Degradation Characteristics of Pinoxaden by *Acinetobacter* and Prediction of Related Genes

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Abstract

In order to improve crop yield per unit area, pinoxaden was widely used in weed control in the field to better degrade the residual pinoxaden in the soil. In this experiment, 14 strains were domesticated and isolated from soil samples using pinoxaden as the only carbon and nitrogen source, and the highly degraded strain was T4P20-1. The effects of different pH values, inoculation amount and culture time on the growth and degradation rate of strain T4P20-1 were studied. The optimal growth conditions of strain T4P20-1 were determined, and the degradation rate reached 92.23%. The morphological characteristics of strain T4P20-1 were determined by optical microscope. The strain T4P20-1 was identified as *Acinetobacter Pittii* by physiological and biochemical characteristics and 16S rDNA homology analysis, and the nucleic acid sequence was submitted to Gentbank to obtain the accession number MW350066. Through gene sequencing, the whole genome framework of strain T4P20-1 and database annotations of GO, KEGG, COG, CAZymes and PHI were obtained. Meanwhile, genes related to herbicide degradation were predicted by gene functional annotations. There was a negative correlation between herbicide and seed germination rate, bud length and root length of maize susceptible to subsequent crop. In conclusion, the strains screened in this study have certain remediation effects on soil contaminated by pinoxaden. It provides a new choice for the degradation of pinoxaden in the future. It can increase crop yields and reduce food security risks.

Introduction

Pinoxaden is an endosuction conductive phenyl pyr pinoxaden herbicide discovered, developed and produced by Syngenta. Its molecular formula is C₂₃H₃₂N₂O₄. Its chemical structure formula (Figure S1).

After entering the environment, pinoxaden is easy to degrade and metabolize. There are more than 20 kinds of metabolites, which can be detected in animals and plants. 5% EC is a slightly toxic herbicide, which has moderate irritation to white rabbit skin and eyes. pinoxaden is difficult to photolysis, difficult to leach in soil, easy to be degraded, adsorbed and volatilized, has low biological toxicity to the environment, has a certain impact on the tested plants [1, 2], and is easy to be degraded in water sediment [3]. The median lethal concentrations of 5% pinoxaden EC to rainbow trout, Daphnia, algae, birds and silkworm were 24 mg / L, 4 mg / L, 4.17 mg / L, 4780.3 mg / kg and 4000 mg / L respectively. It has low toxicity to organisms in the environment and medium toxicity to aquatic organisms such as algae, which has a certain impact on the tested plants [4, 5].

Material And Methods

Experimental materials

Test soil sample: Soil sample number (Table S1). After screening, the test soil sample was stored in the refrigerator at 4°C.

Reagent: 95% pinoxaden (Syngenta crop protection Co., Ltd., Switzerland); Chromato-graphic grade methanol (Shandong Yuwang, chromatographic pure); Dichloromethane (Tianjin Guangfu, analytical purity).

Medium: basic inorganic salt medium (MSM): K_2HPO_4 1.79 g, KH_2PO_4 0.45 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, NaCl 0.4 g, distilled water 1000 mL, pH 7.0. Solid medium: 1.5 ~ 2.0% AGAR powder.; Luria Bertani (LB) medium: tryptone 10.0 g, yeast extract 5.0 g, NaCl 10.0 g, distilled water 1000 mL, pH 7.0, 121 °C, autoclave for 15 min.

Mother liquor configuration: weigh 0.5g of pinoxaden standard, put it into a 10mL volumetric flask, fix the volume with chromatographic grade methanol, and pass 0.45 μL organic filter membrane, keep for standby.

Preparation of bacterial suspension: select the purified single colony, inoculate it into the test tube containing 5mL LB liquid medium, shake it at 30 °C for 160rpm, culture it for 12h, activate it, and take the activated strain 100 μL . Place it in a 96 well plate to measure the OD value, take samples according to the OD_{600} value, place it in a 1.5 mL centrifuge tube, 16,000 G, centrifuge for 5 min, discard the supernatant, add sterile 0.9% normal saline, shake with vortex for 1 min, centrifuge again, discard the supernatant, add sterile 0.9% normal saline, adjust $\text{OD}_{600} = 1.0$ to make a bacterial suspension.

