Screening and UV-induced Mutagenesis Breeding of High-yield Chitinase-producing Strains from Soil

Hongbing QI, Chunquan KANG

School of Life Science &Technology, Lingnan Normal University, Zhanjiang 524048, China

Abstract [**Objectives**] To isolate chitinase-producing strains from soil and enhance their enzyme activity through UV mutagenesis, aiming to obtain high-yield mutant strains. [**Methods**] Using colloidal chitin as the substrate, chitinase-producing strains were preliminarily screened from soil samples collected from Cunjin Bridge Park in Zhanjiang. Target strain A was obtained after a secondary screening based on enzyme activity assay. Its growth curve was determined, and the optimal UV mutagenesis dose was established. Strain A was then subjected to UV mutagenesis, and the enzyme activities of mutant strains were compared with that of the wild type strain. High-activity mutants were selected and their genetic stability over 10 generations was examined. Strain A was identified by 16S rDNA sequence analysis. [**Results**] Three enzyme-producing strains were obtained from the primary screening, among which strain A was confirmed as the target strain in the secondary screening. Four superior mutants were obtained after UV mutagenesis; strains B_{14} and B_{27} exhibited the highest enzyme activities of 0.116 U/mL and 0.114 4 U/mL, respectively, representing 2.76 and 2.71 fold increases over the wild type strain A (0.042 1 U/mL). Both mutants maintained stable enzyme activity after 10 successive subcultures. Strain A was identified as belonging to the genus *Stenotrophomonas* based on 16S rDNA analysis. [**Conclusions**] UV mutagenesis can effectively increase the chitinase activity of strain A. Mutants B_{14} and B_{27} show good genetic stability and possess potential for further research and application development.

Key words Chitin, Chitinase, UV mutagenesis, screening, Identification

0 Introduction

Chitin is a biopolymer and ranks second in abundance in nature, only to cellulose[1]. It is an off-white, irregular solid with stable physicochemical properties. It is sparingly soluble in water, dilute strong acids, organic acids, common organic solvents, and alkalis, but is soluble only in concentrated inorganic strong acids such as concentrated hydrochloric acid and phosphoric acid[2]. Chitin can combine with calcium-containing inorganic salts and various proteins to form diverse structural components of living organisms[3]. In nature, chitin is extremely abundant in crustaceans, accounting for up to 85% of their exoskeletons; the hard exoskeleton composed of chitin effectively protects the bodies of these crustaceans[4].

In recent years, with economic development, human demand for crustaceans has been continuously increasing, and the number of processing plants for crustaceans such as shrimp and crabs has grown, leading to the accumulation of large quantities of chitin-rich crustacean waste. This not only causes environmental damage but also wastes substantial chitin resources[5]. Therefore, many scholars both domestically and internationally have paid increasing attention to the applied research on chitin and its derivatives.

Nevertheless, the inherent stability of chitin makes its efficient utilization through routine physicochemical methods challenging. In contrast, chitinase offers an effective solution, as it can degrade chitin efficiently, is sourced from diverse organisms, and involves environmentally benign processes. Hence, chitinase represents the key to unlocking the full potential of chitin resources. In nature, a wide variety of microorganisms produce chitinase, which catalyzes the hydrolysis of chitin, facilitating its biogeochemical cycling and contributing to ecological equilibrium[4]. The history of chitinase research dates back to 1905, when Benecke isolated a chitin-degrading bacterium and demonstrated its ability to grow on chitin as a sole nutrient source[6]. In a similar vein, Folpmers observed transparent zones around bacterial and fungal colonies cultured on chitin-based media, suggesting the existence of chitinolytic enzymes[6]. Later, chitinase was also detected in plants, viruses, and the intestinal tracts of animals by successive investigators.

Chitinase-producing microorganisms are ubiquitous in nature, with their primary habitats including soil, lakes, rivers, and oceans[7], and they are readily obtainable. Given their strong reproductive ability, large population sizes, high species diversity, and ease of cultivation, these microorganisms can generate substantial quantities of chitinase within a short period, thereby effectively satisfying the demand for chitinase in various applications. Furthermore, microbial chitinases exhibit a relatively stable pH optimum between 4 and 9, and remain active across a temperature range of 20 to 60 ℃, with optimal activity typically occurring at 40 -50 ℃[8]. By hydrolyzing the cell walls of pathogenic fungi, microbial chitinases can markedly suppress fungal growth[9]. Likewise, chitinase can degrade the insect cuticle, offering an effective strategy for pest management and thereby contributing significantly to agricultural protection[10]. In addition, the products derived from chitin hydrolysis, such as chitooligosaccharides, have been shown to inhibit tumor cell proliferation and boost im-

Received: June 25, 2026 Accepted: August 13, 2026

Supported by Zhanjiang Non-funded Science and Technology Research Program (2023B01023) University-level Educational and Teaching Reform Project of Lingnan Normal University (LSJW[2022]154).

