MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN
Faculty of Chemical and Biopharmaceutical Technologies
Department of Biotechnology, Leather and Fur

QUALIFICATION THESIS

on the topic **Study on the influence of mycelium morphology of *Maoyuan Streptomycetes* on TG enzyme synthesis**

First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group BEBT-20
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APPROVE
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Doctor of Technical Science
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_____
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ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Zhang Junhao

1. Thesis topic **Study on the influence of mycelium morphology of *Maoyuan Streptomycetes* on TG enzyme synthesis**
Scientific supervisor Olga Andreyeva, Dr. Sc., Prof.

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EXECUTION SCHEDULE

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	From 01 April 2024 to 11 April 2024	
2	Section 1 Literature review	From 06 April 2024 to 20 April 2024	
3	Section 2 Object, purpose, and methods of the research	From 21 April 2024 to 30 April 2024	
4	Section 3 Experimental part	From 01 May 2024 to 10 May 2024	
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I am familiar with the task:

Student _____ Zhang JUNHAO

Scientific supervisor. _____Olga ANDREYEVA

SUMMARY

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The aim of this study was to investigate the effect of mycelial morphology on the synthesis of glutamine transaminase (TGase) produced by Streptomyces pentadiengensis. Through experiments, we found that $MgCl_2$ stress can significantly increase the production of TG enzymes, and has an important effect on the morphology of bacteria. In this study, we first determined the optimal addition concentration of $MgCl_2$. In addition, $MgCl_2$ stress led to the advance of mycelium germination and spore formation, indicating that the bacteria may activate secondary metabolic regulators earlier under salt stress and promote the synthesis of TG enzymes. We also studied the effects of kaolin on the morphology and TG enzyme synthesis of Streptomyces pentadiengensis. The results showed that kaolin microparticles had the greatest effect on mycelium morphology and significantly improved TG enzyme activity, indicating that the enzyme production capacity of the strain could be effectively enhanced by regulating mycelium morphology. This study provides a new strategy for increasing the yield of TG enzymes, which is of great significance for in-depth understanding of the regulatory mechanism of microbial metabolic performance, and provides a theoretical basis for efficient production of TG enzymes in industrial production. In the future, we will further explore the specific mechanisms of kaolin influence on mycelium morphology and enzyme activity, and apply these strategies to the metabolic regulation of other microbial systems.

Keywords: Glutamine transaminase, Streptomyces pentadiengensis, $MgCl_2$ stress transduction, microparticle, Mycelial morphology

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INTRODUCTION

The aim of this study was to investigate the effect of mycelial morphology on the synthesis of glutamine transaminase (TGase) produced by Streptomyces pentadiengensis. Through experiments, we found that $MgCl_2$ stress can significantly increase the production of TG enzymes, and has an important effect on the morphology of bacteria. In this study, we first determined the optimal addition concentration of $MgCl_2$. In addition, $MgCl_2$ stress led to the advance of mycelium germination and spore formation, indicating that the bacteria may activate secondary metabolic regulators earlier under salt stress and promote the synthesis of TG enzymes. We also studied the effects of microparticles on the morphology and TG enzyme synthesis of Streptomyces pentadiengensis. The results showed that kaolin microparticles had the greatest effect on mycelium morphology and significantly improved TG enzyme activity, indicating that the enzyme production capacity of the strain could be effectively enhanced by regulating mycelium morphology. This study provides a new strategy for increasing the yield of TG enzymes, which is of great significance for in-depth understanding of the regulatory mechanism of microbial metabolic performance, and provides a theoretical basis for efficient production of TG enzymes in industrial production. In the future, we will further explore the specific mechanisms of microparticles' influence on mycelium morphology and enzyme activity, and apply these strategies to the metabolic regulation of other microbial systems.

The relevance of the topic is Effect of mycelial morphology on TG enzyme synthesis of Streptomyces pentadiengensis.

The purpose of the study is effect of mycelial morphology on the synthesis of glutamine transaminase (TG enzyme) produced by Streptomyces pentapentadienes.

The object of the study in this paper is Streptomyces pentadiengensis, a microbe that produces glutamine aminotransferase (TG enzyme). The study mainly focused on the effect of mycelium morphology on TG enzyme synthesis, and the effect of $MgCl_2$ stress and microparticles on mycelium morphology and TG enzyme synthesis.

Based on the in-depth study of Streptomyces pentapentadienes, this paper discusses new strategies to increase TG enzyme production, and provides theoretical basis for understanding the regulatory mechanism of microbial metabolic performance.

The subject of the study: Synthesis of TG enzymes.

Research methods: Experimental design, strain culture, medium preparation, reagents and instruments, data processing and analysis, cell growth measurement, enzyme activity determination, protease activity determination, microscope observation, microparticle influence study, particle size distribution analysis, fermentation culture and enzyme solution preparation, protein mass concentration determination

The scientific novelty: The effects of $MgCl_2$ stress on TG enzyme production, the effects of $MgCl_2$ stress on cell morphology, the effects of microparticles on mycelium morphology and TG enzyme synthesis, the relationship between mycelium morphology and TG enzyme production, the theoretical basis and industrial application, and future research directions were proposed

The practical significance of the results obtained is increase TG enzyme production, gain a deeper understanding of the regulatory mechanisms of microbial metabolic performance, applications in industrial production, optimize fermentation processes, influence of environmental factors on microbial growth, development of novel enzyme preparations, strategies for microbial metabolic engineering, education and research training, international academic exchanges, and socio-economic development

CHAPTER 1
LITERATURE REVIEW

1.1 Introduction to Streptomyces cenoyuanensis

Streptomyces cenoyuanensis belongs to the genus Steptomyces and is a Gram-positive, multicellular, filamentous soil bacterium [1]. The main methylnaphthoquinones include MK-9 (H6), MK-9 (H8) and some species also contain MK-9 (H4). It has a very complex morphological differentiation cycle, and its morphological characteristics mainly include the formation of highly branched base filaments and gas filaments, and stromal mycelia will develop into air mycelia and spores [2], and most of the mycelia are without transverse septa or fracture. Most species produce chain spores that do not swim and are often colored, smooth, or spiny. In addition to its special morphological differentiation, Streptomyces pentadiengensis also has a remarkable feature that it produces secondary metabolites of great significance for a wide range of applications. Most domestic and foreign studies have proved that the morphological differentiation of Streptomyces pentadiengensis is closely related to the biosynthesis of various secondary metabolites and certain enzymes. Streptomyces pentadiengensis produces a substance called ponidocidin, and its main value at this stage lies in its taxonomic studies and its use as a model strain.

1.2 Introduction to glutamine transaminase

TGase, full name of Transglutaminase, also known as transglutaminase, is an enzyme that can catalyze cross-linking reactions between or within proteins. Its molecules are formamide on glutamic acid residues and ξ- amino on lysine residues in proteins. This enzyme can make protein molecules from small to large. Forms a powerful gel that changes the physical and chemical properties of the protein. For example, it improves the adhesion, thickening,

emulsifying, foaming, gelling and emulsifying stability of the protein. TG enzymes are widely distributed in mammals, fish, plants and microorganisms, and have different physiological functions in these organisms. For example, the TGase from plant participated in the formation of cell wall and cytoskeleton, and the TGase from microorganism Bacillus subtilis played a cross-linking protein role and participated in the spore wall assembly process. TG enzymes derived from mammalian plasma participate in the cross-linking of blood fiber molecules. Tissue TG enzymes play an important role in multiple physiological processes when blood clots, and excess and loss of TG enzymes are associated with many human diseases [**Error! Reference source not found.**].

1.3 Research progress of glutamine transaminase at home and abroad

With the development of biotechnology, the production technology of TG enzymes is also improving, including the application of microbial fermentation and genetically engineered expression systems, aiming to achieve more efficient and lower cost production. The research and development of TG enzymes in China has just started, but some progress has been made in improving enzyme activity and stability. In contrast, the international research on TG enzymes is more in-depth. The researchers will investigate the catalytic mechanism of TGase and how to optimize its catalytic efficiency and substrate specificity by changing the structure of the enzyme. The researchers also modified the enzyme molecules through genetic engineering and protein engineering techniques to improve the catalytic efficiency and thermal stability of TG enzymes, such as enhancing enzyme activity and thermal stability through deletion mutations and saturation mutations. TG enzymes are not only widely used in the food industry, but also in medicine, cosmetics and other fields. In addition, there are already some mature commercial products in the world, such as Japan's MTG. Moreover, new functions of TG enzymes

have been discovered in recent years, such as their role in promoting the progression and spread of prostate cancer.

In the 1980s, the cross-linking effect of TG enzymes on food proteins was reported by Ikura et al. The latest research results were innovative. They created a new idea and used TG enzymes from mammals to cross-link various food proteins such as soybean protein and casein, thus significantly improving the product functionality of these proteins. For example, protein solubility, gelatinability and emulsification [4]. However, its price is extremely expensive and its utilization rate is also very inefficient, which greatly limits the application of TG enzymes in the food industry and becomes a new pain point [5]. There is an important milestone in the food industry, that is, the successful isolation of microbial source TG enzymes from Streptomyces, which improves the price and utilization rate of TG enzymes, and enables the large-scale production and gradual commercialization of TG enzymes [6].Not only in the food industry, but also in the study of gene expression and construction of engineered strains, researchers build engineered strains and optimize expression conditions or gene transfer breeding to improve the yield and activity of TG. For example, two methods of vector-free DNA electric transfer and protoplast fusion of Streptomyces centaeus were established to enable gene transfer and recombination, thereby improving the yield of TG-producing strains and obtaining better strains. After high-throughput screening, strains that combine the advantages of the two series and are more conducive to industrial production were obtained [**Error! Reference source not found.**].

1.4 Application of glutamine transaminase

1.4.1 Application in dairy products

First of all, in terms of laws and regulations, on May 5, 2022, the National Health Commission issued a notice, which mentioned the use of TG enzymes in flavored fermented milk and unmatured cheese, and specified the

maximum amount of use, which greatly promoted the application of TG enzymes in dairy products.