Enrichment, domestication, culture, separation and purification of highly effective degrading bacteria

The pinoxaden degrading strain was acclimated and isolated by enrichment culture method. 5g soil sample, 25mL inorganic salt liquid medium and an appropriate amount of pinoxaden were added to a 50 mL triangular flask to make the concentration of pinoxaden in the triangular flask reach 200 mg / L. it was cultured in a constant temperature shaking table at 30 °C 180 R / min. 2.5 mL of culture was taken every 5 days and inoculated into a new inorganic salt liquid medium, At the same time, the content of pinoxaden in the culture medium was increased by 200 mg / L and domesticated for 5 weeks until the concentration of pinoxaden in the culture medium reached 1000 mg / L. the enriched cultures were gradient diluted with sterile water (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8}) and coated in the inorganic salt solid medium added with pinoxaden. Culture in a constant temperature incubator at 30 °C for 3 days, select colonies with different colors and shapes for purification, add the purified strain to the LB medium containing 1 mL for shaking culture, when the OD value reaches 1.0, 16,000 G, centrifuge for 5 min, discard the supernatant, add 70% glycerol, suck and mix well, and store at - 20 °C.

Determination of growth curve, addition recovery rate and degradation rate of highly degrading bacterial strain

Take 20 μL of cultured bacterial suspension was added to 180 μL of pinoxaden inorganic salt medium (concentration of pinoxaden was 25 mg/L, 50 mg/L, 75mg/L) and LB liquid medium, and four replicates were tested every 12h. Bioscreen was used for 72 h. The strain growth curve was plotted.

Add 1 mL of culture medium, 0.25 g NaCl and 5 mL CH₂Cl₂ into 10 mL centrifuge tube respectively, and shake on vortex for 1 min; Take out the upper layer and put it into a new 10mL centrifuge tube, add 1mL CH₂Cl₂, vortex oscillate for 1min, take the lower organic phase and place it in the first corresponding centrifuge tube, continue to add 1mL CH₂Cl₂ into the second 10mL centrifuge tube and oscillate for 1min, take the organic phase and place it in the first centrifuge tube; Add 0.2g anhydrous Na₂SO₄ to the combined first centrifugal tube and shake, put the upper liquid into a 10 mL volumetric flask, put the volumetric flask into a 50 °C water bath, and evaporate CH₂Cl₂; Dilute to 10mL with chromatographic grade methanol, shake with ultrasonic for 30min, pass through 0.45 µm organic filter membrane, put it in 2mL centrifuge tube, store it in refrigerator at 4 °C and wait for measurement.

Add 5 mL of pinoxaden inorganic salt liquid medium (25 mg / L, 50 mg / L, 75 mg / L) and 50 mL into the test tube respectively bacteria suspension, mark the liquid level before shaking, and culture it in a constant temperature shaking table at 30 °C 200 rpm for 5 days. CG in the blank control group shall be cultured together with the test tube receiving bacteria. Before sampling, the liquid level shall be supplemented according to the records, and the culture solution shall be extracted for high performance liquid chromatography (the chromatographic column is INERTSIL ODS-3 5UM(5µm, 4.6×250 mm), the injection volume is 20 µL), and its degradation ability is measured. Set the liquid chromatographic conditions of pinoxaden (column temperature: 40 °C; flow rate: 1.0 mL / min; mobile phase: acetonitrile: water (0.05% phosphoric acid aqueous solution) = 65:35 (V: V); detection wavelength: 260 nm). After the above conditions are set, take the mother liquor of pinoxaden and dilute it to 12.5 mg / L, 7.5 mg / L, 5 mg / L, 2.5 mg / L and 1 mg / L respectively, and draw the standard curve of pinoxaden, HPLC analysis was carried out according to the chromatographic conditions. The determination of the addition recovery rate and the calculation formula of the degradation rate are as follows:

Addition recovery (%) = (measured / addition) × 100% (1)

Degradation rate (%) = [1 - (treatment measured / control measured)] × 100% (2)

Identification of highly degrading bacterial strain

Gram staining: carry out Gram staining on the strain, and then determine the characteristics of the strain under the oil microscope of the optical microscope. Refer to the three-step Gram staining [6–8], prepare LB solid medium, dilute and coat the purified strain on the medium respectively, and observe the size, edge, transparency, color, shape and other characteristics of the single colony after cultured in the incubator at 30 °C for 72 hours.

