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Delftia lacustris SW-1, a Strain with Degradation Potential for Aniline in Weakly Acidic Industrial Wastewater

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Abstract

In this study, a weak acid-resistant strain SW-1 was isolated from the aeration tank of the sewage treatment plant at Shanxi Normal University and identified as *Delftia lacustris*. It exhibited a high capability for degrading aniline up to 1500 mg/L. Under optimal conditions (pH 6.0, 30˚C), 1000 mg/L of aniline was nearly completely degraded within 32 hours. When utilizing sodium acetate, glucose, and folic acid as co-metabolic carbon and nitrogen sources, the strain demonstrated enhanced degradation efficiency for aniline. RT-qPCR analysis indicated that the strain degrades aniline via the meta-cleavage pathway. This finding could provide a theoretical basis and practical material for the treatment of aniline pollution. The adaptability of strain SW-1 to diverse cultivation conditions signifies its potential for degrading aniline in weak-acid industrial wastewater.

Keywords

Aniline Degradation, Weak-Acid Wastewater, Co-Metabolism, Degradation Pathway

1. Introduction

Aniline is commonly used in the production of azo dyes and in the manufacturing of chemical products such as pharmaceuticals, insecticides, and rubber chemicals [1]. Due to the incomplete degradation of azo dyes in the treatment of printing and dyeing-related wastewater, a significant amount of dye intermediates re-

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mains, which are the main source of aniline in this wastewater [2] [3]. Aniline easily adheres to colloidal organic compounds and is difficult to degrade naturally [4]. It primarily accumulates and pollutes soil and aquatic ecosystems [5]. This is because aniline is volatile and can enter the air as vapor, causing air pollution, which then contaminates water bodies [6]. It changes the physical or chemical properties of water, alters biological populations, and poses a threat to aquatic ecology [7] [8]. The ecological balance is difficult to restore in a short period, and aniline eventually seeps into the soil, where it is challenging to degrade quickly [9] [10]. Therefore, eliminating aniline pollution is imperative.

Biodegradation is a commonly used method for removing organic pollutants, offering advantages such as low cost, simple operation, and no secondary pollution to the environment [11] [12]. However, there are still certain problems with the biodegradation of aniline [13]. For example, the aniline-degrading bacterium *Acinetobacter calcoaceticus*, isolated by researchers, can effectively degrade aniline at low concentrations with external carbon and nitrogen sources, but its degradation is inhibited at higher concentrations of aniline. While some species can biodegrade aniline efficiently in wastewater containing high concentrations of aniline in alkaline conditions or at low temperatures [14]-[16], there has been little research on the effective degradation of aniline in weak-acid environments. Additionally, co-metabolism research plays an important role in the biodegradation process [17], but there are almost no reports on the co-metabolites of aniline. Therefore, developing bacterial resources and finding suitable co-metabolites that can efficiently degrade aniline is expected to play an important role in the removal of aniline pollutants.

In this study, a promising bacterial strain for the efficient biodegradation of aniline was successfully isolated and identified as *D. lacustris* SW-1. The characteristics and the pathway of aniline biodegradation by this newly isolated *D. lacustris* SW-1 were further investigated, and a new biodegradation pathway of aniline by *D. lacustris* SW-1 was preliminarily confirmed. This study is significant for both basic research and environmental science applications, as it helps to expand the biological resources for aniline degradation while addressing the problem of low aniline degradation rates.

2. Materials and Methods

2.1. Strain Isolation, Enrichment, and Domestication

Strain SW-1 was isolated from sludge samples collected from the aeration tank of the sewage treatment station at Shanxi Normal University, China. The activated sludge was mixed with nutrient broth medium, which contained (per liter): 3.0 g beef extract and 5.0 g peptone, and was cultured on a shaking table at 30˚C for 24 hours. The bacterial suspension was then prepared and added to 100 mL of inorganic salt liquid medium at a 2% inoculum volume. The ingredients of the inorganic salt medium were (per liter): 1.0 g NaCl, 1.31 g $K_2HPO_4 \cdot 3H_2O$, 1.0 g $KH_2PO_4 \cdot 3H_2O$, and 10.0 mL trace element solution. The trace element solution contained 0.5 g $MgSO_4$, 0.17 g $MnSO_4 \cdot 4H_2O$, 0.12 g H_3BO_3, 0.12 g $ZnSO_4 \cdot 7H_2O$, and 0.3 g $FeSO_4 \cdot 7H_2O$ in 1 L of distilled water. Aniline was used as the sole source of carbon, nitrogen, and energy. The concentration of aniline was gradually increased to 1000 mg/L, and strain cultivation was conducted with a 7-day domestication cycle. The acclimated bacterial suspension was then spread on inorganic salt solid medium (1000 mg/L aniline) and cultured at 30˚C until single colonies formed for the detection of the degradation rate.