Hongbing QI, doctoral degree, associate professor, research fields: microbial fermentation.

munity[11]. Therefore, these attributes endow chitinase with great potential for diverse applications in the food, agricultural, environmental, and chemical industries[12-13].

Despite the widespread distribution of chitinase-producing microorganisms in nature, their relatively low enzyme activity remains a major bottleneck for industrial applications[14]. To address this issue, the present study was designed to isolate and identify target strains with comparatively high enzyme activity from soil, followed by UV mutagenesis breeding, with the goal of generating mutant strains that exhibit markedly enhanced chitinase production.

1 Materials and methods

1.1 Materials

1.1.1 Soil sample. Soil samples were obtained from Cunjin Bridge Park, Chikan District, Zhanjiang, Guangdong Province.

1.1.2 Media and reagents. The NB liquid medium was prepared by dissolving 18 g of nutrient broth powder in distilled water, making up to 1 L, and adjusting the pH to 7.0. The primary screening solid medium contained 3 g peptone, 1 g yeast extract, 0.5 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.7 g KH_2PO_4, 15 g agar, and 50 mL of 5% (*w/v*) colloidal chitin, with the final volume adjusted to 1 L with distilled water and pH adjusted to 7.0. The basal solid medium consisted of 5 g beef extract, 5 g peptone, 1 g yeast extract, 3 g NaCl, 15 g agar, and 50 mL of 5% colloidal chitin, made up to 1 L with distilled water, pH 7.0. The slant solid medium was composed of 5 g beef extract, 2 g peptone, 1 g yeast extract, 0.3 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 15 g agar, and 0.7 g KH_2PO_4, with distilled water added to a final volume of 1 L and pH adjusted to 7.0.

N-acetyl-D-glucosamine (analytical grade) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd.; chitin (analytical grade) was obtained from Shanghai Macklin Biochemical Co., Ltd.; the bacterial genomic DNA extraction kit was sourced from Beijing Solarbio Science & Technology Co., Ltd.; and PCR primers were supplied by Invitrogen Corporation Shanghai Representative Office.

1.1.3 Reagents. Preparation of colloidal chitin[15]: Colloidal chitin was prepared as follows: 5.0 g of chitin powder was mixed with 100 mL of concentrated hydrochloric acid in a 250 mL beaker, and the mixture was stirred with a glass rod to form a yellow liquid. The beaker was then sealed with a stopper and stored at 4 ℃ for 24 h. After standing, the mixture was centrifuged at 4 000 r/min for 8 min. The supernatant was collected and neutralized to pH 7.0 with 40% NaOH solution and distilled water. The neutralized suspension was then centrifuged at 6 000 r/min for 10 min. The resulting milky white, viscous precipitate was collected as colloidal chitin.

Preparation of McIlvaine buffer: 1.92 g of citric acid solid was weighed and dissolved in 100 mL of distilled water; 1.42 g of disodium hydrogen phosphate was weighed and dissolved in 100 mL of distilled water. Finally, 100 mL of 0.1 mol/L citric acid solution was mixed with 62.5 mL of 0.1 mol/L disodium hydrogen phosphate solution to obtain the McIlvaine buffer.

Preparation of DNS reagent[16]: DNS reagent was prepared by dissolving 3.15 g of 3, 5-dinitrosalicylic acid in 500 mL of distilled water with constant stirring. The solution was then placed in a 45 ℃ water bath, and 100 mL of 0.2 g/mL sodium hydroxide solution was added slowly. After complete dissolution, 91.0 g of potassium sodium tartrate tetrahydrate, 2.50 g of phenol, and 2.50 g of anhydrous sodium sulfite were added successively, with thorough stirring between additions. The resulting mixture was stirred until all components were fully dissolved, then allowed to cool to room temperature and brought to a final volume of 1 L with distilled water. The prepared reagent was stored in an amber bottle and left at room temperature for 7 d prior to use.

