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The Preliminary Study on Screening and Application of Phthalic Acid-Degrading Bacteria

Honghao Zhang[1], Lin Yang[1], Rubing Xu[2], Yuxiao Sun[3], Yong Yang[1], Yanyan Li[2*]

[1]College of Life Sciences, Hubei University, Wuhan, China
[2]Tobacco Research Institute of Hubei Province, Wuhan, China
[3]Xuan'en Branch Company of Enshi Tobacco Corporation, Enshi, China
Email: 1011795220@qq.com, *yanyanli0025@126.com

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Abstract

Phthalic acid is a main pollutant, which is also an important reason for the continuous cropping effect of tobacco. In order to degrade the phthalic acid accumulated in the environment and relieve the obstacle effect of tobacco continuous cropping caused by the accumulation of phthalic acid in the soil. In this study, phthalate degrading bacteria B3 is screened from continuous cropping tobacco soil. The results of biochemical identification and 16sDNA comparison show that the homology between degrading bacterium B3 and *Enterobacter* sp. is 99%. At the same time, the growth of *Enterobacter hormaechei* subsp. B3 and the degradation of phthalic acid under different environmental conditions are studied. The results show that the environment with a temperature of 30˚C, PH of 7, and inoculation amount of not less than 1.2%, which is the optimal growth conditions for *Enterobacter* sp. B3. In an environment with a concentration of phthalic acid not exceeding 500 mg/L, *Enterobacter* sp. B3 has a better effect on phthalic acid degradation, and the degradation rate can reach 77% in 7 d. The results of indoor potting experiments on tobacco show that the degradation rate of phthalic acid by *Enterobacter* B3 in the soil is about 45%, which can reduce the inhibitory effect of phthalic acid on the growth of tobacco seedlings. This study enriches the microbial resources for degrading phthalic acid and provides a theoretical basis for alleviating tobacco continuous cropping obstacles.

Keywords

Phthalic Acid, Degrading Bacteria, Rhizosphere Soil

1. Introduction

Due to the limitations of cultivated land area and tobacco growing conditions,

the problem of continuous cropping obstacles in tobacco agriculture has become increasingly prominent [1]. Continuous cropping obstacles lead to a decrease in the yield and quality of tobacco leaves, exacerbate soil-borne diseases, and severely affect the sustainable production of tobacco [2]. The relevant studies have indicated that allelopathy is one of the main causes of continuous cropping obstacles in tobacco, where allelopathic substances inhibit the growth and development of plants by being produced and released by the plant roots into the surrounding environment [3].

Phthalic acid, as one of the main allelopathic substances in tobacco fields, can reduce the root activity of tobacco plants and inhibit their growth [4]. At present, more than 10 phenolic acids, such as phthalic acid and cinnamic acid, have been isolated from root exudates of crops prone to continuous cropping disorders [5]. Moreover, once phthalic acid enters the environment, it may be absorbed by organisms and humans through various pathways, reducing food security and causing various diseases [6]. In low-oxygen soil environments and sediments, the half-life of phthalic acid and its derivatives can extend to several months or even years [7]. Therefore, finding beneficial microorganisms that can effectively degrade phthalic acid is crucial for addressing phthalic acid pollution issues [8].

Currently, degradation methods for phthalic acid and its derivatives include physical and chemical methods [9], as well as biological methods [10]. Physical methods for degrading phthalic acid carry the risk of secondary pollution; therefore, biological methods are primarily employed for its degradation. Research has demonstrated that phthalic acid can be decomposed and absorbed as a nutrient by microorganisms, with high degradation efficiency, low cost, and environmental friendliness. Thus, microbial degradation is considered the most economically effective biological method for degrading phthalic acid [11]. Significant progress has been made by many researchers in screening and isolating phthalic acid-degrading microorganisms, such as *Ochrobactrum* sp. [12], *Bacillus* sp. [13], and *Paracoccus* sp. [14].

Microorganisms in soil environments are regarded as the largest repository of biological diversity in nature, harboring more efficient microorganisms for degrading phthalic acid and esters [15]. Most of the previous studies are limited to laboratory environments, and there is little research on the degradation effects of degrading bacteria in soil environments that have been seldom reported. This study aims to provide a basis for alleviating continuous cropping obstacles in tobacco by screening for efficient phthalic acid-degrading microorganisms in soil and determining their application effects and factors influencing degradation efficiency.

2. Materials and Methods

2.1. Materials

2.1.1. Culture Media

Basic Salt Medium: Per liter, containing $(NH_4)_2SO_4$ 2.0 g, $MgSO_4$ 0.2 g, $CaCl_2{\cdot}2H_2O$

0.01 g, $FeSO_4$ 0.001 g, $Na_2HPO_4 \cdot 12H_2O$ 1.5 g, KH_2PO_4 1.5 g.

LB Medium: Per liter, containing NaCl 10 g, yeast extract 5 g, tryptone 10 g.

PDA Medium: Per liter, containing 200 g potato infusion, 20 g glucose.

Gause No. 1 Medium: Per liter, containing soluble starch 20 g, NaCl 0.5 g, KNO_3 1 g, $K_2HPO_4 \cdot 3H_2O$ 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g.

Beef Extract Peptone Medium: Per liter, containing beef extract 3 g, peptone 10 g, NaCl 5 g.

MacConkey Agar Medium: Per liter, containing peptone 5 g, glucose 10 g, KH_2PO_4 1 g, $MgSO_4$ 0.5 g, crystal violet 0.03 g, chloramphenicol 0.1 g.

2.1.2. Screening of Degradation Bacteria

Soil samples were collected from Xuan'en County, Hubei Province, China, where tobacco had been continuously cultivated for over 15 years. Ten grams of soil samples were placed in sterilized Erlenmeyer flasks, and basic culture medium was added to a total volume of 100 ml. The flasks were then shaken overnight at 30˚C and 180 rpm to prepare bacterial suspensions. Ten milliliters of the bacterial suspensions were separately added to Gause No. 1 medium (for actinomycetes), MacConkey agar medium (for fungi), and beef extract peptone medium. The medium was pre-added with the allelochemicals phthalic acid to give a final concentration of 100 mg/l.