Biochemical identification: use biochemical identification tube for identification, inoculate the purified strain into nutrient broth medium, culture at 37 °C for 18–24 hours, and absorb 50 µL broth cultures were added into micro vials and cultured in 35–37 °C incubators. For identification of physiological and biochemical characteristics, refer to Berger's Manual for identification of bacteria (Ninth Edition) [8] and manual for identification of common bacteria [9].

16S rDNA sequence identification: 16S rDNA universal primers were used to amplify the DNA of the isolated strain. General primers (Table S2), reaction systems and circulation conditions (Table S3) for strain identification. PCR products were detected by 1% agarose gel electrophoresis. Finally, the purified PCR product was sent to bioengineering Shanghai company for gene sequencing. The 16S rDNA sequence was stored in the ribosomal database: <http://rdp.cme.msu.edu/index.jsp>. And submit it to NCBI to obtain the login number.

Determination of growth characteristics of highly degrading bacterial strain under different pH, inoculation amount and culture time

Pick out the purified single colony, inoculate it into a test tube containing 5 mL LB liquid medium, shake it at 30 °C, 160 R / min, culture it for 12h, activate it, and take the activated strain 100 μ 50. Put it into a 96 well plate to measure OD₆₀₀, take samples according to the OD₆₀₀ value, put it into a 1.5 mL centrifuge tube, 5000 R / min, centrifuge for 5 min, discard the supernatant, add sterile 0.9% normal saline, shake with vortex for 1 min, centrifuge again, discard the super-natant, add sterile 0.9% normal saline, adjust OD₆₀₀ = 1.0 to make a bacterial suspension. Determine the growth characteristics of high-efficiency degrading bacteria strain at different pH: take 10% bacterial suspension and add it to the inorganic salt liquid medium containing 75 mg / L of pinoxaden at pH = 6.0, 7.0 and 8.0 for three repetitions. Set one without strain as the control group, test it every 12 hours, test it with Bioscreen for 72 hours, and draw the strain growth curve.

Repeat the above steps to determine the growth characteristics of high-efficiency degrading bacteria strain under different inoculation amounts: Take 1%, 5% and 10% bacterial suspension and add it to the liquid medium containing 75 mg / L pinoxaden inorganic salt for three repetitions. Set one without adding strain as the control group, detect it every 12 hours, detect it with Bioscreen for 72 hours, and draw the strain growth curve.

Repeat the above steps to measure the growth characteristics of high-efficiency degrading bacteria strain under different culture times: take 10% of the bacterial suspension and add it to the liquid medium containing 75 mg / L pinoxaden inorganic salt for three repetitions, set one without adding the strain as the control group, detect it every 12 hours, detect it with Bioscreen for 72 hours, determine the residue by HPLC and calculate the degradation rate, The growth curve and degradation curve of the strain were drawn.

Determination of degradation capacity of highly degrading bacterial strain

The bioassay test method of strain degradation ability is as follows: the strain with high degradation rate is connected to the pinoxaden inorganic salt medium with sensitive concentration, shake culture at 30 °C, and applied to the sensitive crop corn seeds according to the above method. This is the treatment group. In addition, sterile water is set as the blank group, and the herbicide inorganic salt medium without

bacteria is set as the control group. After each batch of treated corn seeds grew for 6 days, the germination rate, bud length and root length were observed and recorded.

Whole genome sequencing of degrading strain

Genomic DNA was extracted by SDS. DNA purity and integrity were analyzed by agarose gel electrophoresis, and DNA concentration was quantified by Qubit. Each sample contained 1 µg of 300 bp DNA fragment randomly crushed with covaris ultrasonic crusher. Complete DNA library was prepared by terminal repair, A-tail addition, sequencing linking, purification, PCR amplification and other methods. After the library was constructed, Qubit2.0 was used for pre-liminary quantification, diluted to 2ng / uL and Agilent 2100 was used to detect the library insert fragment. After the inserted fragment met the expectation, q-PCR method was used to accurately quantify the effective concentration of the library to ensure the quality of the library. After the library was identified, the whole genome of strain T4P20-1 was sequenced using Illumina-Hiseq platform according to the requirements of effective concentration and target data volume. All high-quality paired reads are assembled into many scaffolds using SPAdes (V3.13.0) software. Finally, scaffolds larger than 500bp were selected for follow-up analysis. The specific steps of genomic component prediction are as follows: 1) Use Prokka (1.13.7) software to retrieve relevant coding genes. 2) Use RepeatMasker (V4.0.9) to predict the scattered repeat sequence. 3) Transport RNA (tRNA) genes were predicted by tRNAscan SE. Ribosomal RNA (rRNA) genes were analyzed with rRNAmmer (1.2). 4) Protein signal peptides were predicted by SignalP (V5.0 software). And TMHMM (v2.0) (<http://www.cbs.dtu.dk/services/TMHMM/>) using the tools of the annotations, whether to contain transmembrane protein sequence structure choice across with signal peptide structure and membrane structure protein as a secreted protein. GeneWise is used to look for pseudogenes. We used four databases to predict gene function. They are GO, KEGG, COG and CAZymes.

