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Isolation, Degradation Performance and Field Application of the Metolachlor-Degrading Fungus *Penicillium oxalicum* MET-F-1

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Abstract: Metolachlor is extensively used and the most persistent chloroacetamide herbicide, thereby which its metabolites have been frequently detected in soils and surface and groundwaters. Microbial degradation is predominantly responsible for the removal of metolachlor from soil and water. However, few microbial strains reported previously are highly efficient in degrading potentials for metolachlor. We isolated the fungal strain MET-F-1 from an activated sludge, characterized as *Penicillium oxalicum*, which could degrade 88.6% of 50 mg/L metolachlor coupled with 0.1% glucose plus 0.1% yeast extract within 384 h under optimal conditions. Compared with metabolites produced by previously isolated microorganisms, different degradation products, i.e., MOXA, M2H, and MDES, detected by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), were produced through hydrolytic and reductive dechlorination by MET-F-1. This is the first report on the degradation of metolachlor by *Penicillium oxalicum* sp. Furthermore, field plot experiments using the wheat bran inoculum method were performed and demonstrated good metolachlor-degrading activity of this strain. This study serves as a steppingstone to promote MET-F-1 strain usage as a promising agent for metolachlor-contaminated soil remediation.

Keywords: metolachlor; *Penicillium oxalicum* sp. MET-F-1; co-metabolism; microbial degradation; field plot experiment

1. Introduction

The application of herbicides in agriculture increases crop yields but also threatens the terrestrial and aquatic ecosystems [1,2]. Metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methylethyl] acetamide) is one of the most commonly used herbicides, accounting for approximately 4.2% of the global herbicide usage [3]. It is a highly selective chloroacetamide pesticide which is widely used to control broadleaf grasses and annual weeds in the pre-emergence period of more than 70 different crops, such as corn, soybeans, and cotton, which are the main crops in China [4,5]. Meanwhile, metolachlor is not degraded easily, and its half-life in the field ranges from 22 to 205 days [6,7]. It is worth noting that metolachlor exhibits moderate toxicity, leading to detrimental effects on human liver cells [6,8]. Additionally, in previous studies, high detection rates of metolachlor in surface and groundwaters have been observed because of its higher water solubility (530 mg/L, 20 °C) compared with other chloroacetamide herbicides [9–11].

As mentioned above, it is essential to improve the degradation efficiency of metolachlor in the field. Previous studies have shown that the dissipation of herbicides, including metolachlor, mainly occurs through biodegradation [3,12,13]. Microorganisms can degrade pesticides as sources of nutrients or energy, or co-metabolize organic contaminants with other nutrients [3,14]. For example, Munoz et al. [15] showed that the addition of energy resources could accelerate metolachlor degradation by the yeast strain *Candida xestobii*, especially in minimal medium plus sucrose and yeast extract. However, the degradation pathway by this strain was not speculated, as no metabolic products were detected by high-performance liquid chromatography (HPLC) for its low sensitivity. Additionally, the fungus *Ch. Globosum* had the ability to degrade or co-metabolize metolachlor through dealkylation of the nitrogen atom and subsequent dehydrogenation of the ethyl substituent [16]. Besides fungi, several bacteria have been observed to remove chloroacetamide herbicides from soil, mainly by C-dealkylation and N-dealkylation [17,18]. However, the degradation rates of chloroacetamide herbicides by these microorganisms were 24–60% after 5–120 days of inoculation. Furthermore, the environmental factors, including the pH value, moisture content, and concentration of herbicide, also affect biodegradation [12,15,19]. However, while the biodegradation potentials of these isolated microorganisms are evaluated in the laboratory under controlled environmental conditions, they are seldom studied in the field.

The aim of this study was to isolate and identify a strain with good performance on metolachlor degradation from an activated sludge and to propose a possible pathway of metolachlor degradation. Meanwhile, the environmental conditions for the growth of the fungal strain were optimized. Moreover, to verify the effectiveness of the strain in a complex field environment, the immobilized fungal inoculum was prepared for a field plot experiment. This work not only reports a new fungal strain with metolachlor-degrading potential but also provides a strong reference for its field application.

2. Materials and Methods

2.1. Target Source for Screening Functional Microbes

The activated sludge used to isolate microbial strains was obtained from the pesticide sewage treatment tank in Tianjin Boke Technology Co., Ltd. Yum. After being air-dried in a shaded place, the sludge was dark brown and powdery and had a pungent smell, with a pH of 6.75, organic matter content of 73.66 g kg^{-1}, total nitrogen content of 2.4 g kg^{-1} and total phosphorus of 1.54 g kg^{-1}. The soil texture consisted of 64.1% sand, 25% silt, and 10.9% clay.

