PROTEIN CHARACTERISATION OF MUCUS IN *MYTELLA STRIGATA*

DISSERTATION SUBMITTED TO ST. TERESA'S (AUTONOMOUS) COLLEGE ERNAKULAM IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF

DEGREE OF MASTER OF SCIENCE IN ZOOLOGY

SUBMITTED BY

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CERTIFICATE

This is to certify that the dissertation entitled "**Protein Characterisation of Mucus in *Mytella strigata***" is an authentic work carried out by **KARTHIKA K BABU**, (Reg no: SM23ZOO004) during the Academic year 2023-2025, under the external guidance of Dr. V P Limna Mol, Assistant Professor, Department of Marine Biosciences at the Kerala University of Fisheries and Ocean Studies (KUFOS) and under internal guidance of Dr. Jean Mary Joy, Assistant Professor, Department of Zoology, St. Teresa's College (Autonomous), Ernakulam for the partial fulfilment of the requirement of the Degree of Master of Science in Zoology from St. Teresa's College (Autonomous), Ernakulam.

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I hereby declare that this dissertation entitled **"Protein Characterisation of Mucus in *Mytella strigata*"** submitted to Mahatma Gandhi University, Kottayam in the partial fulfillment for the award of Master of Science in Zoology, is a record of original project work done by me, and no part thereof has been submitted to any other course. To the best of my knowledge, this project does not include any content that has been previously published or written by someone else, unless proper acknowledgement has been given to the original source.

KARTHIKA K BABU

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LIST OF ABBREVIATIONS

SL.NO	ABBREVIATION	EXPANSION
1	Eg	EXAMPLE
2	&	AND
3	°	DEGREE
4	Ppt	PARTS PER MILLION
5	ml	MILLILITRE
6	Rpm	ROTATION PER MINUTE
7	°c	DEGREE CELCIUS
8	µl	MICROLITRE
9	g	GRAM
10	Ph	POTENTIAL OF HYDROGEN
11	mg	MILLIGRAM
12	%	PERCENTAGE
13	nm	NANOMETRE
14	µg	MICROGRAM
15	kDa	KILODALTON

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ABSTRACT

This study discusses the biochemical characterization of *Mytella strigata's* mucus proteins in terms of physiological function, environmental significance, and potential biomedical application. Mucus secretion in bivalves plays a critical role in immune defence, adhesion, and environmental acclimation. Proteins were precipitated with acetone and analysed by the Bradford assay, which confirmed a high protein concentration (27.23 µg/mL). SDS-PAGE showed specific protein bands in the range of 65–100 kDa, suggesting the presence of structural, immune, and enzyme proteins. UniProtKB protein characterization indicated the presence of heat shock proteins (HSPs), metabolic enzymes, and adhesins, which reflect their functional diversity in stress response and cellular homeostasis. The presence of antimicrobial peptides and glycoproteins suggests potential application in wound healing and antimicrobial therapeutics. The findings highlighted the ecological adaptation of *Mytella strigata* and further support the future potential of its protein sources in the area of biotechnology and environmental monitoring. Molecular interaction, protein modification, and large-scale protein extraction methods should be the focus of future research to realize their full application potential.

INTRODUCTION

Bivalves are a group of marine invertebrates, oyster, mussel, clams. Due to special ecological significance and physiological features, they hold fascinating research potential. They live in a wide range of habitats, from deep seas to intertidal areas, and have evolved various survival strategies to cope with the harsh conditions. One of the most amazing of these is the secreted mucus, a critical compound to the survival of the organism. In addition to providing physical defense, the produced mucus of bivalves is essential to immunity, environmental adaptation, and even communication with the surrounding. Due to this, bivalve mucus and its biological constitution have been the target of extensive research, particularly when it comes to possible applications in the biomedical field as well as in ecological monitoring.

Bivalves' ecological role relies on the mucus secreted by them. Hypobranchial gland is the key structure responsible for secreting mucus in Bivalves. The gland is glandular in nature and is a part of anatomy of this species. In bivalves, the mucus consists of protein-polysaccharide complexes known as mucopolysaccharides, which have been classified on the basis of acidity level, ranging from neutral to acidic. Its key function is to provide protection to the bivalve against environment-induced challenges, i.e., infection, desiccation, and predators. Mucus, for instance, aids in the formation of a slimy protective film on the surface of the bivalve that prevents pathogenic microbes from attaching on the organism's surface. Mucus plays a role in both adhesion and pathogen defence (Beninger & St-Jean., 1997). It is a good adhesive that several bivalves, e.g., mussels, use to settle on hard substrata in turbulent aquatic environments. Because it is capable of adhering to substrata, bivalves get to remain stable in areas where tidal currents and wave action is high. Also, mucus serves as a bivalve's lubricant when it is moving within its environment, lowering the friction when it is crawling on the sea bed or when it is digging.