2.2. Strain Characterization and 16S rRNA Identification

The strain was further characterized using biochemical and molecular methods. The cell size, shape, colony morphology, motility, and Gram stain reactions were observed using a scanning electron microscope (SEM, SU8100, HITACHI, Japan). The genomic DNA of the bacterial strain was extracted from a fresh bacterial culture using the Ezup Pillar bacterial genomic DNA Extraction Kit (Sangon Biotech Co., Ltd, Shanghai, China). A portion of the 16S rRNA gene was amplified with a pair of forward (5'-AGAGTTTGATCCTGGCTCAG-3') and reverse (5'-CTACGGCTACCTTGTTACGA-3') primers. The amplified products were purified using agarose gel electrophoresis, and sequencing was carried out at Sangon Biotech Company (Shanghai, China). The nucleotide sequence was submitted to the GenBank database for comparison and identification using the BLAST software package (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rRNA gene sequence of the newly isolated strain SW-1 has been deposited in GenBank under the accession number OQ651225. For comparison, the complete 16S rRNA gene sequence of the reference strain *Delftia lacustris* (GenBank: AP0255556) was used. A maximum likelihood phylogenetic tree was generated by the neighbor-joining method using MEGA 7.

2.3. Growth and Aniline Degradation

A liquid inoculum of strain SW-1 was cultured in 500 mL flasks containing 100 mL of liquid nutrient broth medium. The flasks were kept on a rotary shaker at 160 rpm at 30˚C. The cultivated bacterial solution was centrifuged at 6000 rpm for 10 minutes, resuspended with physiological saline, and all initial cell densities were controlled by adjusting OD_{600} to around 0.8. The bacterial suspension was prepared and stored at 4˚C for future use. The bacterial suspension of strain SW-1 was inoculated at 2% into an inorganic salt medium containing 500 mg/L, 1000 mg/L, 1500 mg/L, 2000 mg/L, 2500 mg/L, 2800 mg/L, and 3000 mg/L of aniline and incubated at 30˚C at 160 rpm. Samples were taken every 4 hours to measure the concentration of aniline and calculate its degradation rate. The sample with an aniline concentration of 1000 mg/L was selected to measure the absorbance value at OD_{600} to represent the growth rate of strain *D. lacustris* SW-1. All experiments were carried out in triplicate sets to ensure data consistency.

2.4. Effect of pH Values, Temperature, Shaker Speed, and Inoculation Amount on Aniline Biodegradation

The evaluation of different environmental factors for strain SW-1 was conducted using the optimal aniline concentration obtained from the tests described in Section 2.3. The factors tested included: pH range: 4.0 - 9.0 with increments of 1.0 pH unit; Incubation temperature range: 15˚C - 40˚C with increments of 5˚C; Shaker speed range: 0 - 200 rpm with increments of 40 rpm; Inoculation amounts: 0.25%, 0.5%, 1%, 2%, 3%, and 4%; In each test, both aniline concentrations and cell growth were investigated. All tests were performed in triplicate.

2.5. Study on the Co-Metabolism of Aniline Degradation by Strain *D. lacustris* SW-1

The study was carried out using the optimal aniline concentration obtained from the results of the tests described in Section 2.3. The experimental setup included adding 1 g/L of different carbon sources (maltose, glucose, sodium acetate, lactose, sucrose), 1 g/L of different nitrogen sources (ammonium sulfate, ammonium chloride, urea, ammonium bicarbonate), 1 g/L of different vitamins (vitamin B_1, vitamin B_2, vitamin C, folic acid), and 200 mg/L of different phenols (catechol, phenol) to each group of inorganic salt liquid medium. In each experimental group, the medium without the corresponding additive was used as the blank control. After 28 hours of cultivation, the OD_{600} value of bacterial growth and the aniline degradation rate were measured and analyzed.

In this study, catechol and phenol were chosen as exogenous phenolic compounds for co-metabolism experiments, mainly because of their crucial roles in the microbial degradation pathways of aromatic compounds. Phenol is a key intermediate metabolite in the aniline degradation pathway, whereas catechol acts as a central node for aromatic ring cleavage, commonly involved in the aerobic degradation of various aromatic compounds, including aniline and phenol. Therefore, investigating the co-metabolic effects of these two compounds on aniline degradation by the strain aids in elucidating the complete degradation pathway and determining whether the exogenous addition of key intermediates can enhance the removal of the initial substrate (aniline) by priming downstream metabolic steps or alleviating the accumulation of critical intermediate products.

2.6. RT-qPCR Essay

The primer and probe sets used for the qPCR are shown in (**Table 1**). The qPCR assay was performed in triplicate for each template DNA extracted from the activated sludge samples or microorganisms using the Real-Time PCR System (Shanghai Personal Biotechnology, China). To each PCR tube, 10 μL of 2× SYBR real-time PCR pre-mixture was added, followed by 1 μL of cDNA, and 0.4 μL each of forward and reverse primers (final concentration, 10 μM each). Finally, RNase-free water was added to a total volume of 20 μL. The thermal profile per cycle was as follows: 10 min at 95˚C for pre-denaturation, 15 s at 95˚C for denaturation, and 40 s at 60˚C for annealing and extension. Forty cycles were programmed per run. The experimental data were analyzed using the $2\text{-}\Delta\Delta CT$ method to calculate the gene expression levels according to the Formula (2-1).

Table 1. Shows the sequences of primers used for RT-qPCR analysis of aniline degradation genes.