2.2. Chemicals and Media

Reference standards, including analytical grade standard metolachlor, metolachlor ethane sulfonic acid (MESA, 98.5% purity), metolachlor oxanilic acid (MOXA, 98.3%), metolachlor-2-ethoxy (M2E, 97.7%), metolachlor-2-hydroxy (M2H, 98.6%), metolachlor mercapturate (MMER, 98.2%), and metolachlor deschloro (MDES, 97.6%), were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Technical metolachlor (98.1%) was provided by Boke Technology Co., Ltd. Yum, Tianjin, China. Preparation of 720 g/L metolachlor EC was provided from Tianbang Chemical Co., Ltd., Jinan, China. Inorganic chemical reagents, such as NaCl, $NaNO_3$, K_2HPO_4, and $MgSO_4{\cdot}7H_2O$, were all of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. Peptone, beef extract, sucrose, and other organic reagents were obtained from Solarbio Technology Co., Ltd., Beijing, China.

Enrichment medium (ER, g/L deionized water, pH 7.0): Peptone 5.0, beef extract 3.0, and NaCl 5.0; Czapek Dox agar medium (CDA, g/L deionized water): $NaNO_3$ 3.0, K_2HPO_4 1.0, $MgSO_4{\cdot}7H_2O$ 0.5, KCl 0.5, $FeSO_4$ 0.01, sucrose 30.0, and agar 20.0; and Minimal salts medium (MSM, g/L deionized water, pH 7.0): $(NH_4)_2SO_4$ 1.0, K_2HPO_4 1.5, NaCl 1.0, $MgSO_4{\cdot}7H_2O$ 0.4 were prepared. The above media were sterilized in an autoclave at 121 °C for 20 min.

2.3. Isolation of Metolachlor-Tolerant Strains

For the isolation of metolachlor-tolerant strains, 10 g of the target soil was added into an Erlenmeyer flask containing 100 mL of ER medium with 50 mg/L metolachlor under sterile conditions. Then, the Erlenmeyer flask was rotated on the shaking table with a frequency of 150 r/min at 30°C. After 5 days, 10 mL of cultures were transferred to another 100 mL of fresh ER medium in which the concentration of metolachlor was 100 mg/L. After another 5 days, this step was repeated until the concentration of metolachlor increased to 200 mg/L. One milliliter of the final enriched culture was diluted to 10 mL with distilled water in a 10 mL centrifuge tube and serially diluted by 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. Of each diluted solution. 1 mL was spread onto CDA plates with 100 mg/L metolachlor for 3 days at 30 °C. Single colonies with different morphological characteristics were selected to be inoculated on the plates in which the concentration of metolachlor was 100 mg/L. Single colonies were then purified three times using the plate streak method. Four single colony strains with good growth were selected and stored in CDA medium after purification.

2.4. Screening and Identification of Metolachlor-Degrading Strains

To evaluate the metolachlor-degrading potentials, the four strains were cultured in MSM medium to logarithmic phase (until the OD600 value reached 0.8). Then, 10% inoculation amount ("10% inoculum volume" refers to the volume ratio of the inoculum to the inoculated culture medium) was determined in MSM with a metolachlor concentration of 50 mg/L. These treatments were set as follows: 0.2% glucose, 0.2% yeast extract, and 0.1% glucose plus 0.1% yeast extract were added, respectively, before sterilization, and MSM medium without inoculation was used as control. Each treatment was performed in triplicate. The specific settings for treatments are shown in Table S1. Subsequently, the liquid samples were collected regularly at 0 h, 2 h, 48 h, 96 h, 168 h, 216 h, 336 h, and 384 h to evaluate the metolachlor-degrading potentials of the four tolerant strains. Finally, strain MET-F-1 was isolated for its good ability to degrade metolachlor, and its liquid samples were determined for the metabolites of metolachlor to predict the possible degradation pathways.

Molecular biological identification of strain MET-F-1 was conducted using ITS rDNA sequencing analysis. The strain MET-F-1 was cultured in CDA medium in an incubator at 30 °C for 5 days, and then its total genomic DNA was extracted using Aidlab DN14 Fungal Extraction Kit (Aidlab Biotechnologies Co., Ltd., Beijing, China) as per the protocol. The extraction and amplification of DNA were performed according to a previously described method by Chen et al. [20]. The polymerase chain reaction (PCR) primers used for ITS rDNA amplification were ITS4 (5′ -TCCTCC-GCTTATTGATATGC-3′) and ITS5 (5′-GGAAGTAAAAGTCGTAACAAG-3′). The amplification system contained ddH_2O, 19 μL; PCR mixed buffer, 25 μL; upstream primer, 2 μL; downstream primer, 2 μL; gene samples, 2 μL (the first four components were mixed and transferred into a PCR tube, and the gene samples were added at last). The amplification conditions were as follows: an initial denaturation of 3 min at 95 °C, unwinding at 94 °C for 1 min, renaturation at 54 °C for 40 s, extension at 72 °C for 1 min, amplification for 35 cycles, and a final extension step at 72 °C for 10 min. The products were preserved at 4 °C and sent to Tsingke Biological Technology Co., Ltd. (Beijing, China) for sequencing. Sequencing results were compared with the NCBI database sequence using BLAST.