Bivalve’s mucus sensitivity to external factors is yet another intriguing phenomenon. Stressful environmental pressures, i.e., pollutant exposure, temperature or salinity change, or the presence of pathogenic bacteria, might stimulate enhanced mucus secretions. Mucus is an excellent indicator of environmental stress due to this adaptive response. Because bivalves are filter feeders, they continuously come into contact with the ambient water, and any change in the water's quality, e.g., pollutant increase or toxin level, might trigger a stronger mucus

response (Jorgensen, C. B. 1996). It is suggested that bivalves might be excellent bio indicators of pollution levels and water quality in coastal ecosystems because enhanced mucus secretions might be a defence mechanism to environmental stressors.

Bivalve mucus is full of potential in terms of biomedical research and application over and above its ecological role. Mucus produced by bivalves contains high concentrations of bioactive compounds, ranging from proteins and peptides to enzymes, many of which have therapeutic potential (Davies & Hawkins; 1998). Such substances have been of special importance due to potential therapeutic values, especially in the creation of new drugs and medical devices.

Antibacterial qualities of bivalve mucus represent some of the most fascinating features of mucus (Ahmad et al., 2018). Mucus is a strong defense mechanism against a wide range of infections because it contains antimicrobial peptides, which have a central role in the innate immune system of bivalves (Allam.et.al., 2016). With the ability to break down bacterial membranes and disrupt their structure, such peptides have the capability to kill pathogenic microbes. Due to the ability to kill off infections, researchers are investigating the possibility of using antimicrobial compounds extracted from bivalve plants as a source to develop new antibiotics or topical remedies to cure infections, particularly in light of a surge in antibiotic resistance.

Besides, bivalve mucus also consists of proteins with anti-inflammatory and wound-healing activities. Such bioactive molecules possess the potential to heal tissues and alleviate inflammation by influencing the immune system (sousa et al., 2020). For medical uses, it is desirable that such activities be present, especially in tissue regeneration and wound healing. The wound-healing activities of bivalve mucus make it a potential therapeutic solution to treat burns, chronic wounds, and other skin disorders where recovery should be accelerated and improved.

In addition, bivalve mucus is also suggested to be a source of bio adhesives, which could be used in medicine. Certain mucus proteins have been shown to be potential candidates to be applied as surgical adhesives because they bond well to surfaces, even to biological tissues. The bio adhesives could be used as implant and medical device glues, wound sealants, or as agents to repair damaged tissues.

Maximizing the ecological and therapeutic potential of bivalve mucus involves an appreciation of its biochemical structure. Proteins within bivalve mucus facilitate its bioactivity and also confer structure to it in terms of viscosity and pliability. The area of current research into the nature of bivalve mucus is the isolation and characterization of its proteins.

Proteins are separated from the mucus by a series of protein extraction techniques. For instance, the ability of precipitation in acetone to separate proteins from other chemicals without denaturation renders it a popular technique in marine biology (Campos et al., 2012). The Bradford test, which quantifies protein concentration via colorimetric change, is widely used to quantify the protein content. The simplicity and sensitivity of the technique allow researchers to accurately quantify the protein content in mucus samples (Nguyen & Harvey,1994).

Proteins are then separated based on molecular weight by methods such as SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) after extraction and quantification. The size and quantity of proteins in the mucus are shown in detail by this electrophoretic method (Singh et al., 1991). After that, researchers can identify major proteins, immune-related substances, enzymes, and structures like myosin and lectins, and investigate their possible functions. Determination of the role of these proteins in bivalve physiology and possible application in the area of biomedicine is contingent upon understanding the molecular weight distribution and functional characteristics.

Mytella strigata, also known as Charru mussel, is a bivalve. Charru mussel is native to Panama, Argentina, Brazil and Venezuela but is an invasive species in South-eastern US, Singapore, Thailand, India (Yuan et al., 2016).

Table 1: Taxonomic classification of *Mytella strigata*

Domain	Eukaryota
Kingdom	Animalia
Phylum	Mollusca
Class	Bivalvia
Order	Mytilida

Family	Mytilidae
Genus	*Mytella*
Species	*M. strigata*

Mytella strigata can survive in salinities ranging from 2 ppt (parts per thousand) to 22.5 ppt. They can also withstand large salinity changes over long and short time scales (Rice et al., 2016). M. *strigata* differ from other species by having terminal umbo, slightly angled in dorsal area and laterally concave in ventral area (Beltrao et al.,2022). The species is of high nutritional value, hence can be used as animal feed (Prakashan et al., 2023). *Mytella strigata* commonly inhabiting estuarine environments is the focus of this study. *Mytella strigata's* ecological flexibility makes it a good species to be studied on the topic of mucus production.

