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Elucidating the kinetics and mechanisms of tetramethrin biodegradation by the fungal strain *Neocosmospora* sp. AF3

Wen-Juan Chen[1], Xiaofang Luo[1], Xuanrui Zhang[1], Kalpana Bhatt[1], Shao-Fang Chen[1], Mohamed A. Ghorab[2,3], Xiaofan Zhou[1] and Yaohua Huang[1*]

Abstract

Tetramethrin is a common pyrethroid insecticide, but there is limited knowledge about its degradation kinetics and mechanisms. In this study, a novel fungal strain, *Neocosmospora* sp. AF3, was obtained from pesticide-contaminated fields and was shown to be highly effective for degrading tetramethrin and other widely used pyrethroids. The AF3 strain completely removed 10 mg/L of tetramethrin from mineral salt medium in 9 days. The first-order kinetic analysis indicated that the degradation rate constant of the AF3 strain on 50 mg/L tetramethrin was 0.2835 d^{-1} (per day), and the half-life was 2.45 days. A response surface model analysis showed that the optimal degradation conditions for the AF3 strain are a temperature of 33.37 ℃, pH of 7.97, and inoculation amount of 0.22 g/L dry weight. The Andrews nonlinear fitting results suggested that the optimal concentration of tetramethrin metabolized by the AF3 strain is 12.6073 mg/L, and the q_{max}, K_i, and K_s values were 0.9919 d^{-1}, 20.1873 mg/L, and 7.8735 mg/L, respectively. The gas chromatography–mass spectrometry (GC–MS) analysis indicated that *N*-hydroxymethyl-3,4,5,6-tetrahydrophthalimide, chrysanthemic acid and tetrahydrophthalimide are the main intermediates involved in the metabolism of tetramethrin by the AF3 strain. Furthermore, this strain was shown to effectively degrade other pyrethroid pesticides including permethrin, beta-cypermethrin, chlorempenthrin, fenvalerate, D-cyphenothrin, bifenthrin, meperfluthrin, cyfluthrin, and deltamethrin within a short period, suggesting that *Neocosmospora* sp. AF3 can play an important role in the remediation of pyrethroid contamination. Taken together, these results shed a new light on uncovering the degradation mechanisms of tetramethrin and present useful agents for developing relevant pyrethroid bioremediation strategies.

Keywords Tetramethrin, *Neocosmospora* sp., Degradation kinetics, Degradation mechanisms

*Correspondence:
Yaohua Huang
huang-yaohua@scau.edu.cn
[1] Integrative Microbiology Research Centre, College of Plant Protection, South China Agricultural University, Guangzhou 510642, China
[2] Wildlife Toxicology Lab, Department of Animal Science, Institute for Integrative Toxicology (IIT), Michigan State University, East Lansing, MI 48824, USA
[3] Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616-8741, USA

Introduction

Tetramethrin is a type of synthetic pyrethroid insecticide that is widely used for pest control in agriculture, household, and public health due to its effectiveness against a wide range of pests [1]. Pyrethroids are structurally based on natural pyrethrin extracted from *Chrysanthemum cinerariaefolium* and have the characteristics of broad-spectrum insecticidal activity and low toxicity to mammals [2]. Due to their high efficiency and lower environmental impact, pyrethroids have become a popular alternative to organochlorines

and organophosphates. However, frequent and extensive use of pyrethroids has raised concerns about their environmental impact and their effects on non-target organisms, especially aquatic animals and beneficial insects [3, 4]. Compared with natural insecticide pyrethrin, pyrethroids have a more persistent half-life in the environment [5, 6].

Although the reasonable use of pyrethroid insecticides is generally considered safe for humans and animals, they are still toxic to a wide range of insects, including pollinating insects and predators of pests [7, 8]. Pyrethroids disrupt the nervous system of insects by binding and prolonging the activation of voltage-gated sodium channels, leading to paralysis and death [9, 10]. Pyrethroids have been shown to be neurotoxic and reproductive-toxic to aquatic organisms, such as fish and invertebrates [11–13]. They also have negative impacts on beneficial non-target organisms such as bees, earthworms, and soil microorganisms, which play important roles in ecosystem services and nutrient cycling [14, 15]. Furthermore, some studies suggest that pyrethroids can enter lakes through agricultural or residential runoff and can persist in sediment and water for a long time [16]. Moreover, pyrethroids remaining in sediment and water can pose potential risks to human health through aquatic food webs [17].

The properties of the natural environment are important factors that determine the fate of pyrethroids in the environment [18]. Pyrethroids are dynamically influenced by factors such as the temperature, humidity, and soil pH. Microorganisms play an indispensable role in the degradation of pyrethroids, and a large number of studies have identified bacterial and fungal species that can degrade pyrethroids under laboratory conditions or in soil [19]. However, the effectiveness of microbial degradation may vary depending on the specific type of pyrethroid and environmental conditions. *Staphylococcus succinus* HLJ-10 showed a degradation rate of 62.5% against tetramethrin as the sole carbon source after 7 days of cultivation [2]. Meanwhile, Bhatt et al. reported that *Sphingomonas trueperi* CW3 removed 60.5% of tetramethrin in MSM medium after 7 days [6].

To date, there have been no reports of tetramethrin degradation by fungal strains. In this study, a novel fungal strain, *Neocosmospora* sp. AF3, was isolated from contaminated soil. This is the first report on pesticide degradation by microorganisms from the genus *Neocosmospora*. We investigated the potential of the AF3 strain to degrade pyrethroids and their metabolic pathways. Additionally, the optimal concentration and culture conditions for the degradation of tetramethrin by the AF3 strain were analyzed with response surface technology and the Andrews equation. The results suggest that the AF3 strain could be a potential candidate for pyrethroid-contaminated bioremediation applications.

Materials and methods

Chemicals and culture medium

Mineral Salt Medium (MSM) containing $Na_2HPO_4{\cdot}12H_2O$, 1.5 g; $(NH_4)2SO_4$, 2 g; $MgSO_4{\cdot}7H_2O$, 0.2 g; $CaCl_2{\cdot}2H_2O$, 0.01 g; KH_2PO_4, 1.5 g; and $FeSO_4{\cdot}7H_2O$, 0.001 g in 1 L of distilled water, was used to detect the pyrethroid degradation of the AF3 strain. Potato Dextrose Agar (PDA) medium consists of 20 g/L of glucose and 200 g/L of potatoes. If it is necessary to make a solid medium, 15–20 g/L agar powder can be added to the above formula. The pH value of MSM was adjusted to 7.0 ± 0.2, and all media were autoclaved at 121 ℃ for 20 min before use. The Milli-Q Ultrapure Water System (Millipore, USA) was used for the preparation of mobile phases and reagent solvents.

