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Screening and Genome Sequencing of Deltamethrin-Degrading Bacterium ZJ6

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Abstract

Deltamethrin is a pyrethroid insecticide with high insecticidal activity and a wide range of applications. However, with the increased amount and scope of its application, the accumulated toxicity of deltamethrin has gradually raised concerns. In this study, a bacterium strain, which used deltamethrin as its sole carbon source and was named ZJ6 (*Lysinibacillus* sp.-ZJ6), was isolated from soil samples collected from the sewage outlet of a pesticide plant in Tianjin. Based on morphological observations of ZJ6, as well as its physiological and biochemical characteristics and 16S rDNA sequence (Gen Bank Accession No. KU129013), the strain was identified as *Lysinibacillus fusiformis* sp.. A study of the degradation characteristics of ZJ6 revealed that the optimum conditions for shake flask fermentation to degrade deltamethrin by ZJ6 were as follows: pH 7.0, a temperature of 30 °C, a substrate concentration of 100–200 mg/L, an inoculation volume of 10%, and 7 days culturing at 160 rpm. Under these conditions, the degradation rate of deltamethrin by ZJ6 reached 57.2%. Preliminary sequencing of the ZJ6 genome showed that it has a total length of 3,921,852 bp and contains 4567 genes. The average length of each gene in the ZJ6 genome is 859 bp, and these genes account for 84.62% of the total genome length. KEGG metabolic pathway analysis revealed that genes involved in sugar metabolism and metabolism of exogenous chemical substances were significantly enriched in the genome of ZJ6. Comparison with the COG database showed that 2839 of the predicted protein sequences from the ZJ6 genome had COG numbers. Among all protein functions, the number of genes involved in general functions was the highest (372). For the first time, it was found that ZJ6 has relatively strong deltamethrin degradation ability and high value as a subject for further research. In addition, this study provides a reference to guide the preparation of pesticide-degrading bacterial agents and environmental remediation.

Introduction

As the second largest category of pesticides behind organophosphorus pesticides, pyrethroids currently account for approximately 20% of the global pesticide market and are applied to 1/3 of the total area in China that uses chemical pesticides. Deltamethrin is a pyrethroid pesticide with strong insecticidal activity. Due to its efficiency, low cost, and good light stability, deltamethrin is widely used to control a wide range of pests. Deltamethrin accounts for 80.6% of all pesticides used on vegetables grown in Beijing [1]. However, the toxicity of accumulated deltamethrin has raised increasing concern as usage of deltamethrin has increased and its application scope has widened [2–5]. In recent years, the number of annual production-related and non-production-related poisoning cases has gradually increased, and deltamethrin is currently a factor in 79.5% of all pyrethroid pesticide poisoning cases [6].

Xiangxiang Hao and Xiaoqing Zhang are co-first authors.

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Microbial remediation has become a mainstream technology for cleaning pesticide residues because of its simple method of operation, absence of secondary pollution, low energy consumption, and excellent degradation properties. Studies have shown that, at pH 7.0 and in a basal medium supplemented with 50 mg/L deltamethrin as the sole carbon source, the degradation rates of deltamethrin by *Enterobacter cloacae* and *Pseudomonas fluorescens* reached 62.82 and 43.25%, respectively, after 7 days of culturing at 30 °C on a shaker [7]. At 37 °C and in a medium with an initial pH of 7.0 and a substrate concentration of 20 mg/L, the highest degradation rate of deltamethrin by *Acinetobacter calcoaceticus* was 58.27% [8]. At 30 °C, pH 7–8, and with adequate ventilation, the growth rate of *Bacillus licheniformis* was adequate, and the degradation rate of deltamethrin by this microbe was 61.7% after 5 days of culturing [9]. After culturing at 25 °C and 80 rpm for 14 days, the removal rate of 250 mg/L cypermethrin by *Pseudomonadaceae* was approximately 66.7% [10]. In addition, the degradation rates of 50 mg/L fenvalerate, deltamethrin, cyhalothrin, fenpropathrin, cypermethrin, and permethrin by *Alcaligenes* sp. YF11 were 5.06, 8.01, 3.04, 9.24, 2.00, and 3.84 μmol/L (L·h), respectively [11]. After 3 days of culturing at 30 °C and pH 7.0, the degradation rates of 100 mg/L bifenthrin, cyhalothrin, and cypermethrin by *E. cloacae* w10j15 were 52.43, 50.76, and 56.89%, respectively. After 3 days of culturing, the degradation rate of 100 mg/L cypermethrin by *Rhodococcus* strain CDT3 was 84.24% [9]. The primary sources of pesticide-degrading microorganism include screening and isolation of strains with high degradation efficiency from pesticide-contaminated soil, directional cultivation of good strains, and mutagenesis, breeding, and construction of engineered strains [12]. Detailed information is shown in Table S1.