The gradient pressure domestication method was employed for cultivation, enriching the concentration of phthalic acid in the culture medium from 100 mg/l gradually to 700 mg/l. Solid culture medium containing 500 mg/l phthalic acid was prepared, and the tolerant degradation bacteria were initially isolated using the dilution spread plate method. Colonies showing good growth, clear morphology, and distinct characteristics were selected, and single colonies were isolated using the streak plate method, thus obtaining the phthalic acid-degrading bacteria.

2.2. Identification of Degradation Bacteria

2.2.1. Physiological and Biochemical Identification of Bacteria

Reference: "Bergey's Manual of Systematic Bacteriology", 9th edition.

2.2.2. Molecular Biological Identification

Bacterial strains were cultured on LB agar medium for 8 hours. A volume of 100 μl of bacterial suspension was transferred to a sterilized 1.5 ml centrifuge tube. After boiling in a water bath for 15 minutes, the suspension was centrifuged at 12,000 rpm for 2 minutes, and the supernatant was collected as the template for 16S rRNA gene amplification by PCR.

The PCR reaction system comprised 5 μl of DNA template, 1 μl each of primers 27F/1492R, 1 μl of dNTP (10 mmol/l), 5 μl of Ex Taq buffer (10×), 1 μl of Ex Taq, and ddH_2O added to make up the volume to 50 μl.

Primer sequences:

27F: 5'-AGAGTTTGATCCTGGCTCAG-3';

1492R: 5'-GGTTACCTTGTTACGACTT-3'.

The PCR amplification program included initial denaturation at 94˚C for 3 minutes, followed by denaturation at 94˚C for 1 minute, annealing at 61˚C for 1 minute, extension at 72˚C for 1 minute, for a total of 30 cycles, and final extension at 72˚C for 5 minutes. The reaction was terminated by cooling to 4˚C for 30 minutes. PCR products were analyzed by 1% agarose gel electrophoresis, and sequencing was performed by Wuhan Qingke Biotechnology Company. The obtained sequences were compared with the 16S rRNA sequences in the GenBank database.

2.3. Degradation Efficiency Determination

Equal volumes of ethyl acetate were added to the bacterial suspension for liquid-liquid extraction. The ethyl acetate layer was then evaporated using a rotary evaporator, and the residue was dissolved in water. After filtration through a 0.22 μm membrane filter, the solution was subjected to liquid chromatography-mass spectrometry (LC-MS) to determine the concentration of phthalic acid.

Liquid chromatography conditions: Column: Agilent ZORBAX SB-Aq (250 mm × 4.6 mm); Mobile phase: 5 mmol/L H_2SO_4; Flow rate: 500 μL/min; Injection volume: 20 μL.

2.4. Analysis of Factors Affecting Degradation Efficiency

Single colonies of bacterial strains were inoculated into LB medium and cultured overnight. The bacterial cells were collected by centrifugation at 5000 rpm for 2 minutes, resuspended in inorganic salt medium, and adjusted to an OD600 of approximately 1 as a seed solution. The seed solution was inoculated into 50 mL of LB medium, and different cultivation conditions were set, including different temperatures (18˚C, 30˚C, 37˚C, 45˚C), pH levels (4, 5, 6, 7, 8, 9), concentrations of phthalic acid (100 - 1000 mg/L), and inoculation volumes of bacterial strain 3 (0.4%, 0.8%, 1.2%, 1.6%, and 2.0%). Samples were taken after 48 hours of cultivation for OD600 measurement (for the effect of inoculation volume on growth status, samples were taken every 8 hours). Each experiment was performed in triplicate.

2.5. Effect of Degradation Bacteria on Tobacco Seedling Growth

Pot experiments were conducted in the greenhouse of Hubei University. Tobacco seeds with uniform size and appearance were selected, soaked in water for 12 hours, then disinfected with 70% alcohol for 1 minute, and rinsed repeatedly with sterile water. The treated tobacco seeds were sown in seedling pots, and after reaching the five-leaf stage, the tobacco seedlings were transplanted. Uniform-sized tobacco plants were selected and transplanted into flowerpots (9 × 9 × 12 cm) filled with sterilized soil. Degradation bacteria B3 were fermented under optimal conditions for later use. Three treatments were set up: PA, PA + B3, and CK. In the PA treatment, 50 mL of 2 g/L phthalic acid solution and 5 mL of wa-

ter were added to each pot. In the PA + B3 treatment, 50 mL of 2 g/L phthalic acid solution and 5 mL of bacterial suspension of strain B3 (obtained by picking a single colony of strain B3 from an activated plate, overnight cultured in LB medium, centrifuged to remove the supernatant, and resuspended in an equal volume of basic salt medium) were added to each pot. The CK treatment involved adding 55 mL of water to each pot. After four weeks of cultivation in a constant temperature and humidity greenhouse, the plant height, stem diameter, fresh and dry weights of roots, stems, and leaves of tobacco seedlings were measured. Each treatment was replicated three times.

2.6. Detection of Soil Degradation Efficiency of Degradation Bacteria

Soil samples from the tobacco seedling growth area were placed in sterile water and shaken overnight. After filtration to remove insoluble substances, the phthalic acid content in the water was detected using liquid chromatography. Liquid chromatography conditions were the same as described in Section 2.3.

3. Results and Analysis

3.1. Screening and Physiological-Biochemical Characteristics of Degradation Bacteria

A degradation bacterial strain, B3, exhibiting favorable degradation efficiency towards phthalic acid, was obtained through the dilution spread plate method. The degradation rate of phthalic acid by strain B3 was determined to be 70%. The colony morphology of strain B3 on agar plates was circular, milky white in color, and slightly elevated (**Figure 1**). Further observation and study were conducted on the physiological and biochemical characteristics of strain B3, the results of which are presented in **Table 1**.