1.1.4 Main instruments. The following instruments were used in this study: ZQTY-90S fully controlled shaking incubator (Shanghai Zhichu Instrument Co., Ltd.), TG16-WS benchtop high-speed centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd.), 752N UV-Vis spectrophotometer (Shanghai Yidian Analytical Instrument Co., Ltd.), DYY-6C electrophoresis apparatus (Beijing Liuyi Biotechnology Co., Ltd.), WFH-201B dark-box UV transilluminator (Shanghai Jingke Industrial Co., Ltd.), and A300 thermal cycler (Hangzhou LongGene Scientific Instrument Co., Ltd.).

1.2 Methods

1.2.1 Preliminary screening of chitinase-producing strains. Fresh soil samples were weighed and added to 200 mL of sterile physiological saline, thoroughly mixed, and allowed to stand for 5 min. The supernatant was collected and filtered through gauze. Then, 5 mL of the filtrate was inoculated into sterilized NB liquid medium and incubated in a constant-temperature shaker at 30 ℃ and 180 r/min for 24 h. Subsequently, 1 mL of the bacterial suspension was transferred into a test tube containing 9 mL of sterile water for 10-fold serial dilution. Aliquots of 0.1 mL from each dilution series were spread onto the primary screening solid medium and incubated at 30 ℃ in a constant-temperature incubator for 5 d. Single colonies that formed transparent zones were selected, and the diameter of the transparent zone (D) as well as the colony diameter (d) were recorded.

1.2.2 Preparation of the standard curve for N-acetylglucosamine. 0.1 g of N-acetylglucosamine was weighed and dissolved in 100 mL of distilled water to prepare a 0.1% standard sugar solution. Then, 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mL aliquots of the standard solution were transferred into nine graduated test tubes with stoppers, numbered 1 to 9, and the volume in each tube was made up to 2 mL with distilled water. After thorough mixing, 1 mL of freshly prepared DNS solution was added to each tube, and the tubes were heated in a boiling water bath for 10 min, then cooled to room temperature under running water, and finally diluted to 10 mL with distilled water. Using tube No. 1 (blank) as the control, the absorbance of each tube was measured at 540 nm. A standard curve was plotted with the

amount of N-acetylglucosamine (mg) on the *x*-axis and the absorbance on the *y*-axis[17].

1.2.3 Assay of chitinase activity. The strains obtained from the preliminary screening were subjected to a secondary screening based on enzyme activity, in order to obtain the strain with the highest enzyme activity. One unit of chitinase activity was defined as the amount of enzyme required to produce 1 mol of N-acetylglucosamine reducing sugar per minute at 50 ℃ and pH 7.0[5]. Each strain was inoculated into NB liquid medium and cultured in a constant-temperature shaker at 32 ℃ and 180 r/min for 3 d, after which the enzyme activity of the fermentation broth was determined[4].

The detailed procedure was performed as follows[4]: A 5 mL aliquot of the fermentation broth was centrifuged at 6 000 rpm for 10 min. One milliliter of the supernatant was then combined with 1 mL of buffer and 1 mL of 5% colloidal chitin. The reaction mixture was incubated at 50 ℃ in a water bath for 60 min, and the reaction was stopped by heating in a boiling water bath for 5 min. After cooling with tap water, the mixture was centrifuged at 10 000 rpm for 5 min. To the supernatant, 1 mL of DNS reagent was added, and the mixture was heated at 100 ℃ for 10 min, followed by cooling under running water. The solution was then centrifuged at 5 000 rpm for 5 min, and the supernatant was collected and made up to 10 mL with distilled water. The absorbance was recorded at 540 nm. The enzyme activities of the strains were computed using the formula given below, and the strain exhibiting the highest activity was chosen as the experimental strain for further study.

Enzyme activity calculation Equation: Enzyme activity (U/mL) $= m \times n \times 1\,000/(M \times T \times V)$ (1)

where m is the amount of N-acetylglucosamine, M is its molecular weight (221.21), n is the dilution factor, T is the reaction time, and V is the volume of the enzyme solution.

1.2.4 Determination of the growth curve of the target strain. To determine the growth curve, 1 mL of the target strain inoculum (pre-cultured to the logarithmic phase) was transferred into fresh NB medium and incubated at 32 ℃ with shaking at 180 rpm. At 4-h intervals, aliquots were withdrawn, and the optical density at 600 nm (OD_{600}) was recorded. The growth curve was then plotted based on the measured absorbance values[18].