This study was conducted with the goal of improving biological control methods for pyrethroid pesticides. Soil samples from the sewage outlet of an abandoned pesticide plant were studied to identify bacterial strains capable of degrading deltamethrin, followed by assessment of the degradation characteristics of the identified strains. Subsequently, the shake flask culturing conditions of the strain were optimized, after which bioinformatics analysis was conducted. This study provides a theoretical basis for the development of efficient engineered strains, preparation of bacterial agents capable of degrading pyrethroid pesticides, and improved methods of environmental remediation.

Materials and Methods

Collection and Treatment of Soil Samples

A total of 150 soil samples were collected near the sewage outlets of five major abandoned pesticide plants in Wuqing, Tianjin, and Zhengzhou, in Henan province, China. The samples were collected from the surface soil at different distances from the sewage outlet at a depth of 10–20 cm. All samples were stored at 4 °C before analysis.

Preparation of Culture Media

The basal medium, enrichment medium, Luria–Bertani (LB) medium, LA medium, physiological and biochemical culture medium, and observation reference medium were prepared in accordance with the methods reported by Zhu [13]. Hexane (chromatographic purity), acetone (analytical grade), and the deltamethrin standard (25 g/L) were purchased from Beijing AOBOX Biotechnology Co., Ltd. and Dingguo Changsheng Biotechnology Co., Ltd.

Isolation and Screening of Bacteria Strains

Primary Screening

Preparation of mixed bacteria mother liquor: a sample consisting of 10 g of mixed soil was transferred under sterile conditions into the enrichment medium, followed by the addition of 100 mL sterile deltamethrin at a concentration of 50 mg/L. After adding an appropriate amount of glass, the sample was incubated at 30 °C for 7 days on a shaker operating at 160 rpm. The resulting liquid was collected as the mixed bacteria mother liquor. Subsequently, the enrichment medium containing 100 mg/L deltamethrin was inoculated with the mother liquid at a 1:10 dilution and cultured for 7 days. In the next step, the newly collected bacteria broth was transferred into sterile liquid basal medium containing 150 mg/L deltamethrin and cultured for another 7 days. This procedure was performed three more times with the deltamethrin concentration increased successively from 150 to 200 and 250 mg/L. Finally, 0.5 mL of the medium from the last incubation was subjected to gradient dilution with the basal medium and 0.1 mL of each bacterial broth with 10–4, 10–5, and 10–6 dilutions, respectively, was transferred in triplicate onto plates containing the LA medium, which were incubated for 48 h at 30 °C. Bacterial strains with different morphological characteristics were selected and subjected to isolation and purification. Aliquots of the purified strains were stored at 4 °C and − 20 °C. At the same time, 0.1 mL of cream containing deltamethrin was coated onto a plate of LA culture medium, after which 0.1 mL of mother liquor

was streaked onto the same plate. The plates were prepared in triplicate and incubated at 30 °C for 48 h. Bacterial strains with different morphological characteristics were isolated and purified. Aliquots of the purified strains were stored at 4 °C and − 20 °C.

Secondary Screening

The strains obtained from the primary screening were inoculated into LB medium and cultured for 24 h at 30 °C on a shaker operated at 160 rpm. Subsequently, the bacteria were inoculated in a 10% ratio into an inorganic salt medium containing 100 mg/L deltamethrin and cultured for 7 days at 30 °C on a shaker operated at 160 rpm. The control was the inorganic salt medium containing deltamethrin but no bacteria.