3.2. Identification of Degradation Bacterium Strain B3's 16S rDNA

The genomic DNA of strain B3 was utilized as a template for PCR amplification, and the resulting PCR product was sequenced to obtain the 16S rDNA sequence of strain B3, with a fragment length of 1466 bp. The obtained 16S rDNA gene sequence underwent a BLAST homology comparison on NCBI, revealing a 97% homology with bacteria of the *Enterobacter hormaechei* subsp. (**Figure 2**).

3.3. Effect of Environmental Conditions on the Degradation of Phthalic Acid by Strain B3

3.3.1. Impact of Temperature on the Growth of Strain B3 and Its Degradation of Phthalic Acid

The growth of strain B3 and its degradation of phthalic acid under temperature conditions ranging from 18˚C to 45˚C are illustrated in **Figure 3**. The results indicate that with the increase in cultivation temperature, both the cell density of strain B3 and its degradation rate of phthalic acid initially increase and then decrease. The optimal growth temperature for strain B3 is 30˚C, and both too high

Figure 1. Morphology of degrading bacteria B3.

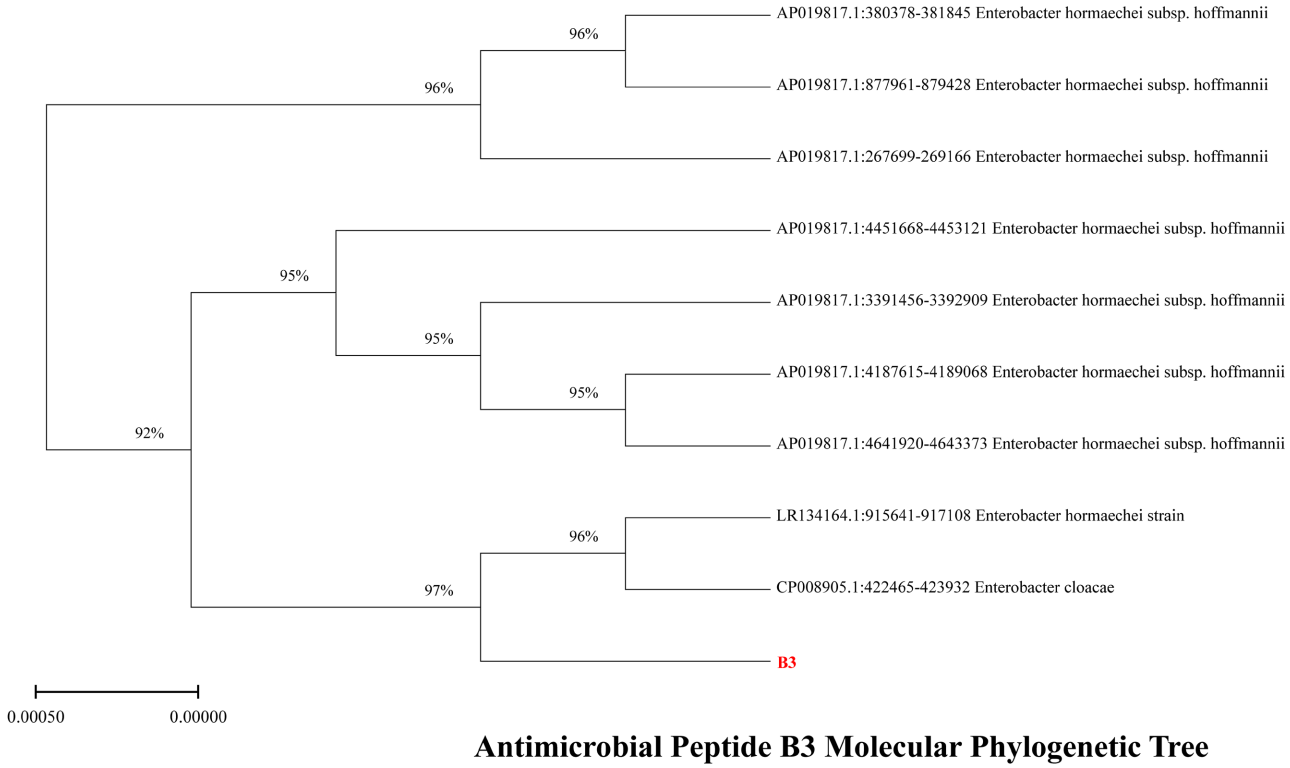

Figure 2. Phylogenetic tree of 16S rDNA of strain B3.

Table 1. The main physiological and biochemical characteristics of degrading bacteria B3.

Bacterial Properties	Result	Bacterial Properties	Result
Gram Stain	−	Sucrose	−
Glucose	+	D-Fructose	+
D-Galactose	+	Trehalose	−
L-Arabinose	+	Mannose	+
Maltose	−	D-Xylose	−
D-Ribose	−	Rhamnose	−
Sorbitol	−	Sarcosine	+

Continued

Mannitol	–	Casein	+
Ethanol	–	Ammonia Production	+
D-Malate	+	Cellulose Decompose	+
Citraconate	+	Lecithin Enzyme	+
D-Alanine	+	Starch Hydrolysis	+
D-Tryptophan	+	Gelatine Liquefication	–
D-Tartrate	–	V-P Test	–
Glycolate	–	Denitrification	–
Pantothenate	–	Sarcosine	+

Note: + denotes positive, – denotes negative.