2.5. Optimization of Metolachlor Degradation Performance of Strain MET-F-1

Strain MET-F-1 was inoculated into 150 mL Erlenmeyer flask containing 50 mL of MSM medium for expanding culture. The metolachlor residues and the mycelial dry weights were determined regularly. To optimize the performance of strain MET-F-1, the effects of a metolachlor concentration of 5–100 mg/L, pH of 5.0–7.5, temperature of 20–35 °C, and culture time of 0–140 h on its metolachlor-degrading potential were investigated using a completely randomized design.

2.6. Metolachlor-Degrading Potential of Strain MET-F-1 in Field Trials

Strain MET-F-1 was treated as the microbial inoculum in a solid-state fermentation system for field trials. The preparation process of the microbial inoculum was as follows. In a sterile environment, the spore suspension of strain MET-F-1 in the logarithmic phase was inoculated with the sterilized wheat bran at a dry w/w ratio of 1:5 (the spore suspension: wheat bran). The moisture content of inoculum was adjusted to 60 ± 5%. The starch content of wheat bran was 17%, the pH was 7.3, and the organic matter content was 65.5 g/kg. Then, the inoculum was stirred well and regularly mixed until the wheat bran appeared light green. At this time, the number of viable fungi reached about 2.5 * 108 CFU/g by the dilution plate counting method.

To test the metolachlor-degrading potential of strain MET-F-1, the field plot experiment was carried out in a corn field in Wuqing, Tianjin Municipality, China (117°11′ E, 39°22′ N) in October 2019, with temperatures ranging from 17 to 33 °C. The soil was classified as fluvo-aquic, and its chemical and physical properties were as follows: pH was 7.65, organic matter content was 23.97 g/kg, total nitrogen content was 1.4 g/kg, total phosphorus was 0.94 g/kg, and the relative percentages of sand, slit, and clay were 84.1%, 15%, and 0.9%, respectively [21]. The application of 720 g/L metolachlor EC at the recommended dosage (1.8 kg/ha) resulted in a theoretical initial concentration of metolachlor in the soil of field plot. The field was then divided into three treatment plots: SM (application of 720 g/L metolachlor EC), SMB (application of 720 g/L metolachlor EC and wheat bran at a ratio of 4 kg/20 m^2), and SMBF (application of 720 g/L metolachlor EC and the microbial inoculum of strain MET-F-1 at a ratio of 4 kg/20 m^2). The area of each plot was 20 m^2 and each treatment was set in triplicate. The wheat bran and microbial inoculum were manually broadcast and ploughed evenly. These plots were watered using a sprayer every 3 days to keep surface soil moisture levels at 30% [22]. Subsequently, surface soil samples were collected periodically at 0 (2 h), 3, 5, 7, 10, 15, and 30 days for the determination of metolachlor residue.

2.7. Determination of Metolachlor and Its Metabolites in the Liquid and Soil Samples

Metolachlor and its metabolites in the liquid and soil samples were analyzed as follows. Two milliliters of the liquid sample or 2.0 g of the soil sample was placed in a 50 mL polypropylene centrifuge tube containing 10 mL of acetonitrile, and 2.0 g of sodium chloride. The tube was vortexed for 1 min and centrifuged at 6654× *g* for 5 min. Then, 1 mL of the supernatant was filtered through a 0.22 µm filter. The concentrations of metolachlor and its metabolites were detected by ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) as described in a previous study [21]. Briefly, UPLC-MS/MS was operated in electrospray ionization (ESI) in both positive and negative ion modes. The electrospray ionization (ESI) parameters were set as follows. The capillary voltage was 3.5 kV, nebulizer was 7.0 bar, the flow of desolvation gas was 10 L/min, the flow of cone gas was 2.5 L/min, and the desolvation temperature was 400 °C. The separation of the compounds was conducted using an ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, Waters Technology, New York, NY, USA).

3. Results and Discussion

3.1. Isolation and Identification of Metolachlor-Degrading Strain

The four metolachlor-tolerant strains were obtained via enrichment culture from the activated sludge of the pesticide plant (Supplementary Materials, Figure S1). Metolachlor is more recalcitrant toward biodegradation than other chloroacetanilide herbicides due to its longer alkyl chain on the amide nitrogen [18]. Biodegradation of metolachlor is thought to proceed through a co-metabolic process in which other carbon sources participate [15,23,24]. Considering this, some treatments of co-metabolic culture in MSM medium were designed, using which MET-F-1 was found to have the good ability to degrade metolachlor, whereas the other three strains had no obvious effects on the degradation of metolachlor.

Colonies of MET-F-1 grown on CDA medium were round and tiled, with white edges and a grey-green center (Figure 1A). Conidial structures were produced in large quantities and spore surfaces were lamellar. As shown in Figure 1B, the conidia were observed to be oval, smooth, and fastigiated, in chains with the sizes of 3.2–8.3 × 2.5–5.0 µm by the microscope. Comparing the ITS rDNA gene sequence of MET-F-1 with the GenBank sequence in NCBI, 99% sequence homology to *Penicillium oxalicum* was observed. A dendrogram based on known representatives of *Penicillium* species is presented in Figure 2. Based on the genetic and morphological characteristics, strain MET-F-1 was identified as *Penicillium oxalicum*.

