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 **Analysis of the degradation potential of chitin by marine bacteria**
First (Bachelor's) level of higher education
Specialty 162 "Biotechnology and Bioengineering"
Educational and professional program "Biotechnology"

Completed: student of group
BEBT-21
Dong Xinjie

Scientific supervisor
Olena Okhmat, Ph.D.,
Assoc. Prof.
Reviewer
Iryna Voloshyna, Ph.D.,
Assoc. Prof.

Kyiv 2025

KYIV NATIONAL UNIVERSITY OF TECHNOLOGIES AND DESIGN

Faculty: Chemical and Biopharmaceutical Technologies
Department: Biotechnology, Leather and Fur
First (Bachelor's) level of higher education
Specialty: 162 Biotechnology and Bioengineering
Educational and professional program Biotechnology

APPROVE
Head of Biotechnology, Leather and Fur Department, Professor,
Dr. Sc., Prof.
_____Olena MOKROUSOVA
«___»_____2025

ASSIGNMENTS
FOR THE QUALIFICATION THESIS
Dong Xinjie

1. Thesis topic **Analysis of the degradation potential of chitin by marine bacteria**
Scientific supervisor Ph.D., Assoc. Prof. Olena Okhmat

approved by the order of KNUTD "05" March 2025, № 50-уч

2. Initial data for work: assignments for qualification thesis, scientific literature on the topic of qualification thesis, materials of Pre-graduation practice

3. Content of the thesis (list of questions to be developed): literature review; object, purpose, and methods of the study; experimental part; conclusions

4. Date of issuance of the assignments 05.03.2025

WORK CALENDAR

№	The name of the stages of the qualification thesis	Terms of performance of stage	Note on performance
1	Introduction	until 11 April 2025	
2	Chapter 1. Literature review	until 20 April 2025	
3	Chapter 2. Object, purpose, and methods of the study	until 30 April 2025	
4	Chapter 3. Experimental part	until 11 May 2025	
5	Conclusions	until 15 May 2025	
6	Draw up a bachelor's thesis (final version)	until 25 May 2025	
7	Submission of qualification work to the supervisor for feedback	until 27 May 2025	
8	Submission of bachelor's thesis to the department for review (14 days before the defense)		
9	Checking the bachelor's thesis for signs of plagiarism (10 days before the defense)		Similarity coefficient ____% Citation rate ____%
10	Submission of bachelor's thesis for approval by the head of the department (from 7 days before the defense)		

I am familiar with the task:

Student _____ Dong Xinjie

Scientific supervisor _____ Olena OKHMAT

Abstract

Dong Xinjie. Analysis of the degradation potential of chitin by marine bacteria. – Manuscript.

Qualification thesis on the specialty 162 «Biotechnology and Bioengineering». – Kyiv National University of Technologies and Design, Kyiv, 2025.

Chitin is the most abundant biological resource in the ocean, with an extremely high annual output. Its degradation products play an important role in fields such as medicine and the food industry. The traditional degradation methods of chitin cause serious waste of resources and have a high degree of environmental pollution. However, biodegradation is green, environmentally friendly and highly specific, and thus has attracted the attention of many scholars. In marine ecosystems, many microorganisms can degrade chitin by secreting glycoside hydrolase, and the products are chitin oligosaccharides or monosaccharides. Among them, marine bacteria are the main players in degrading chitin, and the contribution of *Pseudoalteromonas* is particularly significant. This article takes *Pseudoalteromonas* sp. M58 as the research object and conducts sequencing and comprehensive analysis of its genome. The sequencing results show that the genome contains one complete circular chromosome with a length of 3604,843 bp and one circular plasmid with a length of 824,954 bp, with a total of 3,929 coding genes. In the coding sequences, 2,740 (69.7%), 3,073 (78.2%), and 3,514 (89.4%) genes were classified in the GO, COG, and KEGG databases, respectively. The alignment results with the protein sequences of the reported genes indicated that the strain *P*. sp. M58 contained a series of enzymes and proteins related to chitin degradation and transmembrane transport, suggesting that *P*. sp. M58 has the potential to degrade chitin. This thesis enriches the research results on chitin degradation by *Pseudoalteromonas*, and is of great significance for the effective utilization of chitin resources.

Key words：Chitin; degradation potential; Pseudoalteromonas; genomic analysis; metabolic pathway.

TABLE OF CONTENTS

INTRODUCTION

Chitin is the most abundant biomass resource in the ocean and exists in the form of crystals in nature. Although the annual production of chitin in the global water environment is approximately 10^{11} tons, no significant accumulation of chitin has been detected in the sediments, indicating that Marine bacteria play an important role in the degradation of chitin. Chitin is secreted by crustaceans and has good biocompatibility with the cells of organisms. At the same time, it is non-toxic and can be decomposed by organisms. However, although there are many types of microorganisms capable of degrading chitin in the ocean, the potential of many species of the genus *Pseudomonas* Marine to degrade chitin is still not fully understood, which limits the development of their effective utilization. The degradation products of chitin, such as chitin oligosaccharides and N-acetylglucosamine, have broad application prospects in the fields of medicine, food industry, etc. However, chitin is difficult to purify and chemically modify. Traditional chemical degradation methods are not only inefficient but also cause serious environmental pollution. This study analyzed the chitin degradation potential of *P*. sp. M58, enriching the research results on chitin degradation by *Pseudomonas* Marine and revealing its degradation mechanism, which is of great significance for the effective utilization of chitin resources.

The research method adopted in this thesis is to obtain the research results through specific experiments. The strain of bacteria applied in the experiment was *P*. sp. M58, which was isolated from seawater samples collected in the Mariana Trench in July 2020. The experimental strains were cultivated using the prepared TYS medium, then screened, and finally cryopreserved with liquid nitrogen. Whole-genome sequencing of the strains was conducted to obtain the basic genomic information. Functional annotation of the genes was carried out using multiple databases to understand the biological processes involved in the genes and their products. To further predict the chitin metabolic pathway in *P*. sp. M58, we conducted a local BLAST comparison of its protein sequence with the protein

sequences reported to be involved in the chitin degradation process, determined the enzymes and transport proteins involved in the degradation process, and thereby drew the metabolic pathway map.

Ultimately, according to the research results, *P*. sp. M58 does indeed have the ability to degrade chitin. And the chitin metabolic pathway that exists endoscopically was obtained. Firstly, the primary degradation products of chitin, GlcNAc and chitin oligosaccharides, enter the peritroplasm space respectively through the transport proteins NagP and TBDR. In the pericplasm, chitosan oligosaccharides are degraded by β -N-acetylglucosaminase to GlcNAc, and GlcNAc enters the cytoplasm through NagX. Intracellular GlcNAc is phosphorylated by AmgK to GLCNAC-1P, and GLCNAC-1P is then converted by GlmU to UDP-GlcNAc. Then, under the action of MurA, UDP-GLCNAc-allyl pyruvic acid is generated and is eventually converted into UDP-MurNAc by MurB. Metabolic end products are closely related to the synthesis of the cell wall.

The structure of this thesis is divided into four parts: background introduction, research methods and experimental equipment, research results and analysis, and summary and outlook of achievements.

The relevance of the topic is Whole genome sequencing and genetic analysis.

The purpose of the study is to study the chitin degradation potential of Marine bacteria *P*. sp. M58, enriching the research results of chitin degradation by *Pseudomonas* Marine, revealing its degradation mechanism, and providing important reference value for the effective utilization of chitin resources.

Research methods the whole genome sequencing

The scientific novelty whole genome sequencing

The practical significance of the results obtained is An in-depth study of this process will enrich the research results on the degradation of chitin by *Pseudomonas* is of great significance for the development and utilization of chitin.

CHAPTER I
LITERATURE REVIEW

1.1 OVERVIEW OF CHITIN

Chitin, also known as chitin, methylamine, etc., is a widely distributed biological polymer in nature and also the most abundant biomass resource in the ocean[1]. Chitin is a linear polysaccharide composed of N-acetylglucosamine (GlcNAc) as the basic unit and connected by β-1, 4-glycositic bonds[2] , with the molecular formula $(C8H13O5N)_n$[3]. It is widely present in the cell walls of fungi, the exoskeletons of arthropods, and other crustaceans[4]. It is a strong fortress. Endow countless organisms with elasticity and structural integrity.

Chitin exists in nature in the form of crystals and is called crystalline chitin. They usually exist in the form of three crystalline isomers, namely α -chitin, β -chitin and γ -chitin[5]. Among them, the content of α -chitin is the most abundant. Its structure is composed of two antiparallel sugar chains, so it has high structural stability and is widely distributed in the shells of shrimps, crabs and crustaceans of arthropods such as insects. β -chitin has two parallel sugar chains in the same direction and is mainly found in the cartilage of squids and octopuses, as well as in the shell matrix of snails and shellfish. However, γ -chitin contains the above two arrangements and is distributed in fungi and silkworm pupae[6,7].

1.2 DEGRADATION METHODS OF CHITIN

Crystalline chitin, which exists naturally in nature, is poorly soluble. Despite its wide distribution and abundant content, the degradation and utilization efficiency of chitin by microorganisms is low, leading to problems such as resource waste and environmental pollution. Therefore, in industry, various methods are often used to degrade crystalline chitin and prepare it into relatively loose colloidal chitin, which is convenient for the decomposition and utilization by

microorganisms. The degradation methods of chitin include physical methods, chemical methods and biological enzymatic methods[8].

Physical methods usually employ mechanical grinding, ultrasonic waves and other measures to destroy the structure of chitin in order to achieve the effect of degradation. Its advantages are convenient operation and easy control of conditions, but it also has limitations such as low efficiency and high equipment cost. It is usually used in combination with the chemical method.

