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Discovery of a New Thermostable Chitinase from Streptomyces No. 6

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Abstract: Chitinases are a class of hydrolases that hydrolyze the β-1, 4-glycosidic bonds between N-acetyl-D-glucosamine (GlcNAc) in chitin. Among chitinases, thermophilic chitinases and thermostable chitinases have received extensive attention in recent years due to their ability to tolerate higher temperatures and maintain enzyme stability for a longer period of time. However, it has previously been observed that not all chitinases are thermostable and there are few studies about chitinases isolated from Streptomyces No. 6. Hence, the properties of the chitinase of Streptomyces No. 6 were investigated in this study. In this experiment, the enzyme activity was explored by a single-factor experiment, and the enzymatic properties were detected by a 3,5-Dinitrosalicylic acid (DNS) assay. At the same time, the fermentation conditions of Streptomyces No. 6 producing chitinase were optimized to improve the yield by a single-factor experiment and a response surface experiment. The results showed that the chitinase of Streptomyces No.6 can remain active at high temperatures (65°C). The optimal activity of the enzyme is obtained at a temperature of 65°C, a pH value of 5.0, and the substrate of colloidal chitin. Moreover, in this study, a Box-Behnken Design (BBD) was applied to increase the yield of chitinase. The optimization process showed that the enzyme activity reached 6.3756 U/mL at an inoculum size of 2%, fermentation time of 144 h, and pH of 5.5 (as optimum conditions). These results provided theoretical support for the production of chitinase for biotechnological and industrial applications.

Keywords: Streptomyces No. 6, Chitinase, Medium Optimization, Response Surface Method, Enzyme Properties

Introduction

Chitin is composed of β-1,4-linked N-acetyl-D-glucosamine (GlcNAc) units, an insoluble linear biopolymer that is the second most abundant biopolymer and plays a role in a variety of organisms as a major structural component (Tsurkan *et al*., 2021). Many different types of invertebrates, such as sponges, mollusks, nematodes, arthropods, and fungi, include chitin in their extracellular matrix. Chitin fibers are interconnected to form three possible crystalline structures, namely α-, β-, or γ-chitin (Moussian, 2019). As mentioned in the literature review, such as chitin oligosaccharides, chitin monosaccharides, and their derivatives have various industrial benefits (Le and Yang, 2019). The traditional method of degrading chitin depends on chemical methods, which have problems such as environmental pollution, low yield, low product purity, and an uncontrollable reaction process (Naveed *et al*., 2019). Therefore, microbial biotransformation of chitin using chitinase has become an important process and has gained the interest of researchers all over the world.

Chitinase is a glycoside hydrolase that hydrolyzes chitin by cutting β-1,4 glycosidic bonds to degrade it into low molecular weight products such as Chitooligosaccharides (COS) and GlcNAc. Chitinase has potential application prospects in many fields, such as the production of COS or Glc NAc in the food industry and the production of antifungal agents against pathogenic organisms in the agricultural field (Du *et al*., 2020). High temperatures have the potential to improve the solubility of hydrophobic chemicals in industrial settings, inhibit microbiological contamination, and facilitate environmentally friendly biodegradation processes (Mathew *et al*., 2021). Chitinase is used to remove shellfish waste from the seafood industry and dump it into the water. In the management of

shellfish waste, the desalination and deproteinization of chitin waste require higher temperatures. When chitinases are used, these chitin raw materials can be converted into COS instead of the low-quality heterogeneous mixes that are produced by thermochemical methods. According to the CAZy database (http://www.cazy.org/), most of the chitinases can be classified into two families, Glycoside Hydrolases (GH) families 18 (GH18) and 19 (GH19), according to the amino acid sequences of their catalytic regions (Kozome *et al*., 2022). The primary producers of GH-18 chitinase are plants, animals, insects, fungi, bacteria, and mammals. The GH-19 chitinase is mostly found in higher plants, but rarely in microorganisms. In recent years, with the deepening of chitosan application and research, chitosan detection techniques have been constantly updated and developed.

Streptomyces is known as a major producer of chitinase (Okazaki *et al*., 2004). Therefore, Streptomyces is considered a favorable strain for chitinase production. However, there are few studies about chitinase isolated from Streptomyces No. 6 (Jones, 1992). Despite the discovery of many chitinase-producing microorganisms, as well as the efforts of many researchers to study the structure of chitinases and their functional characteristics, few commercial chitinases have actually been used in industry. In this study, it is proposed to optimize the fermentation conditions of Streptomyces No. 6, a high-producing chitinase strain, by using the traditional plate culture and shake flask fermentation culture methods to further improve the enzyme-producing ability of the strain. It provides certain scientific theory and practical application value for the industrial production of chitinase.