1.2.5 Determination of the optimal UV irradiation dose. To prepare the bacterial suspension for UV mutagenesis, 50 mL of the activated culture of the target strain was centrifuged at 4 000 rpm for 15 min. The harvested cells were washed twice with 30 mL of sterile physiological saline, with centrifugation between washes, and finally resuspended in sterile physiological saline to a final volume of 50 mL. The suspension was then thoroughly vortexed to ensure uniformity.

10 mL of the bacterial suspension was placed in a sterile Petri dish with a diameter of 9 cm. The dish was positioned 60 cm directly below a vertical UV lamp, and the UV light was turned on for irradiation. The irradiation times were set to 0, 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 sec, and the corresponding dishes were labeled as No. 0 to No. 10[19]. Under red light conditions, the UV-irradiated bacterial suspension was serially diluted to 10^{-5}, and then 50 μL aliquots were spread onto plates. After labeling, the plates were incubated at 30 ℃ in the dark for 24 h. The number of single colonies on each plate was counted. Using the sample No. 0 as the control, the lethality rate of the bacteria was calculated according to the following Equation:

Lethality rate (%) = [1 − (Number of viable colonies after UV irradiation/Number of viable colonies in the control)] × 100% (2)

To determine the optimal mutagenesis dose, a lethality curve was constructed by plotting the lethality rate against UV irradiation time. The irradiation time that yielded a lethality rate in the range of 70% − 80% was chosen as the optimal dose[13].

1.2.6 UV mutagenesis. To perform UV mutagenesis, a bacterial suspension of the wild type strain was prepared. Ten milliliters of this suspension was transferred into a sterile Petri dish and exposed to UV irradiation at the predetermined optimal dose[20-21]. After treatment, the mutagenized suspension was serially diluted to 10^{-5} under red safety light, and 50 μL aliquots were spread onto primary screening agar plates. The plates were labeled and incubated in the dark at 30 ℃ for 48 h[22]. Following incubation, colonies were examined for morphological features, and the diameters of both the transparent zone (H, mm) and the colonies (C, mm) were measured. Subsequently, individual mutant colonies obtained from the primary screening were picked and inoculated onto basal medium, then further incubated at 30 ℃ in the dark for 24 h.

1.2.7 Genetic stability test. To evaluate genetic stability, the mutant exhibiting the highest enzyme activity was serially subcultured on basal medium using the streak plate method. Each subculture was incubated for 24 h, and the process was repeated for 10 consecutive generations. The enzyme activity of the 10^{th}-generation culture was then measured and compared with that of the original mutant[22].

1.2.8 Morphological identification of the target strain. To identify the morphological properties of the target strain, the purified wild type strain A was plated onto chitin-containing solid medium and cultured at 30 ℃ for 36 h in a constant-temperature incubator. After incubation, the colonial morphology was recorded. The strain was then characterized by Gram staining and examined under a light microscope to determine its Gram reaction.

1.2.9 Molecular identification of the target strain. (i) DNA extraction of the strain. 1 mL of bacterial culture was taken and centrifuged at 12 000 r/min for 1 min. The supernatant was discarded, and this step was repeated once. Then, 200 μL of Solution A was added to resuspend the cell pellet, followed by the addition of 20 μL of RNase A (10 mg/mL). After thorough mixing, the mixture was incubated at room temperature for 15 − 30 min. Subsequently, 20 μL of proteinase K (10 mg/mL) was added, mixed well, and incubated at 55 ℃ for 30 − 60 min until the sample was completely digested (as indicated by a clear and viscous bacterial

suspension).

200 μL of Solution B was added and mixed thoroughly. Then, 200 μL of absolute ethanol was added and mixed well. The resulting solution and precipitate were transferred to a spin column and allowed to stand for 20 min. After centrifugation at 12 000 r/min for 2 min, the flow-through was discarded, and the spin column was placed back into the collection tube. Subsequently, 600 μL of wash solution (wash solution : absolute ethanol = 1 : 4) was added to the spin column, followed by centrifugation at 12 000 r/min for 1 min. The flow-through was again discarded, and the column was returned to the collection tube. The column was then centrifuged at 12 000 r/min for an additional 2 min. After centrifugation, the spin column was left open at room temperature for several minutes, then transferred to a new microcentrifuge tube. 50 – 200 μL of elution buffer preheated at 65 ℃ in a water bath was added dropwise onto the center of the membrane. After standing at room temperature for 5 min, the column was centrifuged at 12 000 r/min for 1 min. The eluate was reapplied to the same spin column, allowed to stand at room temperature for 2 min, and centrifuged again at 12 000 r/min for 2 min, yielding high-quality bacterial genomic DNA.