Chemical methods often complete degradation through operations such as acid hydrolysis or alkali treatment. Under high-temperature conditions, treatment with strong acid can break the β-1,4- glycosidic bonds to generate oligosaccharides or monosaccharides[9]. However, due to the generation of a large amount of acidic waste liquid during the process, the degree of pollution to the environment is strong. And the purity of the product is not high. Alkaline treatment involves converting chitin to chitosan through deacetylation under strong alkaline conditions. The production process consumes a lot of energy and is prone to causing a large waste of concentrated alkali. The generated chitosan is a mixture with different degrees of deacetylation and has a relatively low quality.

The biological enzymatic method usually employs the combined action of endonuclease chitinase, exonuclease chitinase, deacetylase and chitanase for degradation[10], and the specific degradation pathway is shown in the following figure. Enzymatic degradation has mild reaction conditions, high product specificity, is environmentally friendly, conforms to the concept of green development, and is the main direction of future development.

1.3 CHITIN DEGRADATION PRODUCTS AND APPLICATION VALUE

Chitin exists in nature in the form of crystals[11]. Its special structural properties give it high stability. It is not easily soluble in water and organic solvents and other liquids[12], and is difficult to be utilized by living organisms. However, its

degradation products are soluble and can be easily biodegraded and utilized[13]. Previous studies have found that monosaccharides with a degree of polymerization of 1 and chitosan oligosaccharides with a degree of polymerization of 2-6 have good water solubility and physiological activity[14,15].

Chitosan oligosaccharides, also known as chitosan oligosaccharides or chitosan oligomers, possess various biological activities such as antibacterial and anti-tumor properties, and have good biocompatibility with biological cells. At the same time, they are non-toxic and can be decomposed by organisms. Therefore, they have extensive applications in fields such as medicine, food, agriculture, and environmental protection[16.] Current studies have shown that chitosan oligosaccharides possess various biological activities such as antibacterial, anti-inflammatory and anti-cancer effects, and can be applied in clinical treatment and the biomedicine industry[17]. In the food industry, chitosan oligosaccharides are often used as preservatives, food additives and nutritional supplements because they can supplement the nutrients needed by the human body and defend against bacterial and fungal infections. In agriculture, chitosan oligosaccharides can be used as fertilizers and pesticides. They can serve as a source of nutrients needed for plant growth and development, and at the same time help resist the invasion of harmful microorganisms[18]. In addition, chitin is the most abundant organic carbon source in the ocean. The degradation and metabolism of chitin by Marine microorganisms help promote the material cycle of the Marine ecosystem[19.]

1.4 METABOLISM OF CHITIN

1.4.1 DEGRADATION OF CHITIN BY MARINE MICROORGANISMS

The ocean covers approximately 71% of the Earth's surface and is the "blue heart" of the Earth. Its ecological environment is complex, featuring low temperature, high pressure, oxygen deficiency and other characteristics. The ecological balance is easily affected by multiple factors. In view of the principle of survival of the fittest, Marine microorganisms have evolved a set of characteristics

adapted to the environment. Therefore, there are diverse genetic resources within them that are waiting to be explored, demonstrating rich functional potential. For a long time, Marine microorganisms have played an extremely important role in Marine ecosystems and the biochemical cycle of the Earth. Chitin is a widely distributed biological polymer in nature[20] and is also the most abundant nitrogen-containing natural organic compound on Earth, second only to proteins. Although the annual production of chitin in the global water environment is 1,011 tons, no significant accumulation of chitin has been detected in the sediments, which indicates that Marine bacteria play an important role in the degradation and recycling of chitin[21].

In the ocean, many microorganisms have the ability to degrade chitin. At present, existing reports have revealed that the microorganisms that degrade chitin in the ocean are mainly in the bacterial domain. This includes groups such as *Pseudoalteromonas*, *Vibrio*, *Alteromonas*, *Streptomyces*, and *Flavobacterium*[22, 23].

1.4.2 STUDY ON THE METABBOLIC PATHWAY OF CHITIN DEGRADATION BY Γ -PROTEOBACTERIA

The process of chitin degradation by γ -Proteobacteria can mainly be summarized into three stages, namely extracellular recognition and extracellular enzymatic hydrolysis, transport of oligosaccharides and monosaccharides, and intracellular metabolism. Firstly, chitin-binding proteins such as CBP21 or lipoproteins recognize and adsorb chitin in the water environment. CBP21 has monooxygenase activity for degrading chitin and works together with chitin endonucleases and chitin exonucleases extracellular to degrade chitin into chitin oligosaccharides. Secondly, through specific pore proteins in *Vibrio* or TBDR in *Shivella*, it is introduced into the peritroplasmic space via OM, further degraded by N-acetylglucosaminidase into GlcNAc, and then transported to the cytoplasm by transporter. Finally, it is successively converted into fructose-6-p, NH3 and acetic

acid by the action of GlcNAc kinase (PTS), GLCnac-6-p deacetylase and GlcN-6-P deaminase, thereby completing metabolism[24] (Fig. 1.1)

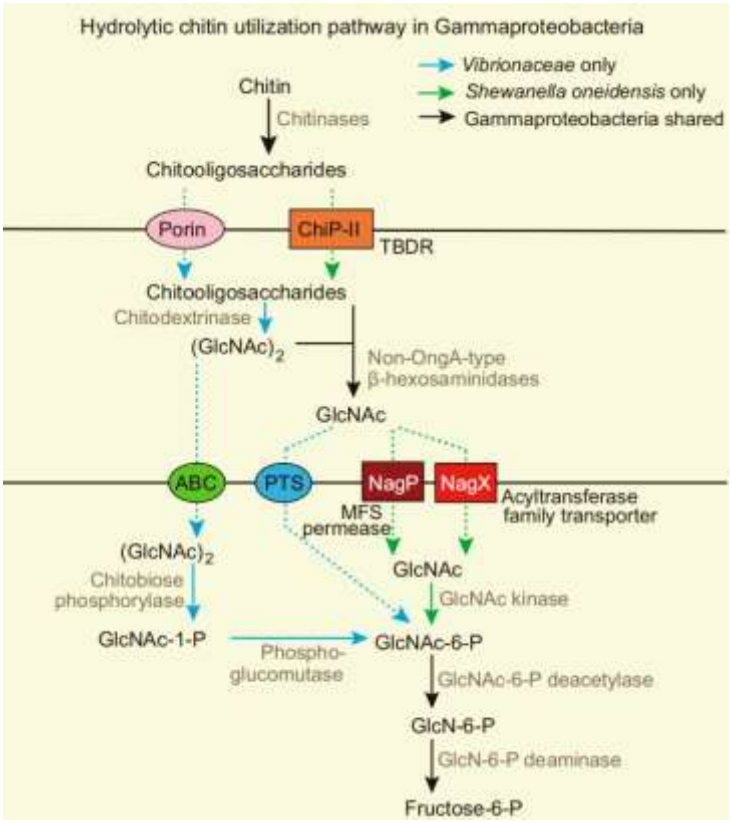

Figure 1.1 – Metabolic pathway diagram of Chitin degradation by γ-Proteobacteria[24]

1.4 *PSEUDOMONAS* GENUS

Pseudomonas is a genus of bacteria endemic to the ocean and has a relatively high abundance, accounting for approximately 2% to 3% of the total bacterial abundance in the upper layer of seawater[25]. It belongs to the *Pseudoalteromonas* family of the Alteromonas order in the γ -Proteobacteria class. This family includes two genera, namely *Pseudoalteromonas* and *Algicola*. *Pseudomonas* alternation has a wide distribution range in the global Marine environment, and is found in low-temperature sea areas such as the Pacific Ocean, the Antarctic and Arctic, as well as tropical and temperate sea areas. For example, categories such as *Pseudoalteromonas* haloplanktis and *P. prydzensis* isolated from low-temperature sea areas, and *P. rubra* in tropical sea areas. Meanwhile, it is also the dominant

force in chitin degradation in the Marine environment. By decomposing the remains of crustaceans, the chitin attached to their surfaces can be degraded, recycled and reused. This not only benefits the maintenance of the Marine micro-ecological environment but also provides a rich carbon or nitrogen source for Marine microorganisms[26]. Based on phenotypic and genome-wide analysis, *Pseudomonas* aeruginosa can be classified into two categories: pigment-producing species and non-pigment-producing species. Compared with non-pigmented species, the genomes of pigmented species contain a large number of biosynthetic gene clusters (BGCs). Both of these two types of species have the genetic potential to produce a variety of glycosylhydrolases, and the pigment-producing species in particular have a strong chitin degradation mechanism, which contains a variety of chitin hydrolases[27].

1.5 RESEARCH PROGRESS ON CHITIN DEGRADATION BY *PSEUDOMONAS AERUGINOSA*

The research on the degradation of chitin by *Pseudomonas aeruginosa* has received high attention from many scholars. To this end, they adopted various technical means to explore the chitin metabolic potential and mechanism of *Pseudomonas aeruginosa* from different entry points and strains. In 2019, in the experiment conducted by Paulsen et al., in the genomic sequencing analysis of 157 *Pseudomonas* strains, it was found that 102 strains had the potential to degrade chitin, that is, they had chitinase encoding genes. All 62 genomes of *Pseudomonas* pigmentogenes encode the chitinase genes of the GH18 and GH19 families. 95% of the genomes of non-pigmentogenes encode the chitinase genes of the GH18 family, including the N-acetylglucosaminidase of the GH20 family and the lytic polysaccharide monooxygenase (LPMO) of the AA10 family[28]. After analysis, it was found that almost all the chitin-degrading strains tested contained the chitin-degrading gene cluster (CLC) [29], which encodes ChiA type GH18 chitinase, ChiC type GH18 chitinase and LPMO.

1.6 INTRODUCTION TO ENZYMES RELATED TO CHITIN DEGRADATION

1.6.1 SOURCES ANDCHARACTERISTICS OF CHITINASE

Chitinase is the general term for enzymes that can specifically catalyze the cleavage of glycosidic bonds, thereby degrading chitin into chitin oligosaccharides (COS), chitosan oligosaccharides or N-acetylglucosamine. Chitinase is widely present in various organisms. Among them, chitinase derived from bacteria has received the most attention.