Statistical analysis methods

SPSS 20.0 statistical software was used for statistical analysis. The Duncan's multiple range test was used. The significance level passed the LSD homogeneity test. All tests were subjected to three biological repetitions, and the significance level was $p < 0.05$. The differences between the treatment group and the blank group of the control group were analyzed and compared to find the correlation with the addition of microbial degrading bacteria.

Results

Enrichment, domestication, culture, separation and purification of highly effective degrading bacteria

Enrichment culture was carried out through the inorganic salt medium added with pinoxaden, and the bacterial strains with transparent circles in the medium added with herbicide in the in-organic salt were initially screened out, and further purification was carried out on LB solid medium. Forty-five highly degrading strains (Table S4) were isolated from soil samples planted with rice, corn, wheat, potato,

cotton, peanut and apple in Heilongjiang and Shandong provinces, among which 14 strains were capable of degrading pinoxaden.

Determination of growth curve of efficient degrading bacterial strain

The Bioscreen growth curve analysis instrument was used to determine the effect of different initial concentrations of pinoxaden on the growth of the strain, and the growth curve of bacterial strains efficiently degraded by pinoxaden for 72 h was plotted (Figure S2). After screening, only strain T4P20-1, strain T4P20-3 and strain T8P20-2 had growth trend under the condition that the initial concentration of pinoxaden was 25 mg / L, and strain T1P20-3 Strain T3P20-1 had a growth trend when the initial concentration of pinoxaden was 50 mg / L. Therefore, the above six strains were selected for follow-up experiments.

Determination of addition recovery rate of highly degrading bacterial strain

The linear regression equation of pinoxaden herbicide standard is $y = 342906x + 41167$, and its correlation coefficient is $R^2 = 0.9993$. To measure the recovery rate of pinoxaden (Figure S3), which is 92.42% – 108.26%. The addition recovery of strain meets the requirements of national standard (80 ~ 120%), which proves that the above herbicide extraction and HPLC detection method is feasible.

Determination of degradation rate of highly degrading bacterial strain

High performance liquid chromatography was used to measure the degradation ability of degrading bacteria in inorganic salt medium, and strains with high degradation rate were screened for subsequent experiments (Fig. 1), strains T4P20-1, T4P20-3 and T6P20-1 had good degradation effect on pinoxaden with an initial concentration of 75 mg / L within 5 days, and the degradation rates were 93.87%, 93.89% and 97.40% respectively.

Identification of highly degrading bacterial strain

Colony morphology of strain T4P20-1 (Fig. 2). Observe the colony morphology of strain T4P20-1 on LB medium. The colony presents milky white, opaque, round, neat edge, smooth surface, raised center, Gram staining is red, and the shape is short rod-shaped bacteria. It is judged that the strain is gram-negative.

Physiological and biochemical reaction results of highly degrading bacterial strains (Table S5). Compared with Dong Xiuzhu's Manual for identification of common bacteria [9] and Ber-ger's Manual for identification of bacteria (Ninth Edition) (Buchanan R.E. et al., 1984), the test results are basically the same.

1.7% agarose gel electrophoresis was performed on the amplified products of 16S rDNA of the highly degraded strain (Fig. 2), and the size of the gene fragment was about 1400 bp.

The comparison results of blast of strain T4P20-1 on NCBI showed that the 16S rDNA sequence of strain T4P20-1 had high homology with *Acinetobacter* (*Acinetobacter*). Combined with the morphological and biochemical identification results, the strain was identified as *Acinetobacter pittii*. It was submitted to GenBank for login number MW350066. Phylogenetic tree was obtained (Fig. 2).