Catechol bis Oxygenase	Primers	Sequence 5' → 3'
Catechol 1,2-di Oxygenase	*cat*A-F1	CCGCTATCTTTACAGGTACATCA
	*cat*A-R2	TCAACTAACGGGGCAGGTTA
	*cat*A-F1	CGTGAACGCCCATAGAGAAT
	*cat*A-R2	TGGTGACCAGATGCCAGAAA
Catechol 2,3-bis Oxygenase	*tadC1*-F1	AGCACTACGAAGAGGTCCTG
	tadC1-R1	GCCTCCAGATCCTCGTCTTT
	*tad*C2-F1	TATTTCAAGACCCGCTCCGA
	*tad*C2-R2	TGTCAAAGTCTTCCAGCGTG
	*tad*C2-F1	CAGGGCACGACGATTTACTT
	*tad*C2-R2	TTGCGGTCGTGGTAGAAGAT
Internal reference	16S-F	AAAGCCTGATCCAGCAATGC
	16S-R	GATTAACGCTCGCACCCTAC

$$Ratio\left(\frac{test}{calibrator}\right) = 2^{-\left[\Delta C_T(test) - \Delta C_T(calibrator)\right]} \quad (2\text{-}1)$$

2.7. Determination of Aniline

The content of aniline was determined by N-(1-naphthyl) ethylenediamine azo spectrophotometry (GB/T 11889-1989). To avoid temperature effects, all experimental reactions were conducted in a 25˚C water bath. The standard curve of aniline is shown in (**Figure 1**).

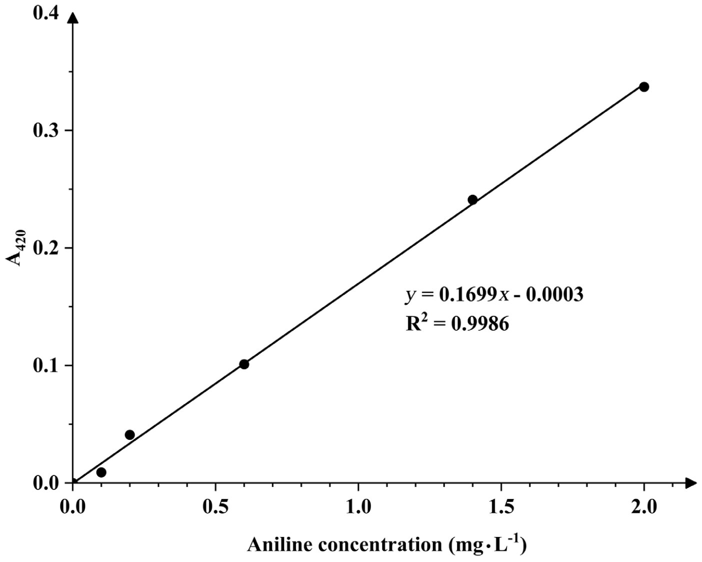
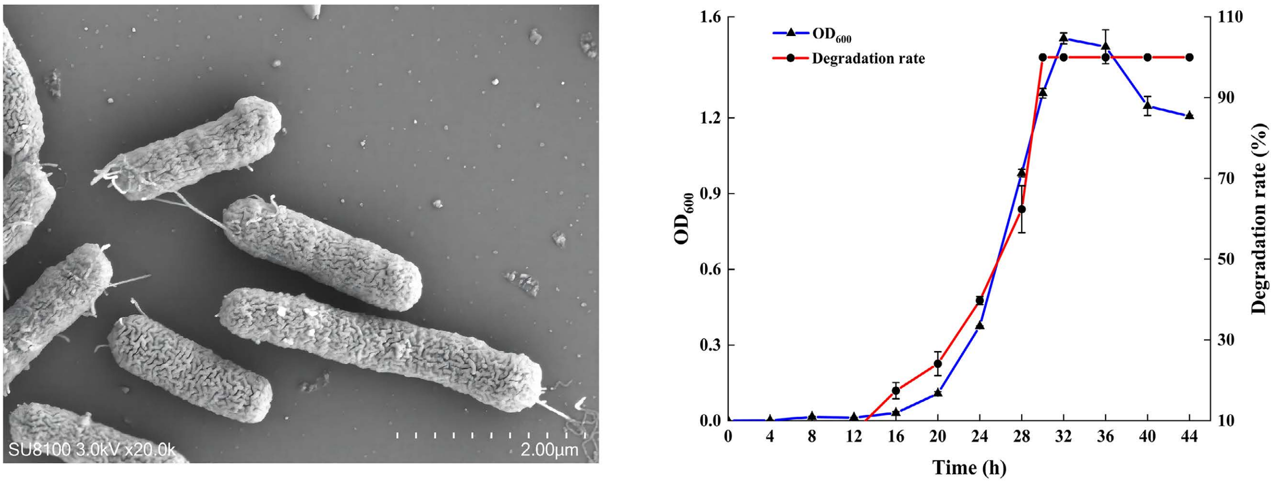


Figure 1. Standard curve of aniline concentration (All data figures were created by Origin).