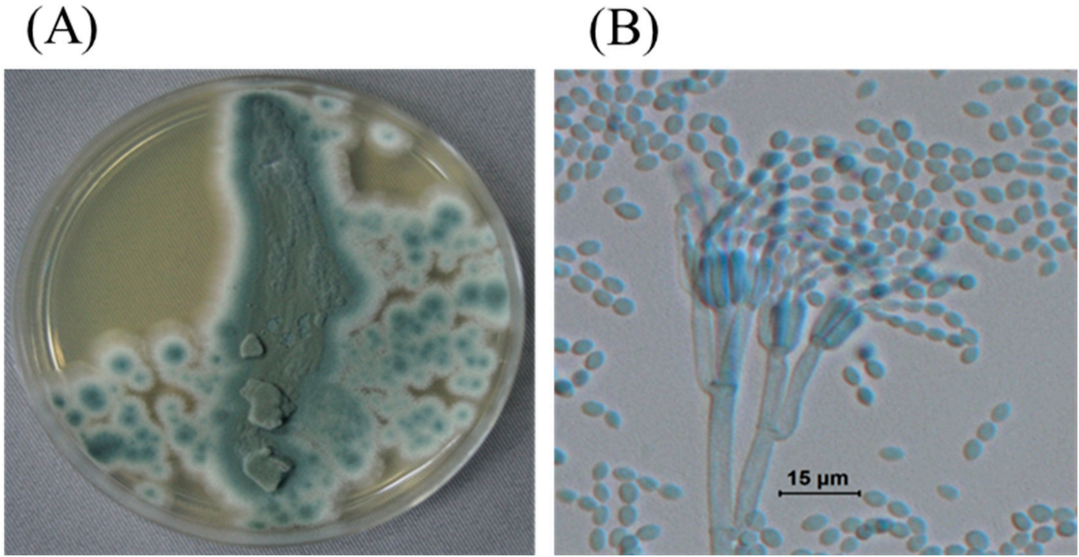

Figure 1. Colony morphology (**A**) and microscopic characteristics (**B**) of metolachlor-degrading strain MET-F-1.

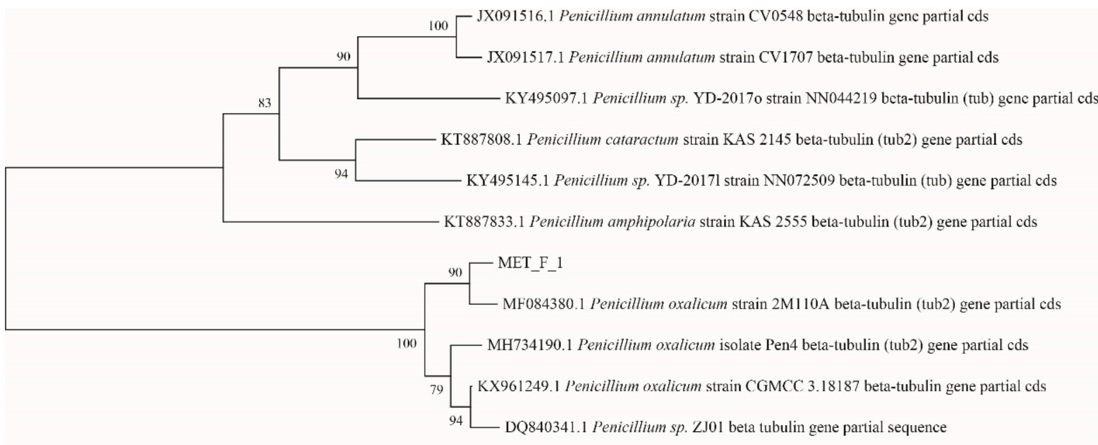

Figure 2. Dendrogram showing the ITS rDNA gene similarity of strain MET-F-1 to several type strains in genus *Penicillium*. Bootstrap values (%) are given at the nodes. The scale bars represent 0.020 substitutions/site.

3.2. Optimization of the Metolachlor-Degrading Performance of Strain MET-F-1

To obtain the optimum degradation condition parameters for this strain, the effects of initial concentration of metolachlor, pH, temperature, and culture time on its metolachlor-degrading potential were investigated by determining metolachlor residues, and the mycelial dry weights were measured using a completely randomized design (Figure 3).