AIM

The aim of this study is to investigate the biochemical composition of *Mytella* strigata's bivalve mucus. The main purpose of this study is to gain knowledge about the ecological and physiological role of these proteins, mostly immune function, metabolic processes, adhesion mechanisms, and environmental stress responses.

OBJECTIVE

- To separate protein from the mucus by appropriate biochemical method
- To the determination of the extracted protein by the Bradford method
- To resolve and characterize protein components by SDS-PAGE
- To identify potential protein functions through comparison of molecular weights with known proteins in the UniProtKB database

RELEVANCE OF THE STUDY

The mucus proteins' characterization takes centre stage in this research since the proteins play a significant part in immune defence, disease pathogenesis, and mucosal protection. The extraction and quantification of the proteins using biochemical methods such as the Bradford assay and analysis of the protein samples using SDS-PAGE yield valuable information about the molecular characteristics and structure of the proteins. A key aspect of this research is the alignment of the protein samples against the known protein data in the UniProtKB database to ascertain potential protein function based on molecular weight and sequence similarity.

REVIEW OF LITERATURE

Bivalves

Since they are filter feeders, marine bivalves are essential to preserving the equilibrium of the ecosystem. By eliminating impurities, surplus nutrients, and particles from the water, they aid in controlling its quality. Their feeding behaviour affects the availability of vital components in aquatic environments and aids in the cycling of nutrients (Dame R.F et al., 2011). The effect of salinity in bivalve physiology helps to determine the best saline condition for bivalve health and productivity (Pourmozaffar et al., 2020). Bivalve filter feeders are crucial to coastal and estuarine environments. They support habitat creation, water filtering, and nutrient cycling. Their presence promotes ecological stability and enhances water quality, both of which are critical for managing and conserving marine life (Dame R. F 2013).

Mucus production in bivalves

The mucus in *Mytella falcata* bivalves' gills traps and expels toxic substances, allowing them to deal with pollution. As a protective mechanism against pollutants, increased mucus production impacts breathing and general health. Comprehending this response yields valuable insights for managing aquatic ecosystems and monitoring pollutants (David, J.A et al., 2009). In *Patella vulgata*, mucus production is essential for attachment, defence against predators, and environmental adaption. It involves energetic costs and advantages that affect the physiology of the organism as a whole. Gaining knowledge about mollusc mucus secretion helps one appreciate both its ecological and functional relevance (Davies, M.S et al 1990). Bivalves' mucin structure and function are essential for feeding, protection, and environmental adaption. The production of mucin has changed over time to promote ecological interactions and improve survival. These molecular substances aid in adaptations connected to mucus, which deepens our comprehension of bivalve physiology (Prezant, R.S 1990).

Importance of Bivalve Mucus in Ecological and Biomedical Research

The ecological and medicinal ramifications of studying bivalves' mucus extraction and protein analysis have drawn a lot of attention. Numerous physiological functions, such as

adhesion, immunological defence, motility, and environmental interactions, depend on bivalve mucus (Beninger & St-Jean, 1997). Furthermore, it is recognized that bioactive molecules with potential uses in biotechnology and medicine are present in the biochemical makeup of mucus (Davies & Hawkins; 1998). Mucus proteins are essential for many marine creatures, and recent research has shown that they contain antibacterial, anti-inflammatory, and wound-healing qualities (Ahmad et al., 2018). In order to discover new bioactive compounds with possible therapeutic applications, a great deal of study has been done to comprehend the molecular components of bivalve mucus. Ecological studies have also examined the capacity of bivalves to create mucus in response to environmental stressors, demonstrating how these animals adjust to changing water conditions and contaminants. Mucus proteins' potential in environmental monitoring applications is further supported by their versatility, since they may serve as bio indicators of pollution levels and water quality (Holovkov et al., 2023). Analysis of the nutritional makeup of *Mytella strigata* from Kerala, India's Cochin Backwater has shown that it has the potential to be a valuable food source. Its nutritional value is derived from its protein, fat, and mineral content, which qualifies it for use in aquaculture and human consumption. Knowing its nutritional makeup encourages sustainable gathering methods and supports its use in the seafood sector (Prakasan, S et al., 2023). The invasive charru mussel has been studied for its nutritional potential, with particular attention to its fatty acid composition for use as aquaculture feed. Packed with vital fatty acids, it provides aquafeed with a reliable substitute protein source. Evaluating its nutritional composition aids in determining whether it is suitable for supplementing the diets of fish and shellfish while resolving ecological issues associated with its invasiveness (Kutako, M et al., 2024).