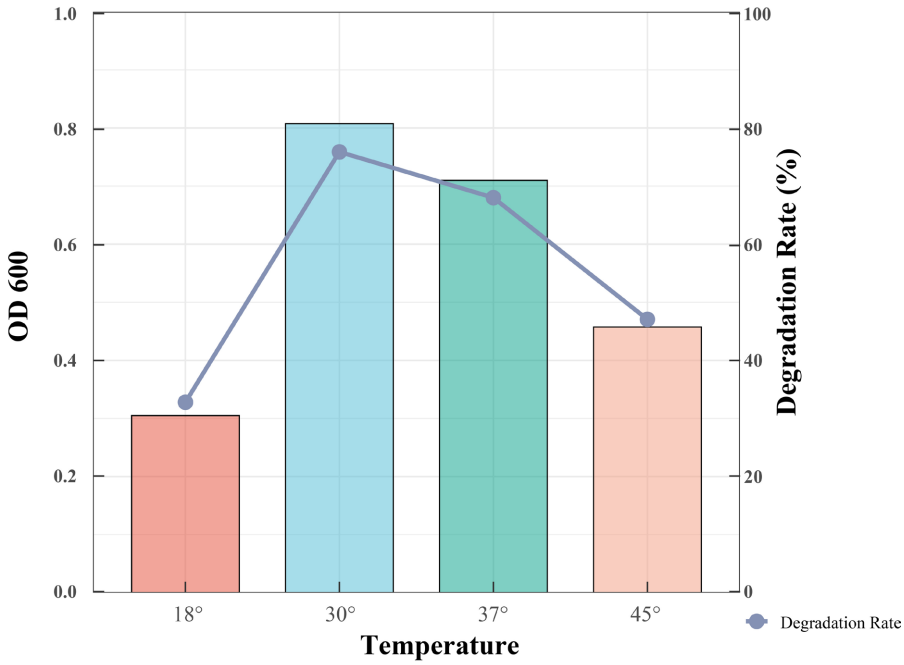

Figure 3. Effect of temperature on the growth and degradation rate of strain B3.

and too low temperatures inhibit the growth of the strain. At the optimal temperature, strain B3 exhibits the highest degradation rate of phthalic acid, indicating a positive correlation between the growth of strain B3 and its degradation efficiency.

3.3.2. Impact of pH on the Degradation of Phthalic Acid by Strain B3

The growth of strain B3 and its degradation of phthalic acid under pH conditions ranging from 4 to 9 are shown in **Figure 4**. It is observed that the growth of strain B3 is optimal at pH values between 7 and 8. Moreover, at pH 8, strain B3 exhibits the highest degradation rate of phthalic acid. This suggests that strain B3 thrives in neutral to slightly alkaline environments, and alkaline conditions favor the degradation of phthalic acid by the strain.

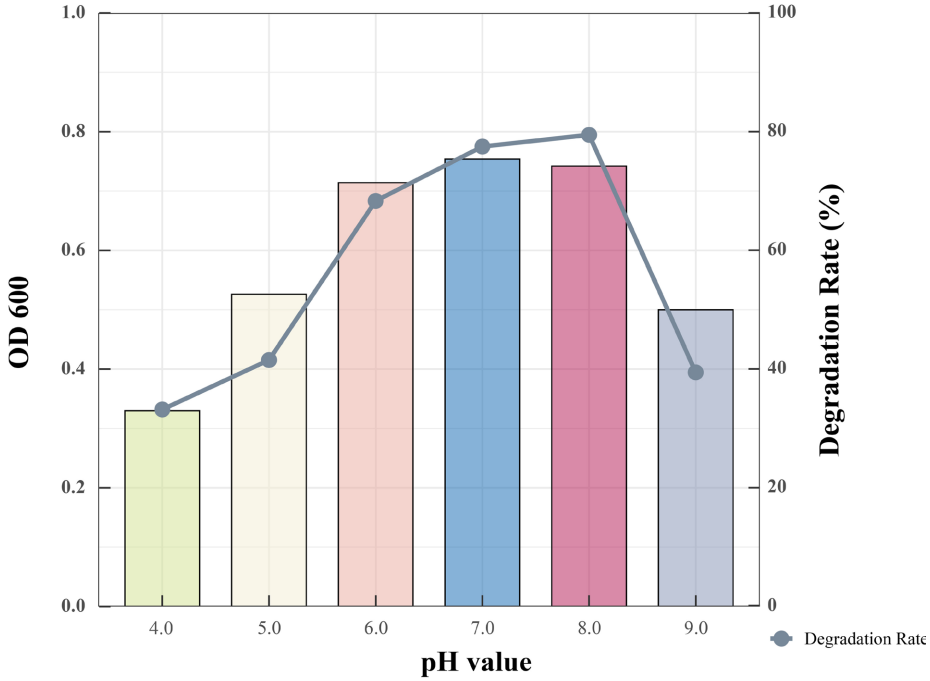

Figure 4. Effect of pH on the growth and degradation rate of strain B3.

3.3.3. Impact of Initial Concentration of Phthalic Acid on the Degradation of Phthalic Acid by Strain B3

The growth of strain B3 and its degradation rate of phthalic acid under initial concentrations of phthalic acid ranging from 100 to 1000 mg/L are illustrated in **Figure 5**. The results indicate that with increasing initial concentrations of phthalic acid from 100 to 500 mg/L, the degradation rate of phthalic acid by strain B3 gradually increases. At an initial concentration of 500 mg/L, both the cell density of strain B3 and its degradation rate of phthalic acid reach their maximum. However, when the initial concentration ranges from 500 to 700 mg/L, both the cell density and degradation rate of strain B3 gradually decrease before stabilizing. Overall, when the initial concentration of phthalic acid exceeds 700 mg/L, the growth of strain B3 is inhibited, and its degradation rate decreases accordingly. This indicates that strain B3 exhibits more effective degradation of phthalic acid at lower concentrations.

3.3.4. Impact of Inoculation Volume on the Growth of Strain B3

In **Figure 6**, it can be observed that when the inoculation volume is greater than 1.2%, there is no significant effect on the growth of strain B3. However, when the inoculation volume is less than 1.2%, there is a certain inhibitory effect on the growth of strain B3 before 72 hours, after which this inhibitory effect disappears.