The main protein in dairy products is casein, which is a good substrate for TG enzymes, so the reported cross-linking effects on food proteins are concentrated on milk proteins. However, the whey protein lacks lysine residues and glutamic acid, and the whey protein is difficult to cross-link. TG enzymes sometimes do not react biochemically with whey proteins because only casein is involved in cross-linking when TG enzymes are added to untreated dairy products. Because of this cross-linking characteristic of casein, the main target of TG enzyme application in dairy products gradually emerged.

TG enzyme plays an important role in the production of yogurt. In the process of adding to yogurt, TG enzyme can be added together with or before the starter culture, and its main function is to increase the strength of gel and reduce the release of whey [8-10]. The superior property that casein can cross-link enables TG enzyme to be applied to dairy products containing casein. In the process of yogurt production, TG enzyme can promote the interaction of milk protein, reduce the use of emulsifiers and stabilizers, and improve the viscosity and gel strength of products, thus obtaining yogurt products with high yield. TG enzyme also improves texture and taste, it reduces whey precipitation, and greatly improves the stability and water holding capacity of yogurt. In the production process, TG enzyme can also enhance the gel system of dairy products, greatly reducing or replacing the use of emulsifiers and stabilizers, and it enhances the gel system of dairy products. Until now, there have been many studies on the effect of TG enzyme on yogurt, and many articles have been published.

1.4.2 Application in baked goods

The application of TG enzymes during dough processing helps to improve the overall quality of bread. Gluten protein is a key protein in wheat

flour that determines the properties of dough, and the disulfide bond between it enhances the network structure, which is beneficial to the quality of bread, and has an important impact on bread and other baked foods [11]. Because gluten protein can be crosslinked by TG enzyme to form polymer, its network structure is enhanced and its rheological and physical and chemical properties are changed [12]. Gottmann and Sprossler [13] have shown that the use of TG enzyme can enhance the stability of dough, so as to increase the volume of bread and have better internal structure [14]. TG enzyme can also enhance the elasticity of bread crumbs, increase the pastry part of pastry bread and increase the volume of croissants [15-16]. Not only in this respect, TG enzymes also enhance the structure of foods, delay the aging process, and extend the shelf life of baked foods; Improves the chewability and overall taste of baked goods, making bread and other foods more delicate and softer; Enhances the natural function of foods such as gluten and reduces or eliminates the use of other additives; It can also facilitate the research and development of new baked goods without losing texture and taste.

1.4.3 Applications in the processing of textile and leather products

In textile and leather products, protease, amylase and lipase have a wide range of applications, they regulate and optimize textile and leather products, greatly improve the quality of products, and are welcomed by the majority of researchers [17-18]. In contrast, the application of TG enzymes in textiles and leather products is minimal, and some domestic and foreign researchers are also trying to break through the technology to form the latest research results. For example, with wool textile modification, enzymatic dyeing, denim finishing, biological polishing, anti-felting finishing and so on.

Textiles are often treated with proteases, which are very powerful and greatly improve their shrinkage resistance and performance. But can not only see its functional advantages, protein hydrolysis control is extremely difficult,

if the hydrolysis of excessive may lead to textile fiber surface damage, so that the quality and concentration of textiles greatly reduced. This greatly limits the application of protease in the optimization process of textiles. In order to solve this problem, Cortez et al. found that TG enzymes can reduce the contractility of textiles and maintain or increase the concentration of fibers. It was also pointed out that the TGase derived from guinea pig liver and Streptomyces pentadiengensis were applied to the textile treated with protease, and the results showed that the fiber strength after treatment was increased by about 25% compared with the control sample. This proves that TG enzyme can reduce proteolysis and improve fiber strength. Du et al. also pointed out that TG enzymes derived from Streptomyces hygrowater can enhance its solubility and reduce the decline in tensile strength when treating wool [19].

In the processing of leather products, the treatment of filler is an indispensable step, which refers to the addition of material to the gap of the leather fiber, so as to make the leather surface smoother, and these fillings will continue to exist in the subsequent processing. In general, the stuffing used in traditional processes is mostly a variety of natural products, such as glucose, flour and resin. But Taylor et al. found that casein and gelatin were very effective as fillers. The use of glutaraldehyde crosslinked high polymerization white gelatin as a leather filling material, even after the washing process, can remain inside the leather. The treatment of casein-filled leather with microbial TG enzymes also achieved a similar durability effect, confirming that TG enzymes have become a new and more cost-effective research direction [20].

1.5 Research progress of TGase production by fermentation of Streptomyces pentadipentadienyces

The production of TG enzymes is closely related to the growth environmental conditions of microorganisms, such as carbon source, nitrogen source, temperature, pH, dissolved oxygen, inducers and repressors, which will

make it change. Therefore, the production of TG enzyme can be increased rapidly through the regulation of environmental factors. Since 1989, when Ando et al. first discovered that actinomycetes produce glutamine transaminase, researchers around the world have been working to increase the production of this enzyme by optimizing culture conditions. They explored a variety of factors including the composition of the medium, temperature, pH, oxygen supply, etc., to find the best culture environment to get more TG enzymes. These studies not only enhance the understanding of the production mechanism of TG enzymes, but also provide important technical support for industrial production. Through these efforts, the yield of TG enzymes has been significantly improved, making its application in the food industry and other fields more extensive and practical. By 1996, Zhu et al. [21], a researcher at TNO Food and Nutrition Research Institute in the Netherlands, revealed the significant effects of specific amino acids on cell proliferation and TG enzyme secretion through in-depth analysis of amino acid metabolic pathways. Their research points out that certain amino acids play a crucial role in the cell culture process. On top of the medium formulation previously developed by Ando et al. Zhu et al. optimized the composition of the medium by adding appropriate amounts of histidine, isoleucine and methionine. This improvement significantly increased the production of TG enzymes during batch fermentation, and even increased by up to four times. This discovery is of great significance for improving the production efficiency of TG enzymes, and provides a valuable reference for the subsequent microbial fermentation process. In 1997, in the study of Junqua et al. [22], Streptoverticillium cinnamoneum was cinnamoneum, a microbial strain, to produce TG enzyme. Through experiments, they found that casein was most beneficial to TG enzyme production when it served as a nitrogen source. In addition, they also found that under the specific conditions of 3.12% glycerol as a carbon source and 3.84% casein as a nitrogen source, the activity of TG enzyme could achieve

a threefold increase, and the resulting enzyme activity reached a maximum of 0.331 U/mL. This study provides important parameters for the optimization of TGase production, which will help to promote the development of related biotechnology.

In Taiwan Ocean University, Professor Wu Jiewen [23] described in detail the fermentation and culture conditions of Streptoverticillium ladakanum for the first time. According to his research, it was proved that the most suitable culture temperature was set at 28 °C, and the culture cycle was 4 days, and the stirring speed during the fermentation process was controlled at 100 to 150 revolutions per minute. Professor Wu also found that the addition of methionine or glycine had a positive effect on the synthesis process of TG enzymes. In terms of the selection of nitrogen source, yeast paste was proved to be the best choice to promote the production of TG enzymes, and the enzyme activity obtained under this condition was the highest, reaching 1.8 U/mL. After adding 22 mg/kg colistin, the yield increased by 30% and the enzyme activity reached 2.1 U/mL. These findings provide important fermentation parameters for the application of Streptoverticillium ladakanum in TGase production.

In China, Jiewen Wu of Taiwan Ocean University reported for the first time the fermentation culture conditions of Streptoverticillium ladakanum: The culture temperature was 28 ℃ for 4 days, the culture speed was 100-150 r/min, and the addition of methionine or glycine was conducive to TGase synthesis. Yeast extract was the best nitrogen source, and its enzyme activity reached 1.8 U/mL. After adding 22 mg/kg colistin, the yield was increased by 30% and the enzyme activity reached 2.1 U/mL. It was also studied by Academician Chen Jian and his research team at Jiangnan University's Center for Future Food Science, who used precise molecular design and advanced genetic engineering techniques to successfully develop a variant of TG enzyme with stronger heat resistance, namely TGm2. Compared to the original strain, TGm2 exhibits a

longer half-life and higher specific activity at 60 °C, which marks a significant improvement in thermal stability. The research team further achieved high expression of TGm2 in Streptomyces pentagenicum, a food-grade safe strain, and finally obtained the recombinant strain smY2022-TGm2, which secreted TG enzyme activity up to 61.7 U/mL. This achievement not only highlights the broad application prospects of TGm2 in industrial scale production, but also provides a better enzyme preparation for food processing and other fields.

Other domestic scholars have also studied the fermentation production of TG enzymes and achieved some results. For example, Meiying Zheng et al. studied the fermentation production process using Streptoverticillium mobaraensis as the starting strain. Although each has achieved an increase in production, but the mechanism of the increase in production is still not very clear. Following the previous research, many domestic scholars have conducted in-depth research on the fermentation production technology of TG enzymes, and obtained a series of research results. For example, Zheng Meiying et al. [24] selected Streptoverticillium mobaraensis as the research object and systematically studied its fermentation production process. Through different process optimization strategies, these studies have made their own progress in increasing the yield of TG enzymes. However, despite the increase in production, there is a lack of comprehensive and in-depth understanding of the detailed mechanisms by which these optimization measures work to increase production. This shows that many details and principles of the TG enzyme fermentation production process need to be clarified by further scientific research.

1.6 Relationship between glutamine transaminase and mycelial morphology of Streptomyces pentaenycens

The production of glutamine transaminase is closely related to the morphological development of mycelium of Streptomyces mobaraensis.