3. Results

3.1. Isolation and Characterization of the Strain

The sentence starts here. Five strains were isolated using a multistep enrichment and screening technique based on morphological distinctions. One of them, named SW-1, was purified for further tests. The colony of this bacterium on aniline inorganic salt solid culture medium is circular, milky white, and opaque, with a smooth surface and flat edges. The size of the strain is 0.4 - 0.6 μm × 2.5 - 3.0 μm, and it has a rod-shaped morphology. The morphology of the strain under SEM is shown in (**Figure 2(a)**).



(a)

(b)

Figure 2. Scanning electron micrographs of cells of strain SW-1 with a magnification of 20.0 k (a). *D. lacustris* SW-1 growth and aniline degradation curve. Values shown are means from triplicate experiments ± standard deviation (b).

The second paragraph starts here. The time courses of aniline degradation rate in the medium and cell growth are shown in (**Figure 2(b)**). The lag phase lasted 20 hours, during which there was a slight increase in aniline degradation. In the logarithmic phase of cell growth, the aniline biodegradation rate increased sharply. A similar trend was observed in the OD_{600} curve. More than 99.98% of 1000 mg/L aniline was degraded within 32 hours, with OD_{600} increasing to 1.514 ± 0.220, indicating that strain SW-1 grew substantially well in the flask. OD_{600} decreased moderately after 36 hours because the substrate became a limiting factor in the metabolic activity of the SW-1 strain.

3.2. Identification of the Strain SW-1 Was Based on 16S rRNA

Strain SW-1 was identified by sequence analysis of the amplified 1423-bp segment of its 16S rRNA gene. The 16S rRNA sequence of strain SW-1 showed 99.86% similarity to the complete sequence of the *Delftia lacustris* 16S rRNA gene (GenBank: AP0255556). The relationship of strain SW-1 to closely related members of Delftia is shown in (**Figure 3**).

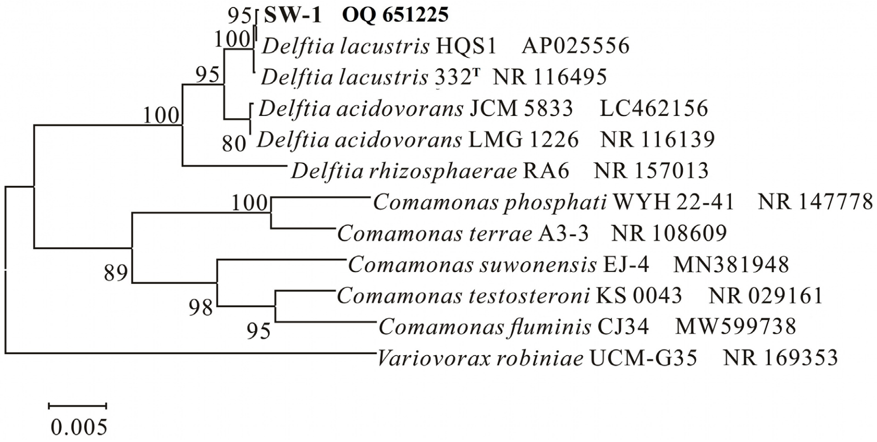


Figure 3. Phylogenetic tree of strain SW-1 based on 1423 unambiguous nucleotides of the 16S rRNA sequence.

3.3. Utilization of Aniline

As shown in (**Figure 4**), strain SW-1 was able to metabolize substrates at both low and relatively high concentrations of aniline. It was found that the isolate was capable of completely removing aniline within 32 hours when the initial aniline concentration was below 1000 mg/L. When the concentration of aniline was 1500 - 2800 mg/L, the degradation rates of strain SW-1 were 93.83% ± 1.57%, 81.99% ± 3.50%, 53.63% ± 3.46%, and 27.23% ± 4.48% at 40 hours, respectively. The degradation rate of strain SW-1 was essentially 0 within 40 hours at an aniline concentration of 3000 mg/L. Based on this comprehensive consideration, an aniline concentration of 1000 mg/L was selected as the focus of subsequent experiments, as strain SW-1 exhibited the highest degradation rate at this concentration.

