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Isolation, Characterization, and Degradation Efficiency Testing of a Paclobutrazol-degrading Bacterium: *Deinococcus arenae*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objective: This study aimed to isolate and characterize a bacterium capable of degrading the pesticide paclobutrazol. The strain was obtained from soil enriched with paclobutrazol, and its degradation ability was assessed using gas chromatography (GC).
Methods: A single strain was isolated from the soil solution of *Ophiopogon japonicus*, a plant exposed to excessive paclobutrazol spraying. Initial isolation utilized dilution and plating methods, followed by separation and purification through the streak plate technique. BLAST analysis revealed the strain's highest similarity to *Deinococcus arenae* SA1, with 100% homology after removing excess sequences. Subsequently, the degradation efficiency of this bacterium on paclobutrazol was evaluated by gas chromatography.

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Results: Quantification using the external standard method yielded a standard curve equation of y=0.0977x-0.412, with a correlation coefficient (R^2) of 0.99842. Testing at different time showed that the *Deinococcus arenae* achieved a degradation rate of 2.49% after three days of degrading paclobutrazol. After five days, the degradation rate increased to 17.58%, reaching 38.66% after seven days, 42.20% after nine days, and 43.87% after eleven days.
Conclusion: The *Deinococcus arenae* strain exhibited a significant degradation effect on paclobutrazol. This finding suggests potential for developing microbial agents to degrade paclobutrazol, promoting sustainable practices in agriculture.

Keywords: Deinococcus arenae; paclobutrazol; microbial degradation; gas chromatography, bacterium capable.

1. INTRODUCTION

Paclobutrazol, a triazole pesticide with a long history of development and extensive research experience, is widely utilized in agricultural cultivation. This pesticide functions by modulating the endogenous hormone levels, particularly by reducing plant gibberellin synthesis, increasing chlorophyll, nucleic acid, and protein levels, and enhancing plant stress resistance. Additionally, paclobutrazol inhibits apical dominance, stimulates lateral bud germination and growth, and possesses fungicidal properties [1,2]. It also exhibits inhibitory effects on various pathogens suppresses weed growth significantly, induces weed dwarfing, and mitigates the negative impact of weeds on crops, thus reducing weed-related damages [3].

Despite its generally safe use, paclobutrazol presents challenges due to its slow degradation,especially in soil, taking approximately 180 to 210 days for 50% degradation[4]. This prolonged persistence post-application can negatively impact the microbial environment, leading to concerns about its environmental persistence. Moreover, the accumulation of paclobutrazol in crop environments may disrupt soil microbial communities, cause oxidative damage to plants, and, through various pathways, accumulated paclobutrazol residues can easily enter the human body via the food chain, posing potential threats to human health, as Paclobutrazol is classified as moderately toxic by the World Health Organizations[5-7]. Hence, its potential environmental and health hazards necessitate careful consideration and management.

Currently, primary methods for addressing pesticide residues include physical, chemical, and biological approaches [8]. Microbial degradation and remediation of agricultural residues stand out due to their advantages, such as minimal secondary pollution, low cost, and simple operation. These methods hold great promise in mitigating the environmental impact of paclobutrazol and other pesticides [9]. This study aimed to isolate and characterize a bacterium capable of degrading the pesticide paclobutrazol. The strain was obtained from soil enriched with paclobutrazol, and its degradation ability was assessed using gas chromatography (GC).

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Experimental soil samples

In March 2022, soil samples were gathered from *Ophiopogon japonicu*s fields subjected to different concentrations of paclobutrazol in Shuimohe Village and Laoma Village, located in Huayuan Town, Santai County, Mianyang City, Sichuan Province of China. These samples were collected with meticulous attention to ensure representative soil compositions from various treated areas. After collection, the mixed soil samples were promptly stored in a refrigerator at 4 ℃ to maintain their integrity and preserve their microbial content for future analysis and experimentation.

2.1.2 Reagents

Paclobutrazol (98%) was purchased from Jiangsu Qizhou Green Chemical. Beef extract, peptone, glucose, agar strips, sodium bicarbonate, sodium dihydrogen phosphate, sodium hydroxide, glycerol and anhydrous ethanol were all analytically pure products manufactured in China.

2.1.3 Medium preparation

NA medium: 1.8g beef extract, 3g peptone,1.5g glucose, 10.8g agar, Distilled water to a volume of 600mL

NB medium: 1.8g beef extract, 3g peptone,1.5g glucose. Distilled water to 600mL.