3.3.5. Degradation of Phthalic Acid by Strain B3 under Optimal Environmental Conditions

Based on the results of the single-factor experiments, the optimal environmental conditions for strain B3 were determined to be pH 7, cultivation temperature

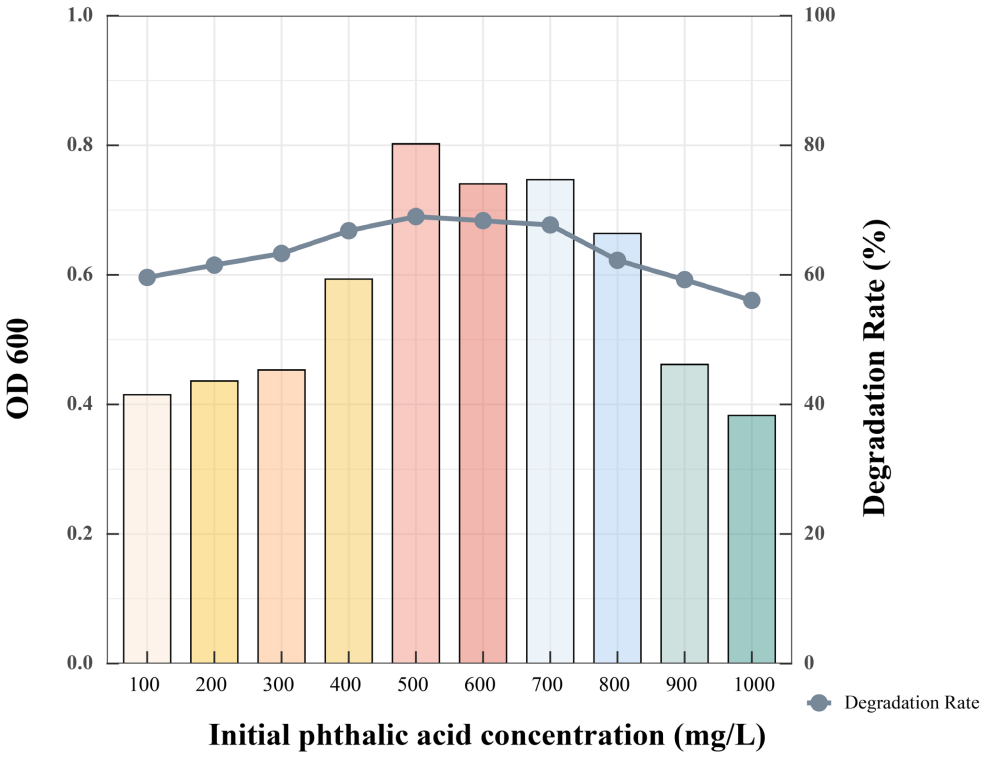

Figure 5. Effect of initial concentration on the growth and degradation rate of strain B3.

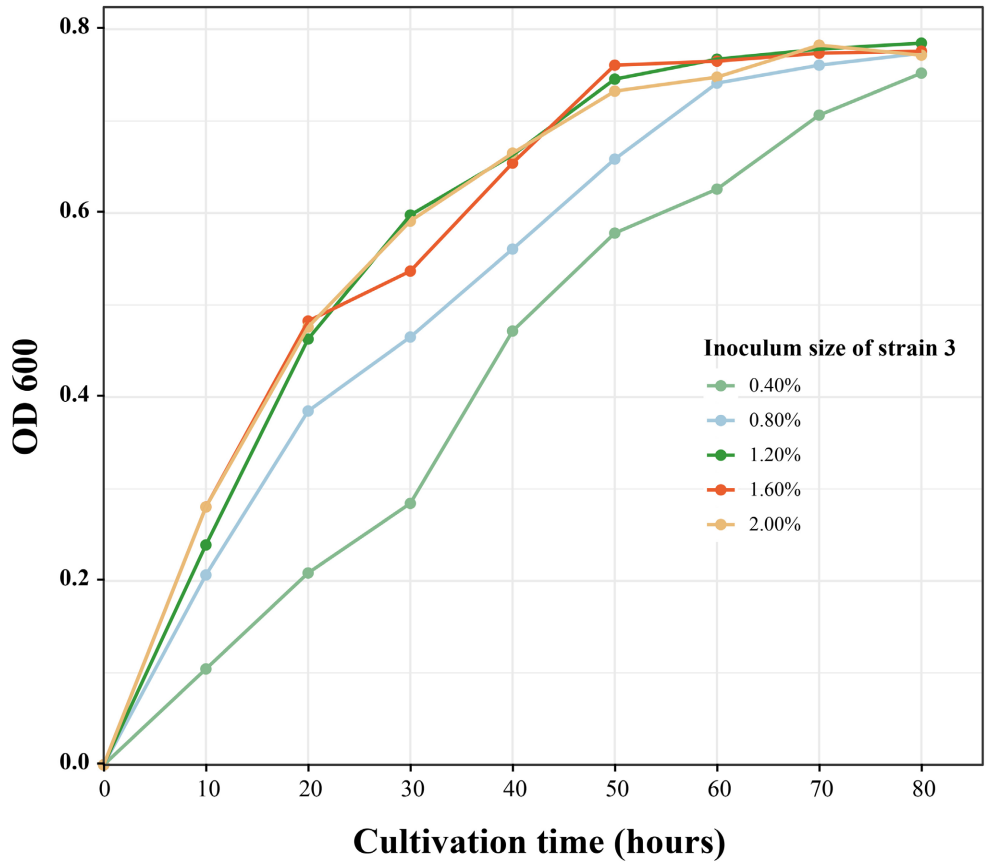

Figure 6. Effect of inoculation amount of strain B3 on strain growth.

30˚C, initial concentration of phthalic acid 500 mg/L, and inoculation volume ≥ 1.2%. The above studies showed that under this optimum condition, the degradation rate of the strain was the highest. Under these optimal conditions, strain B3 was cultured in shake flasks for 7 days, with samples taken every 24 hours. The concentration of phthalic acid in the fermentation broth was measured using high-performance liquid chromatography (HPLC), and the results are shown in Figure 7. As the cultivation time increased, the concentration of phthalic acid in the fermentation broth decreased continuously. Based on the calculations, strain B3 had a maximum degradation efficiency of 77% for phthalic acid in 7 days under the optimal condition.