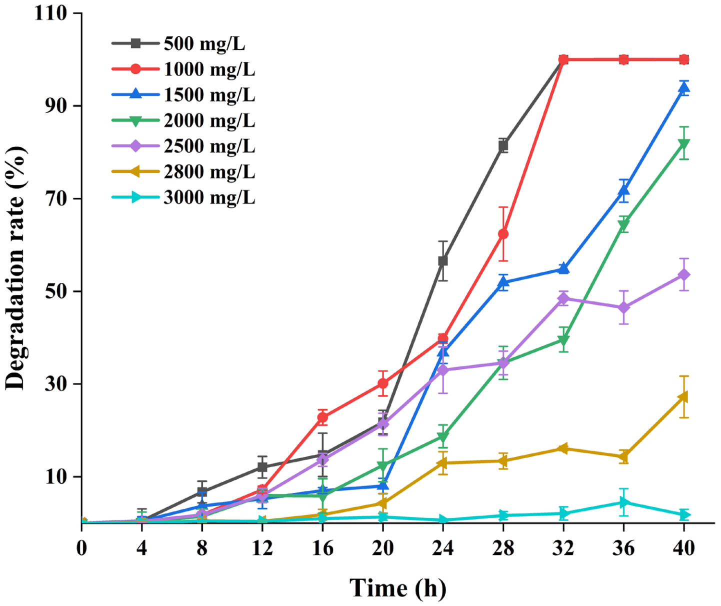
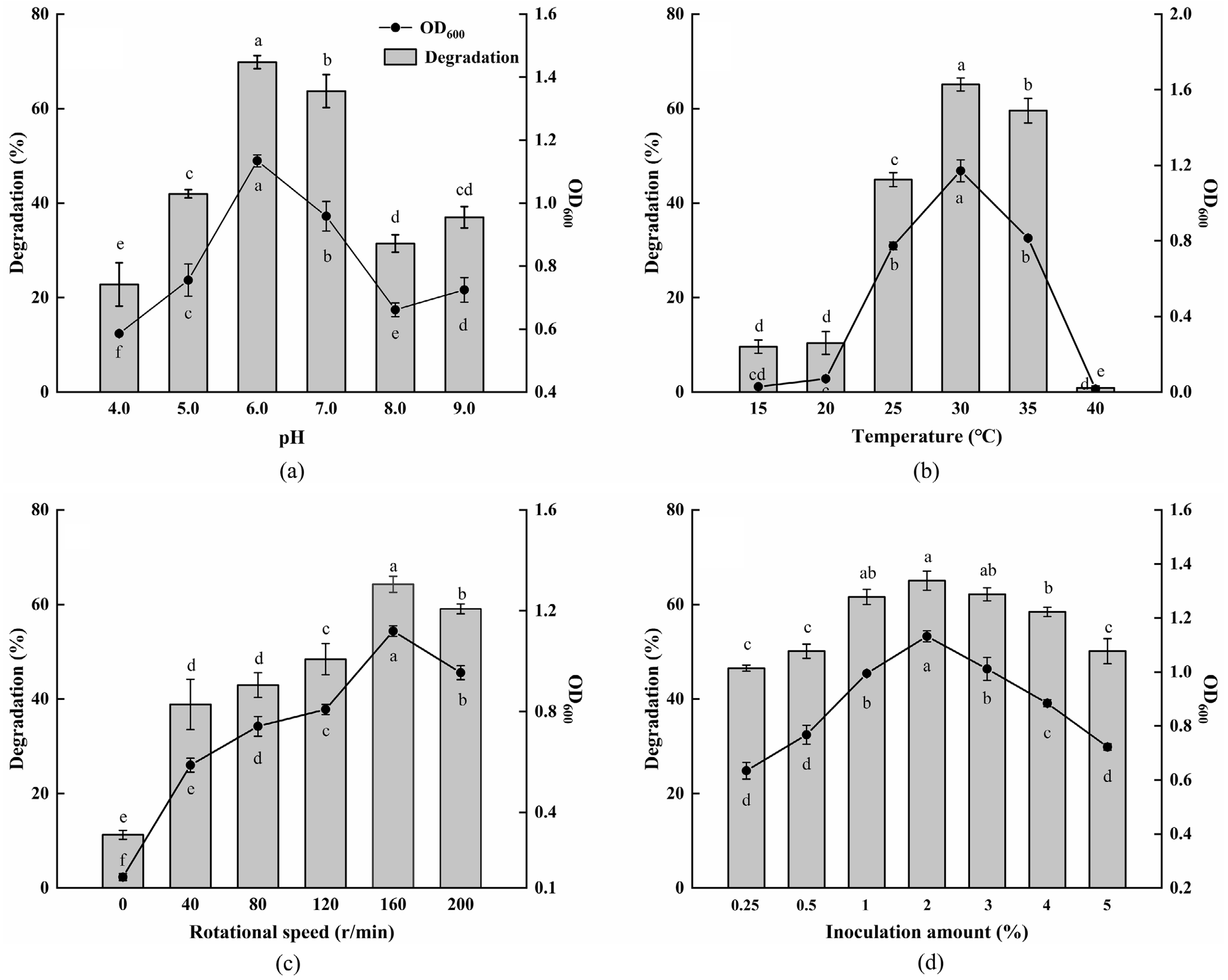


Figure 4. Degradation curves of aniline at different concentrations in strain SW-1. Values shown are means from triplicate experiments ± standard deviation.

3.4. Effect of pH Values, Temperature, Shaker Speed, and Inoculation Amount on Aniline Biodegradation

The results, shown in (**Figure 5**), indicate that strain SW-1 exhibits strong adaptability in both acidic and alkaline environments. At a pH of 6.0, the maximum degradation rate was 69.83% ± 1.41% ($P < 0.05$), and the maximum OD_{600} was 1.134 ± 0.02 ($P < 0.05$). The highest degradation and growth rates of the strain were observed at a temperature of 30˚C ($P < 0.05$), with a degradation rate of 65.11% ± 1.38% and an OD_{600} of 1.171 ± 0.058. At a rotational speed of 160 r/min, strain SW-1 exhibited the highest degradation rate of aniline and OD_{600} ($P < 0.05$), with a degradation rate of 64.27% ± 1.69%. When the inoculation amount was 2%, strain SW-1 showed the highest degradation rate of aniline and OD_{600}, with a degradation rate of 65.04% ± 2.02%.