Chitinase has been widely discovered in various bacteria in the ocean and soil. They secrete chitinase to degrade exogenous chitin to obtain nutrients and meet their own normal metabolic activities. In 2002, Sashiwa et al. first hydrolyzed α -chitin using the crude chitinase preparation from Hydrophila *Pseudomonas* H-2330 and successfully obtained GlcNAc[30]. Recently, genes encoding chitinase have also been identified in *Aquimarina* strains isolated from Marine sponges, corals, sediments and seawater[31].

The wide range of sources of chitinase has led to the diversity of its properties, which are usually reflected in the molecular weight of the enzyme protein, the pH range of enzyme activity and the temperature range (Tab. 1.1). The data in the figure shows that the molecular weights of most chitinases are concentrated between 35 and 55 kDa, with the maximum molecular weight reaching 110 kDa. Some enzymes exhibit high thermal stability. Most chitinases show optimal activity between pH4 and 8[32], while some enzymes have optimal activity under strongly acidic or strongly alkaline conditions.

Table 1.1 – Sources and Biochemical Characteristics of Chitinase [32]

Organism	Expression Host	Molecular Mass (kDa)	Optimal Temperature (°C)	Optimal pH	Activity (U/mg)	Inhibitor	Activator	Refs.
Streptomyces albolongus ATCC 27414	*Escherichia coli* BL21	47	55	5	66.2	Fe^{3+}, Cu^{2+}, Na^{+}, EDTA, SDS	Mn^{2+}, Ba^{2+}, Na^{+}	[57]
Flavobacterium johnsoniae UW101	*Escherichia coli Rosetta-gami 2* (DE3)	35.5	40	6	26.2	Ca^{2+}, WRK, urea, Hg^{2+}	Cu^{2+}	[58]
Trichoderma virens	*yeast Pichia pastoris*	42	37	4.5	NA	NA	NA	[59]
Bacillus licheniformis B307	NA	42	60	6	14.2 U/mL	NA	NA	[60]
Myxococcus fulvus screened from soil	*E. coli* DH5a	26.99	35	8	NA	NA	NA	[61]
Marine bacteria DW2	*Antarctic Escherichia coli*	39.5	30	5	7.3	Cr^{3+}, Ni^{2+}, Fe^{3+}, Mn^{2+}, Cu^{2+}, EDTA, SDS, Hg^{2+}, Ag^{+}	Ca^{2+}, Zn^{2+}, Mg^{2+}, β-mercaptoethanol	[62]
soil of a mangrove tidal flat	*E. coli* BL21 (DE3)	43	45	NA	0.63	SDS, EDTA, Fe^{3+}, Cu^{2+}, Mn^{2+}, Co^{2+}, Ag^{+}, Hg^{2+}	K^{+}, Na^{+}	[63]
actinobacterium *Streptomyces olivaceus* (MSU3)	NA	52	40	8	680.0 IU	Hg^{2+}, Pb^{2+}	Mn^{2+}, Cu^{2+}, Mg^{2+}	[64]
C.shinanensis	*E. coli* BL21DE3-pLysS	58.87	50	7	NA	NA	NA	[65]
Lysobacter sp. MK9-1	*Escherichia coli Rosetta-gami B* (DE3)	NA	55	4.5	12	NA	NA	[66]
Fenneropenaeus merguiensis	*Escherichia coli*	52	40	6	NA	NA	NA	[67]
Thermomyces lanuginosus	NA	18	50	6.5	NA	Cu^{2+}, Hg^{2+}, EDTA	β-ME	[68]
Chitinolyticbacter meiyuanensis SYBC-H1	*Escherichia coli* BL21	110	50	6	4.1	Cu^{2+}, Ni^{2+}, Fe^{3+}	Fe^{2+}, Mg^{2+}, Ba^{2+}, Na^{+}	[69]
Acinetobacter indicus CCS-12	3ZYB medium	50	60	7	480.2	NA	Ca^{2+}, Mn^{2+}, Mg^{2+}, Na^{+}, Fe^{3+}, Cu^{2+}, EDTA and β-mercaptoethanol	[70]
Fenneropenaeus merguiensis	NA	52	40	6	NA	NA	NA	[67]

NA: Not available; EDTA: Ethylenediaminetetraaceticacid; SDS: Sodium Dodecyl Sulfate; WRK: Woodward's reagent K.

1.6.2 CHITINASE

Chitinase consists of chitin exonuclease and chitin endonuclease. Chitin exonuclease is a type of enzyme that successively hydrolyzes the β-1,4 glycosidic bond from the reducing or non-reducing end of the chitin chain[33]. It mainly belongs to the GH18 family and the GH20 family[34]. ChiA and ChiB in the GH18 family are chitin exonuclease. Chitin exonucleases[35], including chitinisaccharidase and N-acetylglucosaminidase[36], usually function within cells and are the key "terminators" in the chitin degradation process[37]. Chitosan glycosidase acts on the non-reducing end and successively hydrolyzes to release chitosan[38]. N-acetylglucosaminase is a type of enzyme capable of hydrolyzing glycosidic bonds containing N-acetylglucosamine (GlcNAc) residues[39.] Its main function is to further decompose chitin degradation products into monosaccharide forms, which can act on specific loci in chitin oligosaccharides to form the product GlcNAc.

Unlike chitin exonucleases, chitin endonucleases are a type of enzymes that can randomly cleave the β-1,4 glycosidic bonds within the chitin chain. They are usually extracellular enzymes. Its core function is to disrupt the chain structure of chitin and generate oligosaccharide fragments, such as chitin trisaccharides and chitin tetrisaccharides. Chitin endonucleases are classified into two glycoside hydrolase families based on amino acid sequence homology, namely the GH18 family and the GH19 family[40]. The GH18 family of chitinases consists of three subfamilies, namely ChiA, ChiB and ChiC. Among them, ChiC is chitin endonuclease, which is further divided into ChiC1 and ChiC2. The GH18 family chitinases exist in organisms such as bacteria, fungi and insects. The GH19 family chitinases mainly exist in plants and some bacteria, and are closely related to defense functions such as resisting fungal infection.

1.6.3 LYTIC POLYSACCHARIDE MONOOXYGENASE (LPMOs)

In addition to the chitin-degrading enzymes mentioned above, some chitin-degrading bacteria also possess lytic polysaccharide monooxygenase, which belongs to the AA10 family. It has been reported that AA10 LPMOs exists in all domains of life, but is mainly found in bacteria and viruses. LPMOs is a copper-containing oxidase. Unlike the common glycoside hydrolases that can only act on a single polysaccharide chain, LPMOs can directly act on the polysaccharide chains on the surface or inside the crystalline polysaccharide, making the substrate structure of the crystalline polysaccharide loose and releasing more polysaccharide chains, thereby enhancing the activity of glycoside hydrolases on the substrate. The principle is to degrade chitin through oxidation, mainly performing its function extracellular. To date, most of the identified LPmos are of the C1 or C1/C4 oxidation type[41]. The mechanism of action is to hydroxyl the glucose units C1 or C4 in the polysaccharide substrate, releasing soluble and insoluble oxidation products[42].

1.6.4 CHITIN DEACETYLASE (CDAs)

Chitin deacetylase is an enzyme that can catalyze the deacetylation of chitin , and its products are chitosan and acetic acid. It belongs to the carbohydrate esterase family (CE4) and requires metal ions as cofactors[43]. In 1974, chitin deacetylase was first discovered in the extract of Mucilomyces rubrina. Subsequent studies found that the conversion of new chitin to chitosan catalyzed by this enzyme is closely related to the synthesis of the cell wall. Chitin deacetylase mainly plays the role of activating the substrate during the chitin degradation process. After deacetylation, the chitin macromolecules are transformed into relatively loose sugar chains, enhancing the degradation activity of chitosanase and thereby promoting the progress of the subsequent degradation pathway.

1.6.5 CHITOSANASE

Unlike the above-mentioned chitinases, the substrate of chitanase is deacetylated chitin. This enzyme usually works in conjunction with deacetylases to complete the degradation of chitin. According to the analysis of amino acid sequences, chitosanases are classified into the GH46, GH75 and GH80 glycoside hydrolase families. Chitin has a large molecular weight and is insoluble in water. To improve this property and make its degradation easier, chitosanase is used to convert the large molecule chitin into sugar chains with a lower degree of polymerization[44]. After the action of chitosanase, free amino groups are produced, the solubility increases, and it is easier to be hydrolyzed, thereby improving the degradation and utilization efficiency of chitin.

1.7 THE PURPOSE AND SIGNIFICANCE OF THIS STUDY

Chitin is the most abundant biomass resource in the ocean and exists in the form of crystals in nature. Although the annual production of chitin in the global

water environment is approximately 10^{11} tons, no significant accumulation of chitin has been detected in the sediments[45], indicating that Marine bacteria play an important role in the degradation of chitin[46]. Chitin is secreted by crustaceans and has good biocompatibility with the cells of organisms. At the same time, it is non-toxic and can be decomposed by organisms. However, although there are many types of microorganisms capable of degrading chitin in the ocean, the potential of many species of the genus *Pseudomonas* Marine to degrade chitin is still not fully understood, which limits the development of their effective utilization. The degradation products of chitin, such as chitin oligosaccharides and N-acetylglucosamine, have broad application prospects in the fields of medicine, food industry, etc. However, chitin is difficult to purify and chemically modify. Traditional chemical degradation methods are not only inefficient but also cause serious environmental pollution. This study analyzed the chitin degradation potential of *P*. sp. M58, enriching the research results on chitin degradation by *Pseudomonas* Marine and revealing its degradation mechanism, which is of great significance for the effective utilization of chitin resources.

Conclusions to chapter 1

1. Chitin is an important biological resource in the ocean. Its structure is a linear polysaccharide composed of N-acetylglucosamine (GlcNAc) as the basic unit and connected by β-1, 4-glycosidic bonds. The degradation products of chitin have rich utilization value. The degradation methods include physical method, chemical method and biological enzyme method. Among them, biological enzymatic degradation is an important direction for the future.