Degradation Performance of the Bacterial Strains

The degradation performance of the bacterial strains was evaluated using a Shimadzu GC-2014 gas chromatograph equipped with an ECD detector in accordance with the method reported by Cheng et al. [14]. In brief, 2 mL of the 7-day bacterial culture and 1 mL of saturated NaCl solution were transferred into a 20-mL centrifuge tube, followed by three cycles of extraction using 4, 4, and 2 mL of petroleum ether, respectively, before the samples were analyzed by gas chromatography. The chromatography conditions were as follows: RTX-5 capillary column, inlet temperature of 250 °C, injection volume of 1.0 µL, ECD detector temperature of 280 °C, column temperature of 250 °C, column flow rate of 1.0 mL/min, and split ratio of 1:20. Qualification was performed based on retention time. Quantitation was performed based on peak area.

Strain Identification

Morphological Identification

The morphological characteristics of the strains, including colony characteristics, Gram staining, and spore staining, were observed under a Motic BA310 digital microscope and a TEScan5136 Scanning Electron Microscope (refer to the methods adopted by Zhu [13]).

Physiological and Biochemical Identification

The physiological and biochemical properties of the strains were identified using the conventional methods described in (Zhu [13]).

Molecular Biological Identification

16S rDNA identification of the bacteria was carried out as follows: the DNA was isolated using a GV-Bacterial Genomic DNA Extraction Kit and amplified using universal primers, i.e., 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGGCTACCTTGTTACGACTT-3′). The PCR reaction system was as follows: 12.5 µL of 2 × Taq PCR Green Mix, 1–5 µL of DNA template, 0.5 µL of primer F (10 µM), 0.5 µL of primer R (10 µM), and nuclease-free water. The total volume was 25 µL. The PCR products were sent to Beijing Liuhe Huada Gene Sequencing Company for sequencing. The analysis of the returned data and construction of phylogenetic trees were carried out according to previously reported methods (Zhu [13]).

Degradation Characteristics of Strain ZJ6

Calculation of the Deltamethrin Degradation Rate

The degradation rate of deltamethrin (%) = (content in blank sample − content in control sample)/content in blank sample × 100.

The Effect of Different Initial pH Values on the Degradation Rate of Strain ZJ6

Activated strain ZJ6 was inoculated at a 10% ratio into inorganic salt basal medium (containing 100 mg/L deltamethrin) with initial pH values of 5, 6, 7, 8, and 9, respectively, and cultured for 7 days at 30 °C on a shaker operated at 160 rpm. Each pH was measured in triplicate. The control was the basal medium (containing 100 mg/L deltamethrin) corresponding to each pH that was not inoculated by strain ZJ6.

The Effect of Substrate Concentration on the Degradation Rate of Strain ZJ6

Activated strain ZJ6 was inoculated at a 10% ratio into inorganic salt basal medium (at pH of 7.0) containing 50, 100, 200, and 300 mg/L of the substrate, respectively, and cultured for 7 days at 30 °C on a shaker operated at 160 rpm. Each substrate concentration was measured in triplicate. The control was the basal medium corresponding to each substrate concentration that was not inoculated by strain ZJ6.

The Effect of Temperature on the Degradation Rate of Strain ZJ6

Activated strain ZJ6 was inoculated a 10% ratio into inorganic salt basal medium (containing 100 mg/L deltamethrin

at pH 7.0) and cultured for 7 days at 30, 35, and 40 °C on a shaker operated at 160 rpm. Each temperature was measured in triplicate. The control was the basal medium (containing 100 mg/L deltamethrin) corresponding to each temperature that was not inoculated by strain ZJ6.

Genome Sequencing of Strain ZJ6

Extraction of Genomic DNA

Genomic DNA was extracted according to previously reported methods (Zhu [13]).

Sequencing and Construction of DNA Libraries

DNA sequencing was performed using an Illumina HiSeq 4000 sequencer.

Results and Discussion

Separation and Identification of Bacterial Strains

After bacterial enrichment, isolation, and purification from the soil samples, five strains (BX32, QX32, ZJ114, ZJ21, and ZJ6) were obtained. The 16S rDNA analysis of these five strains showed that the 16S rDNA sequences of strains X32 and QX32 were highly similar to the 16S rDNA sequence of *Pseudomonas* sp.. In addition, the 16S rDNA sequences of strains ZJ114 and ZJ21 were highly similar to the 16S rDNA sequence of *Achromobacter* sp., and the 16S rDNA sequence of strain ZJ6 was highly similar to the 16S rDNA sequence of *Lysinibacillus* sp. (Annotation No.: KU129013). Among these strains, the role of ZJ6 in deltamethrin degradation has not been reported.