3.4. Influence of Strain B3 on Tobacco Plant Growth and Phthalic Acid Content in Soil

To investigate the degradation of phthalic acid in soil by strain B3 in the natural environment, a pot experiment was conducted. Different treatments were applied to the soil of naturally growing tobacco plants. Subsequently, the heights, stem circumferences, and fresh and dry weights of roots, stems, and leaves of tobacco plants under different treatments (control, PA, PA + B3) were measured, and the results are presented in Table 2. In the PA treatment, the heights and fresh weights of stems and leaves of tobacco plants were significantly lower than those in the control group. In contrast, compared to the PA treatment, the heights and fresh weights of roots and stems of tobacco plants were significantly increased in the PA + B3 treatment. These results indicate that phthalic acid has a significant inhibitory effect on the growth of tobacco plants, while strain B3 can effectively alleviate the growth inhibition caused by phthalic acid.

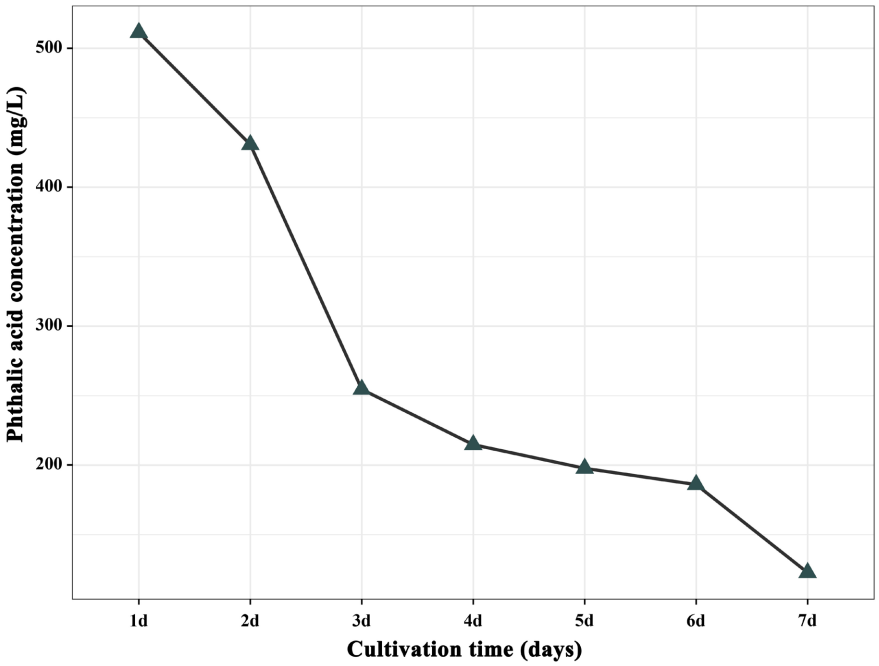

Figure 7. Degradation curve of phthalic acid by strain B3.

Based on liquid chromatography analysis, the content of phthalic acid in soil under different treatments was determined (Figure 8). The results indicate that the content of phthalic acid in the soil of the PA + B3 group decreased by 45% compared to the PA soil (ANOVA, $P < 0.01$), suggesting that strain B3 has a degrading effect on phthalic acid in the soil.

4. Discussion

Escherichia coli plays a crucial role in regulating antibiotic resistance genes, modulating cell membrane permeability, and biological diagnosis and treatment, rendering it a highly versatile and valuable bacterium [16]. However, there is little research on the application of *Escherichia coli* in degrading phthalic acid esters. Previous studies have predominantly focused on screening and isolating

Table 2. Effects of different treatments on the growth of tobacco plants.

Sample	Steam Height	Fresh			Dried		
		Roots	Stems	Leaves	Roots	Stems	Leaves
CK	155.33 ± 6.944a	5.56 ± 0.58b	8.45 ± 0.43a	33.03 ± 0.40a	4.20 ± 1.53	2.10 ± 0.40	11.00 ± 1.02
PA	110.67 ± 11.90b	6.20 ± 0.22b	4.83 ± 0.50b	26.73 ± 1.35b	3.92 ± 0.31	2.94 ± 0.88	11.00 ± 0.84
PA + B3	138.33 ± 4.11a	14.37 ± 0.82a	8.72 ± 0.41a	33.40 ± 0.73a	5.70 ± 0.07	3.04 ± 0.69	12.30 ± 0.08

Note: a and b are a significant letter mark, different letters showed significance at $P < 0.05$.

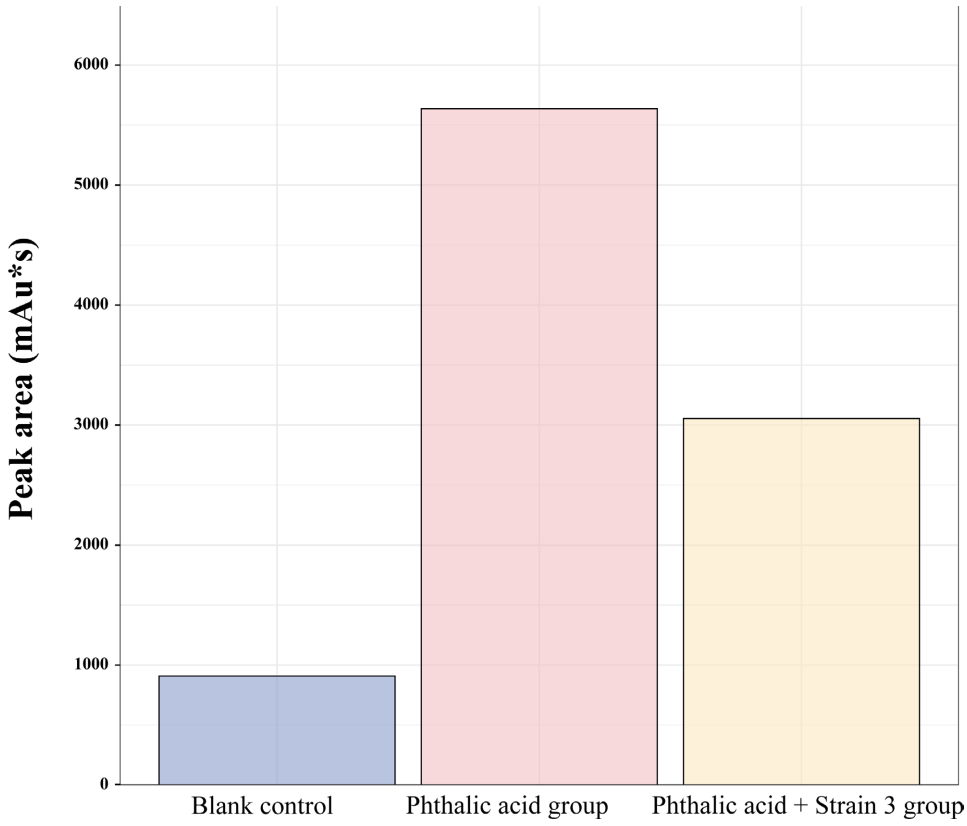

Figure 8. Phthalic acid content in soil.