The pyrethroids used in this study were tetramethrin (purity 98%), fenvalerate (purity 91%), beta-cypermethrin (purity 95%), prallethrin (purity 95%), deltamethrin (purity 99%), D-cyphenothrin (purity 98%), permethrin (purity 97%), chlorempenthrin (purity 94%), meperfluthrin (purity 96%) and cyfluthrin (purity 95%) were commercially acquired from Wuhan Yuancheng Pharm Co., Ltd. (Wuhan, China). Chromatograde acetonitrile and methanol were purchased from Fisher Scientific, USA. Other reagents and agents not specified were of common analytical grades.

Isolation and purification of tetramethrin-degrading microorganisms

The activated sludge used to isolate the tetramethrin-degrading strain was obtained from the agricultural experimental field at South China Agricultural University (SCAU, *N*. 23° 16′ 55″/*E*. 113° 36′ 49″). An appropriate amount of soil samples was placed into an Erlenmeyer flask containing 100 mL of MSM medium, and a final concentration of 100 mg/L of tetramethrin was added. The culture was transferred at 1% for several rounds until the concentration reached 800 mg/L, and then the solution was diluted with sterile water gradient. Medium broth (100 μL) with different dilution grades was spread on the tetramethrin-containing MSM solid plate. Inverted Petri dishes were incubated at 28 ℃ for 3 days to obtain a large number of bacteria of various morphologies as well as a small number of fungi.

Analysis of degradation potential of microbial strains on tetramethrin

The isolated candidate strains were separately added to MSM medium containing 50 mg/L tetramethrin and cultured at 200 rpm and 28 ℃ for 5 days. MSM medium was collected every two days, and the residual amount of tetramethrin was analyzed by high-performance liquid chromatography (HPLC, Waters 2690, USA). One of the fungal isolates, AF3, showed a high efficiency tetramethrin degradation capacity. Therefore, the AF3 strain was placed into MSM containing diverse pyrethroids to analyze the substrate utilization range, including fenvalerate, beta-cypermethrin, prallethrin, deltamethrin, D-cyphenothrin, permethrin, chlorempenthrin, meperfluthrin, and cyfluthrin.

The biomass of the AF3 strain at different periods was characterized by high-speed centrifugation and weighing after water removal, and the residues of pyrethroids were monitored by HPLC. In this study, all experimental groups were performed in triplicate with uninoculated groups as the controls. The first-order kinetic equation was used to analyze the metabolic process of tetramethrin by the AF3 strain (Eq. 1). Based on the degradation constant k obtained for the first-order kinetic parameters, the theoretical half-life of tetramethrin was estimated from Eq. (2).

$$C_t = C_0 \times e^{-k}, \tag{1}$$

$$t_{1/2} = \ln 2/k, \tag{2}$$

where C_0 is the quantity of tetramethrin at time 0 (mg/L); C_t is the amount of tetramethrin at time t (mg/L); k is refers to the degradation constant (d^{-1}); and t is the degradation time (d); ln 2 is the natural logarithm of 2.

Identification of degradation strains

The mycelial morphology of the fungal the AF3 strain cultured on a PDA plate for five days was observed using an optical microscope (Olympus, Tokyo, Japan). After the mycelia of the AF3 strain were broken by 1 mm glass beads at high speed, the genome of the AF3 strain was obtained by utilizing a fungal DNA extraction kit (OMEGA, USA). The AF3 strain's genome was used as a template, and the ITS region was amplified through the polymerase chain reaction (PCR) using the universal primers ITS1: 5′-TCCGTAGGTGAA CCTGCGG-3′/ITS4: 5′-TCCTCCGCTTATTGATGC -3′. The PCR products were sent to GENEWIZ Co., Ltd (Suzhou, China) for sequencing after being tested using gel electrophoresis. By comparing the ITS sequence similarity of the AF3 strain in the NCBI (National Center for Biotechnology Information) online database, 10 strains with the closest genetic relationships to the AF3 strain were selected to construct a phylogenetic tree.

Concentration range of the AF3 strain metabolizes tetramethrin

The AF3 strain was inoculated into MSM medium containing gradient concentrations of tetramethrin at 10, 25, 50, 100, 200 and 300 mg/L to test its degradation potential. In order to further analyze the optimal degradation concentration of tetramethrin metabolized by the AF3 strain, the Andrews equation was used to nonlinear fit the decomposition process of tetramethrin with diverse concentrations. The Andrews equation is shown as Eq. (3):

$$q = \frac{q_{max} S}{S + K_s + \left(S^2/K_i\right)}, \tag{3}$$

where q is refers to the specific degradation rate of tetramethrin (d^{-1}); S is the residual quantity of tetramethrin (mg/L); q_{max} is the maximum specific degradation rate of tetramethrin (d^{-1}); K_s is the half-rate constant (mg/L); and K_i is the inhibition coefficient of tetramethrin (mg/L).

Model to predict the optimal conditions for tetramethrin metabolism

The analysis of the key factors influencing the degradation of tetramethrin by the AF3 strain and the interaction between different factors was performed using the Box–Behnken response surface program. According to the previous method, three main factors, namely, the temperature, inoculum amount, and pH were chosen as X (independent variables), and the coding values of X are shown in Table S1. The degradation rate of 15 sets of different independent variable treatment combinations after 9 days of culture was used as Y (dependent variable), and the treatment combination was designed by Design Expert (version 12.0, USA) following a random block design. The 15 groups of the dependent variable Y obtained by the treatment were analytically fitted to the following quadratic polynomial equation (Eq. 4) through the response surface regression procedure:

$$Y_i = b_0 + \sum b_i X_i + \sum b_{ij} X_i X_j + \sum b_{ii} X_i^2, \tag{4}$$

where Y_i is the predicted degradation value; b_0 and b_i are the constant and linear coefficient, respectively; b_{ij} and b_{ii} are the interaction coefficient and quadratic coefficient, respectively; and X_i and X_j are variables.