Studies have shown that the generation of TG enzymes is not only related to the viability of bacteria, but also plays a regulatory role in their morphological changes. The function of microbial TG enzymes in the life cycle of Streptomyces centaeniae is still uncertain, but it is generally believed that it may play a role in promoting the covalent cross-linking between proteins, which may involve the formation of the cell wall and envelope of the air mycelium. The results of labeling the cell wall proteins of Streptomyces pentadiengensis verified this claim. The double-labeling technique was used to detect and reveal the formation of several protein polymers with low molecular weight under the catalytic action of TG enzymes, which helped Streptomyces pentadiengensis to exhibit the characteristics of multicellular organisms. Changes in cell wall morphology and thickness can be observed with the growth of Streptomyces pentadiengensis, especially during the development of air mycelium into chain spores.

In one of the studies [**Error! Reference source not found.**], researchers adopted the strategy of introducing ethylenediamine tetraacetic acid (EDTA) into the culture medium to inhibit the TG enzyme activation of key proteases, thus regulating the synthesis process of mature TG enzymes. In this study, the researchers used laser confocal scanning microscope (CLSM) and scanning electron microscope (SEM) to carefully observe the microbial activity and morphological changes during fermentation. The results showed that in the control group, with the increase of TG enzyme production, the cell vitality of Streptomyces pentagenicum experienced a process of first increase and then decrease, and was accompanied by the diversification of cell morphology. In contrast, in the experimental group, after the introduction of EDTA, the production of TG enzyme was blocked, resulting in a continuous decline in cell vitality, and the morphological development of bacteria was also significantly hindered. These results indicate that the synthesis of TG enzymes

has an important effect on the mycelial morphological differentiation of Streptomyces pentadiengensis.

In addition to this, the researchers further revealed the production mechanism behind the high-yield TG enzyme strains by resequencing the genome and in-depth analysis of transcription levels. Compared with the original strain, the expression level of MTG in the high-yield strain was significantly increased in the transcriptional stage, which may be related to the specific variation of the upstream non-coding region of the MTG gene in the high-yield strain. This variation may have a positive effect on the transcriptional activity of MTG genes, thereby increasing their expression at the transcriptional stage [**Error! Reference source not found.**].

Conclusions to chapter 1

Streptomyces cenoyuanensis is a Gram-positive, multicellular, filamentous soil bacterium with a complex morphological differentiation cycle, mainly producing methylnaphthoquinone secondary metabolites, such as MK-9 (H6) and MK-9 (H8). The morphological differentiation of this bacteria is closely related to the biosynthesis of secondary metabolites produced and certain enzymes.

Transglutaminase (TGase), also known as transglutaminase, is an enzyme that catalyzes cross-linking reactions between or within proteins. It can make protein molecules form powerful gels from small to large, and change the physical and chemical properties of proteins, such as improving their adhesion, thickening, emulsification, foaming, gelation and emulsification stability. TG enzymes are widely distributed in mammals, fish, plants and microorganisms, and play different physiological functions in these organisms.

The research progress of TG enzymes at home and abroad shows that the production technology of TG enzymes is constantly improving through the

application of microbial fermentation and gene engineering expression systems to achieve more efficient and low-cost production. China has made some progress in improving enzyme activity and stability, while international studies have explored the catalytic mechanism of TGase in more depth, as well as optimizing its catalytic efficiency and substrate specificity by changing the enzyme structure.

TG enzymes are widely used in the food industry, especially in the production of dairy products and baked goods, which can improve product quality and increase output. For example, in yogurt production, TG enzymes can increase gel strength and reduce whey release; In baked goods, TG enzymes can enhance dough stability, increase bread volume and improve internal structure.

In addition, TG enzymes also have potential applications in the treatment of textile and leather products, such as in the modification of wool textiles, enzymatic dyeing, denim finishing, etc. TG enzyme can reduce the contractility of textile, maintain or increase the fiber concentration, and improve the fiber strength.

Research progress on the production of TG enzymes by fermentation of Streptomyces pentadipentadienyces shows that the production of TG enzymes can be rapidly increased by optimizing the growth environmental conditions of microorganisms, such as carbon source, nitrogen source, temperature, pH, dissolved oxygen, etc. Through these studies, the yield of TG enzymes has been significantly increased, which provides important technical support for its application in food industry and other fields.

Finally, the relationship between TG enzyme and mycelial morphology of Streptomyces pentaenycens was also discussed. It was found that the production of TG enzyme is closely related to the morphological development of mycelia, and may play a role in promoting covalent cross-linking between proteins, involving the formation of cell wall and envelope of aerated mycelia.

Through an in-depth analysis of the genome resequencing and transcription levels of the high-yielding TG enzyme strains, the researchers revealed the production mechanism behind the high-yielding TG enzyme strains.

CHAPTER 2
OBJECT, PURPOSE AND METHODS OF THE STUDY

2.1 Experimental materials

2.1.1 Strains

Streptomyces penicilliformis, preserved in the Faculty of Bioengineering, Qilu University of Technology.

2.1.2 Medium

Slant medium (g/L): soluble starch 20, NaCl 0.5, KNO_3 1, $K_2HPO_4 \cdot 3H_2O$ 0.5, $MgSO_4 \cdot 7H_2O$ 0.5, $FeSO_4 \cdot 7H_2O$ 0.01, AGAR 20, pH 7.5.

Seed medium (g/L): Soluble starch 20, polypeptone 20, K_2HPO_4 2, KH_2PO_4 2, anhydrous Magnesium sulfate 2, Yeast powder 2, pH 7.0.

Initial fermentation medium (g/L): soluble starch 10, polypeptone 30, fructose 10, K_2HPO_4 2, yeast powder 2, anhydrous magnesium sulfate 1, pH 7.0.

Inclined medium: 20% potato boiled solution, 1% glucose.

Basic fermentation medium: 22.6% soybean meal extract, 1.035% peptone, 0.3% KH_2PO_4, 0.8% $Na_2HPO_4 \cdot 7H_2O$, 0.01% NaC1, 0.02% $MgSO_4 \cdot 7H_2O$, 0.005% $CaC1_2$.

2.1.3 Reagents

Ethylene diamine tetraacetic acid disodium (EDTA) analysis Pure Sinopharm Group Chemical Reagent Co., LTD

Casein (dephosphorylation) analysis pure American Sigma-Aldrich Corporation

L-glutamate-γ-monooxime acid chromatography pure Sigma-Aldrich Corporation

CBZ-Gln-Gly analysis pure American Sigma-Aldrich Company

Phenylmethyl sulfonyl fluoride (PMSF) biochemical reagent Sigma-Aldrich Company

Glucose, peptone, soluble starch analysis Pure Tianjin Kemie Ou Reagent Co., LTD

Malt extract analysis pure British OXOID

Glycerin, AGAR powder biochemical reagent Tianjin Kemi Ou reagent Co., LTD

KH_2PO_4, K_2HPO_4 Analysis Pure Xilong Science Co., LTD

$MgSO_4 \cdot 7H_2O$, NaCl analysis pure Shanghai Chemical Reagent Co., LTD

$CaCO_3$ analysis pure Shanghai Lotus chemical reagent factory

Soybean cake powder biochemical reagent commercially available

Yeast paste biochemical reagents Fuyang Hangfu biological products factory

$FeCl_3 \cdot 3H_2O$ Chemical pure Chinese Medicine Group

Hydroxylamine hydrochloric acid biochemical reagent Jiangsu Aikewei Technology Co., LTD

Ultra-low temperature refrigerator American thermal power company

Optical microscope Japan Nikon Corporation

Vertical flow ultra-clean table Shanghai Longtuo Instrument Equipment Co., LTD

Autoclave Shanghai Shen 'an medical equipment factory

Biochemical incubator Shanghai Yiheng Technology Co., LTD

Electric thermostatic water bath Shanghai Yiheng Technology Co., LTD

Ultrapure water meter Millipore company

Ultrafiltration equipment American Millipore company

Talc powder granules Shanghai Maclin Biochemical Technology Co., LTD

Aluminum oxide particles, kaolin particles Shanghai Aladdin Biochemical Technology Co., LTD

Potatoes are bought in the supermarket

Soybean meal, peptone Bioengineering Co., LTD

KH_2PO_4, $Na_2HPO_4 \cdot 7H_2O$, NaC1, $MgSO_4 \cdot 7H_2O$, $CaC1_2$ Sinopremedy Group Chemical Reagent Co., LTD

Ferulate methyl ester Nantong Feiyu Biotechnology Co., LTD

Dimethylformamide, chromatographic pure grade acetonitrile, formic acid, methanol Tianjin Siyou Fine Chemicals Co., LTD

2.1.4 Instruments

High performance liquid chromatograph Shimadzu, Japan

Frozen high speed centrifuge USA Beckman Company

Low speed centrifuge Hunan Xiangyi Co., LTD

Ultrasonic complex frequency cleaner Shanghai Dongda Ultrasonic Instrument Co., LTD

Constant temperature adjustable speed shaking table Shanghai Xinrui Automation Equipment Co., LTD

Stereomicroscopes ZEISS GMBH

Laser particle size analyzer Malvern Instruments, UK

2.2 Experimental methods

2.2 1 Data processing and analysis

Experimental data were obtained through three independent experiments, and the results were presented in the form of mean plus or minus standard deviation (x±SD). SPSS 15.0 software was used for statistical analysis of the obtained data. For the comparison of the two groups of data, an independent sample T-test was performed, and the threshold of statistical significance was set at 0.05. For comparison of data from three or more groups, One-way ANOVA was used and Duncan multiple range test was applied for comparison between groups, again setting the significance level at 0.05.

2.2.2 Determination of somatic cell growth of Streptomyces pentadipentadienes

50 ml of fermentation liquid was filtered, and then distilled water was used to wash Streptomyces pentaenycens left on the filter paper three times. After washing, the Streptomyces pentadiengenium on the filter paper was dried at 105 ℃ until its weight was stable and finally weighed [27].

2.2.3 Determination of TG enzyme activity

This method adopts the spectrophotometric method proposed by Folk and Cole [28].

(1) Configure reagents

Reagent A: 100 mg of N-CBZ-Gln-Gly was dissolved in 2 mL of 0.2 mol/L NaOH, followed by 0.2 mol/L of 4 mL of Tris-HCl buffer at pH 6.0, and 0.1 mol/L of 2 mL of hydroxylamine. 0.01 mol/L reduced glutathione to 2 mL and regulated pH to 6.0.