(ii) PCR amplification and sequencing. The PCR reaction system was composed of 0.6 mL of MightyAmp, 15 mL of Buffer, 0.75 mL of forward primer 27F, 0.75 mL of reverse primer 1492R, 2 mL of template DNA, and 10.9 mL of pure water.

The PCR amplification program was as follows: pre-denaturation at 98 ℃ for 2 min, denaturation at 98 ℃ for 10 sec, annealing at 58 ℃ for 15 sec, extension at 68 ℃ for 1.5 min, for 35 cycles, with a final extension at 68 ℃ for 10 min.

After amplification, the products were subjected to agarose gel electrophoresis. The PCR products with clear bands were sent to Shanghai Lifei Biotechnology Co., Ltd. for sequencing. The sequencing results were submitted to the NCBI database for BLAST analysis, and a phylogenetic tree was constructed using MEGA 6.0 software.

1.2.10 Data analysis. Data processing and significance analysis for differences were performed using EXCEL 2019 and SPSS 25.0, and the phylogenetic tree was constructed using MEGA 6.0 software.

2 Results and analysis

2.1 Preliminary screening of chitinase-producing strains

After enrichment culture of the soil samples, the bacterial suspension was subjected to a series of gradient dilutions and spread onto the primary screening solid medium. Three strains with obvious transparent zones were selected and designated as No. 1, No. 2, and No. 3. Their morphological characteristics were as follows: No. 1 was a pinkish, slightly moist colony with a smooth and shiny surface and no liquefaction at the bottom; No. 2 was a grayish-white, dense colony with moderate luster and slight liquefaction at the bottom; No. 3 was a black, moist colony with dull color and no liquefaction at the bottom. In terms of distribution, strains No. 1 and No. 2 were widely distributed, whereas strain No. 3 was only found on plates at dilutions of 10^{-3}, 10^{-4}, and 10^{-5}, with relatively few colonies.

Plates with transparent zones and well-separated colonies were selected for measurement. The diameters of the transparent zone (D) and the colony (N) were determined for each strain, and the D/N ratio was calculated. The results are shown in Table 1; strain No. 2 exhibited a relatively higher D/N ratio.

Table 1 Transparent zone diameter and colony diameter of each strain

Strain No.	Transparent zone diameter D//mm	Colony diameter N//mm	D/N
1	9.2	5.6	1.64
2	11.3	6.1	1.85
3	4.0	3.0	1.33

On the primary screening plates, enzyme activity cannot be judged solely based on the size of the transparent zone of the colony; the brightness, colony size, and degree of liquefaction at the bottom of the colony should also be taken into comprehensive consideration. Therefore, further enzyme activity identification is required[5].

2.2 Plotting of the standard curve for N-acetylglucosamine

The standard curve is shown in Fig. 1. The N-acetylglucosamine content showed a linear relationship with the absorbance, and the regression equation was $y = 0.523\,9x - 0.001\,4$, with a correlation coefficient $R^2 = 0.998\,5$.

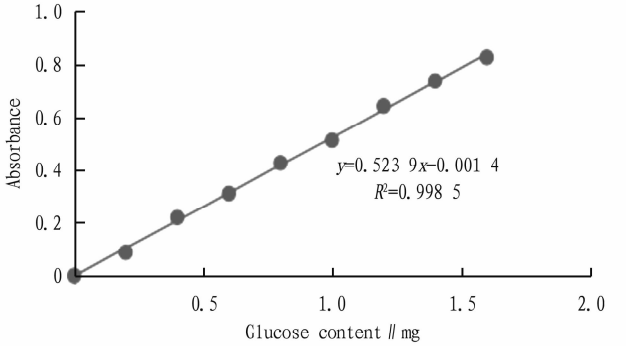


Fig. 1 N-acetylglucosamine standard curve

2.3 Enzyme activity assay of the primarily screened strains

The enzyme activities were determined, and the results are shown in Fig. 2. Within the same time period, strain No. 2 exhibited higher enzyme activity than strains No. 1 and No. 3. Therefore, strain No. 2 was selected as the target strain and designated as strain A.

2.4 Plotting of growth curve of the wild-type strain A The results are shown in Fig. 3. The growth curve could be divided into four phases: 0 – 8 h was the lag phase, 8 – 20 h was the logarithmic phase, 20 – 36 h was the stationary phase, and after 36 h, the culture entered the decline phase. During the logarithmic phase, the bacteria proliferated rapidly, exhibited typical physiological characteristics, and were sensitive to external environmental factors. Therefore, cells cultured for 10 h were selected for subsequent studies.