Materials and Methods

Strain and Cultural Conditions

Streptomyces No. 6 strain was preserved in our lab and grown in medium containing (g/L): Glucose 10; KH_2PO_4 0.2; $(NH_4)_2SO_4$ 0.5; $FeCl_3·6H_2O$ 0.01; $ZnSO_4·7H_2O$ 0.001; $CaCl_2·2H_2O$ 0.01; K_2HPO_4 0.8; $MgSO_4·5H_2O$ 0.2; and agar 15.0 when necessary. The experimental basal fermentation conditions were: Initial pH 7.0, fermentation temperature 30°C, loading volume 400/1000 mL, inoculum volume 0.5%, fermentation cycle 96 h, and rotational speed 200 r/min. The basal medium composition was: Chitin 0.5%, chitosan 0.1%, $(NH_4)_2SO_4$ 0.5 g/L, KH_2PO_4 0.2 g/L, K_2HPO_4 0.8 g/L, $MgSO_4·5H_2O$ 0.2 g/L, $CaCl_2·2H_2O$ 0.01 g/L, $FeCl_3·6H_2O$ 0.01 g/L and $ZnSO_4·7H_2O$ 0.001 g/L.

Chitinase Crude Enzyme Extraction

The fermentation broth obtained after optimized fermentation was pumped through a vacuum pump to draw the supernatant. Then, 80% (w/v) $(NH_4)_2SO_4$ was added under ice bath conditions, stirring while adding and stirring overnight at 4°C. 4°C, 10000 g, centrifuged for 30 min, and the supernatant was taken. The precipitate was dissolved in pH 6.5, 20 mM phosphate buffer, and dialyzed three times in the same buffer for 3 h.

Determination of Hydrolysis Products

5 mL of 1% colloidal chitin was added to 1 mL of the above enzyme solution and reacted at 50°C for 12 h. The supernatant was then extracted by centrifugation. High-Performance Liquid Chromatography (HPLC) was used to evaluate the chitinase hydrolysis products. Agilent Hi-Plex Ca PL1170-6810 (300×7.7 mm) was the chromatographic column, with a temperature of 75°C and a RID detector. The mobile phase consisted of deionized water flowing at a rate of 0.2 mL/min (Yeganeh-Zare *et al*., 2022).

Chitinase Activity Assay

The colloidal chitin matrix was prepared according to the method described by Li *et al*. (2021); and Gao *et al*. (2018). Approximately 5.0 g of chitin was dissolved in 100 mL of HCl (37 %) and placed at 4°C overnight. Then, 300 mL of 50% ethanol was added to the mixture and centrifuged at 8000 g at 4°C for 15 min. Finally, the precipitate was washed twice to a neutral pH with distilled water.

The chitinase activity was determined according to an established method described by Felse and Panda with some modifications and N-acetylglucosamine was used as a standard (Felse and Panda, 1999). The reaction system contained 1.9 mL of 1% colloidal chitin, dissolved in phosphate buffer (pH 6.0), and 0.1 mL of chitinase fermentation broth, which was incubated at 50°C for 30 min. Then, 3 mL of 3,5-Dinitorsalicylic acid (DNS) was added to the reaction system to stop the enzymatic hydrolysis reaction and boiled for 5 min, followed by the addition of 20 mL water to a final reaction volume of 25 mL. Using a spectrophotometer, the absorbance of the reaction system was determined at 520 nm. One unit of chitinase activity was defined as the amount of enzyme catalyzing the release of 1 μmoL of N-acetylglucosamine per minute.

Table 1: Combination of experimental factors in response surface test

	Factor		
Level	Fermentation time (A) / h	Initial pH (B)	Inoculum size(C)/%
-1	120	5.0	1.0
0	144	5.5	1.5
1	168	6.0	2.0

Influence of pH, Temperature, Substrate, Metal Ion and Surface Active Agents on Enzymatic Activity and Stability

The optimal pH for enzyme activity and stability was determined by a standard determination using buffers in different pH ranges. In order to determine the pH stability, the enzyme was incubated with a buffer of pH 3.0~10.0. The residual enzyme activity was determined under standard measurement conditions. The buffers used were as follows: 0.05 m citric acid-citrate ammonium buffer (pH 3.0-5.0), 0.05 m dipotassium hydrogen phosphate-potassium dihydrogen phosphate buffer (pH 6.0-8.0), and 0.05 m glycine-sodium bicarbonate buffer (pH 9.0-10.0). By running the standard test at temperatures between 25 and 80 degrees Celsius, the ideal reaction temperature was found. It was also investigated how metal ions affected the activity of enzymes. 5 mmoL/L metal ions, $ZnSO_4$, $CoCl_2$, $FeCl_3$, $MgSO_4$, $MnSO_4$, $CaCl_2$, $CuSO_4$, and $BaCl_2$, as well as SDS and EDTA, a chelating agent, were added to the reaction mixture. The relative activities of chitinase under different conditions were calculated separately.

Box Behnken Design (BBD) Experimental Design for Optimization of Enzyme Production

Chitinase activity was determined at different fermentation times (72, 96, 120, 144, and 168 h), initial pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5) and inoculation size (0.1, 0.5, 1.0, 1.5 and 2.0%). The three independent variables were inoculum size (C), starting pH (B), and fermentation time (A). The values of the three coded levels of the three components were presumed after preliminary testing; Table 1 shows them. The chitinase activity (U/mL) was the dependent variable. Software called Design-Expert.V8.0.6 was used to create and evaluate the statistical experimental design (Alsadi *et al*., 2022). Finally, the verification experiment was carried out according to the optimal fermentation conditions.