Determination of growth characteristics of highly degrading bacterial strain

The growth test results of bacterial strains efficiently degraded by pinoxaden at different pH values (Fig. 3). The shaking table is 30 °C, 160 rpm, cultured for 72 hours, and the absorbance values of the strains under the conditions of pH = 6, pH = 7 and pH = 8 are sampled every 12 hours. The optimum pH value of strain T4P20-1 is 7. The absorbance value of strain T4P20-1 at 1%, 5% and 10% inoculum were determined by sampling every 12 h, and the optimal inoculum amount of strain T4P20-1 was 10% (Fig. 3). Determination results of growth and degradation capacity of bacterial strains efficiently degraded by pinoxaden during incubation time (Fig. 3). The shaking table is 30 °C, 160 rpm for 72 hours, and the absorbance value and degradation ability of pinoxaden are measured every 12 hours. The logarithmic growth period of strain T4P20-1 is 12–36 hours on the culture medium of inorganic salt and pinoxaden, and its absorbance value is 0.35, When strain T4P20-1 was cultured for 72 hours, the concentration of pinoxaden decreased from 75 mg / L to 5.83 mg / L, and the degradation rate reached 92.23%.

Validation of biodegradation ability of highly degrading bacterial strains by bioassay

Sensitivity of pinoxaden on maize seed germination (Table 1). The concentrations with significant differences in seed germination rate, bud length and root length were selected for subsequent experiments.

By establishing the linear regression equation of the effect of pinoxaden on the seed germination of subsequent sensitive crops, it was found that pinoxaden had a negative relationship with the germination rate, bud length and root length of subsequent sensitive crops. The sensitive concentrations with significant difference on the germination rate and bud length of corn seeds at the level of 5% were 75 mg / L and 100 mg / L. therefore, the pinoxaden with the concentration of 75 mg/L was selected as the follow-up test. Establish a linear regression equation for the test results (Fig. 4). The sensitive concentration equation of pinoxaden on maize seed germination rate, bud length and root length is $y_1 = -5.8329x + 104.44$, and the correlation coefficient is $R^2 = 0.9414$; $y_2 = -0.0893x + 1.956$, correlation coefficient $R^2 = 0.9635$; $y_3 = -0.0639x + 1.4299$, correlation coefficient $R^2 = 0.9145$, where y_1 , y_2 , y_3 and x represent the germination rate, bud length, root length and pinoxaden concentration of corn seeds respectively.

Changes of germination rate, bud length and root length of maize seeds treated by pinoxaden degradation strain T4P20-1 (Table 2). In the pinoxaden culture medium with an initial concentration of 75

mg / L, the bud length of the control group is significantly lower than that of the blank group. After treated with pinoxaden degrading strain T4P20-1, the germination rate and bud length of corn seeds in the treatment group are significantly higher than those in the control group, compared with the control group, The bud length and root length of the treatment group increased by 16.77% and 5.93% respectively. With the addition of degrading strains, it can repair the germination of maize seeds.

Whole genome sequencing of degrading bacteria

A 300 bp library was constructed for each strain. After sequencing on Illumina sequencing platform and data quality control with Trimmomatic(v0.36) software, strain T4P20-1 obtained 6.6×10^6 original sequences, the number of original bases have 1998,668,400, the number of sequences after quality control have 6,656,588, and the number of bases after quality control have 1,996,053,837. The proportion of bases with sequencing quality of more than 20 was 98%. Before assembly, select the middle high-quality sequencing region, take a certain length of K-mer base by base for genome size evaluation, and count that the depth of each 31K-mer is 132 and the frequency of each depth accounts for 0.46%.

Using SPAdes (v3.13.0) software to assemble the sequencing data of each strain after quality control, the sequence file that can reflect the basic situation of the sample genome is obtained, and the assembly quality is statistically analyzed. Finally, the scaffold sequence greater than 500 bp is selected for subsequent analysis. The results are as follows: the total length of all sequences is 9662835 bp; The number of sequences longer than 1200 bp is 139; The total number of scaffold fragments obtained by assembly, if there are chromosomes and plasmids, the total is 364; The maximum sequence length was 627479 bp; The shortest sequence length is 208bp; GC content was 36.62%; The number of n is 148; The length of scaffold N50 is 178012bp; The length of scaffold N90 is 40279bp.

Prokka(1.13.7) software was used to annotate and predict the bacterial genome. The gene results of strain T4P20-1 were as follows: the total length of predicted genes was 8105181 bp the number of predicted genes was 9350, and the total length of coding region accounted for 83.88% of the whole genome.