Comparison of reagent A: The reagent is the same as reagent A except that N-CBZ-Gln-Gly is not added.

Reagent B: 12% trichloroacetic acid, 3 mol/L hydrochloric acid, 5% $FeCl_3 \cdot 6H_2O$ solution was mixed in a ratio of 1:1:1.

(2) Measurement of standard curve

First, a series of L-glutamate-gamma-hydroxamic acid solutions with concentrations ranging from 0 to 100 μmol/L were prepared. 0.5ml of reagent A was mixed with 50μL of L-glutamate-gamma-hydroxamic acid solution at different concentrations and kept at 37℃ for 10 min. Add 0.5 ml of reagent B to the mixture and stir thoroughly. Finally, by measuring the absorbance at 500 nm wavelength, the corresponding standard curve is drawn according to the absorbance value.

(3) Determination of TG enzyme activity

50 μL of enzyme solution was added to 0.5 ml of reagent A and the reaction was carried out at 37 ℃ for 10 min. After the reaction is complete, 0.5 ml of reagent B is added to the mixture to terminate the enzyme activity. Then centrifuge at 8000 RPM for 15 min to remove the formed sediment. After the removal of the sediment, the absorbance of the red substance in the supernatant was measured at a wavelength of 500 nm. The enzyme solution with only reagent A added was used as the control group, and the value of enzyme activity was calculated according to the pre-established standard curve.

2.2.4 Determination of protease activity

At 37 °C, protease activity is measured by an increase in absorbance of 0.01 per hour, which is equivalent to 1 unit of activity (U). In this paper, the activity determination of total protease and metalloproteinase will follow the experimental method described by Hirano et al. [29].

(1) The total activity of proteases was determined by using AZ casein as a substrate dissolved in a phosphate buffer solution of 50 mmol/L (pH 7.0) at a concentration of 2% and reacted at 37 °C for 1 h. After the reaction is complete, the unreacted substrate is precipitated by adding 10% trichloroacetic acid (TCA). Subsequently, the activity of the protease was assessed by measuring the absorbance value at a wavelength of 440 nm.

(2) Metalloproteinase activity is the introduction of 1.25 μmol/L EDTA into the reaction system and the subsequent evaluation of metalloproteinase activity according to the above-mentioned assay procedure. After the reaction lasted for 12 h, enzyme activity was measured. This was then determined by subtracting the activity reading after the addition of EDTA from the total protease activity.

(3) The activity of serine proteinase was measured in accordance with the method of metalloproteinase activity, and 1.25 μmol/L phenylmethyl sulfonyl fluoride (PMSF) was used instead of EDTA.

2.2.5 Morphological changes of Streptomyces pentadiengensis were observed by microscope

The preparation procedure for the microscopic observation of Streptomyces pentadiengensis is as follows:

Bacteria collection: Centrifuge 5 ml of bacteria culture solution by 12000g for 10 min and cool to 4 ℃, collect bacteria precipitation, then wash with PBS three times and centrifuge again to collect bacteria.

Fixation: The bacteria were precipitated in 2.5% glutaraldehyde solution, the pH value was adjusted to 6.8, and fixed at 4 ℃ for 2 h.

Rinse: Use 0.1 mol/L sodium phosphate buffer (pH 6.8) to flush bacterial cells twice.

Dehydration: Firstly, ethanol solution of different concentrations (50% to 90%) is used to dehydrate successively, and each concentration stage lasts for 10 min; Then use anhydrous ethanol to dehydrate three times, also for 10 minutes each time.

Displacement: A mixture of ethanol and tert-butanol (volume ratio 1:1) and pure tert-butanol were used for displacement dehydration, each lasting 15 min.

Drying: The sample was dried by freeze-drying technology for 4 h.

Sticky sample: The dried bacterial powder is fixed on the sample table of the microscope with conductive tape.

Coating: A 100-150 A gold film is formed on the surface of the sample by ion sputtering technology.

Microscope observation: the treated sample is placed under the microscope, and the computer is used to select the best field of view for imaging.

2.2.6 Fermentation culture and preparation of enzyme solution

Set the mass concentration of the microparticles to 0.1, 0.5, 1, 5, 10 and 20 g/L. Microparticles of 0.003, 0.015, 0.03, 0.15, 0.3, 0.6 and 3 g were added to 30 ml medium, respectively. Firstly, the microparticles and liquid medium were sterilized separately for further use. Next, the specific strains were inoculated on the prepared potato glucose AGAR medium and cultured in a constant temperature incubator at 28 °C for 6 days. The spore suspensions were then inoculated into 250 ml triangulated bottles containing 30 ml liquid medium at a predetermined dose (spore concentration approximately 107 CFU/mL) and cultured in a rotating shaker at 28 ℃ and 180 r/min for 7 days. Samples were taken on day 3, day 5 and day 7 of culture. After sampling, the bacterial suspension was centrifuged at a low-speed centrifuge at 3000 r/min for 30 min. The resulting supernatant was the crude enzyme liquid, and it was stored in a refrigerator at 4 ℃ for subsequent use.

2.2.7 Determination of protein mass concentration

According to the Bradford method [30], protein quantification kits were used to measure the protein concentration in the fermentation supernatant. First, the Coomath brilliant Blue stain is restored to room temperature and thoroughly mixed while preheating the spectrophotometer. The standard solution of bovine serum albumin (0 to 6 μL at a concentration of 1 mg/mL) was added to different pores in the enzyme-labeled plates and supplemented with phosphate buffer to 10 μL. Then 190μL of Bradford Coomas Bright Blue dye was added to each well, mixed well and left for 5 to 10 min at room temperature. The absorbance was measured at the wavelength of 595 nm using an enzyme-labeler, the absorbance value of the sample without BSA was set to zero, and the standard curve was drawn with protein concentration (g/L) as the X-axis and absorbance as the Y-axis. In the process of sample determination, a sample of 10 μL fermentation solution was evenly mixed with 190 μL

Coomas bright Blue stain solution, and left at room temperature for 5 to 10 min, and then the absorbance was measured at the wavelength of 595 nm using an enzymoleter. Finally, the protein concentration in the sample was calculated according to the standard curve prepared previously, which was then used to calculate the specific activity of TG enzymes.

2.2.8 Microscopic observation and particle size distribution

First, the colonies of Streptomyces pentadiengensis were observed with stereoscopic microscopy, and then the colonies of Streptomyces pentadiengensis were examined in more detail by XL-30 ESEM scanning electron microscopy after certain pretreatment steps. As for the particle size analysis of Streptomyces pentadiengensis, Hitachi S-3000N scanning electron microscope was used to observe the particles at an accelerated voltage of 20 kV, and finally Mastersizer2000 laser particle size analyzer was used to measure and determine the particle size distribution of the sample.

Conclusions to chapter 2

The second chapter introduces the experimental materials, experimental methods and data processing and analysis in detail.

Experimental materials include:

Strain: Streptomyces penicilliformis, preserved in the School of Bioengineering, Qilu University of Technology.

Media: The components and proportions of inclined medium, seed medium, initial fermentation medium, inclined medium and base fermentation medium are listed in detail.

Reagents: including disodium ethylenediamine tetraacetic acid (EDTA), casein (dephosphorylation), L-glutamate-γ-monohydroxamic acid, etc., are provided by different companies.

Instruments: including high performance liquid chromatograph, frozen high-speed centrifuge, ultrasonic cleaning machine, etc., produced by many companies.

The experimental methods include:

Data processing and analysis: Experimental data were obtained through three independent experiments and presented in the form of mean plus minus standard deviation. SPSS 15.0 software was used for data analysis, and independent sample T-test and One-way ANOVA were used for data comparison.

Streptomyces growth assay: Cell growth of Streptomyces pentadipentadienes is measured by filtration, washing, drying, and weighing.

Determination of TG enzyme activity: The spectrophotometric method proposed by Folk and Cole was adopted, including reagent configuration, standard curve measurement and enzyme activity determination steps.

Protease activity assay: The activity of total protease and metalloproteinase was measured by increased absorbance using AZ casein as a substrate.

Observation of colony morphology: Morphological changes of Streptomyces pentadiengensis were observed by microscope.

Fermentation culture and enzyme solution preparation: particles with different mass concentrations are set, specific strains are inoculated, and crude enzyme solution is prepared by sampling after culture.

Protein mass concentration determination: Bradford method was used to measure protein concentration in fermentation supernatant with protein quantitative kit.

Microscopic observation of colony morphology and particle size distribution: stereoscopic microscopy and scanning electron microscopy were used to observe colonies, and laser particle size analyzer was used to determine particle size distribution.

As a whole, the second chapter provides a detailed list of materials, standardized experimental procedures and accurate data analysis methods for the experimental part to ensure the accuracy and reliability of the experimental results.

CHAPTER 3
EXPERIMENTAL PART

3.1 Experimental procedure and analysis

3.1.1 Effects of salt types on TG enzyme production

Some studies have shown that appropriate salt stress can significantly increase the yield of TG enzymes, but the effects of different types of salt on TG enzymes are also different. Eight different neutral salts were ₄ added to the medium. They were NaCl, Na_2SO_4, $C_6H_5O_7Na_3$, $MgCl_2$, $CaCl_2$, CH_3COONa, KC1, and Na_3PO_4. Finally, the concentration in each medium was 0.2mol /L. The control group without salt was set up and cultured at 30 ℃ and 200 r/min for 96 h. The dry weight of the bacteria (g/L) and the TG enzyme activity in the supernatant (U/mL) were weighed, as shown in Figure 3.1.