bacteria capable of degrading phthalic acid esters and their derivatives from environments such as sludge, artificial wetlands, and river sediments [8]. In order to obtain degradation strains beneficial for alleviating tobacco continuous cropping obstacles, this study screens and isolates phthalic acid ester-degrading bacteria from soil with a long-term history of tobacco cropping. Through physiological and biochemical tests and 16S rRNA identification, the degradation strain B3 is confirmed to belong to the genus *Escherichia coli* (*Enterobacter hormaechei* subsp.). Although there are relatively few reports on the degradation of phthalic acid esters by the genus *Escherichia coli*, some studies have demonstrated that the genus *Escherichia coli* has the capability to degrade various organic substances, such as dissolving mineral elements like phosphorus and potassium, as well as degrading polycyclic aromatic hydrocarbons and heavy metals [17]. Additionally, research has confirmed that the genus *Escherichia coli* can degrade the phthalic acid ester derivative di(2-ethylhexyl) phthalate (DEHP) [18].

Temperature can significantly influence enzyme activity, thereby affecting the growth of strains and their ability to degrade organic compounds [19]. pH is another crucial factor that impacts strain growth. pH can influence enzyme activity, cell membrane permeability, enzyme-catalyzed reaction rates, and other physiological and biochemical properties during the transformation of organic pollutants by strains, thereby affecting the degradation efficiency of strains [20]. The results of this study indicate that the optimal cultivation conditions for strain B3 are a pH of 7, 30˚C, and an inoculum size ≥ 1.2%. Under the condition, strain B3 achieved a 77% degradation rate of phthalic acid esters in 7 d, which is significantly higher than that of other microorganisms such as *Sphingomonas* spp. and *Bacillus* spp. [21]. The phthalate-degrading strain B3 screened in this study had a better degradation effect than Li *et al.* [22].

Upon the addition of phthalic acid esters to the soil, the growth of tobacco seedlings is significantly inhibited. Phthalic acid esters act as phytotoxic substances, inhibiting the growth of tobacco plants [23]. However, following the addition of strain B3, the inhibitory effect of phthalic acid esters on tobacco seedling growth is alleviated. Agronomic traits such as plant height and stem circumference are improved, and the content of phthalic acid esters in the soil is significantly reduced. Therefore, strain B3 may alleviate the constraint of phthalic acid esters on tobacco growth by degrading them in the soil.

5. Conclusion

A phthalic acid ester-degrading strain B3 is isolated from tobacco soil with long-term continuous cropping. Physiological, biochemical, and 16S rDNA analysis confirmed that strain B3 belongs to the genus *Enterobacter hormaechei* subsp. The optimal cultivation conditions for strain B3 are found to be 30˚C, pH 7, with an initial concentration of phthalic acid esters at 500 mg/L, and an inoculum size greater than or equal to 1.2%. Strain B3 exhibited a degradation rate of 77% for phthalic acid esters in liquid culture medium over 7 d, while its degradation effi-

ciency in soil is approximately 45%.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Chen, D., Zhou, Y., Wang, M., Mujtaba Munir, M.A., Lian, J., Yu, S., Dai, K. and Yang, X. (2021) Succession Pattern in Soil Micro-Ecology under Tobacco (*Nicotiana tabacum* L.) Continuous Cropping Circumstances in Yunnan Province of Southwest China. *Frontiers in Microbiology*, **12**, Article 785110. https://doi.org/10.3389/fmicb.2021.785110

[2] Gu, H.S., Guo, L., Zeng, Z.L., Wang, B., Qiu, P., Cao, T.M. and Chen, C.Y. (2013) Research Progress on the Formation Mechanism and Regulation Techniques of Continuous Cropping Obstacles in Tobacco. *Hunan Agricultural Sciences*, **1**, 25-28.

[3] Li, X., Ding, C., Hua, K., Zhang, T., Zhang, Y., Zhao, L., Yang, Y., Liu, J. and Wang, X. (2014) Soil Sickness of Peanuts Is Attributable to Modifications in Soil Microbes Induced by Peanut Root Exudates Rather than to Direct Allelopathy. *Soil Biology and Biochemistry*, **78**, 149-159. https://doi.org/10.1016/j.soilbio.2014.07.019

[4] Zhang, X.L., Pan, Z.G., Zhou, X.F. and Ni, W.Z. (2007) Autotoxicity and Continuous Cropping Obstacles. *Bulletin of Soil and Water Conservation*, **38**, 781-784.