Mytella strigata

In Southeast Asia, *Mytella strigata* is becoming a more invasive marine species that could be harmful to the environment. Its quick expansion could affect local species, change ecological dynamics, and affect native biodiversity. Managing marine biodiversity and creating control plans require an understanding of its invasion patterns and impacts (Sanpanich, K et al., 2019). The nomenclature, morphology, and distribution of *Mytella strigata* and *Mytella guyanensis* in Babitonga Bay, South Carolina, have been investigated. Understanding these species' ecological responsibilities and distinctions can be gained through identification and classification. Their presence adds to our knowledge of bivalve diversity and distribution by reflecting habitat preferences and possible environmental effects (Beltrao, M.C et al., 2022). An ecological analysis of the invasive Charru mussel's spatial distribution in Ashtamudi

Ramsar Lake, Kerala, has been conducted. Its proliferation could change habitat architecture, affect ecosystem dynamics, and affect native biodiversity. Conservation initiatives and the control of invasive species in aquatic environments depend on an understanding of its distribution patterns (Viswanathan, C 2024).

Protein Extraction Using Acetone Precipitation

Acetone precipitation is a well-established method for separating and concentrating proteins in biochemical research. Because it eliminates impurities while maintaining protein structure, this technique works especially well for marine samples (Campos et al., 2012). (Santa et al., 2016) used acetone precipitation to analyse bioactive chemicals in mussel mucus, demonstrating the widespread use of this technique in research involving molluscan secretions. Acetone is a perfect option for protein extraction in marine biological research since it can precipitate proteins while preserving their functional integrity. Researchers like Geret et al., (1998) have also improved the method in recent years by evaluating several precipitation solvents and verifying that acetone is the best option for extracting marine proteins. Acetone precipitation was utilized to extract and purify bioactive proteins with possible medicinal uses in research on various marine invertebrates, confirming the efficacy of this technique (Zhou et al., 2022).

Protein Quantification Using Bradford Assay

Bradford 1976 created the Bradford test, which is still one of the most popular techniques for quantifying proteins in biochemical research. Protein concentrations in biological samples can be quickly and accurately measured using this colorimetric assay, which has a high sensitivity. Mucus proteins have been successfully quantified using this method in marine biology, guaranteeing consistency under test conditions. According to (Gotham et al., 1988), modifications to the Bradford test have been made to increase sensitivity in low-protein samples. (Noble & Bailey,2009) also underlined the significance of normalizing BSA concentrations in protein measurement, also gave an example of how assay results could be impacted by differences in protein standards. In their comparison of protein quantification methods, (Nguyen & Harvey; 1994) found that the Bradford assay is still one of the most accurate ways to analyse the proteins found in marine mucus. Over the years, various modifications have been introduced to improve sensitivity and accuracy, particularly for low-protein samples (Compton & Jones, 1985). Researchers such as (Zor & Selinger 1996) have

further optimized the assay by linearizing its response curve, reducing errors in protein concentration estimations

Protein Characterization Using SDS-PAGE

Because it allows researchers to separate proteins according to their molecular weights, SDS-PAGE is an essential tool for protein characterisation. Bivalve mucus proteins have been extensively analysed in marine biological research using the electrophoretic method, which was first presented by Laemmli in 1970 (Singh et al., 1991). Researchers have been able to identify structural and functional proteins involved in adhesion, immunological response, and biochemical signalling, which has given important insights into the protein composition of marine mucus. Mollusc adhesion proteins were examined using SDS-PAGE in a work by (You et al 2012), which showed molecular weight distributions resembling those found in *Mytella strigata.* (Mann et al., 2018) investigated ways to improve electrophoretic resolution by improving protein band clarity by SDS-PAGE condition optimization. SDS-PAGE was further validated by this appropriate technique for marine protein separation, demonstrating its dependability for characterizing mucus proteins. Sharma and colleagues (2021) also tested with various buffer solutions and gel compositions to enhance the visualization of protein bands, which is essential for recognizing proteins with identical molecular weights. Also used SDS-PAGE to examine mucus proteins from other molluscan species, showcasing the method's adaptability.

Functional and Structural Analysis of High-Molecular-Weight Proteins

Mucus mechanical characteristics and bivalve adhesion depend on large structural proteins like myosin and extracellular matrix constituents. When (Donnelly, E., et al. 2019) looked at high-molecular-weight proteins in marine mucus, they discovered that proteins around 100 kDa help to make mucus elastic and viscous. This study identifies a large adhesive protein (Mfp-1) in mussel foot proteins, which is often around 108 kDa. Proteins between 63-75 kDa might include various functional proteins such as lectins, which are common in bivalve mucus and play a role in immune defence (Allam, B., et al. 2000). This paper describes a C-type lectin of approximately 66 kDa in clams, involved in immune defence.