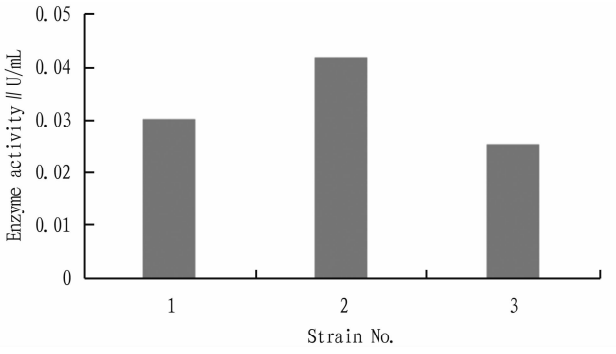


Fig. 2 Preliminary screening of enzyme activity of each strain

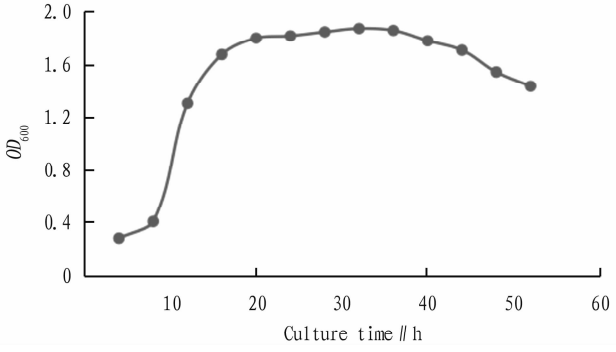


Fig. 3 Growth curve of the target strain A

2.5 Optimal UV mutagenesis dose The effect of different UV irradiation durations on the lethality rate of the target strain is shown in Fig. 4. When the irradiation time reached 240 sec, the lethality rate was 100%. Previous studies have shown that a lower lethality rate in UV mutagenesis is associated with a higher probability of positive mutations[19]. Therefore, an irradiation time of 90 sec, which corresponded to a lethality rate of 70% – 80%, was selected as the optimal UV mutagenesis dose in this experiment.

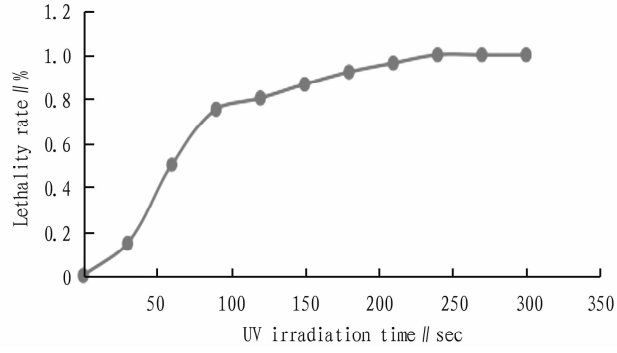


Fig. 4 Lethality curve for target strain A

2.6 Preliminary screening results of the mutagenized strains After UV mutagenesis, 58 colonies with different morphological characteristics were screened. Based on the H/C ratio (transparent zone diameter to colony diameter) of each mutant, four dominant strains with relatively high H/C ratios were selected and designated as B_5, B_{11}, B_{14}, and B_{27}. Their H/C ratios were 2.43, 2.76, 3.04, and 2.83, respectively (Fig. 5). As clearly shown in Fig. 6, the transparent zones of these four dominant mutants were all significantly larger than those of the wild type strain.

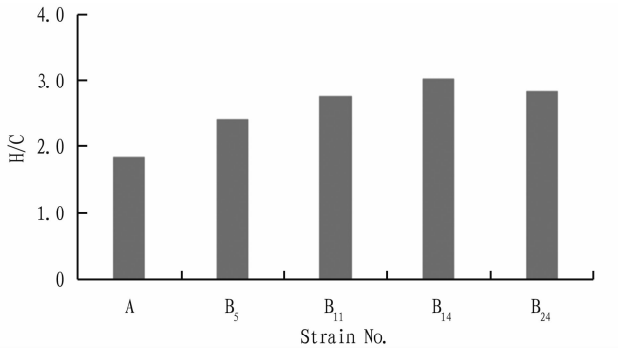


Fig. 5 Ratios of transparent zone diameter to colony diameter of the dominant mutants and wild type strain A