Statistical Analysis

Statistical software SPSS Statistics version 25.0 (SPSS, Inc., Chicago, IL, USA) was used for data analysis in this study. Three independent experiments were performed as one-way tests and the results are shown as the mean ± SD. To evaluate differences between means, a student's t-test was employed, with $p<0.05$ being regarded as substantially different.

Results

Analysis of Hydrolysis Products and Effects of Substrate, Metal Ions, and Surfactants on the Activity and Stability of the Chitinase

To study the hydrolysis of chitin products by chitinase, the hydrolysis products were analyzed by HPLC in this experiment. It can be seen that the fermentation broth sample from 144 h of fermentation contained four sugars in Fig. 1: GlcNAc, (GlcNAc)2, (GlcNAc)3, and (GlcNAc)5. Among hydrolysates, a large amount of GlcNAc, (GlcNAc)3, (GlcNAc)5, and a small amount of (GlcNAc)2 was obtained. Thus, it was assumed that the fermentation of Streptomyces No.6 produced chitinase that could degrade chitin to COS.

From Fig. 2A, we can see the specificity of the chitinase of Streptomyces No.6. As shown, the chitinase activities rank as follows: 100% with colloid chitin, 82.3% with powder chitin, 60% with soluble chitosan, 4% with CMC Na and 0% with glucose. At the same time, we can observe the effect of metal ions and surfactants on chitinase activity in Fig. 2B. It can be seen from the data that Zn_2+, EDTA and Tween-80 had no significant effect on chitinase activity; Co2+, Fe_3+, Mg_2+, Mn_2+, Ca_2+, Cu_2+ and Ba_2+ had an inhibitory effect on chitinase activity, with the surfactant SDS having a significant inhibitory effect on chitinase.

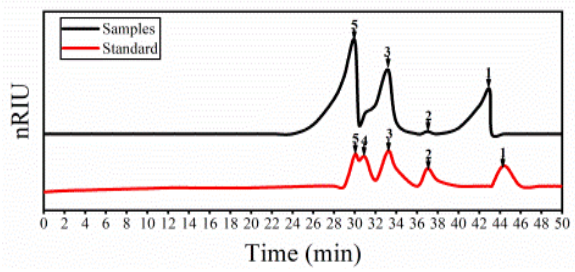
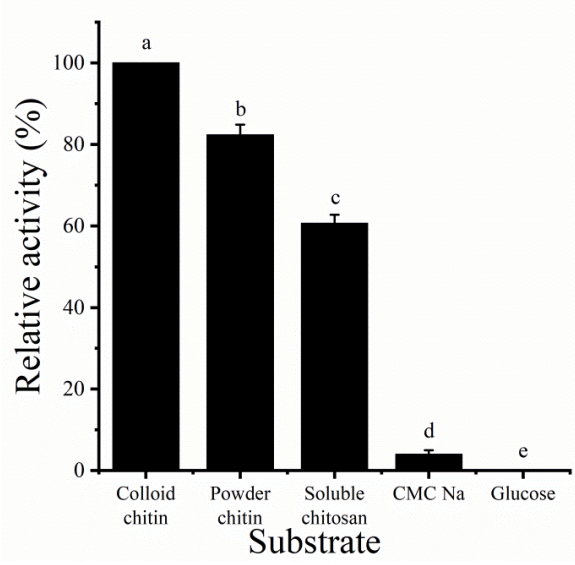


Fig. 1: HPLC analysis of the product of chitin catalyzed by chitinase. Standard: 1: GlcNAc; 2: (GlcNAc)2; 3: (GlcNAc)3; 4: (GlcNAc)4; 5: (GlcNAc)5



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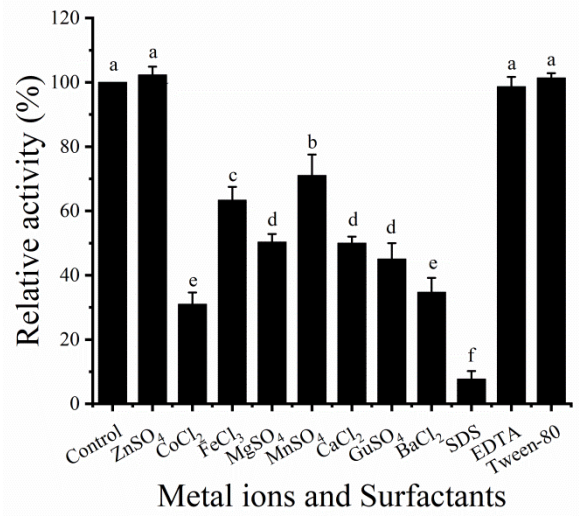