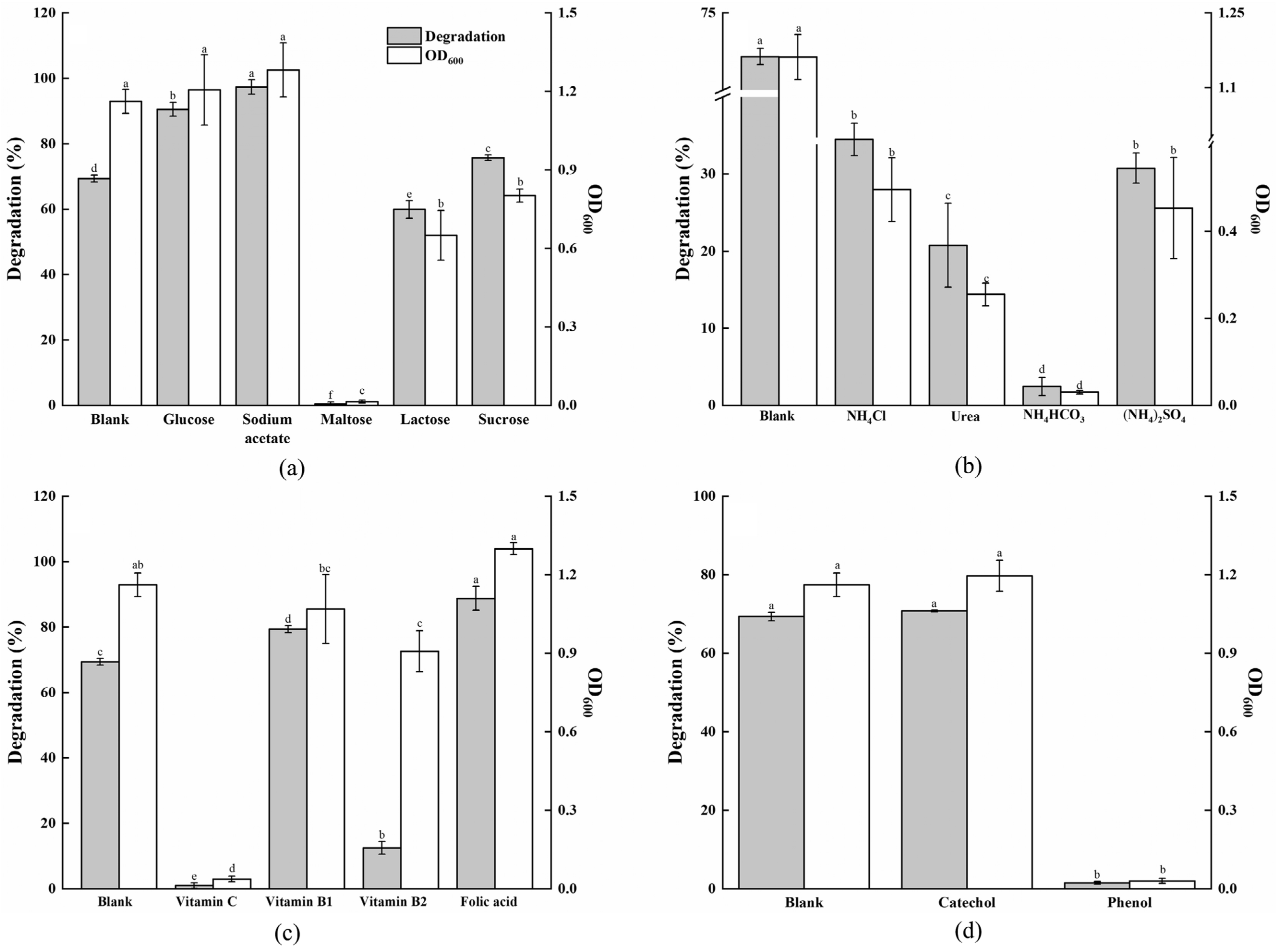
(a) (b)

(c) (d)

Figure 5. The effects of different pH (a), temperature (b), rotational speed (c), and inoculation amount (d) on aniline removal (1000 mg·L⁻¹) by strain SW-1. Values shown are means from triplicate experiments ± standard deviation.

3.5. The Effect of the Co-Metabolic Matrix on Aniline Degradation

For the degradation of aniline, the addition of glucose and sodium acetate improved the degradation rate compared to the blank group (**Figure 6(a)**). The best effect was observed with sodium acetate ($P < 0.05$), achieving a degradation rate of 97.36% ± 2.14%, which is 28.02% ± 2.14% higher than that of the blank group. The addition of co-metabolic nitrogen sources resulted in a certain degree of reduction in the degradation and growth of aniline compared to the blank group (**Figure 6(b)**). The addition of folic acid significantly enhanced the degradation of aniline by strain SW-1, with a degradation rate of 88.77% ± 3.69% ($P < 0.05$), which was 19.43% ± 3.69% higher than that of the blank group (**Figure 6(c)**). The addition of catechol did not significantly affect the degradation of aniline and the growth of the strain compared to the blank group, while the addition of phenol severely inhibited the degradation of aniline and the growth of the strain (**Figure 6(d)**).