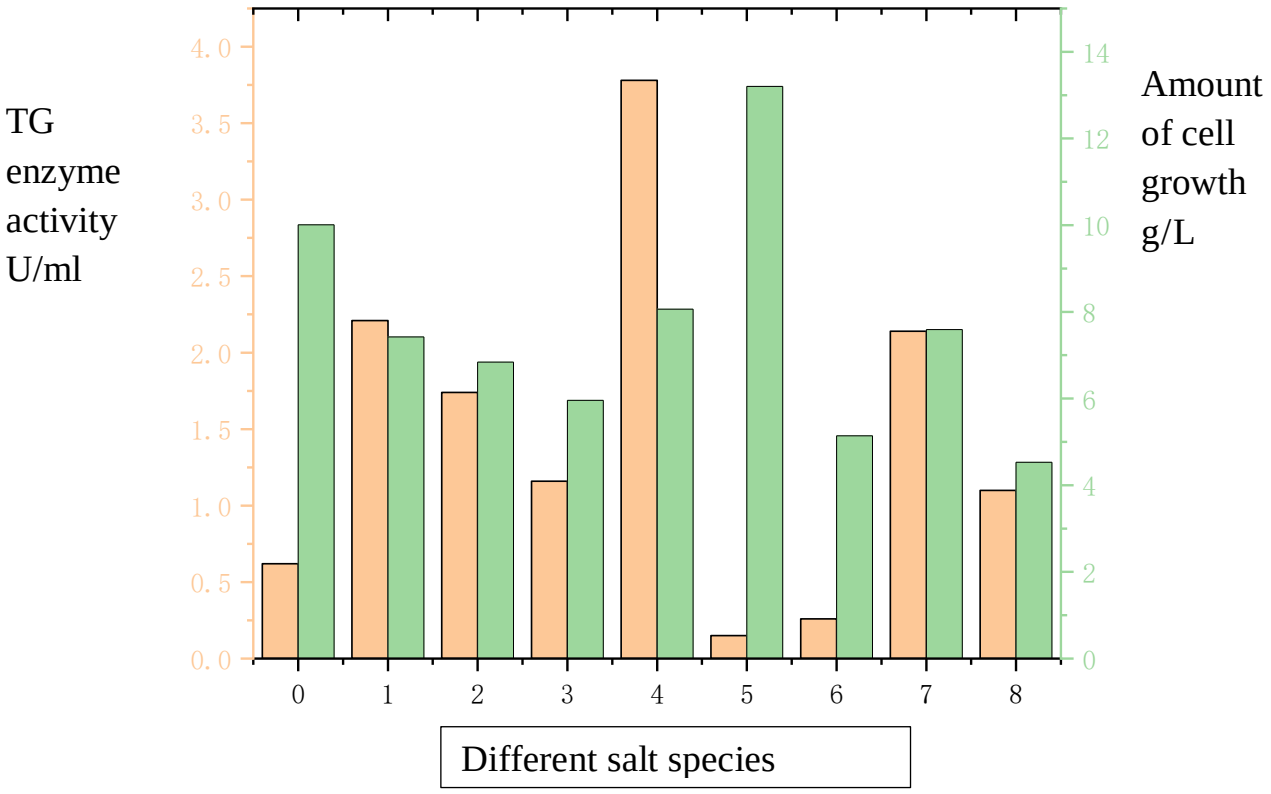

Figure 3.1 - **Effects of different salts on the biomass and TG enzyme production of Streptomyces pentapentadienes**

According to the data in Figure 3-1, adding $CaCl_2$ can increase bacterial biomass, but significantly inhibit the production of TG enzyme ($P < 0.05$). It was also observed that the salts KCl, Na_2SO_4, and $MgCl_2$ reduced the biomass of the bacteria. However, they significantly enhanced the production of TG enzymes. In particular, the addition of $MgCl_2$ significantly increased the TG enzyme yield ($P < 0.05$), which increased the yield from 0.62 U/mL to 3.78 U/mL in the control group. Although the addition of NaCl and Na_2SO_4 both resulted in an improvement in the yield of the enzyme. There was no statistically significant difference ($P > 0.05$). This indicates that Cl^- and SO_4^{2-} did not much to increase the yield of the TG enzyme.

3.1.2 Effect of $MgCl_2$ concentration on TG enzyme yield

Through the above experiments, it is found that $MgCl_2$ has a prominent effect. Now, the influence of $MgCl_2$ concentration on TG enzyme production is further studied, and different concentrations of $MgCl_2$ are added to the medium. The concentrations were 0 mol/L, 0.05 mol/L, 0.1 mol/L, 0.15 mol/L, 0.2 mol/L and 0.25 mol/L, respectively. After fermentation at 30 ℃ and 200 r/min for 96 h, the dry weight of the bacteria was weighed and the TG enzyme activity was measured, as shown in Figure 3.2.

The above results show that $MgCl_2$ can promote the production of TG enzyme, and with the continuous addition of $MgCl_2$, its concentration continues to increase, and the enzyme production first increases and then decreases, with the highest concentration at 0.1 mol/L. When the concentration of $MgCl_2$ reached 0.1 mol/L, the activity of TG enzyme decreased significantly when the concentration of $MgCl_2$ was further increased. These results indicate that $MgCl_2$ is beneficial to the synthesis of TG enzymes. However, high concentration of $MgCl_2$ may inhibit the growth of Streptomyces pentaenycens and thus reduce the production of TG enzymes. The optimum concentration of $MgCl_2$ was determined to be 0.1 mol/L.

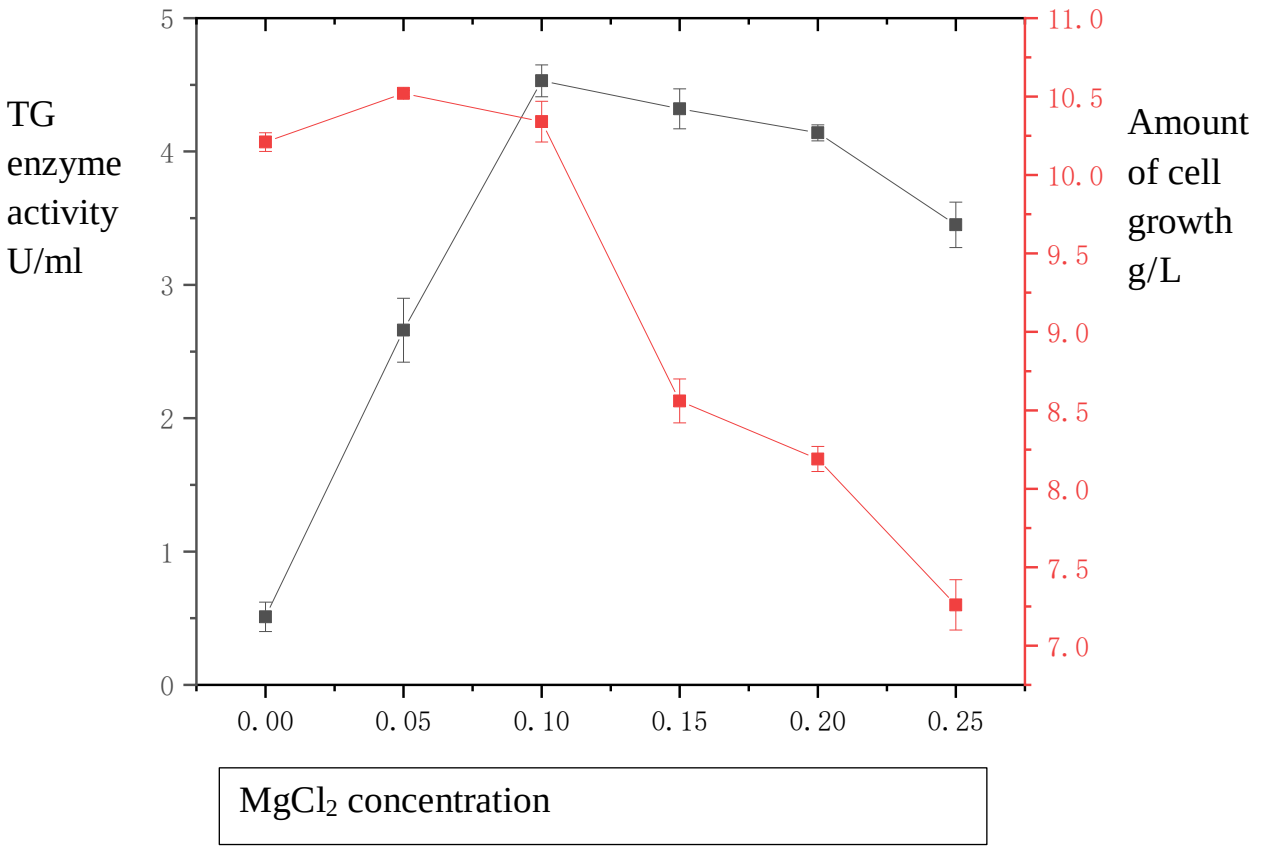

Figure 3.2 - **Effects of $MgCl_2$ concentration on the biomass and TG enzyme production of Streptomyces pentapentadienes**

3.1.3 Effect of $MgCl_2$ stress on fermentation cycle of Streptomyces pentadiengensis

The above experiments proved that $MgCl_2$ had certain effects on the production of TG enzymes and the bacteria of Streptomyces pentaenycens, and speculated whether $MgCl_2$ stress had an effect on the fermentation cycle of Streptomyces pentaenycens. To develop experimental protocols to investigate this hypothesis. The seed medium containing Streptomyces pentaenycens was inoculated into the medium containing 0.10 mol/L $MgCl_2$ at an inoculating rate of 8-10%. The dry weight of the bacteria and the TG enzyme activity of the supernatant were measured and recorded every 12 hours during the fermentation process, and the change curve was drawn according to the recorded values, as shown in Figure 3.3.

Figure 3.3 shows that the growth of Streptomyces pentadiengensis was slow in the early stage of fermentation, and then the bacteria continued to grow until the fermentation time was 108 h, and the cell dry weight of Streptomyces pentadiengensis reached the maximum (11.43 g/L). It also showed that TG enzyme activity could be detected at 24 h of fermentation, and the maturation process of TG enzyme would be synchronized with the growth of bacteria. The enzyme activity peaked at 4.32 U/mL at 108 h of the fermentation cycle. With the further extension of fermentation time, the enzyme activity decreased.