Control carbon source medium: 0.6g sodium chloride, 0.12g magnesium chloride, 0.3g ammonium chloride, 0.012g calcium chloride, 0.3g potassium dihydrogen phosphate, 0.3g sodium dihydrogen phosphate, 10.8g agar, 0.15g paclobutrazol. Distilled water to a volume of 600mL.

Ensure all media were properly mixed, sterilized, and cooled before use.

2.2 Methods

2.2.1 Isolation and purification of bacterial strains

Grind the collected soil sample in a sterilized mortar, diluted it tenfold with sterile water, and spread it onto NA medium. It was incubated at 37℃ for 48 hours. Next, using sterile techniques,selected yellow colonies exhibiting robust growth for further streaking and inoculated onto agar plates for purification. Incubate these plates at 37 ℃ for another 48 hours. Repeated the purification process 3-7 times. Finally, stored the isolated and purified strains in sterilized glycerol tubes.

2.2.2 Microbial identification (16SrDNA sequence identification)

Extracted microbial genome DNA with a bacterial DNA extraction kit (Omega Biotek), conducted PCR experiments (2720 thermal cycler, Applied Biosystems), verified the PCR product using 1% agarose gel electrophoresis (PowerPac 164-5050, Bio-Rad), and sequenced the PCR product (3730XL, Applied Biosystems). The obtained gene sequence was used for BLAST alignment. Utilized MEGA software to draw the phylogenetic tree using the NJ method for tree construction, and the Bootstrap method for phylogenetic testing with 1000 guided replicates and the p- distance model[10,11].

2.2.3 Control carbon source test

Prepared a control carbon source culture medium, sterilized it at 121 ℃ for 20 minutes. Used aseptic techniques to inoculate the previously isolated and purified strain onto the prepared paclobutrazol inorganic salt culture medium. Cultured at 37 ℃ for more than 48 hours.

2.2.4 Gas chromatography analysis

The chromatographic conditions: Utilized Agilent7890B gas chromatograph (Agilent Technology) with the Agilent HP-5 column and a splitless injection mode. The airflow rate was maintained at 300 mL/min, while the flow rates of hydrogen and nitrogen gases were both set at 30 mL/min. The heater temperature was set to 280 ℃, and an injection volume of 1 μL was used. The column oven temperature was initially set to 120 ℃ and then ramped up at a rate of 5 ℃/min until reaching 260℃.

Standard curve: Dissolve 0.0126 g of paclobutrazol standard (99.7%) in acetone, ensuring complete dissolution, and subsequently diluted to prepare concentrations of 1.5 mg/L, 3.0 mg/L, 6.0 mg/L, and 15.0 mg/L. Filtered the solutions through a 0.45-micron microporous filter membrane to remove any particulate matter or impurities. Employed the external standard method to construct a standard working curve, establishing the linear relationship between the peak area measured by GC and the concentration of paclobutrazol.

Prepared a bacterial suspension containing paclobutrazol, set GC measurement nodes at different time points (Day 0, Day 3, Day 5, Day 7, Day 9, and Day 11), and performed gas chromatography analysis. Each sample was repeated three times, and the average peak area is taken as the measurement result.

Sample preparation involved inoculating the strain into a conical flask containing NB medium and incubating it in a shaking bed at 37 ℃ for 24 hours. After cultivation, used an inoculation ring to pick up the bacterial solution and streak plate again to check for contamination before GC measurement. For the analysis, prepared a bacterial suspension containing paclobutrazol and set GC measurement nodes at different time points (Day 0, Day 3, Day 5, Day 7, Day 9, and Day 11). Perform GC analysis on each sample, repeating the process three times. Took the average peak area from each sample as the measurement result.

3. RESULTS

3.1 Isolation and Purification of Bacterial Strains

The screened bacterial strains were streaked onto a plate and then placed in a constant temperature incubator set at 37℃ for 24 hours. Following the incubation period, the isolated and purified strains displayed characteristic features, appearing as red, smooth, circular colonies with intact edges (Fig. 1).

Fig. 1. The purified bacterial strain

3.2 Microbial Identification

After DNA extraction, PCR amplification was conducted, and the resulting sequences were visualized through gel imaging following electrophoresis. Agarose gel electrophoresis photography (Fig. 2) provided evidence of successful amplification of the 16SrDNA region for the sample. The sample exhibited clear and distinct bands at approximately 1500bp. After conducting BLAST comparison on the NCBI official website, it was found that the strain had the highest similarity with *Deinococcus arenae* strain SA1, with a homology of 100.00% after removing the redundant sequence at the beginning and end. Finally, MEGA software was used to create an evolutionary tree, which was drawn using the NJ method and subjected to phylogenetic testing using the Bootstrap method. The number of replicates was set to 1000, and the *p*-distance model was used to create the evolutionary tree (Fig 3 and Biological project number: PRJNA1104564).