Potential Applications of Mucus Proteins in Biotechnology and Medicine

Bivalve mucus proteins may find use in medicine, biotechnology, and environmental research. (Benkendorff, 2010) also discussed on the creation of medicinal agents and bio

adhesives. (Sousa & Hinzmann, 2020) made contributions to studies on mucus protein-based antibacterial effects. The use of mucus proteins as bio indicators for environmental monitoring was emphasized by Viarengo & Canesi, (1991).

Table 2: Mucus bio activity from different bivalves

Species	**Extraction method**	**Bioactivity**	**Reference**
Oyster (*Crassostrea virginica*)	Extraction is done by swabbing and immersion method followed by filtration	Shell formation and maintenance	Espinosa, E.P et al.,2009.
Pinctada (*Pinctada martensii)*	Extraction is done by pipette and filtration process followed by centrifugation	Wound healing	Zeng T, et al.,2024
Clams (*Calyptogena okutanii*)	Extraction is directly from the gill tissue after dissection.	Physical and chemical barrier	Yoshimitsu et al., 2013
Mussel	Extraction is done in distilled water and then successively partitioned with hexane, CH2Cl2 and ethyl acetate to separate different chemical components.	Provide lubrication, trapping food particles.	Sallenave et al 1999

METHODOLOGY

Sampling

Sampling of *Mytella strigata* was done in the Panagad region (latitude: 9.90990° N – 9.90638° N, longitude: 76.31475° E – 76.31514° E) at low tide in May 2024. The site was chosen because of the high concentration of M. *strigata*, which is founded by attaching to a variety of substrates to create enormous colonies. The species survive there because, Panagad Lake is an estuary ecology.

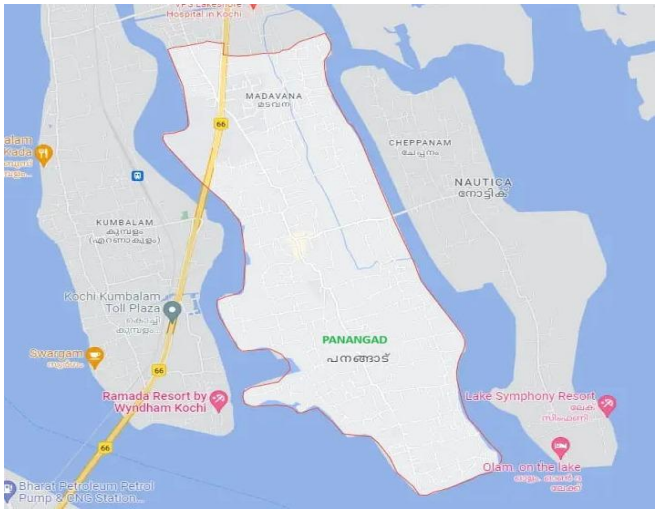

Fig 1: Map showing sampling location

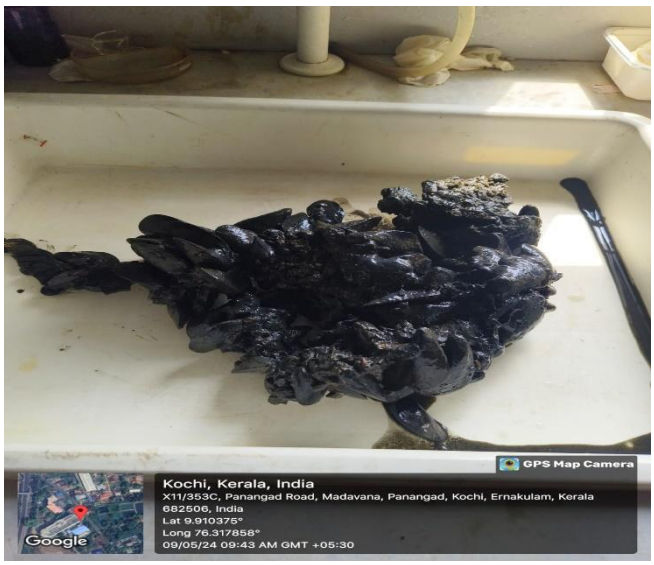

Fig 2: *Mytella strigata* collected from the sampling site

Laboratory set up

Around 50 samples were collected and transported to laboratory. Among these, 30 samples were transferred to an artificial culture system equipped with facilities for proper aeration and salinity regulation to maintain optimal environmental conditions for further study.

Fig 3: Samples provided with aeration

Mucus collection

Two methods were performed for mucus extraction from M.*strigata*. In the first method, three samples were placed separately on Petri dishes, and mucus secretion was stimulated using a sterile needle. The samples were left undisturbed for 2–3 hours to allow mucus accumulation.