GC–MS analysis of tetramethrin degradation by the AF3 strain

To analyze the metabolic pathways of tetramethrin degradation by the AF3 strain, gas chromatography–mass spectrometry (GC–MS) was employed to detect and identify tetramethrin and its metabolites. Periodic samples were collected from MSM medium at different time points, and the metabolites were analyzed in comparison with standard compounds from the NIST (National Institute of Standards and Technology) database.

For sample preparation, 5 mL of the MSM culture medium was periodically collected and centrifuged at 12,000 rpm for 10 min. The resulting clear supernatant (2 mL) was transferred to a 10 mL glass tube, and 4 mL of ethyl acetate was added. The mixture was then sonicated for 20 min, followed by vortexing for 2 min. After vortexing, the sample was allowed to stand in the dark for 30 min, and 2 mL upper organic phase was collected in a 5 mL glass tube. Ethyl acetate was then removed at 35 ℃ using a vacuum centrifugal concentrator. Finally, 1 mL of chromatographic methanol was added to the sample, which was passed through a 0.22 μm membrane to recover tetramethrin and its degradation products [20].

GC–MS analysis was conducted using an Agilent 6890N/5975 system (USA), with helium as the carrier gas at a flow rate of 1.5 mL/min. The temperature program for the GC column consisted of an initial hold at 80 ℃ for 5 min, followed by a ramp of 5 ℃/min to 150 ℃ for 2 min, a further ramp of 10 ℃/min to 200 ℃ for 5 min, and finally a ramp of 20 ℃/min to 250 ℃ for 8 min. The mass spectrometer was operated in full-scan mode with a scan range of 30–500 m/z, and the ion source and transfer line temperatures were set to 230 ℃ and 280 ℃, respectively. An injection volume of 1.0 μL was used for each sample.

Statistical analysis

All experimental data were presented as the mean ± standard deviation (SD) of at least three independent replicates. Statistical analysis was performed using GraphPad Prism (version 9.0, GraphPad Software, USA) and SPSS (version 28.0, IBM Corp., USA). For comparisons between multiple groups, one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test was applied to determine significant differences. A p-value < 0.05 was considered statistically significant.

Results

Isolation of tetramethrin-degrading strains and their degradation potentials

Many morphologically diverse microbial isolates were obtained based on enrichment culture technology. Three fungi were inoculated into MSM medium containing tetramethrin (50 mg/L) at an initial dose of 0.3 g/L dry weight after multiple rounds of purification to test their degradation potential. After incubation at 28 ℃ and 200 rpm for 5 days in the dark, samples were extracted, and the residual concentration of tetramethrin was detected by HPLC. The results showed that the AF3 isolate had obvious tetramethrin removal potential, so the pyrethroid degradation ability was further verified and analyzed.

Based on a preliminary study of tetramethrin degradation by the AF3 strain over 5 days, the degradation period was subsequently extended to 9 days. The degradation

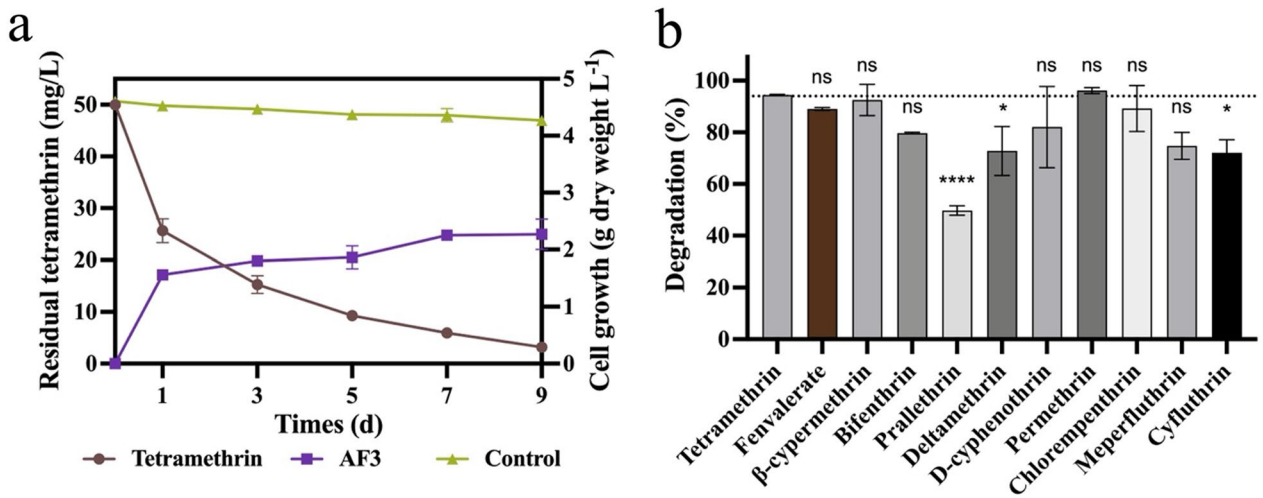

Fig. 1 Degradation potential of the AF3 strain against multiple pyrethroids. **a** Dynamics of the quantity of the AF3 strain and tetramethrin during degradation. **b** Differences in the metabolic activity of the AF3 strain against various pyrethroids

of 50 mg/L tetramethrin by the AF3 strain is shown in Fig. 1a. More than 50% of tetramethrin was removed by the AF3 strain after 24 h of incubation, while no significant change was observed in the control group. Subsequently, with an increase in the incubation time, the amount of tetramethrin decreased slowly, and the degradation rates over 5 and 9 days of incubation were 82.40% and 93.64%, respectively. It can be seen from Fig. 1a that the AF3 strain grew slowly in the late degradation stage, which may be related to an insufficient substrate concentration. At the same time, the AF3 strain can use tetramethrin in the MSM medium as the growth energy source, indicating that the AF3 strain can grow with tetramethrin as the only carbon source. Moreover, no significant decrease in the concentration of tetramethrin was observed in the control group after 9 days of dark culture, suggesting that the AF3 strain can effectively promote the metabolic breakdown of tetramethrin in water. The chromatogram of the degradation of tetramethrin using HPLC is shown in Figure S1.