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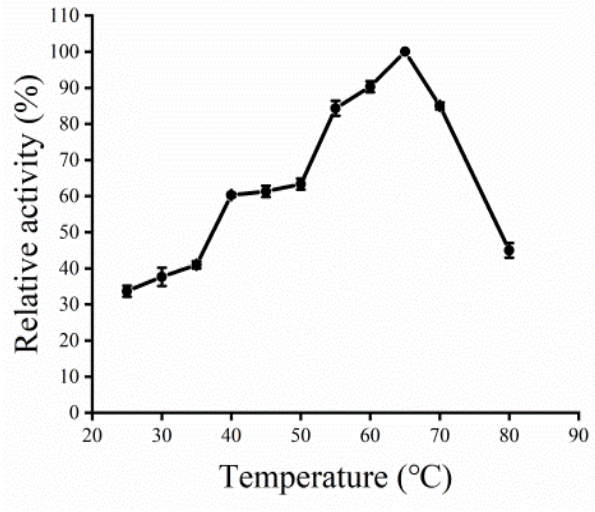
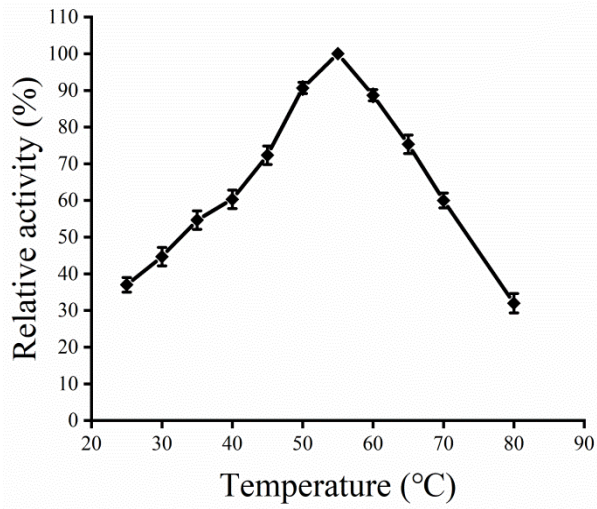


(a)

Fig. 2: The influence of substrate, metal ions, and surfactants on the activity of chitinase. A: Colloid chitin, Powder chitin, Soluble chitosan, CMC Na, Glucose; B: $ZnSO_4$, $CoCl_2$, $FeCl_3$, $MgSO_4$, $MnSO_4$, $CaCl_2$, $CuSO_4$, $BaCl_2$, SDS, EDTA and Tween-80. Different letters represent significant differences ($p<0.05$)



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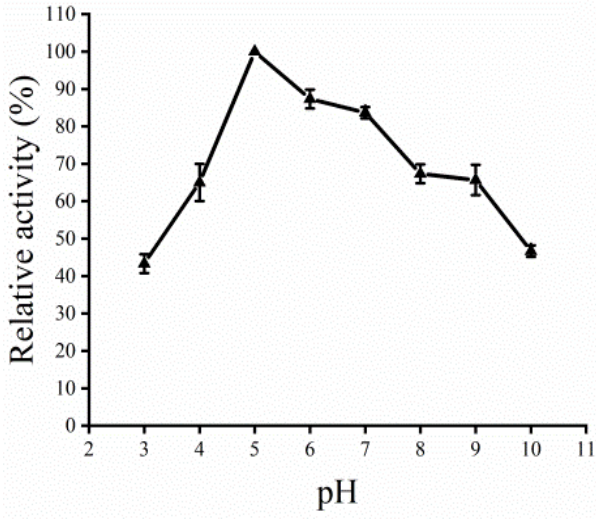


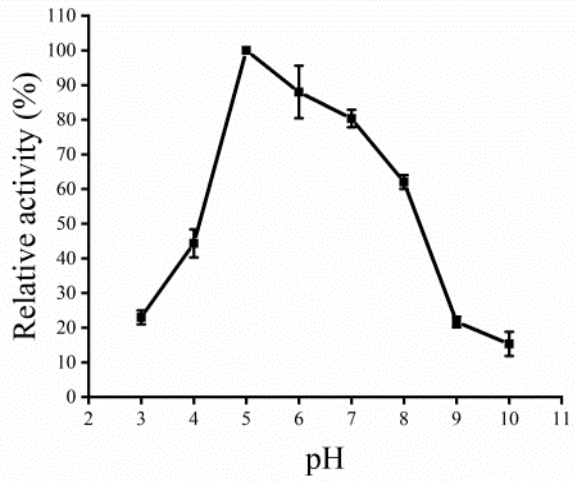
(c)

Effect of Temperature and pH on Enzyme Chitinase Activity

From Fig. 3A, we can see that the chitinase of Streptomyces No.6 had the highest relative chitinase activity at 65°C. The higher optimum reaction temperature prevents contamination by other microorganisms during the reaction and guarantees the safety of the reaction process. When the temperature was higher than 65°C, the activity of chitinase decreased rapidly and significantly, with less than half of the maximum activity when reaching 80°C. Chitinase retains more than 50% of its activity at 35-70°C (Fig. 3B). In the meantime, in the effect of pH on chitinase activity, the results of the correlational analysis showed the higher relative enzyme activity of chitinase at pH 5.0 in Fig. 3C. The activity of chitinase increased with increasing pH when pH <5.0 and gradually decreased with increasing pH when pH >5.0. It can be seen from Fig. 3D that the chitinase activity remained above 40% in the pH range of 4.0-8.0.