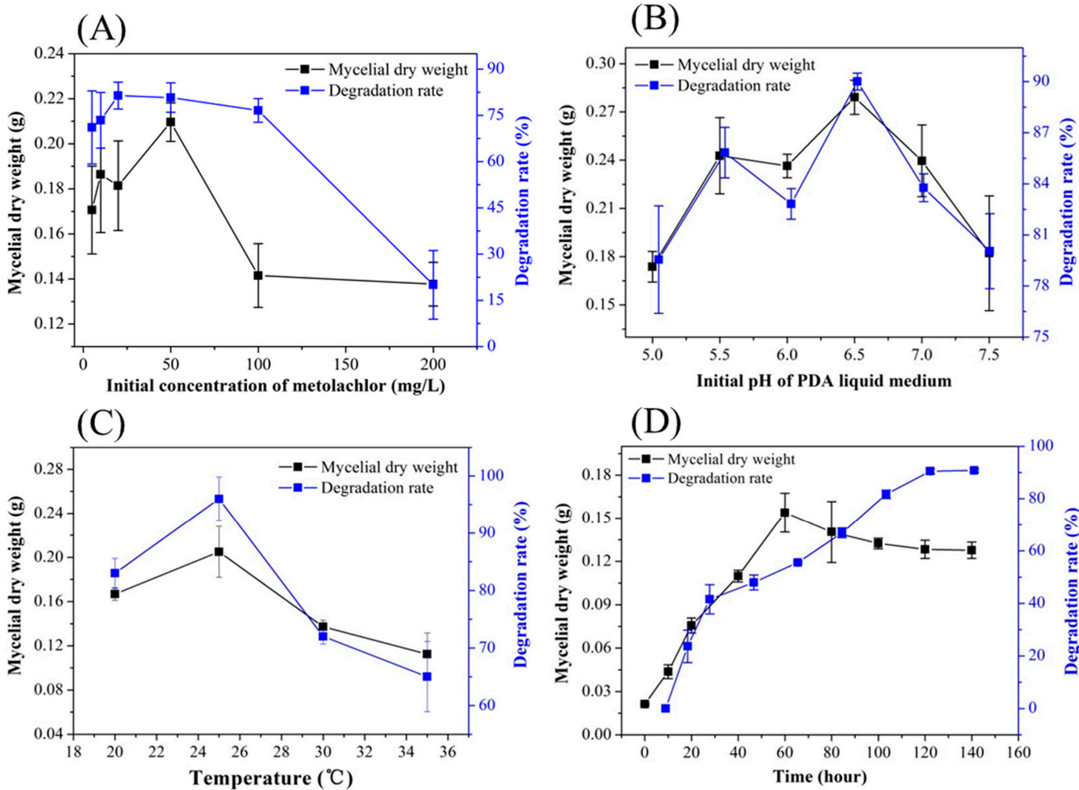

Figure 3. Effects of initial concentration of metolachlor (**A**), initial pH (**B**), temperature (**C**), and time of incubation (**D**) on metolachlor degradation and growth of strain MET-F-1.

Strain MET-F-1 could efficiently degrade metolachlor in a relatively broad range of initial concentration from 5 mg/L to 100 mg/L, with more than 75.0% metolachlor degraded in 72 h (Figure 3A). However, when the initial concentration was set as 200 mg/L, the degradation rate dropped below 20%. These results are consistent with previous studies, wherein a prolonged lag phase was associated with higher concentrations of pesticides [25–27]. This might be caused by an extended acclimation period of functional microbes at higher concentrations [28]. Accordingly, the growth of mycelia was considerably inhibited at higher concentrations, i.e., 100 mg/L and 200 mg/L. The value of pH is an extremely important factor affecting the degradation rate of pesticides [29]. The higher level of the degradation of metolachlor by strain MET-F-1 was observed between pH 5.5 and 7.0, with the optimum pH value of 6.5 (Figure 3B). The mycelia growth rate was positively correlated with the degradation rate of metolachlor. Regarding temperature, another important environmental factor, both the degradation rate of metolachlor and the growth of strain MET-F-1 reached their maximum at 25 °C (Figure 3C). The highest rate of degradation and maximum growth of other pesticide-degrading strains have been observed between 20 °C and 30 °C in previous studies [25,26,30]. As shown in Figure 3D, the degradation of metolachlor reached a stationary phase at 120 h of incubation, whereas the growth of mycelia reached a maximum at 60 h. This indicated that the degradation of metolachlor by strain MET-F-1 was mainly due to co-metabolism in the presence of other nutrient sources, which were preferred by the mycelia [15].

3.3. Co-Metabolism of Metolachlor by Strain MET-F-1

Under optimized environmental conditions, as described above (i.e., pH 6.5 and temperature 25 °C), 10% inoculation amount of strain MET-F-1 was performed in MSM with a metolachlor concentration of 50 mg/L. As shown in Figure 4A, metolachlor had similar degradation rates in the absence of MSM (CK) and other treatments with different nutrients (i.e., MG, MY, and MGY) after 384 h of cultivation, ranging from 22.1 ± 3.7% in the treatment of MGY to 32.0 ± 2.2% in MG, despite some

differences in the degradation process. This suggests that photodegradation and chemical hydrolysis contribute little to the degradation of metolachlor, consistent with previous studies [18,31]. However, as shown in Figure 4B, after inoculation with strain MET-F-1, treatments with nutrients, i.e., MFG, MFY, and MFGY, exhibited significant enhancement in the degradation of metolachlor compared with that in CK, even with 86.6 ± 2.2% in MFGY at 384 h. At the same time, in the treatment of MF, the degradation rate of metolachlor was comparable with that of CK. These results indicated that the fungus is barely able to use metolachlor as a sole source of carbon and nitrogen for growth and instead lived on other nutrient sources, e.g., glucose, yeast extract, and similar mixtures, which are easily utilized.