In the second method, 500 mL of saline water was autoclaved and cooled overnight to ensure sterility. Subsequently, 100 mL of sterile saline water was transferred into three separate beakers, each containing a single sample. The saline solution with the mucus was then recovered after exposure and partitioned into five 30 mL centrifugal tubes. The samples were then centrifuged at 5000 rpm for 15 minutes at 4°C to remove particulate matter and debris and leave a purified extract to be further analyzed.

Fig 4: Mucus collection

Acetone precipitation

Protein precipitation is a common method to purify impurities like salts, detergent, nucleic acids etc. protein concentrate can be obtained by this method of purification from the sample. 30 mL of acetone was added to the sample for protein precipitation, and the sample was frozen overnight at −20°C to facilitate protein aggregation. The incubated sample was then centrifuged at 4°C, 3000 rpm for 20 minutes to remove the precipitated protein.

Protein quantification using Brad Ford Assay

This method is used to determine the concentration of protein in sample (Valipour Nouroozi R et al., 2015). Six centrifuge tubes were prepared to be employed in protein quantification by the Bradford assay. To each tube, varying concentrations of bovine serum albumin (BSA), distilled water, and 3 mL of the sample were added. Five tubes containing varying concentrations of bovine serum albumin (BSA) with an equal amount of the sample were prepared. Tube 1 contained 196.8 mL of distilled water, 3.2 mL of BSA, and 3 mL of the sample. Tube 2 contained 197.6 mL of distilled water, 2.4 mL of BSA, and 3 mL of the sample, followed by Tube 3 containing 198.4 mL of distilled water, 1.6 mL of BSA, and 3 mL of the sample. Tube 4 contained 199.2 mL of distilled water, 0.8 mL of BSA, and 3 mL of the sample, and Tube 5 contained 196 mL of distilled water, 4 mL of BSA, and 3 mL of the sample. Standardized dilution provided proper protein determination by establishing a standard curve based on absorbance values at 595 nm. For the preparation of the blank, 200 mL of distilled water and 3 mL of Bradford reagent were mixed. The absorbance of all samples was measured at 595 nm with the help of a spectrophotometer to quantify protein concentrations based on the standard curve prepared using the dilution of the BSA.

Micro plating for quantifying protein

The microplate assay was performed using Eppendorf tubes to quantify protein concentrations. In each tube, 200 µl of phosphate-buffered saline (PBS)**,** 1 mL of Bradford reagent**,** and 1 mg of the protein sample were added and mixed thoroughly. For the preparation of the blank**,** 200 µl of PBS and 1 mL of Bradford reagent were combined without the protein sample. Using a 1000 µL micropipette, both the sample and blank solutions were loaded into the respective wells of a microplate. The optical density (OD) was recorded using a spectrophotometer to determine protein concentration based on absorbance values.

SDS-PAGE for separation of protein

Sodium dodecyl sulfate polyacrylamide gel electrophoresis is a process used to differentiate proteins based on the mobility in an electric current, either on the basis of polypeptide chain length or molecular weight. It is achieved by adding SDS detergent to denature secondary and tertiary protein structures and maintain the protein in the state of polypeptide chains (Roy et al., 2012).

Materials required

- **Acrylamide** (30% stock): - dissolved 29.2 g acrylamide and 0.8 bis-acrylamide in distilled water and make up to 100ml. Store under dark in amber colour bottle at 4°c) .

- **Resolving gel/ separating gel buffer** pH 8.8, 1.5M tris-HCl: - dissolved 18.17g tris in 75 ml distilled water. Adjust to pH 8.8 with 6 N HCl. Adjust the total volume to 100ml with distilled water and store at 4°c.

- **Stacking gel buffer**, pH-6.8, and 1.0Mtris-HCl: - dissolved 3g tris in 40 ml distilled water, adjust to pH 6.8 with 6N HCl. Adjust the total volume to 50ml with distilled water. Store at 4°C.

- **Electrophoresis buffer** pH 8.3: - dissolved 3g tris, 14.4 g glycine and 1g SDS in 100ml of distilled water. Store at 4°C. Ammonium per sulphate-initiator 10%: - dissolved 0.1 g APS in 1 ml distilled water.

- **TEMED** (NNN'N' Tetramethylenediamine): catalyst.

- **Sample buffer**: - 7.25 ml distilled water + 1.25 ml stacking gel buffer + 1ml glycerol + 0.5 ml β mercaptoethanol + 150 mg SDS and a pinch of bromophenol blue.

- **Staining solution**: -dissolved 200mgcoomassie brilliant blue R 250 in 50ml methanol/ethanol, 7ml acetic acid and 43ml distilled water & filter it.