The degradation rates of deltamethrin by these bacterial strains were measured under identical conditions (Table 1). The deltamethrin degradation rate of newly discovered strain ZJ6 was 57.20%, which was similar to previously reported deltamethrin degradation rates by bacterial strains, which ranged from 43.25 to 66.7% [15–18]. In addition, the deltamethrin degradation rate of strain ZJ6 was slightly higher than the degradation rates of *Pseudomonas* sp. (54.80 and 55.90%), which demonstrated that strain ZJ6 required further study.

Using 16S rDNA extracted from strain ZJ6 as the template, PCR amplification was carried out with primers 1429R and 27F, and a clear band at approximately 1500 bp was obtained, indicating that the length of the 16S rDNA of strain ZJ6 was 1500 bp (GenBank Accession Number: KU129013, Fig. 1a). A BLAST analysis of the 16S rDNA of strain ZJ6 was carried out. Ten bacterial species with relatively high homology to ZJ6 were selected for construction of a phylogenetic tree, as shown in Fig. 1b. It was found that strain ZJ6 was 99% homologous to *Lysinibacillus* sp., which suggested that strain ZJ6 might be a member of genus *Lysinibacillus* sp..

The Physiological and Biochemical Characteristics of Strain ZJ6

The physiological and biochemical characteristics of strain ZJ6 are shown in Table 2.

After further morphological observation, electron microscopy observation, and identification of physiological and biochemical indicators, strain ZJ6 was confirmed as *Lysinibacillus fusiformis*.

The Degradation Characteristics of Strain ZJ6

The Effect of Initial pH on the Degradation Rate of Strain ZJ6

The effect of the initial pH of the culture medium on the degradation of deltamethrin by strain ZJ6 is shown in Fig. 2a. The maximum deltamethrin degradation rate of strain ZJ6 occurred when the initial pH of the culture medium was 7, whereas the degradation rate of strain ZJ6 was obviously decreased when the culture medium was either slightly acidic or slightly alkaline. In addition, when the pH of the culture medium was <7, the degradation rate of strain ZJ6 was slightly higher than that measured at pH >7, which indicated that the degradation activity of the enzymes in strain ZJ6 was highest in a weakly acidic environment.

Table 1 Degradation rates of screened bacteria strains

	KB	BX32	QX32	ZJ114	ZJ6	ZJ21
Deltamethrin content (mg/L)	70.37	31.83	31.04	25.08	30.11	26.73
Degradation rate (%)		54.80	55.90	64.40	57.20	62