2. In Marine ecosystems, there are many microorganisms capable of degrading chitin, among which Marine bacteria are the main players in chitin degradation. Previous studies have revealed that groups such as Vibrio Marine and *Pseudomonas alterniflora* can degrade chitin, but the mechanism by which *Pseudomonas alterniflora* degrades chitin is still not fully understood.

3. The enzymes involved in chitin degradation include chitinase, LPMOs, deacetylase and chitosanase, etc. They play different roles respectively in the process of chitin degradation.

CHAPTER 2
OBJECT, PURPOSE, AND METHODS OF THE STUDY

The purpose of the study is to study the chitin degradation potential of Pseudoalteromonas. sp. M58, enriching the research results of chitin degradation by Pseudomonas Marine, revealing its degradation mechanism, and providing important reference value for the effective utilization of chitin resources.

Object of study – Experimental strain: P. sp. M58, isolated from seawater samples collected in the Mariana Trench in July 2020, is preserved in the laboratory.

Subject of study – To explore the potential of strain M58 in degrading chitin, whole-genome sequencing of strain M58 was conducted.

2.1 EXPERIMENTAL STRAIN

Experimental strain: *Pseudoalteromonas sp.* M58 isolated from seawater samples collected in the Mariana Trench in July 2020 and preserved in the laboratory.

2.2 MAIN REAGENT AND REAGENT KITS

Sea salt and vitamins are purchased from Sigma Company in the United States. Agarose is purchased from Invitrogen Corporation in the United States. The DNA extraction kit was purchased from BioTeke in China. The remaining medicines were all purchased from Sinopharm Group Chemical Reagents Co., LTD (China).

2.3 EXPERIMENTAL INSTRUMENTS

1. Zhicheng Constant Temperature Shaking Incubator (Shanghai Zhicheng Analytical Instrument Manufacturing Co., LTD).

2. Centrifuge GCL-16B (Shanghai Anting Scientific Instrument Factory).

3. UV-1900 Ultraviolet-visible Spectrophotometer (Shimadzu, Japan).

2.4 THE METHOD OF PREPARING THE CULTURE MEDIUM

Table 2.1 – Method of Medium Preparation

Culture medium	Preparation method
TYS medium	0.5% casein, 0.1% yeast powder, artificial seawater

2.5 DATABASE AND MAIN ANALYSIS SOFTWARE

(1) BLAST sequence alignment retrieval tool:

http://blast.ncbi.nlm.nih.gov/Blast.cgi;

(2) EzBiocloud database: https://www.ezbiocloud.net/.

2.6 EXPERIMENTAL METHOD

2.6.1 ACQUISITION OF BACTERIA

Under the condition of 20℃, the experimental strain *P*. sp. M58 was cultured using the prepared TYS medium (0.5% casein, 0.1% yeast powder, artificial seawater). When it is cultured to the logarithmic phase, transfer it to 30 mL of liquid medium at an inoculation volume of 1%. Incubate under the conditions of 25℃ and 180 rpm until OD600≈0.8. Next, wash the bacteria three times with sterilized artificial seawater, then collect the bacteria and quickly freeze them in liquid nitrogen for preservation.

2.6.2 GENOME SEQUENCING AND SEQUENCE ALIGNMENT

Strain *P*. sp. M58 was sent to Beijing Novogene Technology Co., LTD. Whole genome sequencing was performed using PacBio Sequel II/PacBio Sequel IIe and Illumina NovaSeq PE150. And Canu software (https://github.com/marbl/canu/, version: 2.0) for genome assembly reads. At present, the general functional

databases providing annotations mainly include GO, KEGG, COG/KOG, NR, Pfam, CAZy, TCDB and Swiss-Prot.

The operation steps are as follows: Perform Diamond alignment between the predicted gene sequence and the protein sequences in each functional database, and set evalue ≤ 1e-5; Compare and filter the results, and annotate the option with the highest alignment result value for each sequence. The requirements are identity ≥ 40% and coverage ≥ 40%. Calculate the completeness of the sketch using CheckM[47].

2.6.3 Analysis of chitin degradation potential

Use hmmbuild to convert the amino acid sequences of the enzymes related to chitin metabolism that have been reported into hmm format files. Homologous protein sequences were searched in the P.sp.M58 genome using hmmsearch and BlastP, with thresholds of Identity ≥ 30%, Coverage ≥ 70%, and E-value ≤ 1e-10. Compare the protein sequences with the database to retrieve whether there are homologous sequences. If so, it indicates the presence of the enzyme of this metabolic pathway; If not, it indicates that the homology is relatively low or the enzyme has no relevant function. Based on the enzymes involved in the process, a metabolic pathway map is drawn to obtain the degradation path.

Conclusions to chapter 2

1. The strain used in the experiment was *Pseudoalteromonas* sp. M58, which was preserved in the laboratory. The experiment utilized the prepared TYS medium to cultivate the strains, and after screening and washing, they were stored at low temperature in liquid nitrogen.

2. Whole-genome sequencing was performed on the experimental strains, and the sequencing results were assembled and analyzed. Gene function comparisons were conducted using various databases.

CHAPTER 3
EXPERIMENTAL PART

3.1 OVERVIEW OF THE *P*.sp. M58 GENOME

The genome contains one complete circular chromosome of 3,604,843 bp and one circular plasmid of 824,954 bp (Fig. 3.1). The GC content of the genome is 41.77%, and there are 3,929 coding genes. The *P*. sp. M58 genome has 102 tRNA coding genes and 1 rRNA operon, all of which are located on chromosomes. In the coding sequences, 2,740 (69.7%), 3,073 (78.2%), and 3,514 (89.4%) genes were classified in the GO, COG, and KEGG databases, respectively. The COG classification indicates that 8.3% of the genes are involved in amino acid transport and metabolism, 7.2% are involved in transcription, 5.2% are involved in carbohydrate transport and metabolism, 8.0% are involved in translation, ribosome structure and biogenesis, and 8.5% are involved in signal transduction.

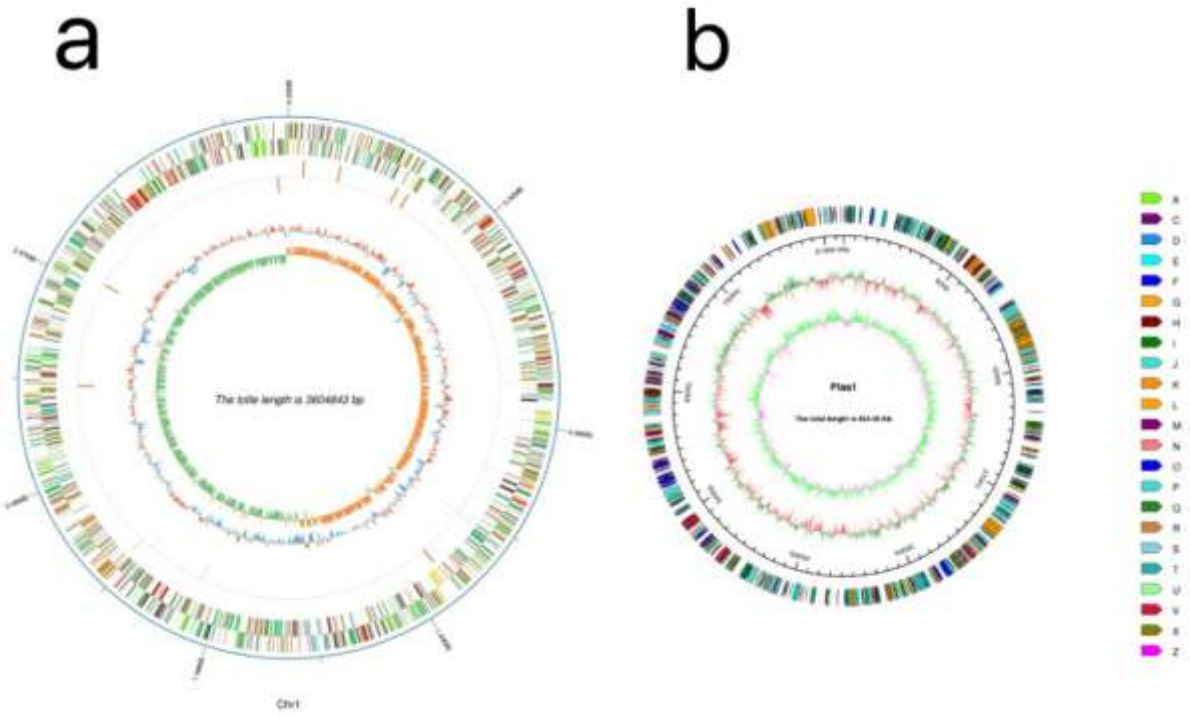

Figure 3.1 – Whole-genome circular atlas

Figure a is the circular map of the chromosome genome, and Figure b is the circular map of the plasmid genome. The bar chart on the far right shows the frequencies of the four bases (A, T, C, and G) in the plasmid genome. Different colors represent different types of base coding, and the depth of the color is determined by the frequency of base occurrence in the genome.

3.2 GENOMIC COMPONENT ANALYSIS

The functional regions contained in the microbial genome are very rich. It not only contains coding proteins and functional Rnas, but also non-coding components such as regulatory sequences, repeat sequences, introns, etc. Thus, related functions such as transcriptional regulation and translational regulation can be accomplished, and some functional regions are also related to the evolutionary diversity of species. The primary step of genomic component analysis is genomic sequencing and assembly. Then, the coding genes, repeat sequences and non-coding genes are predicted and compared and analyzed with the gene sequences in the database, which can identify whether the tested genome contains relevant functional genes. Finally, component statistics were conducted to obtain relevant analysis data such as GC content and the proportion of repetitive sequences in the genome. Annotate the genes, determine their possible functions and predict the related pathways.