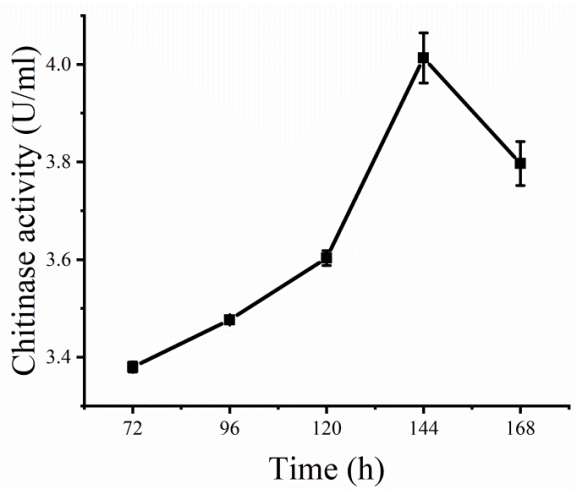
Effect of Fermentation Time, Initial pH, and Inoculum Size on Enzyme Production

We investigated the effect of fermentation conditions on the chitinase activity of the bacterium, our results showed that chitinase activity increased with increasing fermentation time from 72-144 h and then gradually decreased (Fig. 4A). And the highest chitinase activity was achieved at an initial pH of 5.5 (Fig. 4B). Finally, as shown in Fig. 4C, the maximum chitinase activity was 4.003 U/mL when the inoculum size was 2 %.



(d)

Fig. 3: Impact of pH and temperature on chitinase activity. A: Chitinase activity in relation to temperature; B: Chitinase thermal stability; C: Chitinase activity in relation to pH; D: Chitinase pH stability



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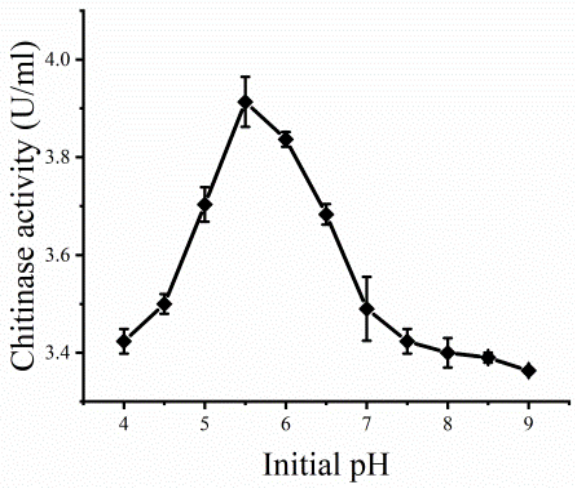

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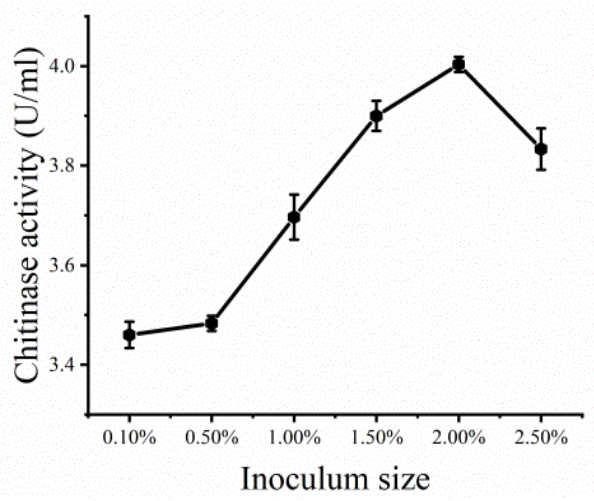

(c)

Fig. 4: Impact of fermentation time, various initial pH values and various inoculum sizes on enzyme production; A: Effect of fermentation time (72, 96, 120, 144, 168 h) on enzyme production; B: Effects of various initial pH (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0) values on enzyme production; C: Effect of various inoculum size (0.1, 0.5, 1.0, 1.5, 2.0, 2.5 %) on enzyme production

Box-Behnken Design (BBD) Experimental Design for Optimization of Enzyme Production

By regression analysis, a regression equation of the fitted quadratic model was obtained as follows:

$$Y = 6.26 - 0.40A - 0.12B - 0.41C + 0.44AB \\ -0.033AC + 0.06BC - 1.21A^2 - 1.51B^2 - 1.06C^2 \quad (1)$$

In order to assess the impact of the three factors, we use multiple linear regression to analyze our data matrix (El Hazzam *et al*., 2022). Table 2 show the actual and anticipated values. As can be seen in Table 2, the results demonstrated a high degree of agreement, with the disparities between the actual and anticipated values being quite minor. Response Surface Methodology (RSM) curves help to understand the comprehension of the process conditions as well as determine the parameters' optimal points. There was a considerable degree of agreement because there were very few differences between the two values (Alsadi *et al*., 2022).

According to the p-value of each factor, considering the interaction between factors, the Analysis of Variance (ANOVA) table of this experiment is shown in Table 3. With a determination coefficient R^2 of 0.9878, they showed a strong connection between the values observed and those anticipated, suggesting that the quadratic model's responses have a significant correlation. The

predicted R^2 of 0.8806 agrees with the adjusted R^2 of 0.9720 (Orak and Yüksel, 2022). The model's relevance and significance were confirmed using the ANOVA. The model was deemed significant based on its F-value of 62.8, $p<0.01$. Furthermore, the model terms are significant, as indicated by the p-values of A, A^2, B^2, C^2, and AB, which are less than 0.05. In addition, the model's validity is supported by the mean square of lack-of-fit, which is 1.75, and a p-value of 0.2949, which indicates no significant value (El Hazzam *et al*., 2022). Thus, it could be deduced that the model was well-fitted to the observed response.