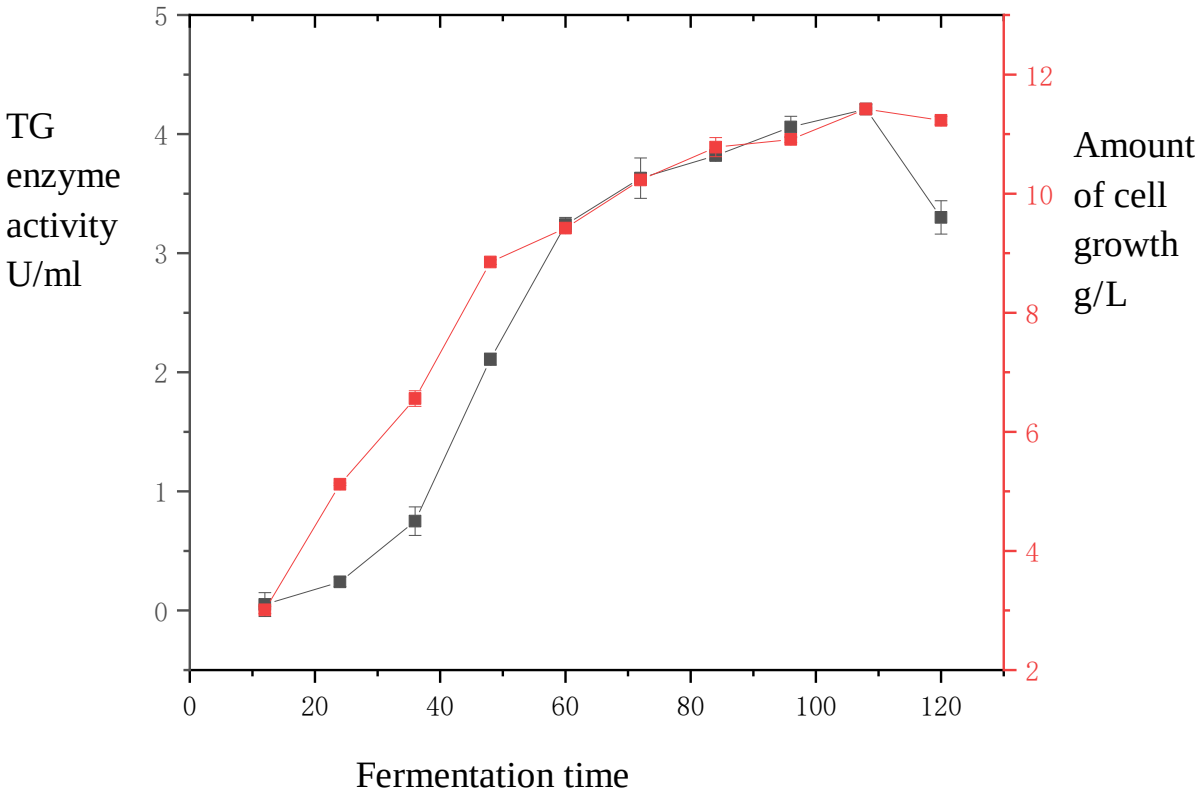

Figure 3.3 - **Changes of biomass and TG enzyme production of Protostreptomyces pentaenycens in $MgCl_2$ medium**

3.1.4 Effect of $MgCl_2$ stress on morphological changes of Streptomyces pentadiengensis

Two groups of media were set up, with or without $MgCl_2$ being the only variable, and cultured under the same conditions. The morphological changes of Streptomyces pentadiengensis in each medium were observed and recorded

every 12 h. According to Figure 3.4, compared with the control group, in the medium of the experimental group at the same time, the form of Streptomyces pentadiengensis was firmer, the surface was rougher, and the bacteria were more and larger. The results showed that the fermentation time of Streptomyces pentaenycens under $MgCl_2$ stress was earlier, and the formation time of its spore mycelia was also earlier. Some proteins are involved in the formation of spore and mycelium of Streptomyces pentaenycens, because the amount of TG enzymes in the $MgCl_2$-stressed medium increases, and then the TG enzymes cross-link with these proteins, promoting the formation of spore, mycelium, pili and other structures.

In this experiment, we paid special attention to the effect of $MgCl_2$ on the growth morphology of Streptomyces pentadiengensis. In order to ensure the accuracy of the experiment, we designed two groups of media, in which the only different variable was the presence or absence of $MgCl_2$, and the experimental group was the medium containing an appropriate amount of $MgCl_2$. All other conditions of the two groups of media, such as temperature, pH, humidity, and light, were kept consistent to exclude other factors that might affect the results of the experiment.

During the fermentation process of Streptomyces pentadiengensis, we observed and recorded the Streptomyces pentadiengensis in two groups of media every 12 hours. Through continuous observation, we noticed significant morphological changes. According to Figure 3.4, it can be clearly seen that in the experimental group where $MgCl_2$ is present, the colony morphology of Streptomyces pentadiengensis is more compact and rougher. In addition, there were more bacteria and larger individuals in the experimental group, indicating that the presence of $MgCl_2$ may promote the growth of Streptomyces pentaenycens.

Through further analysis, it can be inferred that under $MgCl_2$ stress, Streptomyces pentaenycens entered the fermentation growth stage earlier, and

the formation time of spore mycelia was also advanced. This early growth acceleration may be related to the role of $MgCl_2$ in cell metabolism, which affects the activity of TG enzyme, promotes its cross-linking with related proteins, accelerates the growth of cell structures such as spores and mycelia, and thus promotes the growth of Streptomyces pentaenipentaeniae as a whole.

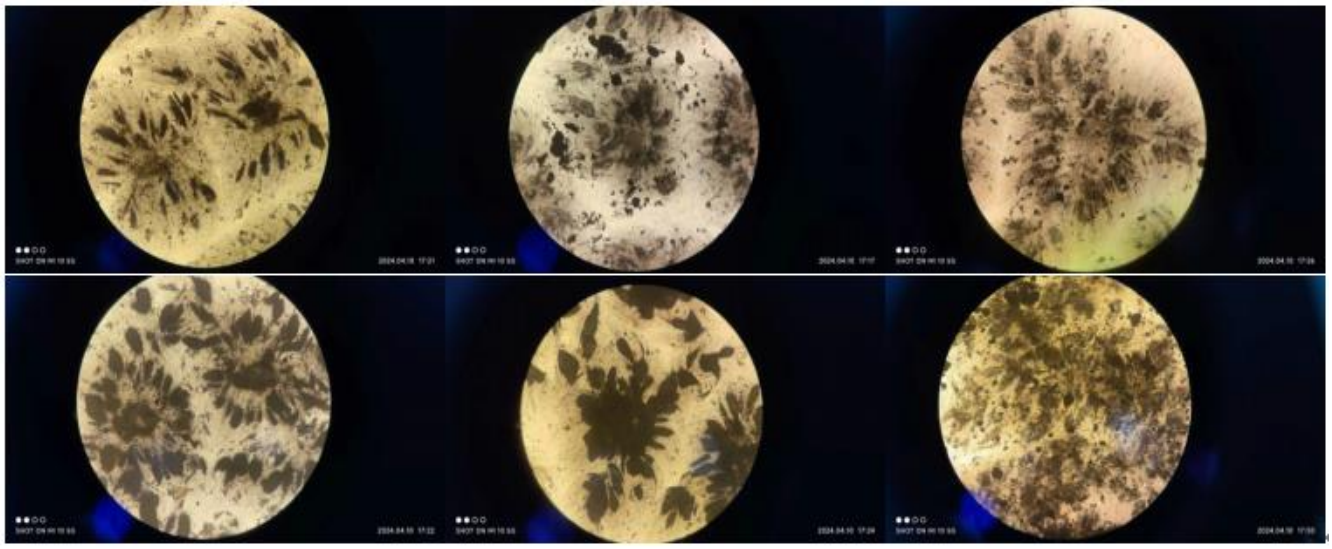

Figure 3.4 - **Streptomyces pentadienes morphology under $MgCl_2$ stress**

3.1.5 Effect of $MgCl_2$ stress on the activity of TG enzyme and protease

Although $MgCl_2$ did increase the production of TG enzymes, it could not be ruled out that Mg^{2+} activated the protease to improve its activity. The activity of TG enzyme, total protease, metalloproteinase and serine protease was determined by adding 0.01 mol/L and 0.1 mol/L $MgCl_2$ into the cell-free initial fermentation medium for 72 h, respectively. After the culture medium was continued for 24 h, TG enzyme activity was measured again, as shown in Table 3.1.

Table 3-1 - **Activity of various enzymes under different $MgCl_2$ concentration**

MgCl2 concentration, mol/L	TG enzyme activity	Total protease activity	Metalloproteinase activity	Serineprotease activity	Enzyme activity after 24 h culture
0	1	1	1	1	0.614
0.01	0.912	1.117	1.079	1.105	0.628
0.1	0.953	1.081	1.068	1.084	0.609

Figure 3.3 shows that $MgCl_2$ significantly increases the activity of total protease, metalloproteinase and serine protease, and significantly decreases the activity of TG enzyme. However, the increase in the activity of other proteases was only about 10%, far less than the two-fold increase observed during fermentation. Although $MgCl_2$ can enhance the activity of protease, this increased activity does not promote the transition of Pro-TGase to mature enzyme. Therefore, $MgCl_2$ does not increase the production of TG enzyme through the activation of protein.