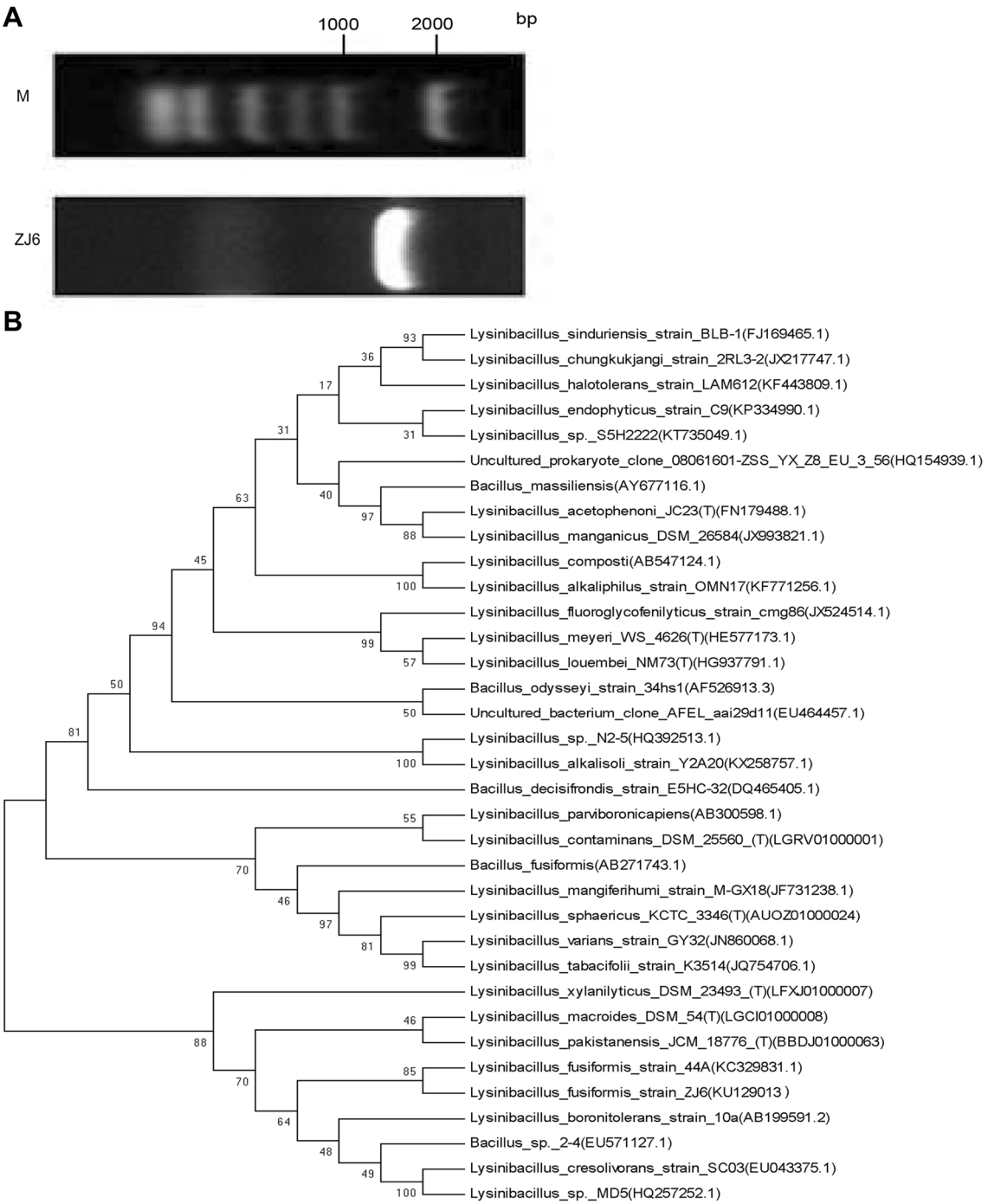

Fig. 1 The 16S rDNA gene of strain ZJ6 and the phylogenetic tree of strain ZJ6 (1) **a** PCR amplification of 16S rDNA gene of strain ZJ6; (2) **b** The phylogenetic tree of strain ZJ6; (3) M *Marker*

The Effect of Substrate Concentration on the Degradation Rate of Strain ZJ6

The effect of substrate concentration on the degradation of deltamethrin by strain ZJ6 is shown in Fig. 2b. The degradation rate of ZJ6 was highest when the substrate concentration was between 100 and 200 mg/L, whereas the degradation rate of ZJ6 was significantly decreased when the substrate concentration was either lower than 100 mg/L or higher than 200 mg/L. When the substrate concentration was relatively low, growth and proliferation of the strain were limited, and the induced secretion of degrading enzymes by the strain

Table 2 Physiological and biochemical characteristics of strain ZJ6

Physiological and biochemical characteristics	ZJ6	*Pseudomonas aeruginosa*
Oxidation and fermentation	−	+
Methyl red test	−	−
Starch hydrolysis test	−	+
Indole test	−	−
Hydrogen sulfide test	−	+
Gelatin test	+	−
Casein hydrolysis test	−	+
Nitrate reduction test	+	+
Catalase test	+	+
Urease test	−	−
Phenylalanine deaminase test	−	+
Mobility test	−	+
Salt resistance test		
1%	+	+
2%	+	+
5%	+	+
7%	−	+
10%	−	−

was insufficient. When the substrate concentration was too high, the growth of bacterial cells was also inhibited, thus affecting the secretion of degrading enzymes and the degradation performance of deltamethrin by strain ZJ6.

The Effect of Culture Temperature on the Degradation Rate of Strain ZJ6

The effect of culture temperature on the degradation of deltamethrin by strain ZJ6 is shown in Fig. 2c. The degradation rate of deltamethrin by strain ZJ6 was relatively high at 28–35 °C and peaked at 30 °C. When the culture temperature was lower than 30 °C or higher than 35 °C, the growth and proliferation of the strain were inhibited, which inhibited secretion of degrading enzymes and significantly reduced the deltamethrin degradation performance of strain ZJ6.