Table 2: Design and results of responses surface experiments for fermentation conditions optimization of Streptomyces No. 6

No.	A (Fermentation time)/h	B (Initial pH)	C (Inoculum size)/ %	Experimental Values Chitinase activity/U/mL	Predicted Values Chitinase activity/U/mL
1	120	5.0	1.5	4.468	4.490
2	168	5.0	1.5	2.986	2.810
3	120	6.0	1.5	3.194	3.370
4	168	6.0	1.5	3.477	3.450
5	120	5.5	1.0	4.408	4.388
6	168	5.5	1.0	3.476	3.654
7	120	5.5	2.0	4.554	4.372
8	168	5.5	2.0	3.489	3.506
9	144	5.0	1.0	3.916	3.918
10	144	6.0	1.0	3.693	3.544
11	144	5.0	2.0	3.539	3.702
12	144	6.0	2.0	3.585	3.596
13	144	5.5	1.5	6.347	6.260
14	144	5.5	1.5	6.353	6.260
15	144	5.5	1.5	6.400	6.260
16	144	5.5	1.5	5.945	6.260
17	144	5.5	1.5	6.267	6.260

Table 3: ANOVA for chitinase production

Sources	Sum of squares	DF	Mean square	F value	F value
Model	25.22	9	2.80	62.80	<0.0001
A	1.28	1	1.28	28.61	0.0011
B	0.12	1	0.12	2.58	0.1522
C	0.013	1	0.013	0.30	0.6023
A^2	6.23	1	6.23	139.58	<0.0001
B^2	9.66	1	9.66	216.49	<0.0001
C^2	4.77	1	4.77	106.87	<0.0001
AB	0.78	1	0.78	17.45	0.0042
AC	4.422 E-003	1	4.422 E-003	0.099	0.7621
BC	0.018	1	0.018	0.41	0.5446
Residual	0.31	7	0.045		
Lack of fit	0.18	3	0.059	1.75	0.2949
Pure error	0.14	4	0.034		
Cor total	25.54	16			
Std. Dev.	0.21		R^2	0.9878	
Mean	4.48		Adj R^2	0.9720	
c.v. %	4.72		Pred R^2	0.8806	
PRESS	3.05		Adeq. precision	21.304	

$p<0.05$ is significant

The resulting 3D response surface plots can predict the chitinase activities at different levels of variables. The strength of the interaction between these three factors can be visually reflected by the shape of the contour lines. The more pronounced the interaction, the more pronounced the elliptical shape of the contour line, and vice versa, the more rounded it is. As we can conclude from Fig. 5, the largest interaction was between fermentation time and initial pH, followed by the interaction between initial pH and inoculum size and the interaction between fermentation time and inoculum size.

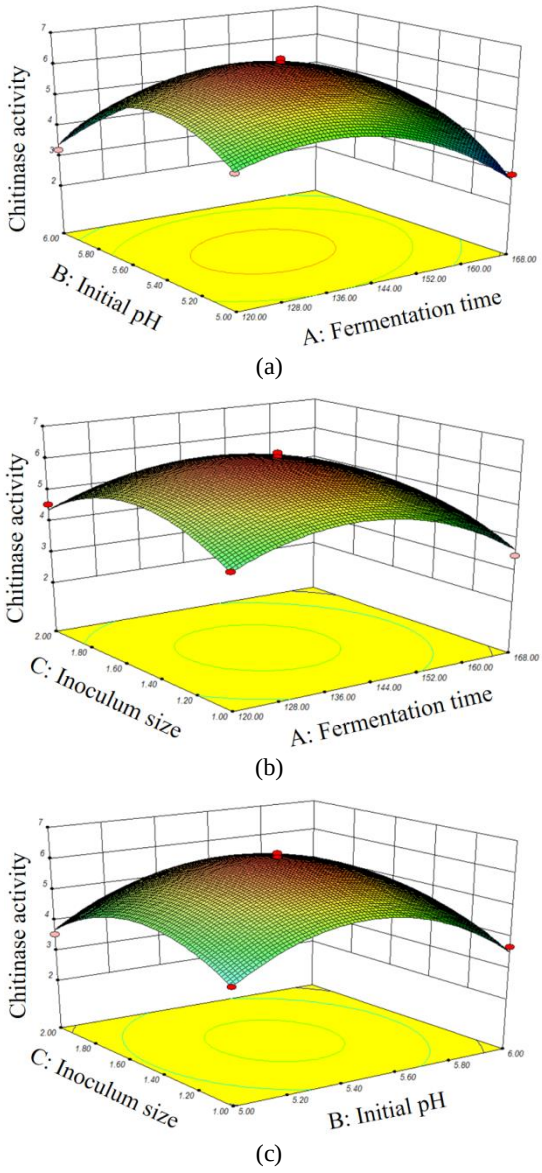

Fig. 5: Response surface plots of the interaction effect between parameters on Chitinase activity during fermentation. A, The effect of the interaction between fermentation time and initial pH; B, The effect of the interaction between fermentation time and inoculum size; C, The effect of the interaction between initial pH and inoculum size