The software tRNAscan-SE was used to predict the tRNA in the genome, and the software rRNAmmer was used to predict the rRNA in the genome. It was found that strain T4P20-1 had 145 tRNAs, 20 types and 19 rRNAs, including 16S rRNAs have 2, 23S rRNAs have 2 and 5S rRNAs have 15.

Through BLAT alignment, the homologous gene sequence was found in the genome, and then the immature termination codon and frameshift mutation in the gene sequence were found by GeneWise The number of pseudogenes of strain T4P20-1 was 5, there were 3 pre phages, and the total length was 72381 bp.

Genomic Island (GI) is a sequence obtained by horizontal transfer. It contains genes related to a variety of biological functions, which can be related to symbiosis or pathogenic mechanism, and the adaptability of organisms. It has an important impact on the evolution of bacteria. According to the difference of genes, gene island can be divided into virulence island and metabolic island. Using the obtained genomic gbk

files, the GI Island sequences contained in the sequenced strains were predicted based on different GI Island prediction software, and the gene island number of strains T4P20-1 was determined as GI_1. The length of gene island is 584230 bp the starting position of gene island is 610554 bp and the ending position of gene island is 26325 bp.

Through alignment, a protein sequence can be annotated into a COG, and each COG cluster is composed of lineal homologous sequences, thus the function of the sequence can be inferred. COG database can be divided into 25 categories according to their functions (Fig. 5), and their specific functions are annotated and classified (Table S6). 7961 genes of strain T4P20-1 are annotated into COG, R: There were 702 genes, accounting for 9.75%, E: amino acid transport and metabolism 714 genes, accounting for 8.97%, K: transcription 688 genes, accounting for 8.64%.

Statistical results of three categories in GO database (Fig. 5). The T4P20-1 gene of strain T4P20-1 has 36 functions, including 8663 gene orders belonging to biological processes, 1496 gene orders belonging to cell components and 6191 gene orders belonging to molecular functions. Among them, the genes involved in biological processes accounted for the most, including metabolic process (17.41%), cellular process (17.92%), localization (4.83%), biological regulation (4.08%), regulation of biological process (3.99%), and response to stimuli (4.83%); In cellular function, extracellular region accounted for 0.10%, membrane 7.04%, protein – containing complex 1.13%, and organelle 0.87%; Among the molecular functions, catalytic activity accounted for 18.20%, structural molecular activity accounted for 0.71%, binding accounted for 12.79%, transporter activity accounted for 3.29%, and transcription regulator activity accounted for 1.79%.

There were 145 metabolic pathways in strain T4P20-1, and there were 4969 annotated genes, 668 genes for amino acid metabolism (12.24%), 330 genes for energy metabolism (6.64%), 330 genes for cofactor and vitamin metabolism (6.34%). Global and overview Fig. 642 genes (12.92%) (Fig. 5), KEGG annotation metabolism of carbohydrate metabolism 681 genes (13.70%) (Table S7), mainly involved in pyruvate metabolism (97 genes), glycolysis/gluconogenesis (85 genes) and propionic acid metabolism (73 genes).

CAZymes annotation was performed on the protein sequence of the whole genome through crypto-code model search, and the annotated profile of carbohydrate active enzyme genes was obtained (Fig. 5). Strain T4P20-1 obtained 315 carbohydrate related enzymes, of which glycoside hydrolase accounted for 17%, glycosyltransferase accounted for 24%, carbohydrate binding enzyme accounted for 17% and carbohydrate ase accounted for 24%.

At present, the key enzymes for degrading bacteria to metabolize herbicides include hydrolase, dehydrogenase, decarboxylase, oxidoreductase, ase, cytochrome P450 and so on. In this study, the whole genome of strain T4P20-1 was analyzed, and the gene function was annotated through KEGG, GO, COG, CAZymes, Swiss Prot, Pfam, Interpro Scan and other databases. According to these notes, we screened the relevant degrading enzymes.

Degradation genes phosphatidylserine decarboxylase (*psd*), tRNA ribosyltransferase (*tgt*), adenylate 5'-phosphate decarboxylase (*pyrF*), uroporphyrinogen decarboxylase (*hemE*), acetal-dehyde phosphate hydrolase (*phnX*), GTP cyclohydrolase (*folE*) and metal dependent hydrolase (*BCG9842\U B0515*) were found in strain T4P20-1.