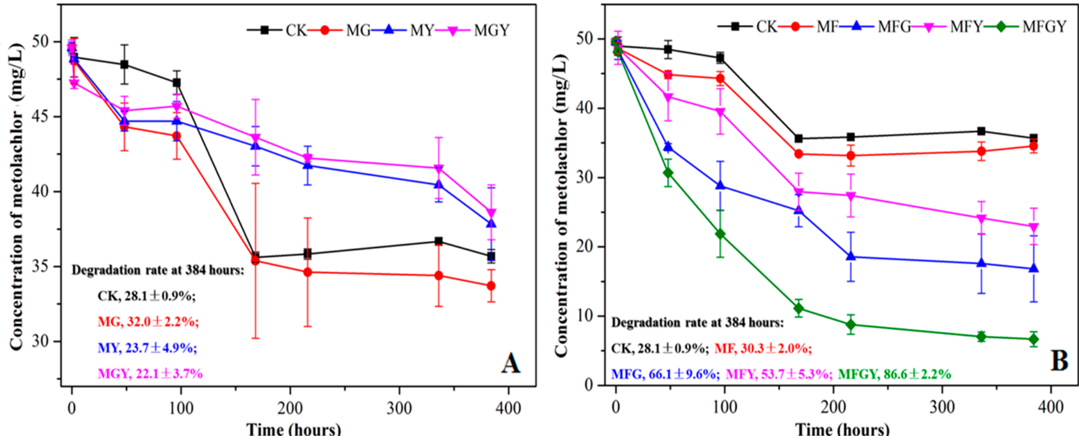

Figure 4. Degradation of metolachlor in different substrates: (**A**) Blank of MSM (Minimal salts medium) and MSM coupled with different nutrients; (**B**) Co-metabolized substrates inoculated with strain MET-F-1 and added with different nutrients. CK, only MSM; MG, MSM added with 0.2% glucose; MY, MSM added with 0.2% yeast extract; MGY, MSM added with 0.1% glucose plus 0.1% yeast extract; MF, MSM inoculated with strain MET-F-1; MFG, MF added with 0.2% glucose; MFG, MF added with 0.2% yeast extract; MFGY, MF added with 0.1% glucose plus 0.1% yeast extract.

This is the first report demonstrating the ability of *Penicillium oxalicum* to co-metabolize metolachlor with other nutrient sources efficiently. Previous reports on degradation characteristics of *P. oxalicum* involved cellulose [32,33], diclofenac [34], triclosan [35], and PAHs [36]. To date, the metolachlor-degrading microorganisms have mainly been fungi, which is consistent with the fact that fungi play key roles in the dissipation process of metolachlor in soil, as reported previously [37,38]. Sanyal and Kulshrestha [39] found that a mixed fungal culture harboring *Aspergillus flavus* and *A. terricola* could reduce 99% of metolachlor with an initial concentration of 100 μg/mL. A yeast strain identified as *Candida xestobii* was isolated by Munoz et al. [15] and could co-metabolize 60% of 50 μg/mL metolachlor with 0.04% of yeast extract within 216 h of incubation. Additionally, reduction of metolachlor degradation in soils by certain fungicides also indicates a central role of fungi in the removal of metolachlor [40].

3.4. Metabolites of Metolachlor Degradation by Strain MET-F-1

The detailed mechanism by which metolachlor is degraded by *P. oxalicum* is unclear due to the unavailability of analytical standards of all possible metabolites and the unobservability of certain transient metabolites. To predict plausible pathways for the biodegradation of metolachlor by strain MET-F-1, we analyzed the liquid MM medium treated in MFGY at 384 h and determined the degradation products of metolachlor by HPLC-MS/MS. Three metabolites (Retention time (Rt) = 1.90 min, 2.33 min, 2.46 min, respectively), along with metolachlor (2.59 min), were presented in the total ion chromatogram (Figure 5A). The metabolites were identified as MOXA, M2H, and MDES based on characteristic fragment ion-pairs by multiple reaction monitoring mode coupled with standard comparison (Figure 5B–D).