Based on the results of single-factor testing and response surface analysis, combined with operational feasibility, the model was validated under the following conditions: Fermentation time of 144 h, initial pH of 5.5, and inoculum size of 1.5%. Three replicates were performed and the average chitinase activity was 6.3756 U/mL, which is close to the predicted value, indicating that the model is reliable and valid. The optimum conditions for the maximum activity of chitinase were determined by the response surface analysis (design-expert 8.0.6) and regression equation. The chitinase activity reached the theoretical maximum value of 6.3054 U/mL, with fermentation condition: 143.5 h of fermentation time, 5.46 of initial pH, and 1.5% of inoculum size.

Discussion

Streptomyces is considered to be a favorable strain for chitinase production. In order to enhance the utilization of chitin, it is necessary to increase the activity of Streptomyces chitinase. The present study was designed to determine the effect of optimal fermentation conditions on the enzyme activity of Streptomyces No.6.

The HPLC-RID system was used to determine the hydrolysis products for this study. The hydrolysis products were found to contain (GlcNAc)5, (GlcNAc)3, (GlcNAc)2 and GlcNAc. These results corroborate the findings of a great deal of previous work that chitinase is capable of utilizing chitin to produce COS (Fu *et al*., 2020; Le and Yang, 2018). This study showed that the chitinase of Streptomyces No.6 was capable of utilizing chitin to produce COS.

At the same time, we found that the chitinase produced by Streptomyces No.6 showed the highest specificity to colloid chitin, followed by powder chitin and soluble chitosan, in the study of enzymatic properties. This indicated that the chitinase has specificities and that its optimal substrate is colloid chitin. The preferable fermentation substrate is colloidal chitin in this study, as previously reported for the chitinase of *Paenibacillus* sp., the chitinase Rchi1602 of Microbulbifer sp. BN3 and the chitinase of Chitiniphilus shinanonensis CsChiE (Du *et al*., 2020; Li *et al*., 2021; Rani *et al*., 2020). The substrate binding specificity of chitinase can be explained by active site structure analysis (Liu *et al*., 2015). Different families of chitinases differ in the number, type, and position of substrate atoms bound to the active site, resulting in different substrate binding specificities. In addition, the current study found that Zn^{2+}, EDTA, and Tween-80 had no significant effect on chitinase activity. However, Zn^{2+} was found to promote the GH19 chitinase of Streptomyces alfalfa (Lv *et al*., 2021). This difference may be due to the different effects on the chitinase activity owing to the difference in Zn^{2+} concentration. These results match those observed in earlier studies that Co^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, Ca^{2+}, Cu^{2+}, Ba^{2+} and SDS moderately or strongly inhibit the activity of chitinase in different bacteria (Pan *et al*., 2019; Deng *et al*., 2019; Du *et al*., 2020; Lv *et al*., 2022; Park *et al*., 2016; Sousa *et al*., 2019; Take *et al*., 2018; Zhang *et al*., 2018). The inhibitory effect of metal ions on chitinase has been widely reported and, as shown for lysozyme, metal ions can form stable complexes with carboxylate groups at the catalytic center of enzymes, resulting in their inhibition (Perkins *et al*., 1979). On the other hand, the chelating agent EDTA did not affect the chitinase activity of Streptomyces No.6, indicating that this chitinase is not a metalloenzyme and therefore does not require metal ions for its activity.

In the meanwhile, the optimum temperature of the chitinase produced by Streptomyces No.6 is 65°C and it remained stable at 35-70°C in this study. The optimum temperature for the chitinase produced by Streptomyces No. 6 is higher than the chitinase SaChiA4 from the Streptomyces albolongus ATCC 27414 strain which was 55°C and the chitinase from the Bacillus cereus strain NK91 strain which was 40°C (Gao *et al*., 2018; Thakur *et al*., 2022). As can be seen from Table 4, the optimum reaction temperature of the chitinase produced by Streptomyces No.6 used in this result was higher than the optimum reaction temperature of other bacterial chitinases. The wider range and higher temperature of thermal stability can ensure both the activity of chitinase and the safety of the reaction process.

The optimum pH of chitinase from different bacteria was quite different. The chitinase of Streptomyces No.6 has higher activity in an acidic environment when the pH is 5.0. However, the optimum pH of chitinase activity from B. subtilis and Streptomyces sp. CS147 was 5.0 and 11.0 (G. C *et al*., 2015; Wang *et al*., 2018). The chitinase activity remained above 40% in the pH range of 4.0-8.0 in this study. In accordance with the present results, previous studies have demonstrated that the optimum pH for most chitinase ranges from 4.0 to 9.0. Like the vast majority of extracellular enzymes, the chitinase of Streptomyces No.6 was an acidic chitin deacetylase with excellent pH stability. Previous studies have shown that the conformation of the enzyme can be altered by pH, causing a change in the structure of the active site in the enzyme molecule (Yang *et al*., 2022). Therefore, in the case of over-acid or over-base, the enzyme activity will be rapidly reduced. We can see that the low optimum reaction pH and high optimum reaction temperature contributed to suppressing other non-experimental reactions.