The Optimal Degradation Rate of Strain ZJ6

The degradation rate of strain ZJ6 was significantly influenced by external factors, including culture temperature, pH, and substrate concentration. The optimum conditions for this strain to reach the highest degradation rate after 7 days of culturing were 10% inoculation ratio, pH 7, 100–200 mg/L deltamethrin, 30 °C, and on a shaker operated at 160 rpm. When the deltamethrin content was 100 mg/L, the highest degradation rate was 57.2%. When the deltamethrin content was 200 mg/L, the degradation rate was 46–53% (Table 3).

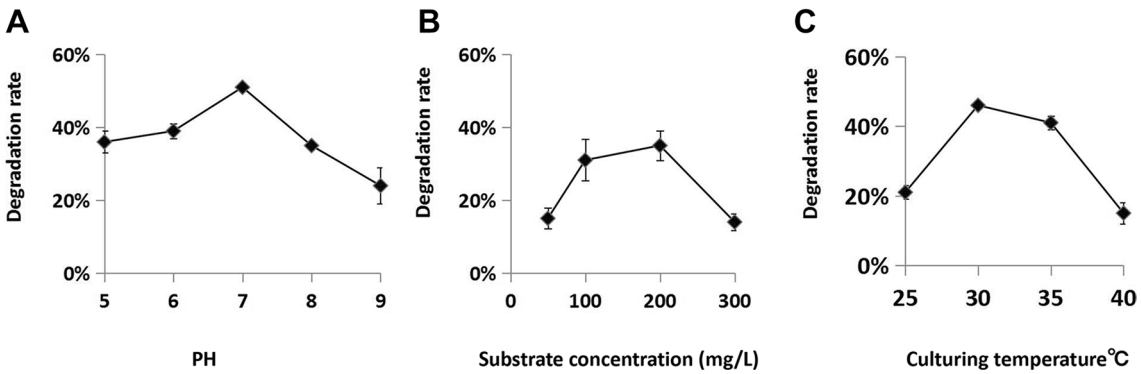

Fig. 2 The effect of initial pH, substrate concentration, and temperature on the degradation rate of strain ZJ6 (1) **a** effect of pH on the degradation rate of strain ZJ6; (2) **b** effect of substrate concentration on the degradation rate of strain ZJ6; (3) **c** effect of temperature on the degradation rate of strain ZJ6

Table 3 Degradation rate of strain ZJ6

Parallel	1	2	3	4	5	6
ZJ6 (mg/L)	90.08	90.54	92.46	86.65	86.36	77.77
KB (mg/L)	189.8	173	171.74	161.63	159.42	144.26
Degradation rate (%)	53	48	46	46	46	46

Table 4 Differences between strains ZJ6 and SW-B9

Item	ZJ6	SW-B9
Sequencing platform	Illumina HiSeq 4000 system	MiSeq system
Genome total length (bp)	3,921,852 bp	4.7 Mbp
GC content in gene region (%)	38.34	37
Intergenic region length (kbp)	712.744	N5 (997.350)
GC content in intergenic region (%)	34.31	
Total DNA gene number	4567	4400
tRNA numbers	109	70
rRNA numbers	34	28
Coding sequence (CDS) & KEGG approach	2839 coding sequences (CDS) were assigned to 40 KEGG pathways	1721 coding sequences (CDS) were assigned to 178 KEGG pathways
Relative minimum distance	Strain ZJ6 (KU129013) has the highest similarity to *L.fusiformis*_strain_44A (KC329831.1), reaching 85%	Relative minimum distance (RSD) of strain SW-B9 shared with *L. fusiformis* RB-21 (GenBank Accession Number JPEF00000000.1)
New discovery	For strain ZJ6, the 40 major genes involved in the metabolism of exogenous chemicals are being cloned and functionally verified	15 candidate isoeugenol monooxygenase genes were found in sw-b9 strain

Preliminary Analysis of the Genome Sequence of Strain ZJ6

After genome sequencing on the Illumina HiSeq 4000 platform, the genome of strain ZJ6-1A was found to contain 4567 genes and to have a total length of 3,921,852 bp. The average length of each gene was 859 bp, and genes accounted for 84.62% of the genome. In addition, 121 tandem repeats with a total length of 21,439 bp were found to account for 0.4626% of the genome. The genome of strain ZJ6-1A also contained 121 satellite sequences, 5 microsatellite sequences, 109 tRNAs, and 34 rRNAs. The results of the genomic analysis of strain ZJ6-1A are shown in Table 4 and Fig. 3a. According to the draft genome sequence of *L. fusiformis* strain SW-B9, 15 candidate isoeugenol monooxygenase genes were identified. However, for strain ZJ6, 40 major genes involved in the metabolism of exogenous chemicals were cloned and functionally verified. The differences between strains ZJ6 and SW-B9 are shown in Table 4.