- **De-staining solution**: - add 7ml acetic acid to 30ml methanol/ethanol and 63ml distilled water

- **Vertical slab-gel electrophoresis equipment**.

- **Acrylamide mixture** (10%) for 25 ml of resolving gel: -9.9 ml, distilled water + 8.3ml, 0% acrylamide + 6.3ml, 0.5 M tris-HCl + .25ml, 10% SDS + .25ml, 10% APS +.01 ml, TEMED.

- **5% stacking gel** for 5 ml: - 3.04ml, distilled water + 0.83ml, 30% acrylamide + .63ml, 0.5 M Tris – HCl(pH-6.8) + 0.05ml, 1% SDS +0.05ml, APS + 0.005ml, TEMED.

Sample preparation

The samples were subjected to centrifugation at 10,000rpm for 20 minutes. After the process the supernatant was discarded and pellet weights were noted.

Table 3: pellet weights of sample

Sample	Pellet weight
K1	35
K2	45
K3	82
K4	90

The entire pellets were again suspended in 100µl buffer solution and were kept in water bath for 15 minutes to denature the protein. Subsequently, those tubes were centrifuged at 10,000rpm for 10 minutes and the supernatant pipetted to a new tube.

Electrophoresis

Electrophoresis was performed to separate proteins present in the sample. A total of 25 µL of supernatant was mixed with 5 µL of sample buffer and loaded into the gel. The gel was run at 150V for 55 minutes, allowing for efficient protein separation based on molecular weight. Following electrophoresis, further analysis was carried out to visualize protein bands and determine their molecular weights by comparing them with a protein ladder. This approach facilitated the identification of distinct protein fractions, aiding in the assessment of sample composition and molecular weight estimation.

Uniprot Database search

A comprehensive search was conducted in the UniprotKB database to identify proteins from Mytella strigata within the molecular weight range of 65 – 100 kDa.

RESULT

Bradford assay

The Bradford assay was used to quantify the amount of proteins contained in *Mytella strigata* mucus that are involved in physiological and ecological processes. With protein concentrations ranging between 4 and 20 µg/mL and the corresponding absorbance values measured at 595 nm, a standard curve was prepared. The accuracy of the Bradford assay was confirmed by the graph produced, which had a linear regression equation y = 0.0051x + 0.0191 with a high correlation coefficient (R2 = 0.9654). The protein concentration of the unknown sample was then estimated to be 27.23 µg/mL using the equation x = (y - 0.0191) / 0.0051 and the absorbance reading of 0.158. This tells us that M. *strigata* mucus contains a large amount of proteins.

Table 4: protein concentration and OD reading

Protein concentration µl/mL	**OD reading at 595nm**
4	0.040
8	0.060
12	0.075
16	0.111
20	0.117

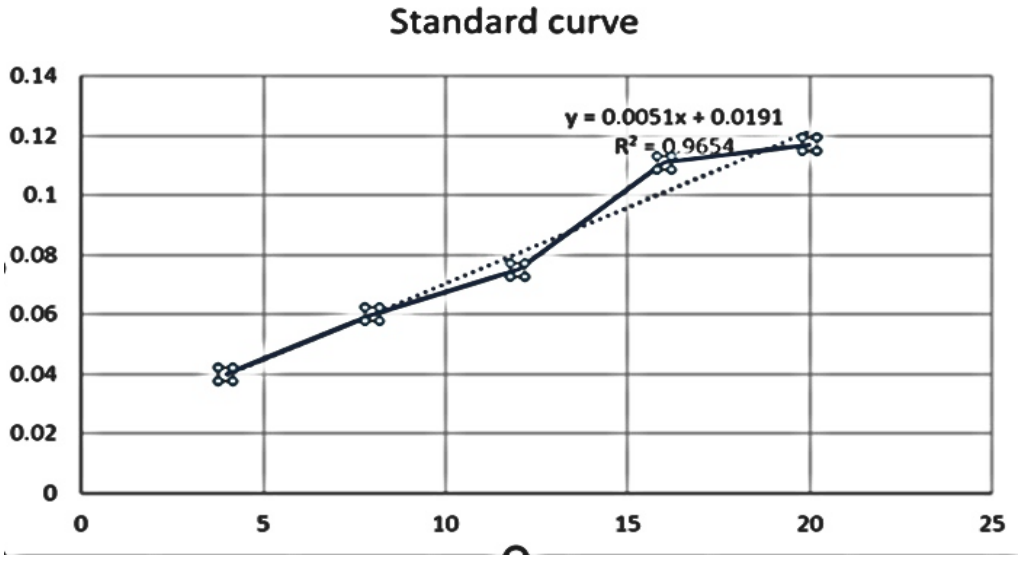

Fig 5: Graph showing Standard curve

SDS

When compared to the protein ladder (lane 5), silver-stained SDS-PAGE gel analysis indicated that a specific protein band is present in lane 4 (K4) in the molecular weight range of 65–100 kDa. This indicates that a protein or group of proteins in this molecular weight range is present in sample K4. The success of the purifying process is demonstrated by the presence of a specific band, which is an indicator of effective protein extraction and separation**.**

