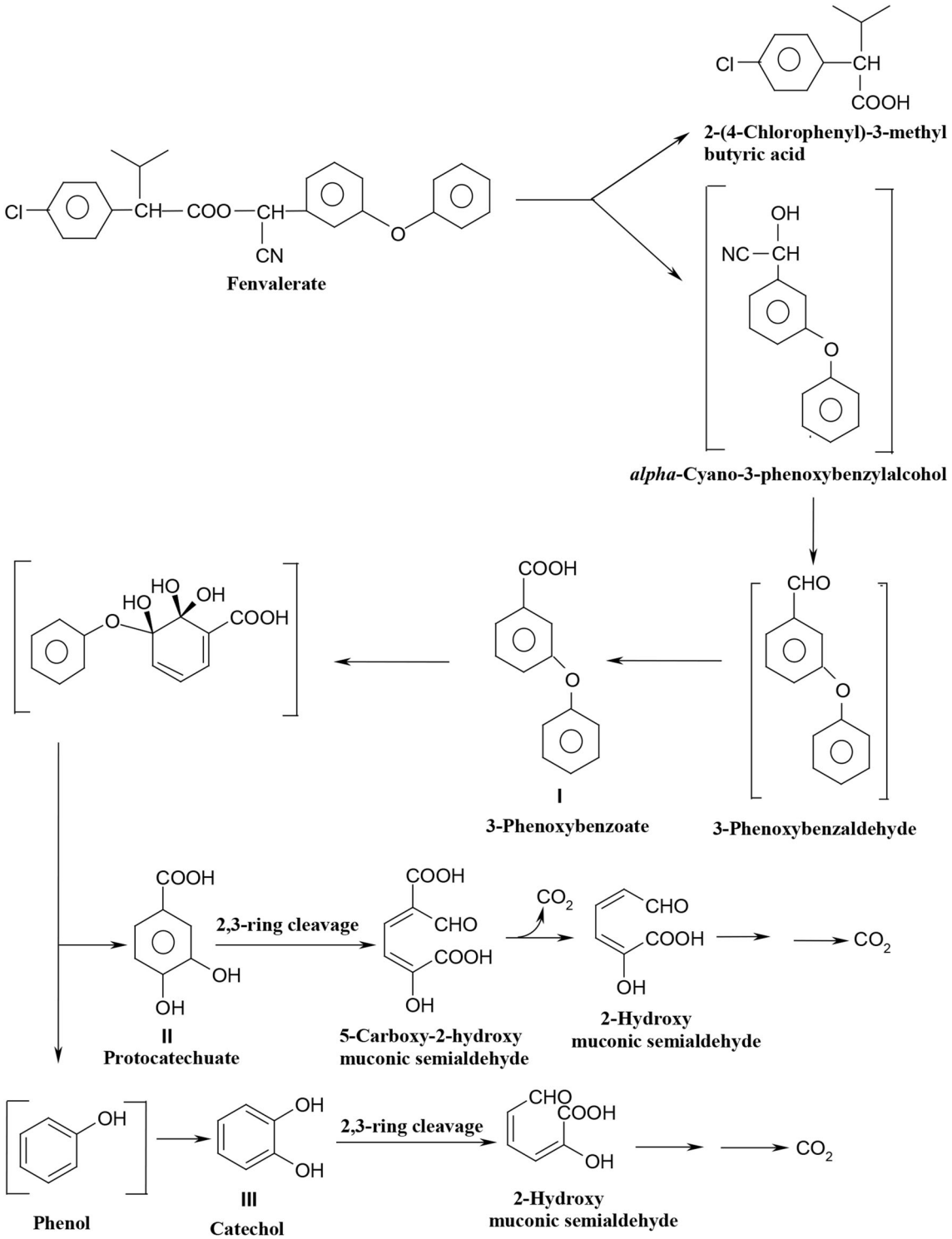

Fig. 4 Proposed pathway for the degradation of fenvalerate in *Bacillus flexus* strain XJU-4

4-hydroxy-3-phenoxybenzoate (Maloney et al. 1988). Though, in strain XJU-4, protocatechuate and catechol were proceeded via *meta*-cleavage pathway under oxidation condition, whereas in strain CPN 1, protocatechuate and catechol are oxidized through *ortho*-ring cleavage (Tallur et al. 2008). Other than bacteria, fungal culture (*Cladosporium* strain HU) was transformed fenvalerate into 3-phenoxybenzaldehyde and α-hydroxy-3-phenoxybenzeneacetonitrile (Chen et al. 2011b). In another study, Zhu et al. (2016) reported in *Aspergillus oryzae* (a

filamentous fungus M-4 strain), 3-phenoxybenzoate was gradually transformed into various metabolites such as phenol, 3-hydroxy-5-phenoxy benzoate, protocatechuate, catechol and gallic acid.

Additionally, strain XJU-4 has also showed its ability to degrade 3-phenoxybenzoate, which is a major degradation by-product, and common to most pyrethroids. Hence, in this study, both fenvalerate and 3-phenoxybenzoate degradation was accomplished by same bacterium; *B. flexus* strain XJU-4. However, further study is necessary to determine fenvalerate degradation pathway proceeds through, 2-(4-chlorophenyl)-3-methyl butyric acid, which might also have an impact on environment.

Moreover, the bioremediation potential of Gram-positive bacterium (i.e., *B. flexus* strain XJU-4) was also explored in soil with fenvalerate (2.5 mM) spiked sterile and non-sterile soil using microcosms approach. *B. flexus* strain XJU-4 significantly degraded fenvalerate in microcosms with sterile and non-sterile soils. However, it was observed that the degradation of fenvalerate in non-sterile soil was slightly faster than sterile soil. These results suggest that indigenous bacteria and biotic conditions could assist the degradation of fenvalerate with strain XJU-4. Hence, the organism was favourable for the use in bioremediation of fenvalerate-contaminated sites. Previously, Chen et al. (2011a), also reported bioremediation of 95% of fenvalerate ($\sim$0.12 mM) by a Gram-negative bacterium, *Stenotrophomonas* sp. strain ZS-S-01 within 9 days. From the results of our study, it indicates that more than 75% of fenvalerate was degraded from contaminated soil than controls. However, it is difficult to assess accurately as bioremediation was significantly influenced by various parameters in the environment (Chen et al. 2011a). But, still bioremediation method is considered to be a primary choice for the remediation of contaminated sites due to its eco-friendly and cost-effectiveness.

Conclusion

Our investigation results suggest that the degradation pathway of fenvalerate by *B. flexus* strain XJU-4 proceeds via 3-phenoxybenzoate to phenol and protocatechuate. Phenol was further biotransformed into catechol. Finally, both protocatechuate and catechol were entered into the *meta*-cleavage pathway. Furthermore microcosm investigations support for the organism could be useful for the decontamination of fenvalerate-contaminated sites. The results would be useful for the environmental authorities towards the management of fenvalerate (a pyrethroid pesticide) contaminated soils/environment.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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