By comparing the genes of strain ZJ6 with KEGG data, the genes were divided into 40 functional categories (Fig. 3b), which were divided into five major groups: genes involved in systemic activity, genes involved in metabolism, genes involved in the regulation of human diseases, genes involved in the regulation of genetic information, and genes involved in cellular metabolism. Among the genes of strain ZJ6, 434 genes were involved in glucose metabolism, 379 genes were involved in metabolism of exogenous chemicals, 418 genes were involved in amino acid metabolism, and 379 genes were involved in transmembrane transport.

The COG database is based on the classification of phylogenetic relationships among the coding proteins in the complete genomes of bacteria, algae, and eukaryotes. By comparison, a protein sequence can be annotated into a specific COG, while each cluster of COGs consists of orthologous sequences, so that the function of a specific sequence can be inferred. By comparing the results of this study with the COG database, it was found that 2839 protein sequences were associated with a COG number. In addition, this study clarified the functions of chromosome-related proteins that corresponded to 22 categories of proteins in the COG database. Therefore, the functions of these chromosome-related proteins were deduced, and the proteins were classified according to their functions. As shown in Fig. 3c, among all protein functions, the number of genes involved in general functions (R) was the highest (372), followed by proteins involved in metabolism. In particular, the number of genes participating in amino acid translation and metabolism (E) was relatively high (331), which indicated that the metabolism of this strain is probably quite active. In addition, 224 proteins were involved in transcription (K).

This study found for the first time that *L. fusiformis* ZJ6 can be utilized for deltamethrin degradation. By optimizing the degradation conditions of deltamethrin by strain ZJ6, it was found that the highest degradation rate of deltamethrin (57.2%) was achieved by culturing strain ZJ6 for 7 days at pH 7.0 with a substrate concentration of 100–200 mg/L, a temperature of 30 °C, and on a shaker operated at 160 rpm. In addition, this bacterial strain can be used to degrade cellulose, methylene chloride, and chlorobenzene, and it can also be used as an algicidal bacterium and an efficient chromium reduction bacterium [19–25].

Current problems: As a simple technology with low energy consumption and no secondary pollution, microbial remediation has become a mainstream practice used for environmental remediation. However, some problems