The process of degradation of tetramethrin by the AF3 strain was evaluated using the first-order kinetic model, and the kinetic parameters are listed in Table 1. In the kinetic model, the determination coefficients R^2 of the treatment group and the control group were 0.9806 and 0.9644, respectively, indicating that the degradation process was consistent with the first-order kinetic model. The degradation rate constants of the treatment group and the control group were 0.2835 and 0.0079, respectively, and the theoretical half-lives were 2.4450 and 87.7402 days. These parameters illustrate that the treatment group supplemented with the AF3 strain could effectively remove tetramethrin and significantly shorten the residual half-life of tetramethrin in water.

In addition, the degradation rate of the AF3 strain for different pyrethroids is shown in Fig. 1b, and the results indicate that the AF3 strain has great degradation potential for a variety of pyrethroids. After 9 days of treatment under the same conditions (50 mg/L), the AF3 strain showed a significant difference ($p < 0.001$) in its degradation effects on permethrin, chlorempenthrin, bifenthrin, fenvalerate, D-cyphenothrin, beta-cypermethrin, meperfluthrin, cyfluthrin, and deltamethrin, with the degradation rates being 96.17%, 92.54%, 89.25%, 89.04%, 82.06%, 79.75%, 74.74%, 72.14%, and 72.79%, respectively, while the degradation rate against prallethrin was less than 50%. The specific degradation kinetic parameters are listed in Table S2. The AF3 strain did not show any substrate preference for commonly used pyrethroid pesticides, indicating that it is suitable for in situ bioremediation of multiple pyrethroid contamination sites.

Table 1 Kinetic parameters of biodegradation of tetramethrin in MSM by the AF3 strain

Treatments	Regression equation	k (d^{-1})	R^2	$t_{1/2}$ (d)
AF3	$C_t = 49.0388e^{-0.2835t}$	0.2835	0.9806	2.4450
Control	$C_t = 48.7392e^{-0.0079t}$	0.0079	0.9644	87.7402

C_t refers to tetramethrin degradation (mg/L); k refers to degradation rate constant; t refers to degradation times; R^2 refers to determination coefficient; d^{-1} refers to per day

Identification of the AF3 fungal strain

A small piece of the AF3 strain was selected and incubated in a 90 mm Petri dish containing PDA medium for 5 days at 28 ℃, and the mycelium and its spore morphology were shown in Fig. 2a, b. The colonies of the AF3 strain are plush-shaped, with neat edges spreading around and increased aerial mycelia in the later stage of culture. The front side of the medium is white mycelium, and the back is pale yellow. Mycelium from the AF3 strain has varying numbers of septa and releases a

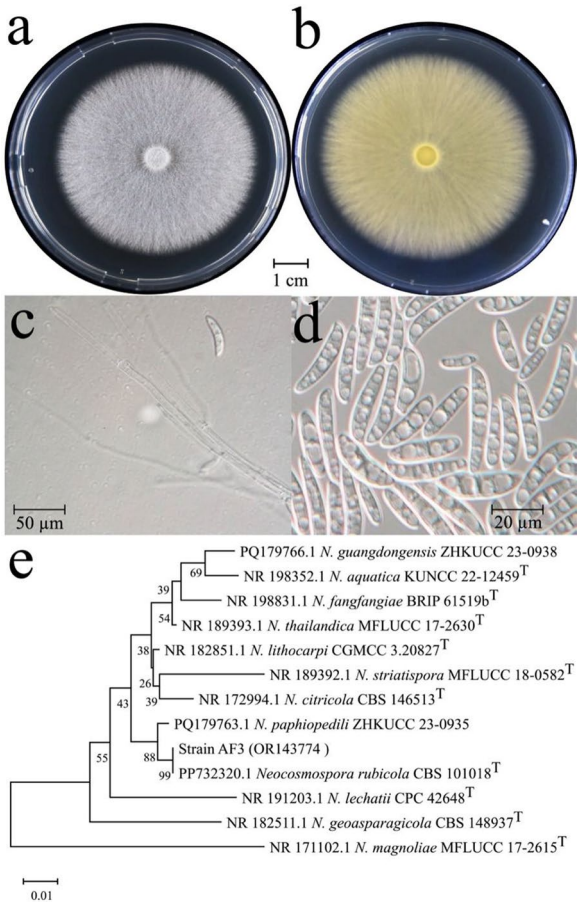

Fig. 2 Identification of the AF3 strain. **a**, **b** Morphology of colonies on the front and back sides of the AF3 strain on PDA plates. **c**, **d** Microstructure of mycelia and conidia of the AF3 strain. **e** Phylogenetic tree constructed based on ITS sequences

large quantity of macroconidium during growth, which is sickle-shaped (Fig. 2c, d).

PCR amplification and sequencing results of the ITS1 and ITS4 primers showed that the ITS region size of the AF3 strain was 545 bp. A phylogenetic tree was constructed based on the ITS sequence of the AF3 strain, and the results indicated that the AF3 strain belongs to the genus *Neocosmospora* and has a high similarity with *N. rubicola* CBS 101018 (GenBank accession number: NR154227.1) (Fig. 2e). The ITS sequence of the AF3 strain was submitted to GenBank and the accession number OR143774 was obtained. Based on the morphological observations and fungal ITS sequence analysis, the AF3 strain was further described as *Neocosmospora* sp. AF3. This is the first report on the degradation of pyrethroid insecticides by fungi of the genus *Neocosmospora*.

Concentration range of the AF3 strain metabolizes tetramethrin

The results of different concentrations of tetramethrin degradation by the AF3 strain are shown in Fig. 3a. At low concentrations (≤50 mg/L), the AF3 strain showed a rapid metabolic capacity on the first day with degradation rates reaching over 30%. When the tetramethrin concentration was higher than 50 mg/L, the degradation rate on the first day was less than 20%. After 9 days of treatment with the AF3 strain, the degradation rates of 10, 25, and 50 mg/L tetramethrin were 100%, 88.12%, and 93.64%, respectively, with no lag period. However, when the concentration was raised to 100–300 mg/L, more than half of the tetramethrin residue was still observed on day 5. After 9 days of incubation, the removal rates of the treatment groups at concentrations of 100, 200, and 300 mg/L were only 73.29%, 59.87%, and 40.10%. The natural degradation rates of different concentrations of tetramethrin are shown in Figure S2.