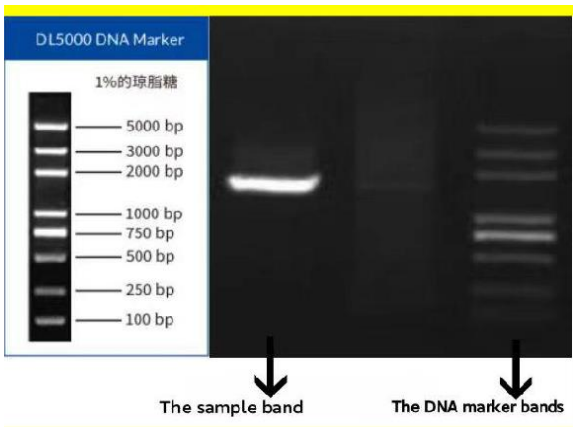

Fig. 2. Agarose gel electrophoresis of the PCR product

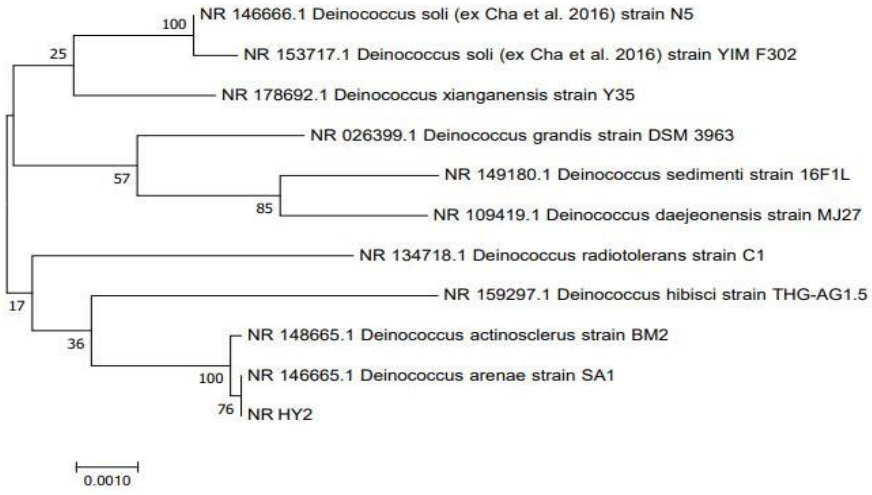

Fig. 3. The phylogenetic tree of the purified bacterial strain HY2

3.3 Control Carbon Source Test

After isolation and purification, the strain was inoculated onto an inorganic salt medium with paclobutrazol as the sole carbon source. After 48 hours of cultivation, the results indicated that the *Deinococcus arenae* strain could utilize paclobutrazol as a carbon source for growth.

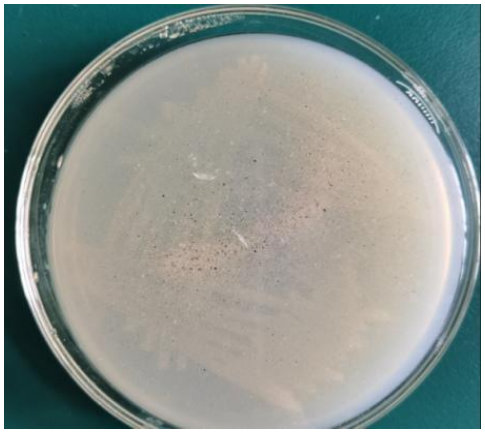

Fig. 4. Control control carbon source diagram of Deinococcus arenae

3.4 Gas Chromatography Analysis Results

The standard curve determination employed an external standard method, quantified by peak area. Concentrations of paclobutrazol used in the determination process were 0 mg/L, 1.5 mg/L, 3.0 mg/L, 6.0 mg/L, 15.0 mg/L, and 30.0 mg/L. After statistical analysis, a linear relationship between paclobutrazol concentration and peak area was established, resulting in the regression equation y=0.0977x-0.412, with a correlation coefficient (R^2) of 0.9984.

The *Deinococcus arenae* demonstrated a notable degradation effect on paclobutrazol, achieving a maximum degradation rate of 43.87% during the experimental period (Table 1).

Peak degradation rates were observed between the 5th and 7th days, indicating enhanceed degradation efficiency (Fig.5). Subsequently, degradation rates declined, possibly attributed to reduced nutrient availability for the strains in the culture medium as cultivation time extended.