[5] Wu, Z., Yang, L., Wang, R., Zhang, Y., Shang, Q., Wang, L., Qin, R. and Xie, Z.K. (2015) *In Vitro* Study of the Growth, Development and Pathogenicity Responses of *Fusarium oxysporum* to Phthalic Acid, an Autotoxin from Lanzhou Lily. *World Journal of Microbiology and Biotechnology*, **31**, 1227-1234. https://doi.org/10.1007/s11274-015-1872-8

[6] Sicińska, P., Mokra, K., Wozniak, K., Michałowicz, J. and Bukowska, B. (2021) Genotoxic Risk Assessment and Mechanism of DNA Damage Induced by Phthalates and Their Metabolites in Human Peripheral Blood Mononuclear Cells. *Scientific Reports*, **11**, Article No. 1658. https://doi.org/10.1038/s41598-020-79932-5

[7] Boll, M., Geiger, R., Junghare, M. and Schink, B. (2020) Microbial Degradation of Phthalates: Biochemistry and Environmental Implications. *Environmental Microbiology Reports*, **12**, 3-15. https://doi.org/10.1111/1758-2229.12787

[8] Hu, R., Zhao, H., Xu, X., Wang, Z., Yu, K., Shu, L., Yan, Q., Wu, B., Mo, C., He, Z. and Wang, C. (2021) Bacteria-Driven Phthalic Acid Ester Biodegradation: Current Status and Emerging Opportunities. *Environment International*, **154**, Article ID: 106560. https://doi.org/10.1016/j.envint.2021.106560

[9] Mo, J., Wang, Z., Xu, W., Li, S. and Yu, Z. (2015) Enhanced Production of Dimethyl Phthalate-Degrading Strain *Bacillus* sp. QD14 by Optimizing Fermentation Medium. *Electronic Journal of Biotechnology*, **18**, 244-251. https://doi.org/10.1016/j.ejbt.2015.03.013

[10] He, L., Gielen, G., Bolan, N.S., Zhang, X., Qin, H., Huang, H. and Wang, H. (2015) Contamination and Remediation of Phthalic Acid Esters in Agricultural Soils in Chi-

na: A Review. *Agronomy for Sustainable Development*, **35**, 519-534. https://doi.org/10.1007/s13593-014-0270-1

[11] Cheng, J., Liu, Y., Wan, Q., Yuan, L. and Yu, X. (2018) Degradation of Dibutyl Phthalate in Two Contrasting Agricultural Soils and Its Long-Term Effects on Soil Microbial Community. *The Science of the Total Environment*, **640-641**, 821-829. https://doi.org/10.1016/j.scitotenv.2018.05.336

[12] Wang, J., Zhang, M.Y., Chen, T., Zhu, Y., Teng, Y., Luo, Y.M. and Christie, P. (2015) Isolation and Identification of a Di-(2-Ethylhexyl) Phthalate-Degrading Bacterium and Its Role in the Bioremediation of a Contaminated Soil. *Pedosphere*, **25**, 202-211. https://doi.org/10.1016/S1002-0160(15)60005-4

[13] Ding, J.M., Wang, C.F., Xie, Z.R., Li, J.J., Yang, Y.J., Mu, Y.L., Tang, X.H., Xu, B., Zhou, J.P. and Huang, Z.X. (2015) Properties of a Newly Identified Esterase from *Bacillus* sp. K91 and Its Novel Function in Diisobutyl Phthalate Degradation. *PLOS ONE*, **10**, e0119216. https://doi.org/10.1371/journal.pone.0119216

[14] Nisha, K.N., Devi, V., Varalakshmi, P. and Ashokkumar, B. (2015) Biodegradation and Utilization of Dimethylformamide by Biofilm Forming *Paracoccus* sp. Strains MKU1 & MKU2. *Bioresource Technology*, **188**, 9-13. https://doi.org/10.1016/j.biortech.2015.02.042

[15] Wang, Z., Huang, W. and Pang, F. (2022) Selenium in Soil-Plant-Microbe: A Review. *Bulletin of Environmental Contamination and Toxicology*, **108**, 167-181. https://doi.org/10.1007/s00128-021-03386-2

[16] Chollet, R., Bollet, C., Chevalier, J., Malléa, M., Pagès, J.M. and Davin-Regli, A. (2002) Mar Operon Involved in Multidrug Resistance of *Enterobacter aerogenes*. *Antimicrobial Agents and Chemotherapy*, **46**, 1093-1097. https://doi.org/10.1128/AAC.46.4.1093-1097.2002

[17] Sonali, J.M.I., Gayathri, K.V., Kumar, P.S. and Rangasamy, G. (2023) A Study of Potent Biofertiliser and Its Degradation Ability of Monocrotophos and Its *in Silico* Analysis. *Chemosphere*, **312**, Article ID: 137304. https://doi.org/10.1016/j.chemosphere.2022.137304

[18] Jin, D.C., Liang, R.X., Dai, Q.Y., Zhang, R.Y., Wu, X.L. and Chao, W.L. (2010) Biodegradation of Di-n-Butyl Phthalate by Rhodococcus sp. JDC-11 and Molecular Detection of 3, 4-Phthalate Dioxygenase Gene. *Journal of Microbiology and Biotechnology*, **20**, 1440-1445. https://doi.org/10.4014/jmb.1004.04034

[19] Tang, W.J., Zhang, L.S., Fang, Y., Zhou, Y. and Ye, B.C. (2016) Biodegradation of Phthalate Esters by Newly Isolated *Rhizobium* sp. LMB-1 and Its Biochemical Pathway of Di-n-butyl Phthalate. *Journal of Applied Microbiology*, **121**, 177-186. https://doi.org/10.1111/jam.13123

[20] Huang, W., Sun, D., Wang, R. and An, Y. (2021) Integration of Transcriptomics and Metabolomics Reveals the Responses of Sugar Beet to Continuous Cropping Obstacle. *Frontiers in Plant Science*, **12**, Article 711333. https://doi.org/10.3389/fpls.2021.711333

[21] Liang, D.W., Zhang, T., Fang, H.H.P. and He, J. (2008) Phthalates Biodegradation in the Environment. *Applied Microbiology and Biotechnology*, **80**, 183-198. https://doi.org/10.1007/s00253-008-1548-5

[22] Li, W., Yang, X., Li, G., Li, C., Xu, Y., Sun, J., Yang, Y., *et al.* (2018) Screening and Application of Phthalic Acid Degrading Bacteria. *Open Journal of Applied Sciences*, **8**, 586-597. https://doi.org/10.4236/ojapps.2018.812047

[23] Guo, Y.L., Li, M.H., Wu, H.T., Yuan, L. and Huang, J.G. (2007) Effects of Tobacco Root Exudates on the Growth and Nutrient Absorption of Tobacco Seedlings. *Journal of Plant Nutrition and Fertilizer Science*, **13**, 458-463.