Discussion

Identification of pinoxaden degrading bacteria

At present, the research on pinoxaden at home and abroad mainly focuses on the determination of its residue and weed control effect. It is reported that the residue of pinoxaden is determined by HPLC-MS and HPLC. Zhu Wenda, Ma Bo and others [10–12] studied the control effect of pinoxaden on weeds in wheat field, However, the research on the degradation of pinoxaden is still blank. In this study, a strain *Acinetobacter pittii* was screened from the long-term polluted soil. Strain T4P20-1 had a degradation rate of 93.87% for pinoxaden with an initial concentration of 75 mg / L in 5 days, which was the first report. Most of the existing literatures reported the research on the remediation of oil pollution by *Acinetobacter*, but there were few reports on the degradation of herbicides in soil by *Acinetobacter*. Yang Fengshan et al. [12] found that the degradation rates of pinoxaden with initial concentrations of 500 mg / L and 5 mg / L were 28.76% and 85% respectively after 7 days of cultivation of *Acinetobacter sp.* TOB1.

Growth and degradation characteristics of pinoxaden degrading bacteria

Because the individual of bacteria is small and difficult to observe, the growth and reproduction of bacteria are usually judged by the number of colonies. Many studies have shown that the growth of bacteria is affected by many external environmental factors, including carbon source, nitrogen source, inorganic salt, growth factor, temperature, humidity, pH, etc. [13-18]. Bacteria also play different roles under different environmental conditions, such as fuel and food processing industry, environmental recycling and purification, pharmaceutical production, etc. [19-22]. This paper mainly studies the bacteria with pesticide degradation ability. According to the data, its degradation ability is also affected by external environmental factors [23,24]. In order to find out the optimal growth and degradation ability of high-efficiency degrading bacterium T4P20-1 under the influence of other external factors, the corresponding experiments were made. The purified T4P20-1 single colony was cultured at different pH, inoculation amount and culture time, and the optimal conditions were obtained through the test results.

Validation of the degradation ability of pinoxaden degrading bacteria

In order to improve crop yield in the field, a series of pesticides, such as insecticides, fungicides, herbicides, cochides, rodenticides and nematocides, have been widely used, but pesticides can also cause

damage to crops to varying degrees in the process of pesticides and pesticides [25-27]. The composition of pesticides and the concentration of pesticides applied will have a certain impact on the growth of crops [28,29]. Applying pinoxaden moiety grass pesticides so this article explores the size concentration is sensitive to the wheat-rice crop seed corn growth, with the in-crease of concentration of pinoxaden moiety grass pesticides, sensitive crops of corn seed germination in the wheat-rice cropping the impact is bigger, the but again to join pinoxaden moiety of grass pesticides after sensitive crop seed corn stubble after adding T4P20-1 high efficiency degradation bacterium, The germination of maize seeds of the latter sensitive crops was improved to a certain extent, and it was concluded that when the concentration of pinoxaden pesticide was 75mg/L, the germination rate of maize seeds of the latter sensitive crops was higher, and the growth of bud length and root length was better. Therefore, the highly efficient bacterial strain T4P20-1 plays an important role in the degradation process.

Degradation genes of pinoxaden efficient degradation bacterial strain

The main reason bacteria can degrade depends on their composition [30]. In order to explore the internal degradation mechanism of the highly degrading bacterial strain T4P20-1 screened in this experiment, the genome sequencing results were analyzed, and the degradation genes in strain T4P20-1 were predicted to be *psd*, *tgt*, *pyrF*, *hemE*, *phnX*, *foIE*, *BCG9842* \ *U B0515*.

Conclusion

In this experiment, strain T4P20-1 with pinoxaden as the only carbon and nitrogen source was domesticated and isolated, and identified as *Acinetobacter pittii*, the serial number on GenBank is MW350066. Through the test to determine the effects of different pH value, inoculation amount and culture time on the growth of strain T4P20-1, it is concluded that when its pH = 7 and the inoculation amount is 10%, the concentration of pinoxaden decreases from 75 mg / L to 5.83 mg / L at 72 h, and the degradation rate reaches 92.23%. The degradation effect is verified by the germination of sensitive corn seeds, and the results show that: the higher the addition con-centration of pinoxaden, the lower the germination rate of corn seeds and the shorter the bud length and root length. However, after the addition of degrading strain, the germination rate, bud length and root length of corn seeds increased significantly, so the highly degrading bacterial strain T4P20-1 can repair the pesticide pollution of pinoxaden. The whole genome framework of the strain was obtained by gene sequencing, and the genome of strain T4P20-1 The total length of predicted genes was 8105181bp the number of predicted genes was 9350, and the total length of coding region accounted for 83.88% of the whole genome. Strain T4P20-1 annotated through databases such as cog, GO, KEGG and CAZymes, and predicted relevant genes related to herbicide degradation. In conclusion, the strains screened in this study have certain remediation effects on soil contaminated by pinoxaden.