3.2 Results and analysis

3.2.1 Effects of microparticles on TG enzymes produced by the fermentation of Streptomyces pentaenycens

As shown in Figure 3.5, it is found that the addition of micro-particles has a significant impact on TG enzyme activity, which is influenced by the combination of micro-particle type, mass concentration and fermentation cycle. According to the data in Figure 3.5 and Figure 3.6, we can observe that on day 3, the TG enzyme activity in the group with added micro-particles was generally higher than that in the control group without added particles. However, on the 5th day, when the mass concentration of talc and alumina

reached a certain value (5 g/L and 1 g/L), the activity of TG enzyme decreased. Until the 7th day, the inhibition effect of high concentration talc powder and alumina on TG enzyme activity was reduced. We can also see from Figure 3.7 that the addition of kaolin significantly improved the TG enzyme production activity of Streptomyces pentadiengensis.

According to the comprehensive consideration of the three figures, kaolin has the most significant effect on enhancing the TG enzyme activity of Streptomyces pentadiengensis. In general, on the 5th day of fermentation, when the mass concentration of micro-particles was 0.5 g/L, the improvement of TG enzyme activity of the three micro-particles reached the highest point.

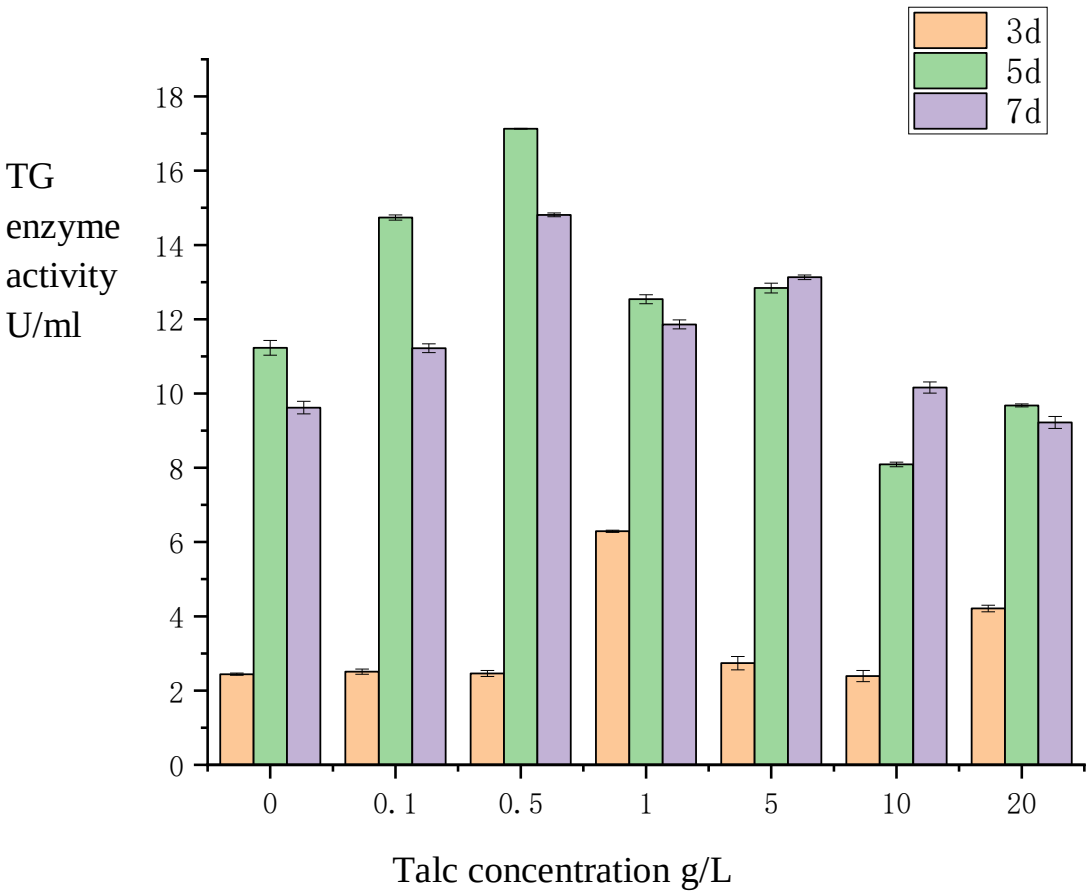

Figure 3.5 - **Activity of TG enzymes at different talc concentrations**

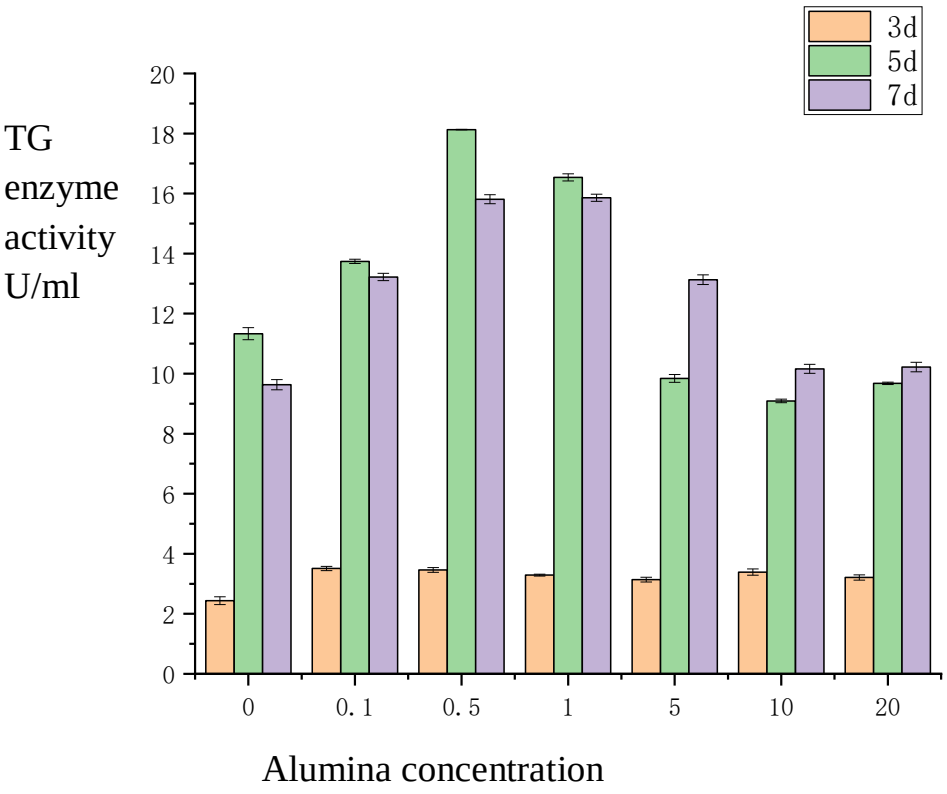

Figure 3.6 - **Activity of TG enzymes at different alumina concentrations**

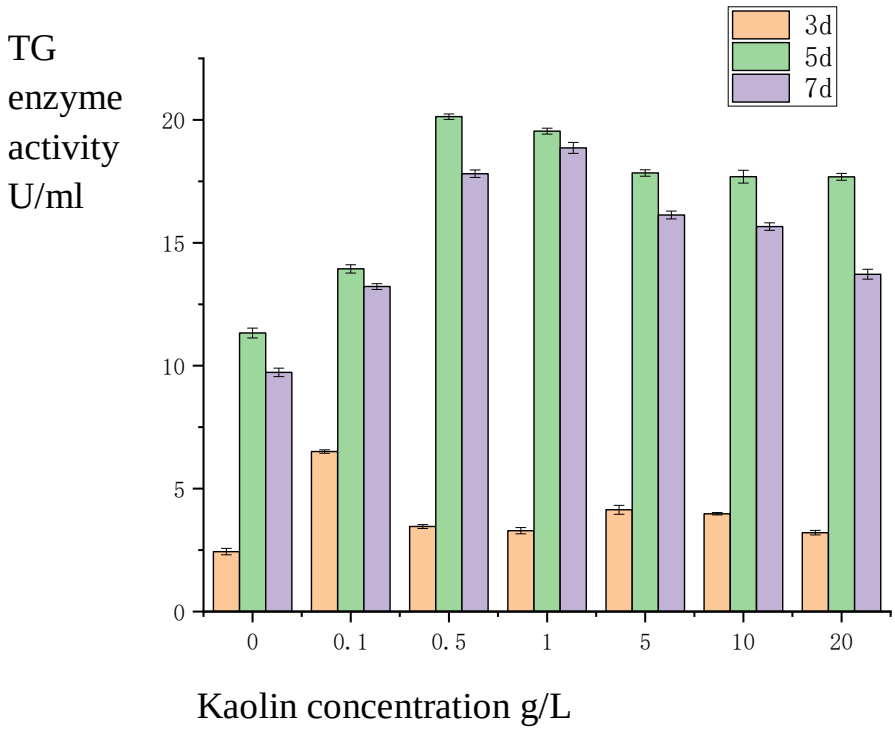

Figure 3.7 - **Activity of TG enzymes at different kaolin concentrations**

3.2.2 Effects of microparticles on the biomass, microflora size and morphology of Streptomyces pentadiengensis

In order to further explore the effects of particulate matter on the biomass, colony size and morphological changes of Streptomyces pentadiengensis, the biomass of Streptomyces pentadiengensis was analyzed in detail on the fifth day of the fermentation cycle. Through the observation in Figure 3.8, we found that although kaolin had a significant effect on enhancing TG enzyme activity, it had a negative effect on mycelium extension. This phenomenon indicates that there may not be a direct relationship between the increase of bacterial biomass and the increase of TG enzyme activity.

In addition, Figure 3.9 shows that talc powder can promote the growth of Streptomyces pentadiengensis at a low concentration (0.5 g/L); however, with the further increase of talc powder concentration, the growth rate of bacteria showed a downward trend. It is not difficult to conclude from further analysis that the mass concentration of particulate matter has a significant impact on the diameter of Streptomyces pentadiengensis, specifically, the higher the mass concentration, the smaller the diameter of the bacteria.