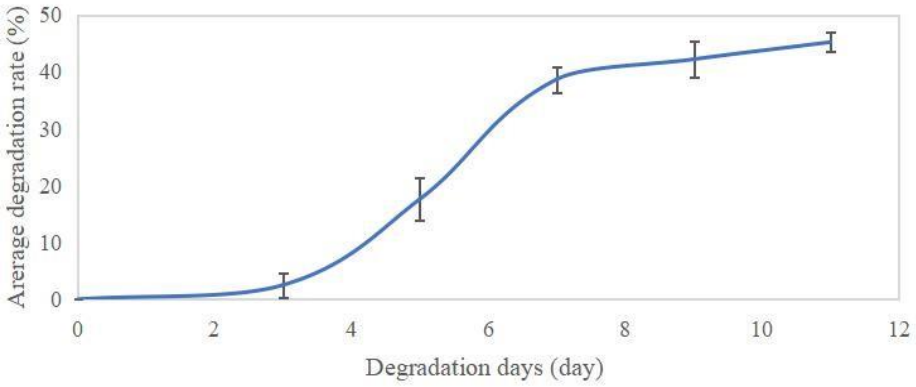

Fig. 5. The degradation efficiency of paclobutrazol by *Deinococcus arenae*

Table 1. GC analysis results

Measurement Days	Concentration of Paclobutrazol (mg/L)	Average Concentration of Paclobutrazol (mg/L)	Degradation Rate (%)	Average Degradation Rate (%)	Standard Deviation
0	6.1051 6.8486 6.8683	6.6073	0 0 0	——	0
3	6.2499 6.7463 6.3323	6.4428	5.41 -2.1 4.16	2.49	2.1637
5	5.9747 5.3342	5.4456	9.57 19.27	17.58	3.7759

Measurement Days	Concentration of Paclobutrazol (mg/L)	Average Concentration of Paclobutrazol (mg/L)	Degradation Rate (%)	Average Degradation Rate (%)	Standard Deviation
	5.0281		23.9		
7	3.735	4.0529	43.47	38.66	2.2698
	4.097		37.99		
	4.327		34.51		
9	3.3829	3.819	48.8	42.2	3.1136
	4.0072		39.35		
	4.0669		38.44		
11	3.422	3.6162	48.21	43.87	1.697
	3.5727		45.93		
	3.854		41.67		

Note: The "concentration of paclobutrazol" data in the table are rounded to four decimal places, while the "degradation rate" data is rounded to two decimal places.

4. CONCLUSION

Microbial degradation is recognized as a highly effective and environmentally friendly method for addressing pesticide residues, with bacteria playing a crucial role, particularly in soil agricultural residue management due to their diverse biochemical effects and susceptibility to mutagenesis[12]. Bacteria typically employ enzymatic reactions or their own biochemical activities to degrade pesticides. Previous research has successfully isolated bacteria capable of utilizing paclobutrazol as the control carbon source, showcasing their potential in pesticide degradation. For examples Qiu Juanping et al.[13] isolated strains of *Pseudomonas* and *Bacillus* capable of degrading paclobutrazol, achieving a degradation rate of 76.9% with a mixed microbial community. Chen et al.[14].

Isolated *Pseudomonas sp.*, demonstrating a degradation rate of 60% paclobutrazol within 2 days. Additionally, Vaz et al. [15] showed that another strain of the same genus *Pseudomonas sp* achieved a 43% degradation rate after 14 days, increasing to 70% after 28 days.

This study employs gas chromatography to evaluate the degradation capabilities of the *Deinococcus arenae* strain isolated from soil excessively treated with paclobutrazol pesticides in *Ophiopogon japonicus* fields. The findings illustrate a progressive degradation effect observed over the experimental period. Initially, after three days of degrading paclobutrazol, the *Deinococcus arenae* achieves a degradation rate of 2.49%. Subsequently, significant enhancement in degradation is noted, with the rate increasing to 17.58% after five days, further rising to 38.66% after seven days, and reaching 42.20% after nine days. The strain maintains its capability, with the degradation rate peaking at 43.87% after eleven days, demonstrating optimal performance.

These results establish a robust theoretical foundation for the potential application of the *Deinococcus arenae* in various domains such as microbial agent production, pesticide research and development, and soil remediation. The gradual increase in degradation efficiency underscores the strain's resilience and efficacy over an extended period, offering promising prospects for sustainable and environmentally friendly practices in agriculture and soil management.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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