3.2.1 CODING GENES

Using GeneMarkS (Version 4.17) (http://topaz.gatech.edu/GeneMark/) software for *P*. sp. M58 genome encoding gene prediction. The statistical information of the gene prediction results is shown in Tab. 3.1, and the distribution of gene lengths is shown in Fig. 3.2. The figure shows the differences in the number of gene fragments from short to long. The selected length range represents the length intervals of important genes in most organisms. Among them, the horizontal axis

represents the length of the gene fragment, and the vertical axis represents the corresponding number of gene fragments. It can be seen from the figure that with the increase of the length of the gene fragments, the number roughly shows a trend of increasing first and then decreasing. Among them, the number of gene fragments with a length of ≥2000 bp is the largest, which is 389.

Table 3.1 – Statistics of Prediction Results of coding genes

Genome size (bp)	Gene number	Gene total length (bp)	Gene average length (bp)	Gene length / Genome (%)
4,429,797	3,929	3,951,225	1,006	89.2

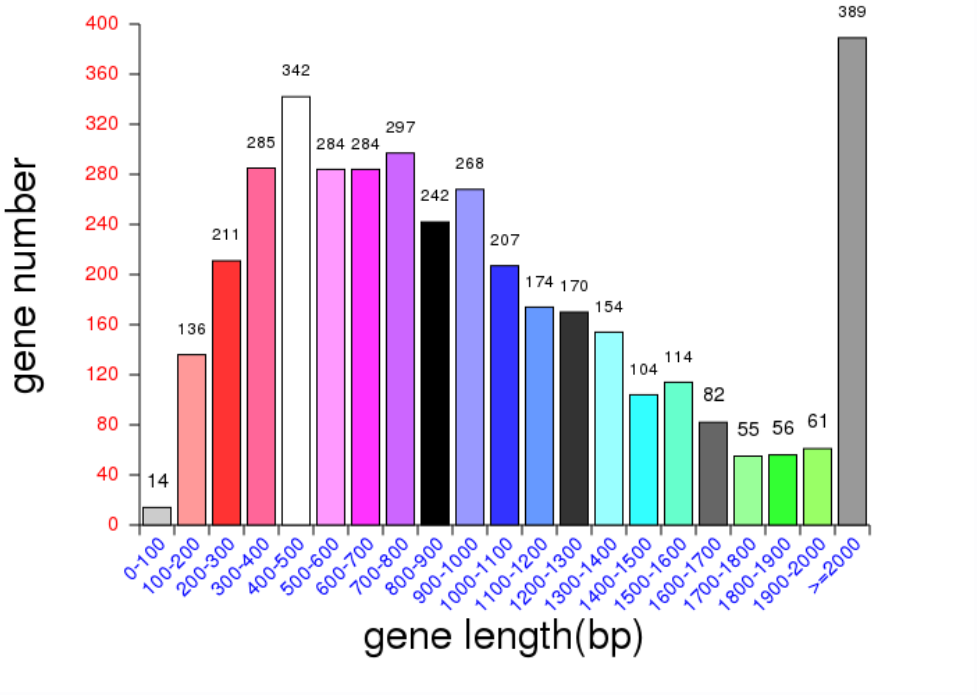

Figure 3.2 – Statistical chart of gene length distribution

3.2.2 NON-CODING RNA

Non-coding RNA (ncRNA) is an RNA molecule that does not participate in encoding proteins, but it has important biological functions by itself. They function by directly participating in the regulation of gene expression or interacting with

other biomolecules. It can affect the transcriptional activity of genes, post-transcriptional regulation, and the silencing and activation of genes.

In microbiology research, tRNA, rRNA and sRNA are mainly the objects of discussion. The prediction of tRNA is usually carried out by scan-SE software (Version 1.3.1). There are two methods for predicting rRNA. One is to find rRNA by comparing it with the rRNA library of the closely related reference sequence. The other is to predict rRNA using the rRNAmmer software[48] (Version 1.2). When predicting sRNA, first compare the annotations with Rfam database[49,50], and then determine the final sRNA using the cmsearch program (Version 1.1rc4) (with default parameters).

The specific data of the number, average length and total length of non-coding Rnas predicted by the software are shown in Tab.3.2.

Table 3.2 – Statistical results of ncRNA after redundancy removal

Type	Number	Average length（bp）	Total length（bp）
tRNA	102	78	7,995
5s(denovo)	9	114	1,026
16s(denovo)	8	1,530	12,241
23s(denovo)	8	2,886	23,092
sRNA	1	105	105

3.3 GENE FUNCTION ANNOTATION

3.3.1 GO DATABASE ANNOTATIONS

The full name of GO is Gene Ontology, which is a standardized resource widely used in biological research, aiming to describe the functions of genes and their products. GO consists of three parts: (1) Molecular Function (MF) : It is used to describe the activity and role of genes and gene products at the molecular level, such as binding to DNA, ATPase catalytic activity, ion transmembrane transport activity, etc. (2) Cellular components (CC) : Used to describe the subcellular localization and macromolecular complexes of gene products, such as nucleoli,

telomeres, and complexes for recognition initiation; (3) Biological processes (BP) : These are used to describe life activities involving gene products, such as mitosis and signal transduction. The three types of statistical results of the GO database are shown in Fig. 3.3. The horizontal axis in the figure represents the items set under the three major categories of GO, and the vertical axis represents the number of genes annotated under this item (including the sub-items of this item). The number of genes in the biological process section is the largest, among which the proportion of genes in metabolic processes, cellular processes and single-organ processes is large. Among the cellular components, cells, cell structures, membranes, and membrane structures contain more gene fragments. In the molecular function section, the number of genes with catalytic activity and binding function accounts for more than half.

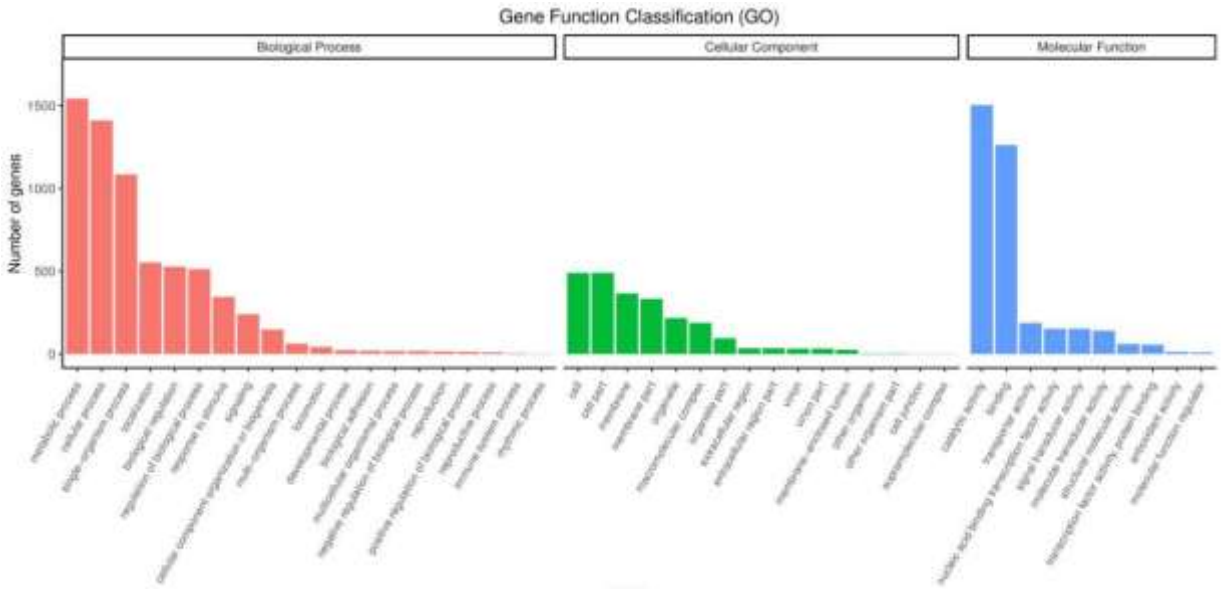

Figure 3.3 – GO functional classification statistics of sample gene function annotation

The three different colors represent the three major categories of GO. The red bar chart represents biological processes, the green one represents cellular components, and the blue one represents molecular functions.

3.3.2 KEGG DATABASE ANNOTATION

KEGG (Kyoto Encyclopedia of Genes and Genomes) is a comprehensive bioinformatics database. This database is mainly applied in research such as metabolic pathway analysis, genomic annotation, and comparative genomics. It integrates data related to genomes, chemistry, system functions and diseases, including KEGG pathways, KEGG GENES, metabolites, KEGG diseases, KEGG drugs, etc. It aims to help researchers understand the molecular mechanisms and advanced functions of biological systems. KO (KEGG ORTHOLOG) is a functional classification system based on orthologous genes, which is used for functional annotation and comparative analysis of the genome. KEGG has established a complete set KO annotation system (http://www.genome.jp/kegg/), which can complete the function of the new genome sequencing species or transcriptome annotation. The statistics of the number of annotated genes in the KEGG database are shown in Fig. 3.4. The numbers on the bar chart represent the number of genes in the annotations, and the vertically arranged legends on the left side show that the database contains major categories with each functional subcategory. The metabolic process contains the largest number of genes, totaling 1,552, while the organism system has the fewest, at 53.