The microparticles not only affect the size of mycelium, but also change the structural characteristics of mycelium. As shown in Figure 3.10, after the introduction of particulate matter, compared with the control group without the addition of particulate matter, the mycelium structure of Streptomyces pentaenycens became looser and the integrity of mycelium was damaged, but the size of the mycelium became smaller and the size distribution tended to be uniform. At the same time, the activity of TG enzyme was significantly enhanced. This increase in activity may be related to the reduction of average particle size of microbiomes caused by the addition of particulate matter, thus making oxygen transfer more efficient, creating favorable conditions for the synthesis of specific proteins, and thus promoting the improvement of TG enzyme activity.

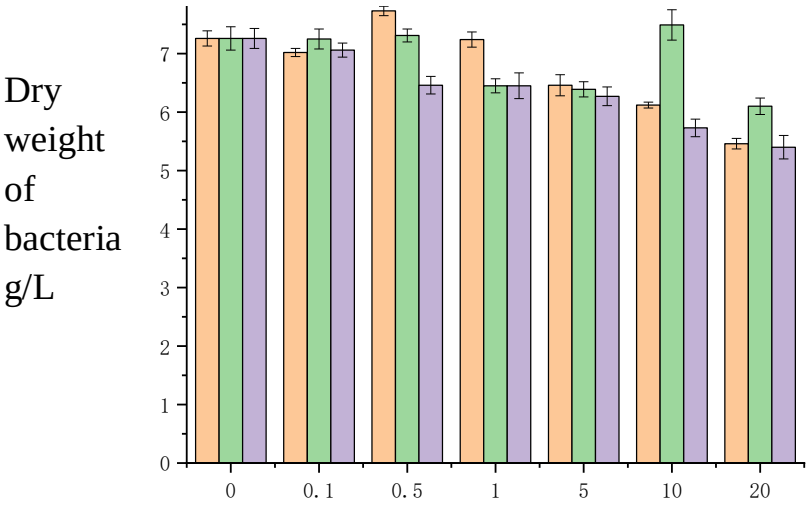

Figure 3.8 - **Dry weight of bacteria under the influence of different concentrations of microparticles**

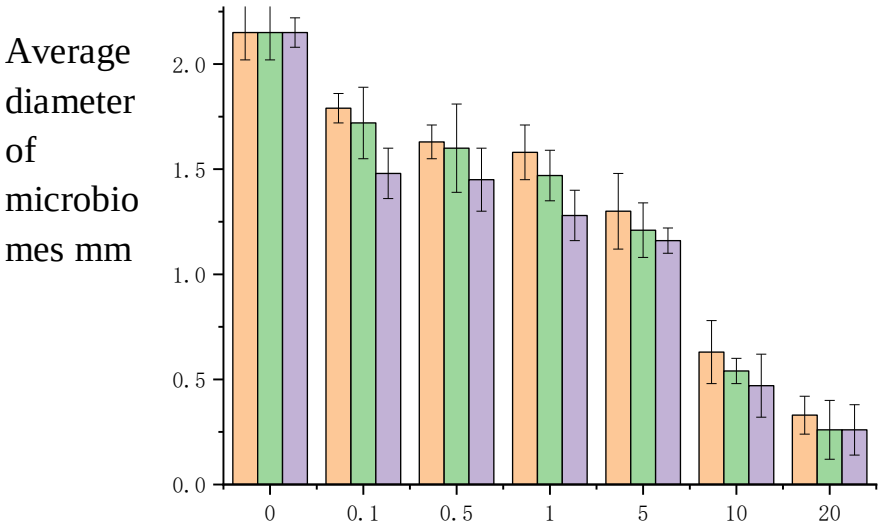

Figure 3.9 - **The average diameter of microflora under the influence of different concentrations of microparticles**

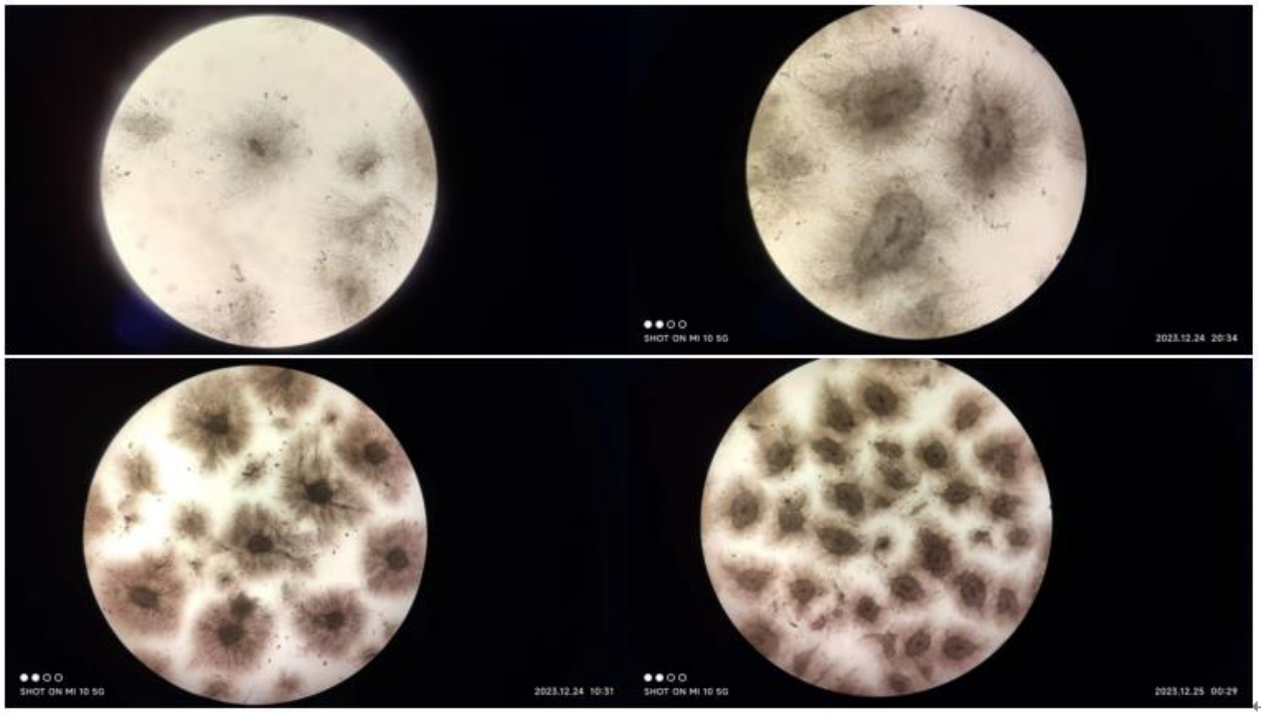

Figure 3.10

Conclusions to chapter 3

(1) Salt more suitable for increasing the yield of the enzyme was studied. We added a variety of salts to the fermentation medium for experiments, and the results showed that $MgCl_2$ was the best choice for increasing the yield of TG enzyme.

(2) Through experimental determination, it was determined that adding 0.1 mol/L $MgCl_2$ in the medium was the best concentration of TG enzymes produced by Streptomyces pentaenycens. When this concentration of $MgCl_2$ was added to the medium, although the growth rate of the bacteria was slightly lower than that of the initial medium without $MgCl_2$, the production of TG enzymes was increased. After 96 h fermentation, the yield of the enzyme reached 4.32 U/mL.

By comparing the growth morphology of the bacteria in the control medium with that in the medium containing $MgCl_2$, it was observed that the bacteria in the $MgCl_2$ medium showed early curvature, branching and amyloidization. In addition, at the last stage of the fermentation process, the

bacteria in $MgCl_2$ medium also formed fimbrial and spore structures not observed in the control group. Thus, the germination and spore formation stage of mycelium occurs earlier, because the mycelium enters a sub-health state under the influence of salt stress, prompting it to activate secondary metabolic regulators earlier. This early activation causes the differentiation process of the bacteria to begin earlier, thus increasing the production of TG enzymes.

These results indicate the effects of three different microparticles on cell morphology and TG enzyme activity in the fermentation process of Streptomyces pentaenycens. The results showed that kaolin microparticles with irregular and sharp surfaces had a significant impact on the mycelial morphology of Streptomyces pentadiengensis, and were the most effective in enhancing TG enzyme activity, suggesting that the level of enzyme activity may be closely related to mycelial morphology. Therefore, the addition of microparticles to the medium to regulate the morphology of mycelia provides a new and effective means for further study and improvement of microbial metabolic performance.

CONCLUSIONS

In this study, the influence of mycelial morphology on the synthesis of TG enzymes produced by Streptomyces pentadiengensis was studied. Through a series of experiments, the research team found that $MgCl_2$ stress can significantly increase the production of TG enzymes, and has an important impact on the morphology of bacteria. The experiment first determined the optimal concentration of $MgCl_2$, and found that appropriate $MgCl_2$ stress can promote mycelium germination and spore formation, which may activate secondary metabolic regulatory factors earlier and promote the synthesis of TG enzymes. In addition, the study also found that the microparticles, especially kaolin microparticles, had a significant impact on the mycelial morphology of Streptomyces pentadiengensis, and could significantly increase the TG enzyme activity. This indicated that the enzyme production ability of the strain could be effectively enhanced by regulating the mycelial morphology.

The results of this study provide a new strategy for increasing the yield of TG enzymes and provide important significance for further understanding the regulatory mechanism of microbial metabolic performance. At the same time, these findings provide a theoretical basis for the efficient production of TG enzymes in industrial production. Future studies will further explore the specific mechanisms by which microparticles affect mycelium morphology and enzyme activity, and consider applying these strategies to metabolic regulation in other microbial systems in order to achieve wider applications and deeper scientific understanding.

Finally, the effects of $MgCl_2$ stress and microparticles on the morphology and TG enzyme synthesis of Streptomyces pentadiengensis were analyzed and discussed in detail, which provided valuable data and insights for subsequent studies. Through comprehensive analysis of the experimental results, this study not only verified the positive effects of $MgCl_2$ and microparticles on TG enzyme production, but also revealed how these factors regulated enzyme synthesis by influencing mycelium

morphology, providing new ideas and methods for future microbial metabolic engineering and industrial applications

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