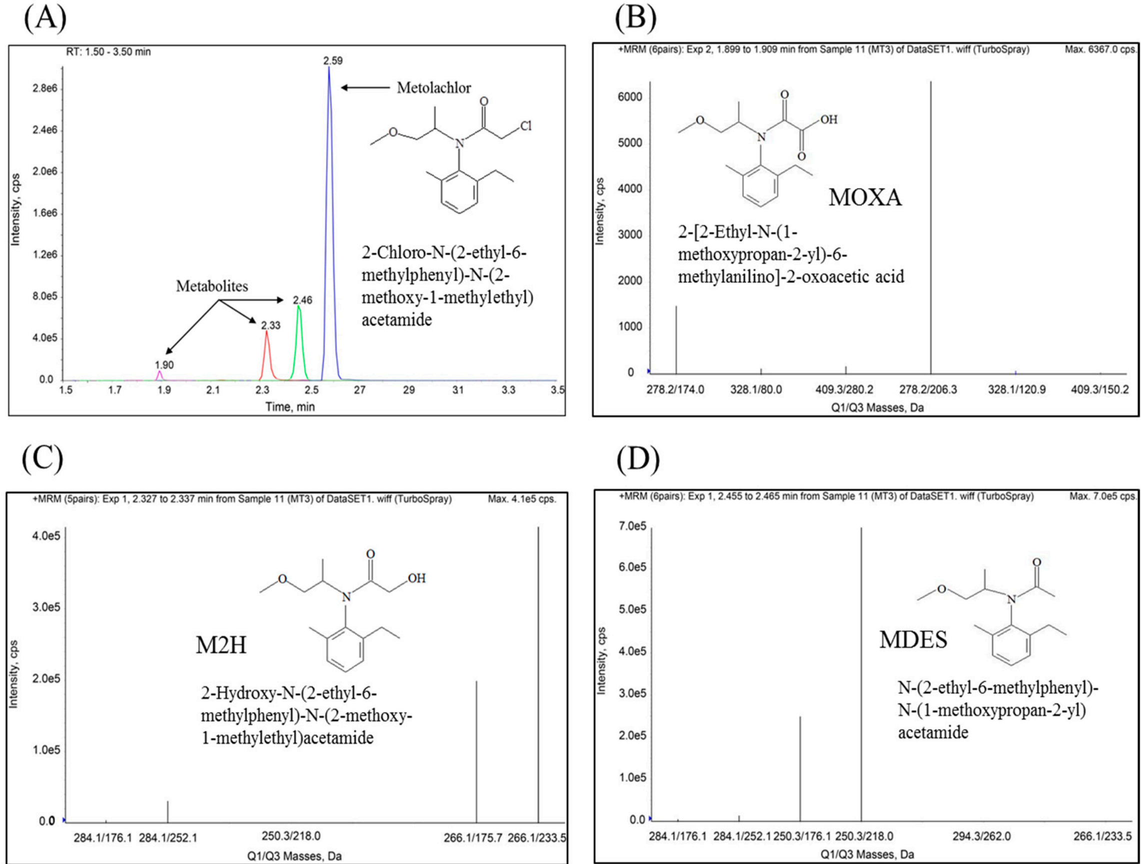

Figure 5. The total ion chromatogram (**A**) and characteristic ions of metabolites (MOXA, M2H, and MDES, (**B**–**D**)) of the liquid sample extracted from the culture of the treatment MFGY at 384 h of incubation. MFGY, MSM inoculated with strain MET-F-1and added with 0.1% glucose plus 0.1% yeast extract.

Because of the presence of strain MET-F-1, all three metabolites in the liquid medium experienced dechlorination, which is generally the first step in the detoxification of aromatic organic pollutants for self-protection of microbial cells [41]. As shown in Figure 6, metabolite M2H was transformed from the parent compound MET by dechlorination, followed by subsequent hydroxylation of the chloroacetyl group, which was then transformed to MOXA by oxidation of the hydroxyl group. Similarly, the degradation of metolachlor by the consortium of four microorganisms, *Streptomyces* sp., *Phanerochaete chrysosporium*, *Rhizoctonia praticola*, and *Syncephalastrum racemosum*, also follows this degradation pathway [42]. Meanwhile, we also observed reductive dechlorination of metolachlor through which MDES was transformed from the parent compound. Likewise, reductive dechlorination of metolachlor with a bacterial consortium in a batch culture has been reported in a previous study [41]. Other researchers have also found that isolated fungi could finally take off the methoxy, chloro, or chloro group from the R groups [15,43].

Figure 6. Proposed metabolic pathway of metolachlor by *P. oxalicum* sp. strain MET-F-1.

3.5. Degradation Performance of Bran Inoculants of MET-F-1 under Field Conditions

The field treatments were performed according to the experimental design and the results of soil analysis are shown in Figure 7. After application with 720 g/L metolachlor EC at the recommended dosage (5.0 mL/20 m^2), the theoretical initial concentration of metolachlor in the soil was 5.0 mg/kg based on the soil density of 1.8 g/cm_3 (dry weight). However, due to the uneven plowing and sampling error in the field environment, the actual initial concentrations of metolachlor in the treatments of SM, SMB, and SMBF were 3.00 ± 0.21 mg/kg, 3.14 ± 0.11 mg/kg, 4.99 ± 0.12 mg/kg, respectively. Nonetheless, this did not affect the evaluation of the degradation trend of metolachlor in each treatment. As shown in Figure 7A, the half-life (DT_{50}) of metolachlor in SMB (14.7 d) was more than twice as long as that in SM (7.1 d), which might be attributed to a significantly higher effect of added wheat bran on the absorption of metolachlor than on the nutrient supply to soil microorganisms. However, in SMBF, DT_{50} of metolachlor sharply decreased to 3.9 d, indicating that wheat bran inoculants of MET-F-1 could greatly accelerate its degradation. Especially in the early stages of treatments, the effect of wheat bran inoculants of MET-F-1 was more evident (Figure 7B). For example, on day 7, the degradation rate of metolachlor in SMBF (88.3%) was 74.2% and 140.0% higher than that in SM (50.7%) and SMB (36.8%), respectively.