2.7 Secondary screening of enzyme activity To identify the mutant with the highest enzyme activity, the enzyme activities of the wild type strain A and the mutant strains B_5, B_{11}, B_{14}, and B_{27} were further compared. As shown in Fig. 7, all four mutants exhibited significantly higher enzyme activities than the wild type strain A ($P < 0.05$). Among them, mutant B_{14} showed the highest enzyme activity. The enzyme activity of mutant B_{27} was slightly lower than that of B_{14}, but the difference was not significant ($P > 0.05$). The enzyme activities of mutants B_5 and B_{11} were significantly lower than that of B_{14} ($P < 0.05$), and the difference between B_5 and B_{11} was also significant ($P < 0.05$). Therefore, mutants B_{14} and B_{27}, which displayed relatively higher enzyme activities, were selected for genetic stability testing.

2.8 Determination of genetic stability To evaluate genetic stability, mutants B_{14} and B_{27}, which exhibited the highest enzyme activities, were serially subcultured for 10 generations. The first-generation cultures were labeled 1-B_{14} and 1-B_{14}, while the tenth-generation cultures were designated 10-B_{14} and 10-B_{14}. Enzyme activities of the initial and final generations were then assayed and compared. As presented in Fig. 8, the enzyme activities of 10-B_{14} and 10-B_{14} did not differ significantly from those of their respective first-generation counterparts, suggesting that both mutants maintained stable enzymatic performance over successive subcultures.

2.9 Identification of the chitinase-producing strain

2.9.1 Culture characteristics of the strain. As shown in Fig. 9, the colonies were approximately circular, 2 – 3 mm in diameter, with a dull, grayish-white color, slightly raised, opaque, and surrounded by obvious transparent zones (hydrolysis zones).

2.9.2 Individual morphological characteristics of the strain. As can be seen from Fig. 10, strain A was short rod-shaped. The Gram staining result showed a red color, indicating that it was a Gram-negative bacillus.

2.9.3 Identification by 16S rDNA sequence analysis. After PCR amplification, the products were subjected to agarose gel electrophoresis. The results are shown in Fig. 11, in which lane B12 corresponds to strain A, and the DNA band size was approximately 1 400 bp.

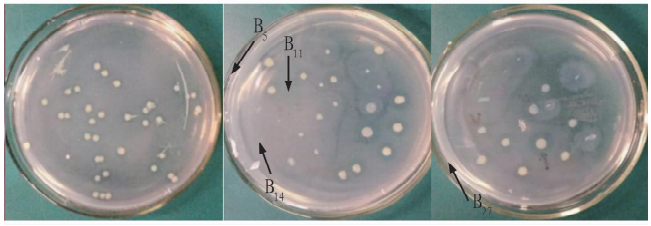


Fig. 6 Media of the control group, mutant group 1, and mutant group 2

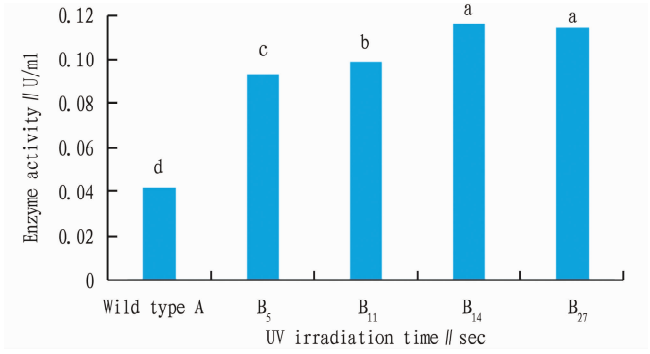


Fig. 7 Comparison of enzyme activity between wild type A and each mutant strain

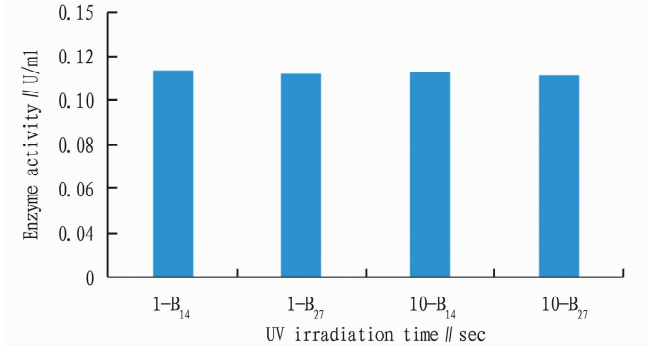


Fig. 8 Comparison of enzyme activities of different strains after 10th generation culture