(a)

(b)

(c)

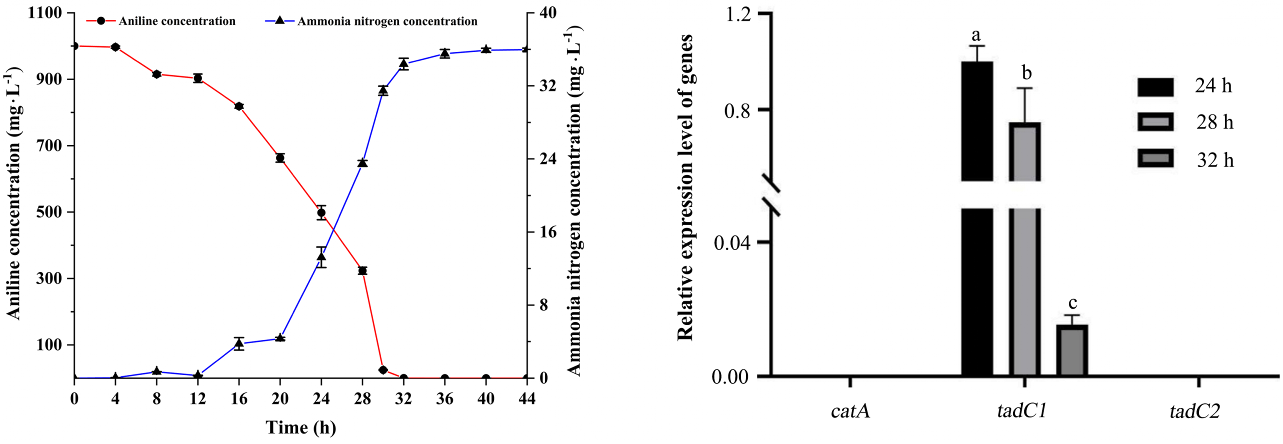
(d)

Figure 6. The effect of co-metabolic carbon source (a), nitrogen source (b), vitamins (c), and phenolic substances (d) on the degradation of aniline and growth of strain SW-1. Values shown are means from triplicate experiments ± standard deviation.

3.6. Exploring the Degradation Pathway of Aniline by *D. lacustris* SW-1

From 0 - 12 hours, strain *D. lacustris* SW-1 is in the adaptation phase, and the concentrations of aniline and ammonia nitrogen remain basically unchanged.

However, from 12 - 32 hours, the concentration of aniline rapidly decreases while the concentration of ammonia nitrogen rapidly increases. After 32 hours, aniline is almost completely degraded by strain SW-1, and the concentration of ammonia nitrogen remains stable (**Figure 7(a)**). The downward trend in aniline concentration is correlated with an upward trend in NH_4^+-N concentration, confirming that the initial step in the degradation of aniline by the strain involves its conversion to catechol, concomitant with the release of ammonia nitrogen. Between 20 - 36 hours, black-brown substances were observed in the culture medium. From 24 - 32 hours, the expression level of the *tadC1* gradually decreases as aniline is degraded (**Figure 7(b)**).



(a) (b)

Figure 7. Changes in the contents of aniline and ammonia nitrogen during the aniline degradation of strain SW-1 (a). Values shown are means from triplicate experiments ± standard deviation. The expression of the catechol dioxygenase gene during the aniline degradation of strain SW-1 ($P < 0.05$) (b).

The observed downregulation of *tadC1* expression during aniline degradation is primarily attributed to substrate depletion and associated transcriptional regulation. During the initial phase (12 - 32 hours), aniline serves as both an inducer and an energy source, activating the transcription of degradation pathway genes, including *tadC1*, to support rapid bacterial growth and metabolism. As aniline is nearly depleted after 32 hours, the induction signal diminishes, leading to reduced transcription of *tadC1* as high-level enzyme synthesis becomes unnecessary. This reflects an efficient resource allocation strategy. Additionally, accumulated intermediates (catechol) or end products (ammonia) may exert feedback inhibition, directly or indirectly suppressing *tadC1* expression to prevent metabolic overload. Thus, the dynamic expression of *tadC1* underscores the precise adaptation of D. *lacustris* SW-1 to substrate availability through coordinated genetic regulation.

4. Discussion

In the preliminary work of this study, multiple methods were used to screen for aniline-degrading strains [18]. Strategies such as direct screening and post-enrichment screening made it difficult to obtain efficient aniline-degrading strains [19] [20]. However, strain SW-1, which efficiently degrades aniline, was obtained through domestication [21]. When the concentration of aniline was 1000 mg·L^{-1}, the highest degradation rate of aniline reached 99.995%, approaching complete degradation. The domestication method is beneficial for microorganisms to better adapt to the toxicity of aniline, enabling the acquisition of efficient aniline-degrading strains [22]. Therefore, when using biological methods to treat toxic pollutants, domestication can be employed to obtain the target strain.