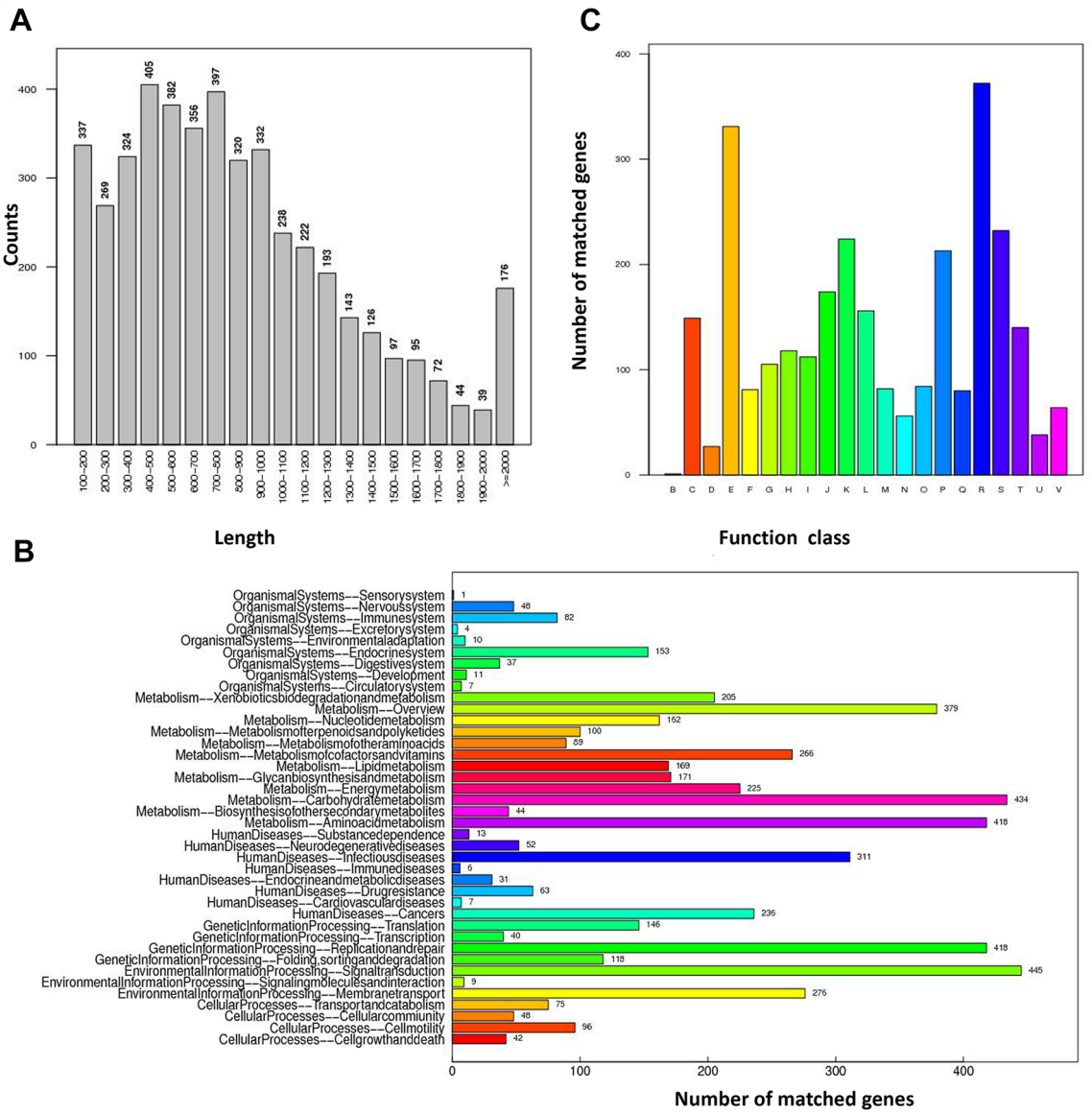

Fig. 3 Genome profile of strain ZJ6 **a** gene length distribution of strain ZJ6; **b** KEGG pathway classification for the genes of strain ZJ6; **c** COG classification for the genes of strain ZJ6 [B: chromatin structure and dynamics (1); C: energy production and conversion (149); D: cell cycle control, cell division, chromosome partitioning (27); E: amino acid transport and metabolism (331); F: nucleotide transport and metabolism (81); G: carbohydrate transport and metabolism (105); H: coenzyme transport and metabolism (118); I: lipid transport and metabolism (112); J: translation, ribosomal structure and biogenesis (174); K: transcription (224); L: replication, recombination, and repair (156); M: cell wall/membrane/envelope biogenesis (82); N: cell motility (56); O: posttranslational modification, protein turnover, chaperones (84); P: inorganic ion transport and metabolism (213); Q: secondary metabolites biosynthesis, transport, and catabolism (80); R: general function prediction only (372); S: function unknown (232); T: signal transduction mechanisms (140);U: intracellular trafficking, secretion, and vesicular transport (38); V: defense mechanisms (64)]

remain regarding current applications of microbial remediation, and significant effort is required to apply laboratory findings to real-world situations. Therefore, future studies of selected degradation bacteria should focus on the following subjects. It is presumed that the enzymes degrading deltamethrin in ZJ6 are primarily involved in the metabolism of exogenous chemicals and include 379 genes. In this study, we conducted experiments aimed at cloning and verifying 40 major genes, with the goal locating genes with a high capacity for deltamethrin to construct highly effective engineered strains.

Study the degradation bacteria that have been selected by our predecessors and study the degradation performance of mixed strains. Study the degradation

mechanism, degradation pathway, and intermediate metabolites of pollutants in degradation bacteria. Study the effect of exogenous enzyme preparations on the degradation rate by deducing the type of degradation enzymes through their degradation mechanisms. Study the degradation performance of degradation bacteria or enzyme preparations for pollutants in simulated soil; Study the degradation performance of degradation bacteria or enzyme preparations for pollutants in environmental soil.

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