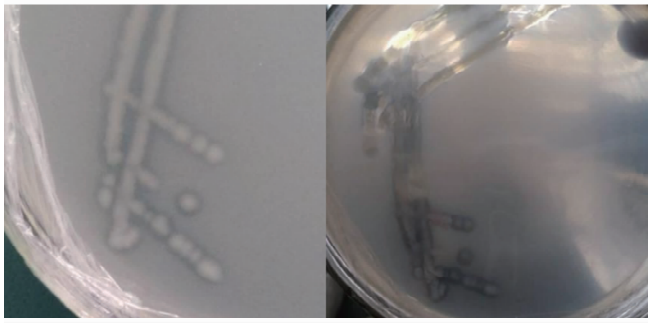


Fig. 9 Culture characteristics of strain A

The PCR products were sequenced by Shanghai Lifei Biotechnology Co., Ltd., and the resulting sequences were submitted to the NCBI database for BLAST analysis. Based on the BLAST results, 16S rDNA sequences of 11 related strains were selected and analyzed using MEGA 6.0 software to construct a phylogenetic tree. The resulting tree is shown in Fig. 12. As can be seen from the figure, strain A clustered in the same phylogenetic branch as *Stenotrophomonas pavanii* strain, indicating that strain A was preliminarily identified as belonging to the genus *Stenotrophomonas*.

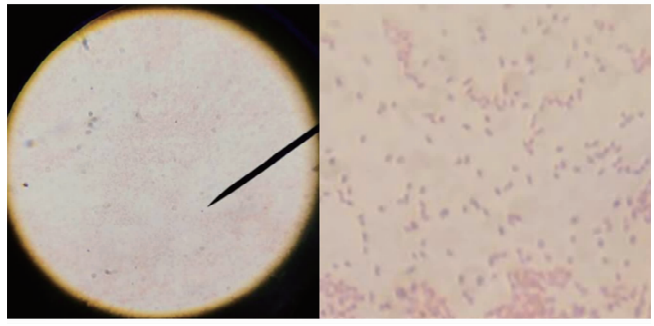
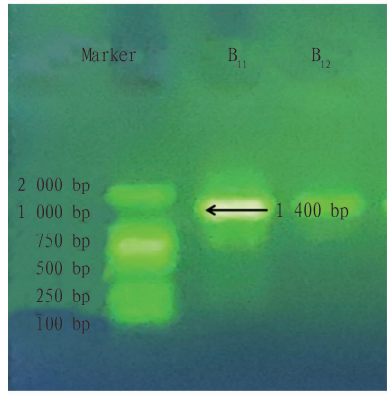


Fig. 10 Gram staining results of the strain



NOTE B12 is strain A.

Fig. 11 Electrophoresis results

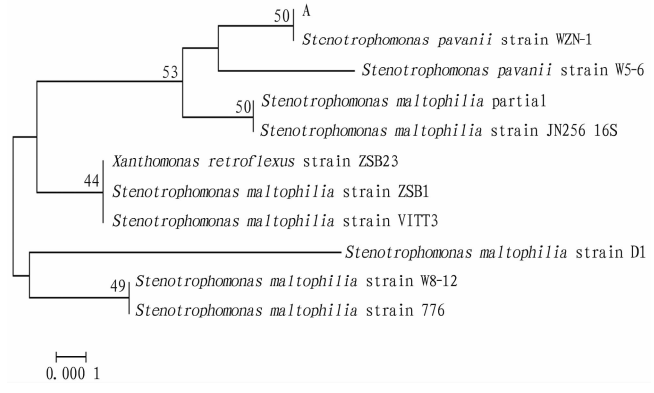


Fig. 12 Phylogenetic tree of 16S rDNA of the strain

3 Conclusions

Through UV mutagenesis, we successfully obtained two mutant strains, B_{14} and B_{27}, which exhibited markedly higher chitinase activities than the parental strain A. The enzyme activities of B_{14} and B_{27} were 0.116 2 and 0.114 4 U/mL, corresponding to 2.76- and 2.71-fold improvements over the wild type, respectively. Both mutants maintained stable enzymatic performance after ten successive subcultures, confirming their genetic stability. Molecular identification further classified strain A within the genus *Stenotrophomonas*.

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Contacts of *Asian Agricultural Research*:

Tel.: USA: (401)515 – 4764

China: 0086551 – 65148112

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