The degradation process of tetramethrin metabolism by the AF3 strain at gradient concentrations was fitted to the Andrews equation shown in Eq. (3). The raw degradative data were nonlinearly fitted by Origin version 9.0 (OriginLab, USA), and finally, the inhibition curves of tetramethrin degradation by the AF3 strain were plotted, as shown in Fig. 3b. According to the k value for the first-order kinetics of tetramethrin degradation, the coefficient of determination (R^2) of the Andrews model fitting results was 0.9971, indicating that the fitting results met the requirements. The maximum specific degradation rate (q_{max}) of tetramethrin metabolized by the AF3 strain was 0.9919 ± 0.22 d^{-1}. The inhibition coefficient (K_i) was 20.1873 ± 6.13 mg/L and the half-rate constant (K_s) of the model was 7.8735 ± 3.83 mg/L. Further calculations of K_i and K_s suggested that the optimal substrate concentration (S_{max}) for degradation of tetramethrin by the AF3 strain is 12.6073 mg/L.

Model predicts optimal conditions of tetramethrin metabolism

Based on the Box–Behnken design of RSM, the experimental results for tetramethrin degradation by the AF3 strain under different combination conditions are listed in Table S3, and the interaction between the three factors on the AF3 strain were simulated according to the results (Figure S3). In addition, a polynomial regression analysis was performed on the raw data, and the quadratic

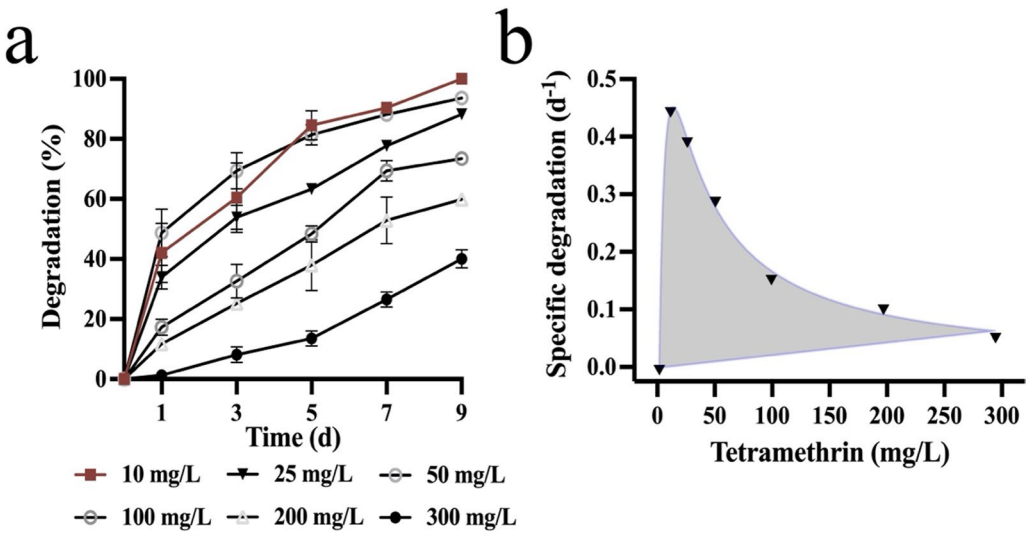

Fig. 3 Analysis of optimal concentration of tetramethrin metabolized by the AF3 strain. **a** Degradation rate of tetramethrin with different concentrations by the AF3 strain. **b** Andrews equation nonlinearly fitting of degradation of tetramethrin

polynomial regression equation was fitted under different combinations of conditions was fitted:

$$Y_1 = 94.39 + 21.86X_1 + 9.64X_2 - 0.38X_3 + 1.22X_1X_2 + 5.73X_1X_3 + 1.16X_2X_3 - 23.55X_1^2 - 2.66X_2^2 - 12.74X_3^2.$$

Table 2 shows the analysis of variance (ANOVA) using the response surface technique to fit the quadratic polynomial regression equation. The analysis results show that the P value of the model was < 0.05, and the F value was 8.26, indicating that the model fits well with the degradation of tetramethrin. The determination coefficient (R^2) and the variation coefficient (C.V.) of the response surface model were 0.9370 and 13.37%, suggesting that the actual degradation value of the model was in good agreement with the predicted value. The results shown in Table 2 further show that the univariate term of temperature (X_1), pH (X_2), and the square term of X_1 (X_1^2) had considerable effects on the tetramethrin degradation ($p < 0.05$) (Figure S4a–c). However, the amount of inoculation (X^3) and the interaction terms of multiple factors had no significant effects on the metabolism of tetramethrin.

Subsequently, by further derivation of the quadratic polynomial regression equation, the optimal coded values of the three variables X_1, X_2, and X_3 were found to be 0.6715, 0.4870 and 0.1164, respectively (Figure S4d–f). Their corresponding uncoded values were determined to be a temperature of 33.37 ℃, pH of 7.97, and inoculation amount of 0.22 g/L dry weight, respectively. Meanwhile, tetramethrin's maximum theoretical degradation rate degraded by the AF3 strain was 103.21% (Figure S4g). In order to more intuitively demonstrate the interactions of various factors with tetramethrin degradation by the AF3 strain, degradative isotherm plots and a multi-dimensional surface model were drawn (Fig. 4). As shown in Fig. 4a–c, the temperature is still the main factor affecting the degradation of tetramethrin by the AF3 strain under the interaction of the two influencing factors. The isotherm plot of Fig. 4d–f shows that when the temperature and inoculum size were coded at 0, the degradation rate increased with an increasing pH value.

Table 2 Analysis of variance for the quadratic tetramethrin degradation model

Source	DF	SS	MS	*F*-value	*P*-value
Model	9	7200.71	800.08	8.26	0.02
X_1	1	3821.41	3821.41	39.44	0.00
X_2	1	743.93	743.93	7.68	0.04
X_3	1	1.12	1.12	0.01	0.92
X_1X_2	1	5.93	5.93	0.06	0.81
X_1X_3	1	131.25	131.25	1.35	0.30
X_2X_3	1	5.38	5.38	0.06	0.82
X_1^2	1	2048.04	2048.04	21.14	0.01
X_2^2	1	26.05	26.05	0.27	0.63
X_3^2	1	599.35	599.35	6.19	0.06
Residue	5	484.48	96.90		
Lack of fit	3	479.23	159.74	60.86	0.02
Pure error	2	5.25	2.62		
Sum total	14	7685.19			