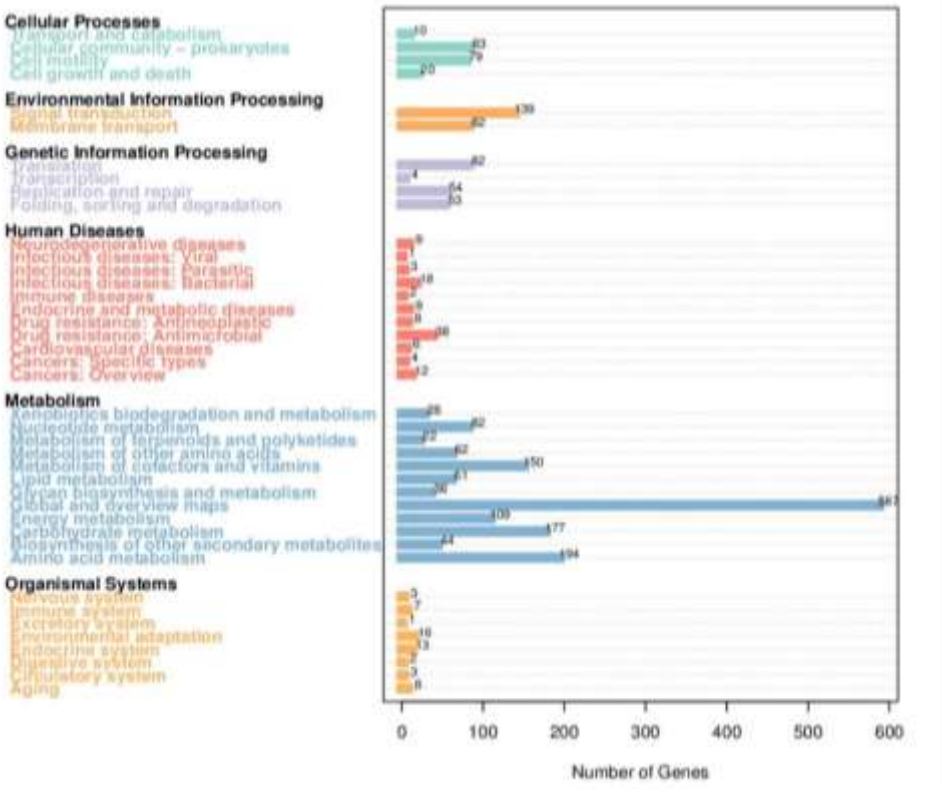

Figure 3.4 – Sample gene function annotation KEGG metabolic pathway classification

Different categories are distinguished by different colors in the picture. Green represents cellular processes, orange represents environmental information processing, purple represents genetic information processing, red represents human diseases, blue represents metabolic processes, and the orange at the bottom represents the body system.

3.3.3 COG DATABASE ANNOTATION

COG (Clusters of Orthologous Genes) is a database developed and maintained by the National Center for Biotechnology Information (NCBI) of the United States. It is constructed by classifying the evolutionary relationships of the coding protein systems of the complete genomes of bacteria, algae, and eukaryotes, aiming to compare the genomic sequences of different species. Classify the genes related to the function as orthologous clusters, thereby inferring the function of the sequence.

COG database (http://www.ncbi.nlm.nih.gov/COG/) gene can be divided into 25 functional category, such as metabolism, cell processes, etc. The statistical results are shown in Fig. 3.5. The horizontal axis in the figure represents the COG function type code, and the legend arranged vertically on the far right is the specific category corresponding to the code. The vertical coordinate represents the number of genes on the annotation. The number of genes annotated for T signal transduction function is the largest, reaching 262, followed by amino acid transport and metabolism, totaling 256. The processing and modification of RNA and the partial annotation genes of the cytoskeleton were the fewest, being 1 and 3 respectively.

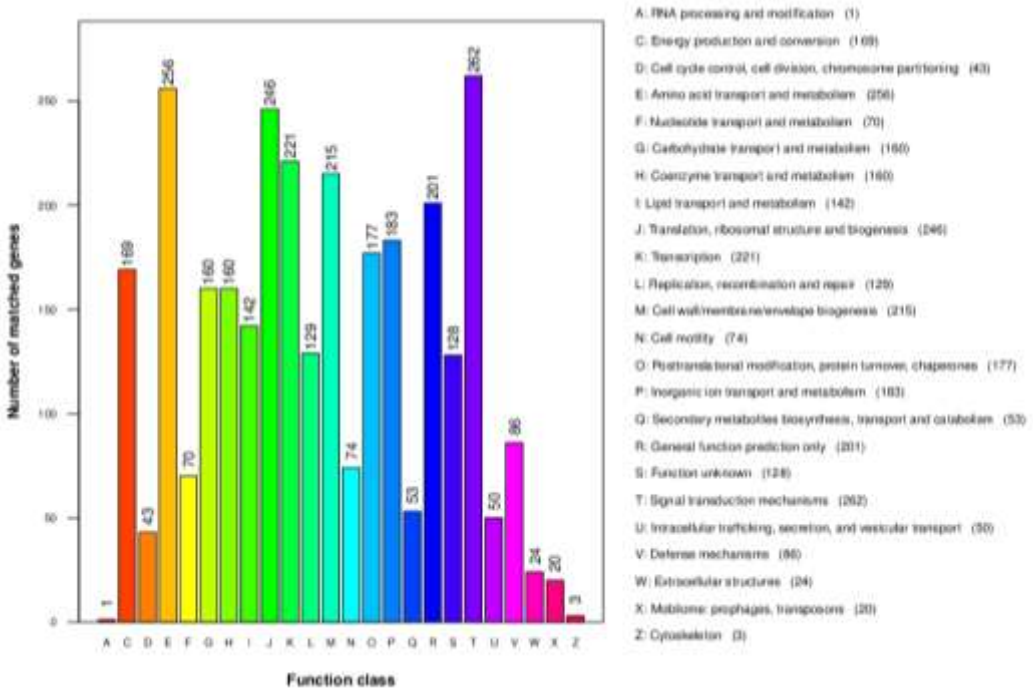

Figure 3.5 – Functional classification statistics of sample gene functional annotation COG

In the figure, A-Z represent different functional classifications respectively and are indicated by different colors.

3.3.4 CAZY DATABASE ANNOTATION

CAZy (Carbohydrate-Active enZYmes Database) is a comprehensive database dedicated to the collection and classification of Carbohydrate Active enzymes. Based on functional and structural characteristics, carbohydrate active enzymes are classified into six major categories: Glycoside Hydrolases (GHs), Glycosyl Transferases (GTs), Polysaccharide Lyases (Polysaccharide Lyases) PLs, Carbohydrate Esterases (CEs), Auxiliary Activities (AAs), carbohydrate-binding Modules (CBMs). The statistics of the number of classification annotations in the CAZy database are shown in Fig. 3.6 as follows. The horizontal axis in the figure represents the classification category of the CAZy database, and the vertical axis represents the number of genes on the annotations. Among them, the enzymes of class GH were the most numerous, with 68, while the enzymes of class PL and class AA were the least, each with 4.

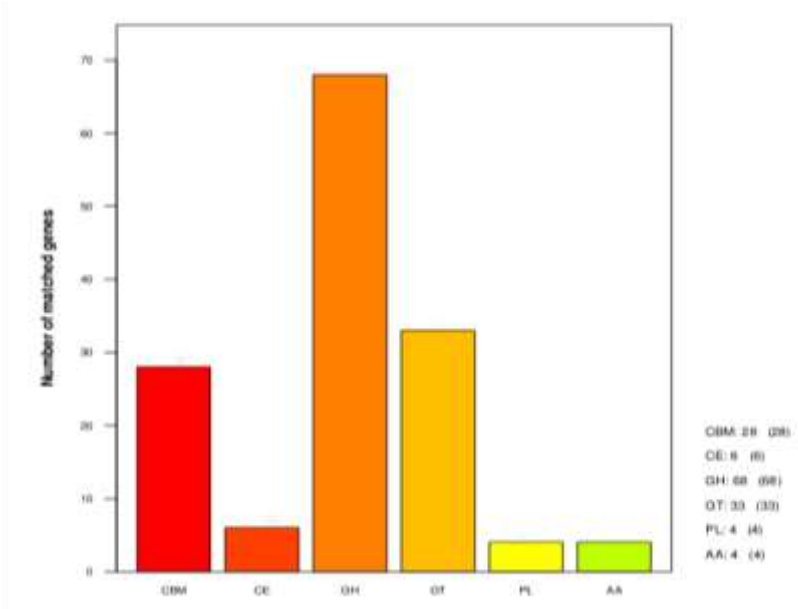

Figure 3.6 – Functional classification of CAZy in the samples and statistics of the corresponding gene quantities

The different colors in the figure represent different categories of carbohydrate active enzymes.

3.3.5 TCDB DATABASE ANNOTATION

TCDB, whose full name is Transporter Classification Database, is a database that focuses on the classification, structure, function and evolutionary relationship of transporter proteins. TCDB is classified into five levels based on function and structure: transport protein major class (V), subclass (W), family (X), subfamily (Y), and specific protein (Z). The first-level statistical results are shown in Fig. 3.7. The horizontal axis in the figure represents the first-level classification type of TCDB, and the vertical axis represents the number of genes on the annotation. The specific information of each classification code and the number of genes it contains are displayed in the rightmost vertical column. The number of active transport protein genes is the largest, reaching 121, while the number of transmembrane electronic vector genes is the smallest, with only 5.

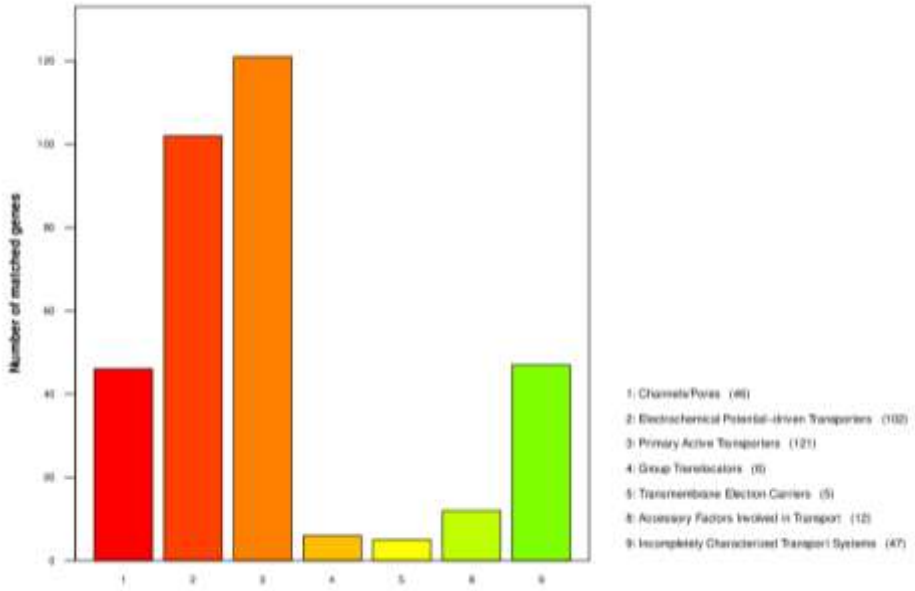

Figure 3.7 –Sample gene function annotation TCDB function classification

3.3 ANALYSIS OF CHITIN DEGRADATION POTENTIAL

To understand the enzymes and transporter proteins related to chitin degradation in strain *P*. sp. M58 and thereby obtain the chitin degradation pathway, we conducted a local Blast comparison of the protein sequence of strain *P*. sp. M58 with the protein sequences reported to be involved in the chitin degradation process. The comparison results are shown in Tab. 3.3, 3.4.