In this study, it was found that when the concentration of aniline was 500 - 2800 mg·L^{-1}, strain SW-1 could degrade aniline within 40 hours, with a maximum degradation rate of 100%. However, when the concentration of aniline was 3000 mg·L^{-1}, strain SW-1 exhibited almost no degradation of aniline, indicating that high concentrations of aniline have an inhibitory effect on the strain. This finding is consistent with previous research results [23]. Many environmental factors affect the growth and metabolic characteristics of microorganisms, such as temperature, pH, and oxygen. Among them, pH can affect the activity of microorganisms and even change the ionization state of functional groups [24] and the three-dimensional state of enzymes [25]. For most microorganisms, temperature changes affect their enzyme activity, and temperatures below optimal values can negatively affect the fluidity of biofilms and hinder the normal operation of transport systems, thereby affecting microbial growth [26]. The results of this study found that strain SW-1 can grow and degrade aniline in weak acidic and alkaline environments, as well as at speeds of 40 - 200 r/min. This indicates that strain SW-1 has strong adaptability to the environment. Studying the aniline degradation characteristics of strain SW-1 can provide a theoretical basis for its application in the actual treatment of aniline pollution.

When exploring the effects of different carbon sources on the degradation of aniline in this experiment, carbon sources such as glucose, sucrose, and sodium acetate were added as co-metabolic substrates [27]. Strain SW-1 showed an increase in the degradation rate of aniline, with sodium acetate having the best effect and reaching the highest degradation rate of 97.36% ± 2.14%. This is consistent with the reported research results on strains that degrade aniline [28] [29]. Strain *D. lacustris* 332T cannot utilize maltose, D-glucose, α-lactose, glycogen, and other carbohydrate substances to grow [30]. When exploring the effects of different nitrogen sources on the degradation of aniline in this experiment, the addition of ammonium sulfate, ammonium chloride, urea, and ammonium bicarbonate to a certain extent inhibited the degradation of aniline and the growth of the strains, which is consistent with previous research results [31]. When studying the effect of adding a co-metabolic substrate on the degradation of aniline by aniline-degrading strains, some studies found that vitamin C was the optimal co-metabolic substrate [32]. However, in this study, it was found that adding vitamin C as a co-metabolic substrate inhibited the growth of strain SW-1 and the degradation of aniline, which differed from previous research results. It is speculated that the reason may be due to the different dosages of vitamin C used in the studies. The previous studies used a dosage ratio of 1:8 and 1:10 for vitamin C and aniline, respectively, whereas this study used a dosage ratio of 1:1. Excessive addition of vitamin C may cause the strain to be unable to utilize it for growth and inhibit the degradation of aniline. Different phenols have different effects on the degradation of aniline, and the results of this study are consistent with this [33]. Therefore, after comprehensive consideration, sodium acetate was selected as the optimal co-metabolic substrate for strain SW-1 to degrade aniline, providing a theoretical basis for the application of strain SW-1 in actual aniline wastewater treatment. In this study, it was found that strain *D. lacustris* SW-1 can grow using carbohydrates, which is different from previous studies. Therefore, further research can be conducted on this aspect in the future.

It is widely accepted in current research that there are two primary pathways for the aerobic degradation of aniline by bacteria: aniline is transformed into catechol through the action of aniline dioxygenase within bacterial cells [34]-[36], with the concurrent release of NH_4^+. Subsequently, catechol undergoes degradation via either the meta-cleavage pathway, the ortho-cleavage pathway, or a combination of both. This study used RT-qPCR technology to measure the gene expression level of *tadC1* (encoding enzyme C23O) in strain *D. lacustris* SW-1, inferring that strain SW-1 can degrade aniline through the meta pathway [37] [38]. It is speculated that the genes *catA* (encoding enzyme C12O) and *tadC2* (encoding enzyme C23O) may not exist in strain SW-1 or their expression levels may be too low. Starting from genomics, transcriptomics, and proteomics, Lee *et al.* [39] conducted in-depth identification of the *Delftia* sp. K82 aniline degradation gene cluster and its specifically induced transcriptome and proteome, thereby verifying that the *Delftia* strain can degrade aniline through ortho and meta-metabolic pathways. Therefore, further in-depth analysis of the aniline metabolism pathway of strain SW-1 can be conducted from the perspectives of metabolomics, genomics, proteomics, and aniline-degrading enzyme activity.

5. Conclusion

The weak-acid-tolerant strain SW-1, identified as *Delftia lacustris*, was isolated from a selective culture using aniline as the sole source of carbon, nitrogen, and energy. Strain SW-1 was able to withstand a relatively wide range of aniline concentrations (0 - 2000 mg/L), pH (5.0 - 7.0), and temperatures (25˚C - 35˚C). Under the optimal conditions for aniline biodegradation (1000 mg/L aniline, pH 6.0, and 30˚C), 1000 mg/L of aniline was almost completely degraded within 32 hours. Moreover, strain SW-1 could efficiently utilize sodium acetate, glucose, and folic acid as co-metabolic sources of carbon and energy. RT-qPCR assay determined that strain SW-1 degrades aniline via the meta pathway.

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Data Availability Statement

Data will be made available on request.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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