X_1 refers to temperature; X_2 refers to pH; X_3 refers to inoculum size; DF refers to the degree of freedom; P value < 0.05 indicates that the model is significant

Analysis of metabolites and metabolic pathways

Samples treated with the AF3 strain were collected every 2 days, and further qualitative analysis of GC–MS was carried out after HPLC detection of tetramethrin and its metabolite concentrations. The results show that significant ion peaks were detected at 25.889 and 26.019 min, with mass-to-charge ratios (m/z) of 315.18 and 316.19, respectively. Based on retention times (RTs), similarity in the NIST library database, and ion fragment characteristics, the compounds were identified as *cis*-tetramethrin and *trans*-tetramethrin. With prolonged treatment time, the amount of tetramethrin decreased obviously and six different intermediate metabolites were found, which are listed in Table S4. The RTs of compounds B, C, D, E, F and G were 7.241, 7.144, 7.214, 12.422, 12.411, and 16.900 min, respectively. According to the NIST database alignment, these six compounds were identified as chrysanthemal, chrysanthemyl alcohol, chrysanthemic acid, *N*-hydroxymethyl-3,4,5,6-tetrahydrophthalimide, tetrahydrophthalimide, and acrylamide, respectively. The mass spectra of the six metabolites are shown in Fig. 5.

A possible metabolic pathway of tetramethrin by the AF3 strain was proposed (Fig. 6). The AF3 strain first decomposed tetramethrin into chrysanthemic acid and *N*-hydroxymethyl-3,4,5,6-tetrahydrophthalimide under the action of hydrolase. Chrysanthemic acid continued to be converted into chrysanthemyl alcohol, and chrysanthemyl alcohol was unstable in the environment and then further oxidized to chrysanthemal. Previous studies have shown that *N*-hydroxymethyl-3,4,5,6-tetrahydrophthalimide is an important secondary metabolite of tetramethrin and is subsequently converted to tetrahydrophthalimide, which is an intermediate of various pesticides, such as Folpet and Phosmet [21, 22]. Tetrahydrophthalimide eventually cleaves to form acrylamide and then it is further broken down. Throughout this degradation process, the toxicity of the metabolites gradually decreases. As illustrated in the Ecosar toxicity analysis results (Figure S5), tetramethrin (Tet) exhibits the

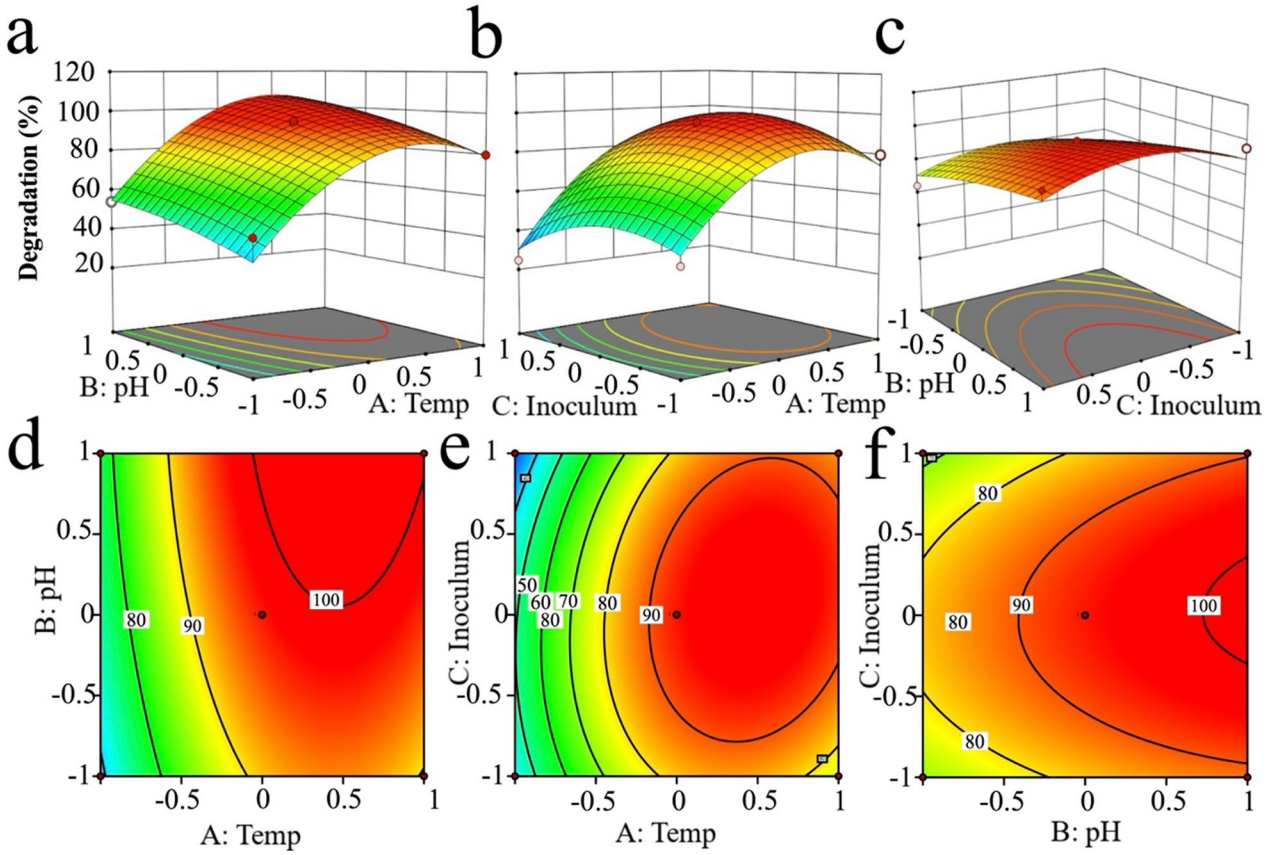

Fig. 4 Effect of multiple factors on the degradation of tetramethrin by the AF3 strain. **a**–**c** A 3D surface model under the influence of two different factors. **d**–**f** Degradative isotherm diagram under the interaction of two different factors