Table 3.3 – The key enzymes and source strains involved in chitin metabolism

Enzyme	Source of enzyme	Query id
chitinase	*Vibrio alginolyticus*	CAC29091.1
beta-N-acetylglucosaminidase	*Aeromonas hydrophila*	BAD00143.1
TBDR	*Pseudoalteromonas distincta*	EGI73602.2
NagX	*Shewanella*	WP_012196773.1
NagP	*Shewanella atlantica*	WP_425470117.1
AmgK	*Caulobacter vibrioides* NA1000	YP_002519022.1
GlmU	*Leptospira interrogans*	XLM39087.1
MurA	*Chlamydia trachomatis*	ABV03254.1
MurB	*Escherichiacoli str*. K-12 substr. MG1655	NP_418403.1
LPMO	*Achaetomiella virescens*	AST24379.1
CDA	*Rhodococcus erythropolis*	AYF60464.1
Porin	*Pseudomonas fluorescens* SBW25	CAI2798997.1

Table 3.4 – Homology comparison of the protein of strain *P*.sp.M58 with the key enzymes known to be involved in chitin metabolism

Query id	Subject id	Identity	Expected value	Score
chitinase	GM001468	54.182	0	860
beta-N-acetylglucosaminidase	GM001468	32.309	2.26E-148	457
TBDR	GM002086	79.373	0	1669
NagX	GM002474	63.784	1.87E-161	455
NagP	GM002652	75.346	0	627
AmgK	GM003641	26.648	9.08E-22	91.7
GlmU	GM004353	33.143	2.55E-27	103
MurA	GM004170	35.526	2.78E-23	91.7
MurB	GM003928	49.688	3.23E-107	313

It can be analyzed based on the comparison results that Strain *P*. sp. The enzymes involved in the chitin degradation process in M58 include chitinase, β -N-acetylglucosaminase, AmgK, GlmU, MurA, and MurB. The transport proteins involved in the degradation process include TBDR, NagX and NagP. Based on this, the metabolic pathway diagram was drawn (as shown in Fig. 3.8).

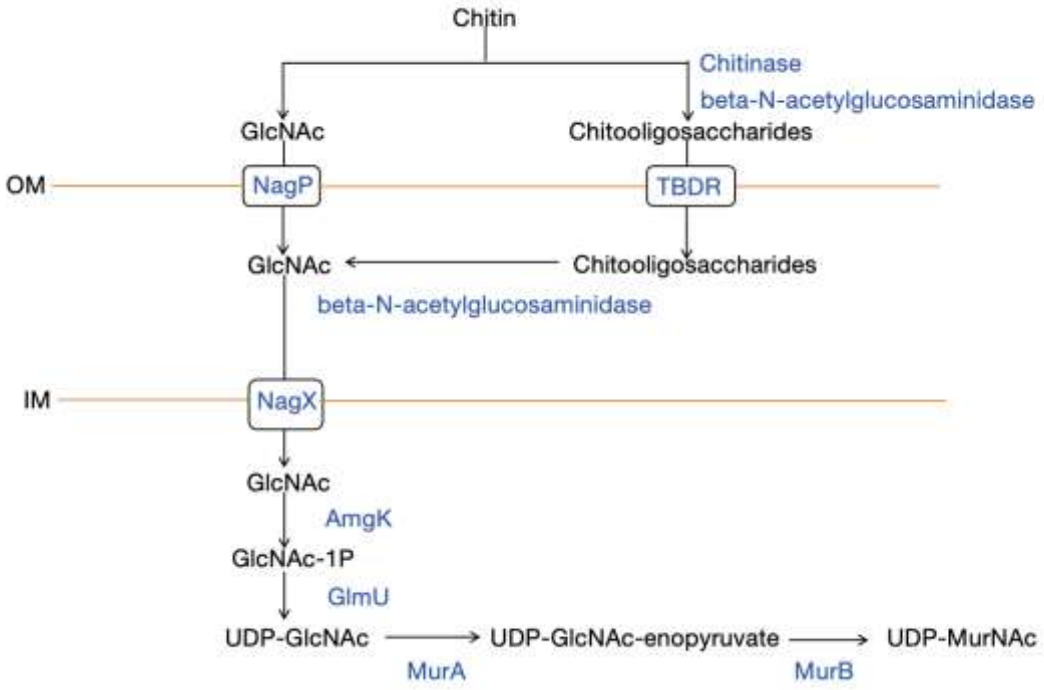

Figure 3.8 – The proposed chitin metabolic pathway in strain *P*. sp. M58

Firstly, the primary degradation products of chitin, GlcNAc and chitin oligosaccharides, enter the peritroplasm space respectively through the transport proteins NagP and TBDR. In the pericplasm, chitosan oligosaccharides are degraded by β -N-acetylglucosaminase to GlcNAc, and GlcNAc enters the cytoplasm through NagX. Intracellular GlcNAc is phosphorylated by AmgK to GLCNAC-1P, and GLCNAC-1P is then converted by GlmU to UDP-GlcNAc. Then, under the action of MurA, UDP-GLCNAc-allyl pyruvic acid is generated and is eventually converted into UDP-MurNAc by MurB. Metabolic end products are closely related to the synthesis of the cell wall.

Conclusions to chapter 3

1. The M58 genome contains one complete circular chromosome of 3604,843 bp and one circular plasmid of 824,954 bp. The prediction results of coding genes and non-coding Rnas were statistically analyzed.

2. The functions of genes were annotated respectively using the GO database, KEGG database, COG database, CAZy database and TCDB database. The annotation results are roughly reflected as follows: the number of genes involved in encoding metabolic processes is the largest, the number of genes involved in encoding GH-type enzymes is the largest, and the number of genes involved in encoding active transporters is the largest.

3. To obtain the chitin degradation pathway, we conducted a local Blast comparison of the protein sequence of strain P. sp. M58 with the protein sequences that have been reported to be involved in the chitin degradation process. Finally, the enzymes and transport proteins involved in the metabolic pathway were obtained, and the degradation pathways existing in the strain were predicted.

CONCLUSIONS

1. This paper takes *Pseudomonas oceanalis* as the research object and conducts whole-genome sequencing of the target strain. The results show that the *P*. sp. M58 genome contains one complete circular chromosome of 3,604,843 bp and one circular plasmid of 824,954 bp. The sequencing results were annotated respectively using the functional databases of GO, KEGG, COG, CAZy and TCDB. The three types of statistical results from the GO database show that the number of genes involved in encoding biological processes accounts for the largest proportion. The annotation results of the comprehensive bioinformatics database KEGG indicate that the number of genes involved in encoding metabolic processes is the largest, while the number of organism systems is the smallest. The COG database performs functional annotation of genes at the level of phylogenetic relationships. Most gene fragments are involved in encoding the signal transduction process and the transport and metabolism of amino acids. The CAZy database classified carbohydrate active enzymes into six categories. The annotation results showed that the number of genes involved in encoding GH-type enzymes was the largest, while PL and AA types were the smallest. The annotation results of the TCDB database show that the number of genes involved in encoding active transporters is the largest, while the number of genes involved in encoding transmembrane electronic vectors is the smallest.
2. To obtain the chitin metabolic pathway, we conducted a local Blast comparison of the protein sequence of *P*. sp. M58 with the reported protein sequences involved in the chitin degradation process, analyzed the enzymes and related transporter proteins required for the degradation process of *P*. sp. M58, and mapped out the proposed chitin degradation pathway in *P*. sp. M58. Thus, it is proved that *P*. sp. M58 have the ability to degrade chitin.

3. The subsequent work will take the various enzymes and proteins discovered as the entry point, further verify the activities of the discovered enzymes through relevant in vitro verification experiments, and analyze their specific functions in the chitin metabolism process. Screen and identify unknown and newly functional enzymes to determine their specific functions and roles in metabolic pathways, and carry out specific biochemical experiments to verify the feasibility of degradation pathways.

REFERENCES

1. Xiaojun Zhang, Jianbo Yuan, Fuhua Li, et al. Chitin Synthesis and Degradation in Crustaceans: A Genomic View and Application [J]. Marine drugs. 2021, 19:153.
2. Jun Li, Ya Ma, Ronghua Zhang, et al. Genomic analysis of *Pseudoalteromonas* sp. M58 reveals its role in chitin biodegradation [J]. Marine Genomics. 2025, 79, 101158.
3. Yanjun Wang. Research on the Chitin Oxidative Degradation Pathway in Marine Bacteria [D]. Tsingtao:Shandong University, 2020.
4. Chuanqi Lu. Screening and identification of Marine chitinase-producing strains, genomic analysis and evaluation of chitinase enzymatic properties [D]. Wuhan:Wuhan Polytechnic University, 2023.
5. AM Dukat, AM Kuznetsova, SD Klyagin, et al. Chitin Degradation by Microbial Communities of the Kandalaksha Bay, White Sea [J]. Microbiology. 2024, 93:61-78.
6. Daniela M. G. da Silva, Filipa R. Pedrosa, M. Ângela Taipa, et al. Widespread occurrence of chitinase-encoding genes suggests the Endozoicomonadaceae family as a key player in chitin processing in the marine benthos [J]. ISME. 2023, 3:109.
7. Sara Skøtt Paulsen. Genomic and phenotypic analyses of chitin degradation and secondary metabolite production in *Pseudoalteromonas* [D]. DTU Orbit. 2020.
8. Zhendong Yang. Identification of *Chitinibacter* sp. Cut -21 and Study on its Chitin degradation mechanism [D]. Guangzhou:South China University of Technology, 2023.
9. Qianqian Xu. Screening of Chitin-efficient degradation strains and application research of pyrolytic polysaccharide monooxygenase [D]. Liaocheng:Liaocheng University, 2022.
10. Feifei Guan. Mining and functional study of Chitin degradation-related enzymes derived from Marine metagenomes [D]. Beijing:Chinese Academy of

Agricultural Sciences, 2020.