Table 4: Comparison of Streptomyces No. 6 chitinases with other bacterial chitinases

Strain	Optimal temp. (°C)	Stability temp. (°C)	Reference
Paenibacillus sp.	50	45	(Du *et al*., 2020)
Exiguobacterium antarcticum	30	25-50	(Lv *et al*., 2022)
Exiguobacterium antarcticum DW2	30	0-50	(Fu *et al*., 2020)
Microbulbifer sp. BN3	60	50	(Li *et al*., 2021)
Chitiniphilus shinanonensis	50	40-60	(Rani *et al*., 2020)
Streptomyces alfalfae ACCC 40021	45	55	(Lv *et al*., 2021)
C. violaceum ATCC 12472	60	40-80	(Sousa *et al*., 2019)
Trichoderma harzianum GIM 3.442	45	30-35	(Deng *et al*., 2019)
Streptomyces albolongus ATCC 27414	55	35-65	(Gao *et al*., 2018)
Paenicibacillus barengoltzii	55	-	(Yang *et al*., 2016)
Paenibacillus sp. D1	50	40-60	(Singh and Chhatpar, 2011)
Streptomyces anulatus CS242	50	30-60	(Mander *et al*., 2016)
Vibrio alginolyticus H-8	40-45	<45	(Ohishi *et al*., 2000)
Chitinolyticbacter meiyuanensis SYBC-H1	50	20-60	(Zhang *et al*., 2018)
Chryseobacterium indologenes	30	-	(Kim *et al*., 2018)
Pseudoalteromonas issachenkonii PAMC 22718	30	0-37	(Park *et al*., 2016)
Streptomyces coelicolor A3(2)	55	<55	(Nguyen-Thi and Doucet, 2016)
Clostridium paraputrificum J4	60	-	(Dohnálek *et al*., 2021)
Microbispora sp. V2	60	-	(Nawani *et al*., 2002)
Streptomyces sp. CS147	60	<50	(G. C *et al*., 2015)
Bacillus subtilis W-118	37	-	(Wang *et al*., 2006)
Streptomyce No.6	65	35-70	Current study

Moreover, the effect of different fermentation parameters (fermentation time, initial pH, and inoculum size) on the chitinase activity of the chitinase produced by Streptomyces No.6 was evaluated and optimized by the BBD of RSM. Initially, the range for BBD optimization of different fermentation parameters was set based on observing single-factor effects on the chitinase activity of the chitinase produced by Streptomyces No.6. The result of the single-factor effect revealed that the chitinase activity was significantly increased when fermentation time, initial pH and inoculum size reached up to 144 h, 5.5 and 2.0%, respectively and afterward, no significant increase in yield was observed upon an increase in fermentation time, initial pH and inoculum size (Alam *et al*., 2021). The increased fermentation time increases the number of bacteria and chitinase contents, resulting in high chitinase activity. Similarly, increased inoculum size increases chitinase activity. Based on the observation of the single-factor effect on the chitinase activity, the range of fermentation time (120-168 h), initial pH (5.0-6.0), and inoculum size (1.0-2.0 %) was selected for optimization by the BBD method. For BBD analysis, a quadratic model was found to be the best fit. In this study, the low value of the percentage residual and the considerable value of R2 supported the high predictive ability of BBD analysis. By response surface methodology, the highest activity of chitinase was 6.3756 U/mL when the fermentation condition was incubation time 144 h, the initial pH 5.5, and the inoculum 1.5%, which provided a certain reference value for microbial utilization of chitin. The striking finding is that Streptomyces No. 6 has a short fermentation time compared with Bacillus pumilus and Humicola grisea ITCC 10360.16, which achieve their maximum yield of chitinase after 7-8 days of fermentation (Kumar *et al*., 2017; Tasharrofi *et al*., 2011).

Conclusion

In this study, the chitinase generated by the fermentation of Streptomyces No. 6 had a high optimum reaction temperature and wide temperature stability. The optimal fermentation conditions were determined by response surface methodology, which provided a certain reference value for the utilization of chitin by microorganisms. In this study, the enzymatic properties of Streptomyces No. 6 chitinase and the optimal fermentation conditions were investigated to improve the activity of chitinase, which provides scientific theories and practical application values for the industrial production of chitinase. It also improves the utilization of chitin and chitosan and promotes the development of the industry as a whole. In the future, we will understand the enzymatic properties of chitinase more accurately by using higher-purity chitinase.

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Author's Contributions

Xiaowen Que and Yonghong Bao: Participate in the whole experimental design, experimental method, result analysis, and manuscript writing.

Qing Liu: Participate in the part of the experimental process and the analysis of results.

Shaoqi Li: Participated in part of the experiment.

Yueping Yang: Ameliorated the manuscript.

Yuanda Song: Participated in experiment design guidance, and result interpretation and ameliorated the manuscript.

Huaiyuan Zhang: Participated in part of the guidance of experimental design and ameliorated the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

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