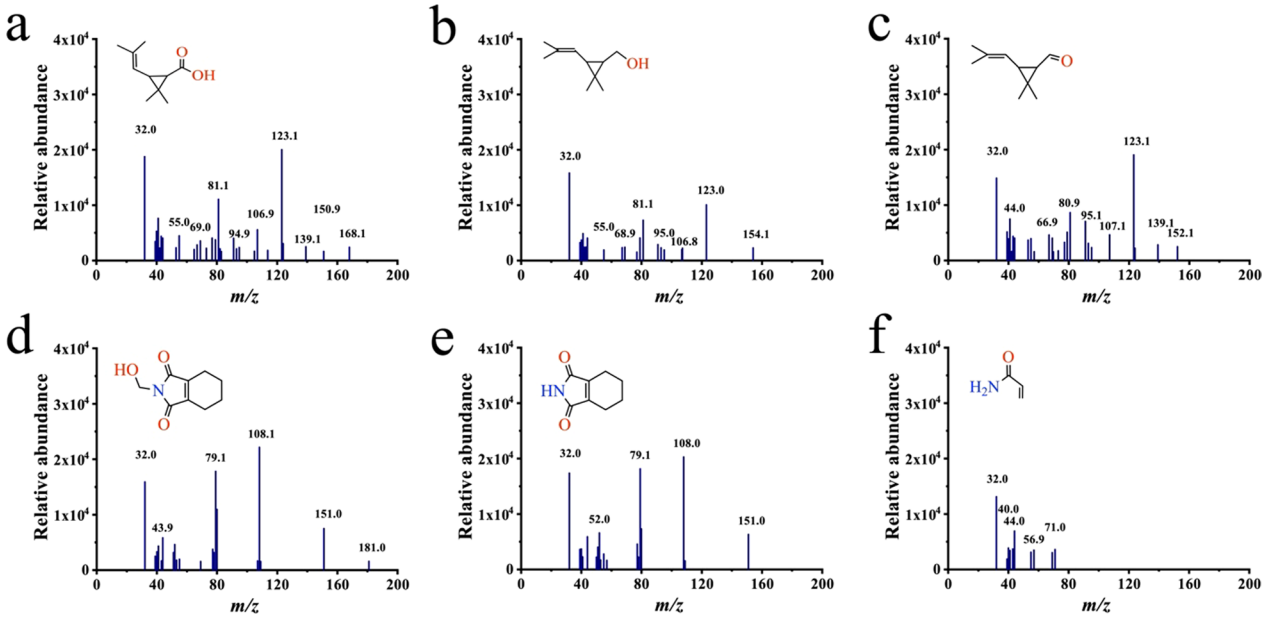

Fig. 5 Mass spectra of intermediate metabolites of tetramethrin matched in the National Institute of Standards and Technology (NIST) database. **a** Chrysanthemic acid, **b** Chrysanthemyl alcohol, **c** Chrysanthemal, **d** *N*-Hydroxymethyl-3,4,5,6-tetrahydrophthalimide, **e** Tetrahydrophthalimide; and **f** Acrylamide

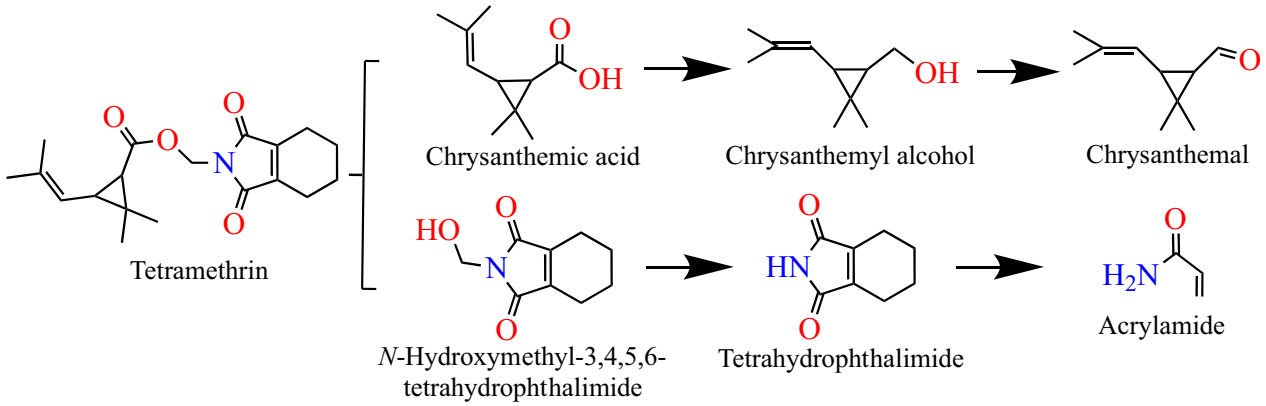

Fig. 6 Proposed metabolic pathway of tetramethrin by the AF3 strain degradation

highest toxicity, while the final metabolite, acrylamide (M6), shows the lowest toxicity. This indicates that the degradation process by the AF3 strain is a detoxification process, thereby reducing its environmental impact. The pathway involves multiple steps, including hydrolysis, reduction, and oxidation, which collectively contribute to the detoxification of tetramethrin.

Discussion

Residual contamination of pyrethroids has been a problem for a long time, and researchers have developed many effective pyrethroid removal methods. The bioremediation of pyrethroids is one of the current research hotspots, in which microorganisms play an important role. Many bacterial isolates have been reported to be highly effective for removing various pyrethroids, including tetramethrin, such as *Gordonia cholesterolivorans* A16, which is capable of thoroughly metabolizing 25 mg/L of tetramethrin in 9 days [23]. Unlike the AF3 strain, the A16 strain has a degradation rate of only 68.3% and 60.7% for D-cyphenothrin and chlorempenthrin, while the AF3 strain has a higher degradation rate. *Staphylococcus succinus* HLJ-10, isolated from farmland contaminated soil on farmland, was reported to be effective for removing 62.5% of 50 mg/L tetramethrin within 7 days [2]. The degradation rate of tetramethrin by the AF3 strain was 88.10% at 7 days, which was significantly higher than that of the HLJ-10 strain.

However, there is currently a lack of research on the fungal degradation of pyrethroids, especially tetramethrin. Birolli et al. obtained a pyrethroid-degrading fungus, *Aspergillus* sp. CBMAI 1829, from seawater that removed only 44.8% of lambda-cyhalothrin from artificial seawater in 14 days [24]. The degradation of beta-cypermethrin by *Eurotium cristatum* ET1 isolated from fermented tea was 57.93% within 8 days [25]. Fungi have stronger resistance and adaptability to adverse environments than bacteria, but the degradation activity of pyrethroid-degrading fungi reported so far has generally been low. The AF3 strain completely eliminated 10 mg/L tetramethrin within 9 days, and the degradation rate of 50 mg/L tetramethrin reached 93.64%, indicating that the AF3 strain had higher metabolic potential than most pyrethroid-degrading fungi.