11. Nyok-Sean Lau, Go Furusawa. Polysaccharide degradation in Cellvibrionaceae: Genomic insights of the novel chitin-degrading marine bacterium, strain KSP-S5-2, and its chitinolytic activity [J]. 2024, 912, 169134.
12. Kou S.G, Peters L.M, Mucalo M.R. Chitosan: A review of sources and preparation methods. Int. J. Biol. Macromol. 2021, 169, 85–94.
13. Chelsea A Hayes, Triana N Dalia, Ankur B Dalia. Systematic genetic dissection of chitin degradation and uptake in *Vibrio* cholera [J]. Environ Microbiol. 2017, 19(10):4154-4163.
14. Tina Keller-Costa, Lydia Kozma, Sandra G. Silva, et al. Metagenomics-resolved genomics provides novel insights into chitin turnover, metabolic specialization and niche partitioning in the octocoral microbiome [J]. Microbiome. 2022, 10:151.
15. Schmitz C, González Auza L, Koberidze D, et al. Conversion of chitin to defined chitosan oligomers: Current status and future prospects [J]. Marine Drugs. 2019, 17(8):452.
16. Taokaew S, Kriangkrai W. Chitinase-assisted bioconversion of chitinous waste for development of value-added chito-oligosaccharides products [J]. Biology. 2023, 12(1):87.
17. Jiawei Xue. Mining and Functional Study of Chitin Degradation Gene in *Pseudomonas sponges* JW229 [D]. Guangdong Ocean University, 2023.
18. Patel S, Goyal A. Chitin and chitinase: role in pathogenicity, allergenicity and health [J]. Biological Macromolecules. 2017, 97:331-338.
19. Zhou J, Wen B, Xie H, et al. Advances in the Preparation and Assessment of the Biological Activities of Chitosan Oligosaccharides with Different Characteristics [J]. Food＆Function. 2021, 12:926-951.
20. Huihui Sun, Li Gao, Changhu Xue, et al. Marine-polysaccharide degrading enzymes: Status and prospects [J]. 2020, 19:2767-2796.
21. Iuliia Pentekhina, Olga Nedashkovskaya, Aleksandra Seitkalieva, et al. Chitinolytic and Fungicidal Potential of the Marine Bacterial Strains

Habituating Pacific Ocean Regions [J]. Microorganisms. 2023, 11: 2255.

22. Takako Hirano, Manabu Okubo, Hironobu Tsuda, et al. Chitin Heterodisaccharide, Released from Chitin by Chitinase and Chitin Oligosaccharide Deacetylase, Enhances the ChitinMetabolizing Ability of *Vibrio* parahaemolyticus [J]. Journal of bacteriology. 2019, 201:e00270-19.
23. Ying Xu, Xiaomin Lan, Minjie Zhou, et al. Whole-genome sequencing and comparative genomics analysis of *Pseudomonas arabinalis* N1230-9, a strain of *Pseudomonas arabinalis* from the surface seawater of the Pacific Ocean [J]. Journal of Microbiology. 2024, 64(6): 1691-1703.
24. Wen-Xin Jiang, Ping-Yi Li, Xiu-Lan Chen, et al. A pathway for chitin oxidation in marine bacteria [J]. Nature Communications. 2022, 13:5899.
25. Sara Skøtt Paulsen, Mikael Lenz Strube, Pernille Kjersgaard Bech, et al. Marine Chitinolytic *Pseudoalteromonas* Represents an Untapped Reservoir of Bioactive Potential[J]. mSystems 4: e00060-19.
26. I. Raimundo, R. Silva, L Meunier, et al. Functional metagenomics reveal differential chitin degradation and utilization features across free-living and host-associated marine microbiomes [J]. Microbiome. 2021, 9:43.
27. Bowman JP. Bioactive compound synthetic capacity and ecological significance of marine bacterial genus *Pseudoalteromonas* [J]. Marine Drugs. 2007, 5, 220-241.
28. Xiyan Wang, Thomas Isbrandt, Mikael Lenz Strube, et al. Chitin Degradation Machinery and Secondary Metabolite Profiles in the Marine Bacterium *Pseudoalteromonas* rubra S4059 [J]. Marine drugs. 2021, 19:108.
29. Wenxin Jiang. Research on the Chitin Oxidative Degradation Pathway in Marine Bacteria [D]. Tsingtao:Shandong University, 2022.
30. Keller-Costa T, Silva R, Lago-Lestón A, et al. Genomic insights into *Aquimarina* sp. strain EL33, a bacterial symbiont of the gorgonian coral Eunicella labiate [J]. Genome Announcements. 2016, 4(4):e00855.
31. Silva SG, Blom J, Keller-Costa T, et al. Comparative genomics reveals complex natural product biosynthesis capacities and carbon metabolism across

host-associated and free-living *Aquimarina* (Bacteroidetes, Flavobacteriaceae) species [J]. Environ Microbiol. 2019, 21(11):4002.

32. Xue-Bing Ren, Yan-Ru Dang, Sha-Sha Liu, et al. Identification and Characterization of Three Chitinases with Potential in Direct Conversion of Crystalline Chitin into N,N, -diacetylchitobiose [J]. Marine Drugs, 2022, 20:165.
33. Wang X, Chi N, Bai F, et al. Characterization of a cold-adapted and salt-tolerant exo-chitinase (ChiC) from *Pseudoalteromonas* sp. DL-6 [J]. Extremophiles. 2016, 20, 167–176.
34. Tsujibo H, Orikoshi H, Shiotani K, et al. A novel family 19 chitinase from the marine-derived *Pseudoalteromonas tunicata* CCUG 44952T: Heterologous expression, characterization and antifungal activity [J]. Biochem. Eng. J. 2015, 93, 84–93.
35. Makhdoumi A, Dehghani-Joybari Z, Mashreghi M, et al. A novel halo-alkali-tolerant and thermo tolerant chitinase from *Pseudoalteromonas* sp. DC14 isolated from the Caspian Sea [J]. Int. J. Environ. Sci. Technol. 2015, 12, 3895–3904.
36. Ling Wang, Ming Xue, Rui Yan, et al. Insights into Chitin-Degradation Potential of *Shewanella khirikhana* JW44 with Emphasis on Characterization and Function of a Chitinase Gene SkChi65 [J]. Microorganisms. 2024, 12:774.
37. Yuriy Mihaylov Stoykov, Atanas Ivanov Pavlov, Albert Ivanov Krastanov. Chitinase biotechnology: Production, purification, and application [J]. Engineering. 2014, 15(1):30-38.
38. Chun Geun Lee1, Carla A. Da Silva1, Charles S. Dela Cruz. Role of Chitin and Chitinase/Chitinase-Like Proteins in Inflammation, Tissue Remodeling, and Injury [J]. Annual of Physiology. 2011,73:479-501.
39. Yang Liu, Zhuhua Chan, Runying Zeng. Purification and characterization of chitinase secreted by *Pseudoalteromonas* sp. DXK012 isolated from deepsea sediment [J]. Journal of Nature and Science. 2015, 1:37.
40. John Onolame Unuofn, Olubusola Ayoola Odeniyi, Omolara Sola

Majengbasan, et al. Chitinases: expanding the boundaries of knowledge beyond routinized chitindegradation [J]. Environmental Science and Pollution Research. 2024, 31:38045–38060.

41. Xiaomin Lan，Minjie Zhou，Jing Wang et al. The function of chitin lyase of *Pseudomonas* arabinalis N1230-9 [J].Journal of Microbiology. 2025.
42. Federica Calderaro, Loes E. Bevers, Marco A. van den Berg. Oxidative Power: Tools for Assessing LPMO Activity on Cellulose [J]. Biomolecules. 2021, 11:1098.
43. Lukas Rieder, Morten Sørlie. Recent Advances in Understanding LPMO Catalysis [J]. Biochemistry. 2023, 62(22):3170-3172.
44. Yan Zhang, Feifei Guan, Ningfeng Wu, et al. Research Progress of Chitin Deacetylase [J]. Biotechnology Bulletin, 2019, 35(11): 179-186.
45. Yong Zhao, Ro-Dong Park, Riccardo A.A. Muzzarelli. Chitin Deacetylases: Properties and Applications [J].Marine drugs. 2010, 8:24-46.
46. Shuting Qiu, Shipeng Zhou, Yue Tan, et al. Biodegradation and Prospect of Polysaccharide from Crustaceans [J]. Marine drugs. 2022, 20:310.
47. Parks DH, Imelfort M, Skennerton CT, et al. CheckM: Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes [J]. Genome Research. 2015, 25(7): 1043-1055.
48. Lagesen K, Hallin P, Rødland E A, et al. RNAmmer: consistent and rapid annotation of ribosomal RNA genes [J]. Nucleic acids research. 2007, 35(9): 3100-3108.
49. Gardner P P, Daub J, Tate J G, et al. Rfam: updates to the RNA families database [J]. Nucleic acids research. 2009, 37(suppl 1): D136-D140.
50. Nawrocki EP, Kolbe DL, Eddy SR. Infernal 1.0: inference of RNA alignments. Bioinformatics. 2009, 25(10):1335-1337