Bioaugmentation for polluted soil remediation is affected by a variety of environmental factors, e.g., temperature, moisture content, soil pH, and energy supply [44–46]. Considering the optimal parameters of strain MET-F-1 described in Section 3.2, a suitable living environment (shown in Section 2.6) was provided for the fungus on the selected field plot. Meanwhile, because of enrichments in organic matters, such as crude protein, crude fat, and crude fiber, and elements including nitrogen, phosphorus, and potassium [47,48], wheat bran could provide sufficient nutrition (resources of carbon and nitrogen) for MET-F-1, which has been frequently used as the carrier of microbial inoculum in previous studies [49–51]. However, the results of the plot experiment provide some references for the field application of strain MET-F-1 due to the complexity and variability of the field environment. Before the large-scale application of this strain, it is inevitable to carry out more field tests under different environmental conditions.

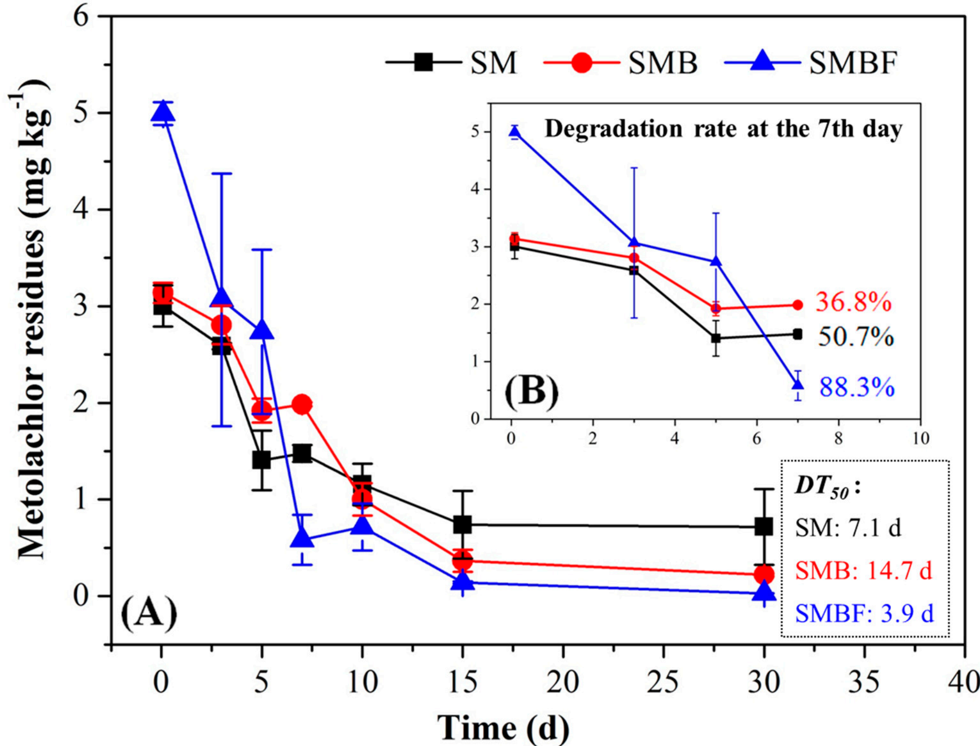

Figure 7. Degradation of metolachlor in field soils by wheat bran inoculants of MET-F-1 within 30 days (**A**), and the degradation curve within 7 days was magnified (**B**). SM, only application with 720 g/L metolachlor EC at the recommended dosage (5.0 mL/20 m^2); SMB, application with 720 g/L metolachlor EC, and the wheat bran at the ratio of 4 kg/20 m^2; SMBF, application with 720 g/L metolachlor EC, and the microbial inoculum of strain MET-F-1 at the ratio of 4 kg/20 m^2.

4. Conclusions

The fungal strain MET-F-1, isolated from activated sludge of a pesticide wastewater treatment plant, was characterized as *Penicillium oxalicum*. This strain was found to be capable of co-metabolizing metolachlor coupled with an additional carbon source, with a greater metolachlor-degrading potential than previously isolated microorganisms. The degradation pathway of metolachlor by MET-F-1 was presented as a combination of hydrolytic and reductive dechlorination. Furthermore, a field plot experiment demonstrated the sufficient metolachlor-degrading activity of this strain.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/23/8556/s1, Figure S1: Colony morphology of the four metolachlor-tolerant strains, Figure S2: The structural formulas and chemical names of parent compound metolachlor and main regular metabolites, Table S1: The representation of meaning for each treatment.

Author Contributions: Writing—original draft, Sample analysis, X.C.; Methodology, Validation, Resources, J.L.; Conceptualization, Methodology, Writing—review & editing, Y.S.; Writing—review & editing, L.Z.; Sample analysis, Software, B.Z.; Writing—review & editing, X.L.; Supervision, Project administration, Funding acquisition, Y.L. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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