Nonlinear fitting based on Andrews equations revealed that the AF3 strain has more outstanding metabolic activity at low concentrations (< 15 mg/L). In actual contaminated environments, the residual level of pyrethroids is usually less than 1 mg/L [26, 27]. A survey of agricultural chemical residues in agro-intensive areas conducted in the United States showed that bifenthrin, cyfluthrin, cypermethrin, lamda-cyhalothrin, permethrin, deltamethrin, and esfenvalerate are widely present in local water bodies with concentrations ranging from 2.19 to 219 μg/kg [28, 29]. This investigation further demonstrated that pyrethroid contamination is a complex type of contamination with a contamination residue level lower than the optimal metabolic concentration of the AF3 strain (12.6073 mg/L), suggesting that the AF3 strain could be a reliable candidate for in situ remediation at actual contamination sites.

The response surface technique has been widely used for the model prediction of optimal conditions [30–35]. Under single-factor conditions, the AF3 strain is significantly affected by the temperature. At suitable temperatures, the degradation rate increases with an increasing pH, which is consistent with previous studies. Different from the Box–Behnken design, Birolli et al. optimized the degradation conditions of cypermethrin by *Bacillus thuringiensis* Berliner using a central composite design, and the results showed that the degradation rate increased from 64.7% to 73.5% as the pH increased from 5.5 to 8.5 at the same concentration and temperature [30]. The bacterial strain *Bacillus megaterium* HLJ7, which was

similarly designed by the Box–Behnken with the AF3 strain, showed very different results from the fungus AF3, with temperature having no significant effect on the degradation of allethrin by the HLJ7 strain [20]. The recently published Gram-positive bacterium *Rhodococcus pyridinivorans* Y6 showed the same response surface model results as the AF3 strain [31]. Temperature and pH have significant effects on prallethrin metabolized with the Y6 strain.

Studies on tetramethrin degradation have been rare so far, and insights into tetramethrin metabolic pathways are limited. In this study, several possible intermediate metabolites were obtained by aligning GC–MS with the NIST library database. Chrysanthemic acid, chrysanthemyl alcohol, and chrysanthemal are typical metabolites of most synthetic pyrethroids and are rapidly metabolized by indigenous microorganisms in the environment [36–40]. *N*-hydroxymethyl-3,4,5,6-tetrahydrophthalimide and tetrahydrophthalimide have been reported as secondary metabolites of tetramethrin [41]. Compared with *N*-hydroxymethyl-3,4,5,6-tetrahydrophthalimide, tetrahydrophthalimide is an important intermediate for a variety of macromolecular organic compounds [42]. The small molecule metabolite acrylamide was also found in the degradation of tetramethrin by *G. cholesterolivorans* A16 [23]. In addition, acrylamide, as an important industrial chemical material, is widely present in the natural environment and has little impact on the environment. Due to the limitations of detection methods, the downstream and final products of acrylamide have not been further explored.

The initial step in the biodegradation of pyrethroids usually involves the hydrolysis of the ester bond [43–48]. In this study, the first step using *Neocosmospora* sp. AF3 was to break down the ester linkage of tetramethrin, producing *N*-hydroxymethyl-3,4,5,6-tetrahydrophthalimide and acid derivatives. This result was consistent with previous studies [49, 50]. This step is crucial for reducing the toxicity of pyrethroids and opening the molecules to further degradation. GC–MS is extensively utilized for detecting and analyzing the secondary metabolites of macromolecular organic compounds; although 3-phenoxybenzoic acid and 3-phenoxybenzaldehyde were not detected in this study, they have been reported in the literature as typical intermediates of type II pyrethroids [51–53]. Pyrethroids are characterized by their unique chiral structure [54–56]. Centered around an α chiral carbon, their parts can be categorized into acid and alcohol groups [57, 58]. Chrysanthemic acid is a common intermediate representing the acid moiety in most pyrethroids [59, 60]. The insecticidal efficacy of pyrethroids is often enhanced by the addition of halogen atoms, such as chlorine and bromine, to the chrysanthemic acid base. It has been reported that chrysanthemic acid and α-hydroxy-3-phenoxy-benzeneacetonitrile are unstable intermediates [16, 61]; it is hypothesized that chrysanthemic acid may first be hydrolyzed to chrysanthemal, before undergoing further ring-opening reactions that eventually lead to its mineralization.

Conclusion

The fungal strain *Neocosmospora* sp. AF3, isolated from pesticide-contaminated experimental fields, can effectively remove a variety of pyrethroid pesticides, and the degradation activity from high to low was tetramethrin, permethrin, beta-cypermethrin, chlorempenthrin, fenvalerate, D-cyphenothrin, bifenthrin, meperfluthrin, cyfluthrin and deltamethrin. Among the currently reported pyrethroid-degrading fungi, the AF3 strain has a superior metabolic capacity. Furthermore, an analysis of its metabolic pathways and degradation mechanisms indicates that the AF3 strain first degraded tetramethrin into non-toxic or low-toxicity chrysanthemic acid and *N*-hydroxymethyl-3,4,5,6-tetrahydrophthalimide through an attack on its carboxylic ester bond. In addition, the AF3 strain has a high degradation efficiency at low concentrations of tetramethrin and higher temperatures, and is also resistant to high amounts of tetramethrin residue and extreme pH environments, suggesting that the AF3 strain could be a candidate for bioremediation in tetramethrin-contaminated areas.

Supplementary Information

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Supplementary Material 1.

Author contributions

All authors contributed to the manuscript revision and approved the submitted version. Conceptualization, Y.H.; methodology, W-J.C. and Y.H.; validation, H.Y.; formal analysis, W-J.C. and Y.H.; investigation, W-J.C. and Y.H.; resources, W-J.C. and Y.H.; data curation, W-J.C. and Y.H.; writing—original draft preparation, W-J.C. and Y.H.; writing—review and editing, X.L., X.Z., K.B., S-F.C., M.A.G., and X.Z.; visualization, Y.H.; supervision, Y.H. and X.Z.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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