Declarations

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Author Contributions Conceptualization: F.Y. and W.Z.; Methodology: F.Y. and C.L.; Software: C.S. and Y.W.; Validation: C.S., Y.W., C.L., and F.Y.; Formal analysis: F.Y., M.Y., and Y.W.; Investigation: C.L., H.F., and C.S.; Resources: C.L. and C.S.; Data curation: Y.W. and C.S.; Writing—original draft preparation: C.S. and F.Y.; Writing—review and editing: Y.W., and C.L.; Visualization: Y.W., and C.S.; Supervision: F.Y., H.F., and C.L.; Project administration: F.Y. and C.L.; Funding acquisition: F.Y. All authors have read and agreed to the published version of the manuscript.

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Data Availability The data presented in this study are openly available on FigShare at doi: xxx.

Code Availability Yes.

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

Consent to Participate Informed consent for participation was obtained from all participants included in this study.

Consent for Publication Yes.

Ethical Approval There are no ethical issues involved in this study.

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Tables

Table 1. Bioassay results of the sensitive concentration of maize seed to pinoxaden in the last sensitive crop

Concentration of pinoxaden (mg/L)	Budding rate (%)	Buds long (cm)	Root length(cm)
0	93.33 a	2.33a	1.08a
5	91.67 a	2.17ab	1.02a
25	88.33 ab	2.01abc	0.98a
50	84.17 ab	1.96abc	0.85 a
75	79.17 bc	1.71bc	0.72a
100	72.50 c	1.67c	0.54b

Duncan method is used for significance analysis. Each column contains different letters, indicating that the difference is significant, and the difference is at the significant level of 0.05.

Table 2. Seed germination test results of pinoxaden after strain treatment

Group	Maize seeds treated with pinoxaden by strain		
	The budding rate (%)	Buds long (cm)	Root length (cm)
The blank group	93.33a	2.33a	1.08a
The control group	72.50a	1.67b	0.54a
Treatment group	83.33b	1.95ab	0.86a

Duncan method is used for significance analysis. Each column contains different letters, indicating that the difference is significant, and the difference is at the significant level of 0.05.

Figures

Figure 1. Degradation rate of pinoxaden by degrading strain. (a), (b) and (c) represent the degradation rates of different concentrations of pinoxaden by T4P20-1, T4P20-3 and T6P20-1 respectively. (□ :25mg / L; ▨ : 50mg / L; ▩ : 75mg / L).

Figure 1

See image above for figure legend.

Figure 2

Identification of species of bacteria. ((a) Morphological identification of highly degrading bacterial strain T4P20-1. (A): Optical microscope; (B): Gram stain. (b) Agarose electrophoresis of 16S rDNA of a highly degrading bacterial strain T4P20-1. M: marker DL2000; 1: 16S PCR product. (c) Phylogenetic tree of strain T4P20-1.)

Figure 3. (a), (b) and (c) were the growth measurements of bacterial strain T4P20-1 with high efficiency of pinoxaden degradation at different pH values, inoculation amount and culture time, respectively. ((a): —◆—: pH 6.0; —■—: pH 7.0; —▲—: pH 8.0. (b): —◆—: 1%; —■—: 5%; —▲—: 10%. (c): —◆—: residue —■—: OD₆₀₀).

Figure 3

See image above for figure legend.

Figure 4

Identification of species of bacteria. ((a) Morphological identification of highly degrading bacterial strain T4P20-1. (A): Optical microscope; (B): Gram stain. (b) Agarose electrophoresis of 16S rDNA of a highly degrading bacterial strain T4P20-1. M: marker DL2000; 1: 16S PCR product. (c) Phylogenetic tree of strain T4P20-1.)

Figure 5

Whole genome sequencing of degrading bacteria. ((a) COG functional annotation classification of strain T4P20-1. (b) Go functional annotation classification of strains. (c) KEGG functional annotation classification of strains. (d) CAZymes functional annotation classification of strains.)

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