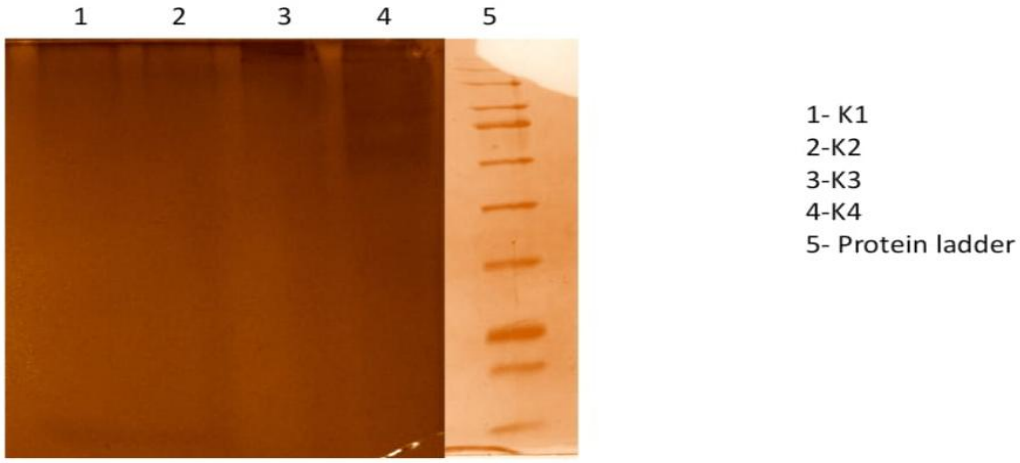

Fig 6: Silver stained SDS-Gel showing Protein bands

Protein characterisation by UniProtKB

The Uniport result shows the protein size and structure. The amino acid length was approximated to be 600 - 900 amino acids. Which is feasible for a number of domains or subunits. With the present limited sequence data on UniProtKB due to incomplete genome annotation, *Mytella strigata's* proteins in the molecular weight range of 65-100 kDa have their function in the organism's physiology. With comparison of homologous proteins in closely related bivalve species researchers could predict potential roles like post-translational modifications, adaptations and cellular locations. The proteins can be capable of being involved in a variety of biological processes.

Table 5: Molecular weight and protein type

Molecular weight (kDa)	Protein type
245 kDa	Structural protein (ECM Protein)
180 kDa	Adhesion protein
135 kDa	Membrane transporters
100 kDa	HSP
75 KDa	Enzymes
63 kDa	Metabolic enzymes, Actin binding proteins
48 kDa	Glycoproteins
35 kDa	Common globular proteins
25 kDa	Small regulatory proteins
20 kDa	Immune defense proteins, Anti-microbial peptides
17 kDa	Small peptides

DISCUSSION

The study's findings offer important new information on the molecular makeup and functions of mucus proteins that were isolated from *Mytella strigata*. Numerous physiological processes that are necessary for the organism's survival and adaptability in dynamic marine environments are suggested by the examination of proteins with molecular weights between 65 and 100 kDa. The discovery of metabolic enzymes, immune-related proteins, stress response proteins, and structural elements demonstrates the diverse roles that these proteins play in cellular stability, adhesion, and environmental resilience.

Bradford assay results for *Mytella strigata* mucus and *Saccostrea glomerata* demonstrate significant protein concentration and biological relevance differences. In the case of M. *strigata,* the assay with known protein concentrations ranging from 4 to 20 µg/mL provided a standard curve with regression line equation $y = 0.0051x + 0.0191$ and an R^2 value of 0.9654, indicating high accuracy. The protein concentration in the mucus was found to be 27.23 µg/mL, indicating a moderate protein presence that might be engaged in physiological and ecological functions such as defence, lubrication, and adhesion. In S. *glomerata,* much higher protein concentrations were obtained, with the value for the crude samples being 780.8 ± 22.4 mg/mL, and fractionated samples varying from 414.6 to 616.0 mg/mL. This indicates a high protein richness, suggesting its role in immune defence and antimicrobial action. The significant difference in the findings highlights the biological diversity in protein expression in marine invertebrates, with S. *glomerata* having a surprisingly high number of bioactive proteins, making it a rich source for antimicrobial and biofilm-inhibitory research (Summer et al., 2024).

The protein analysis comparison of *Mytella strigata* mucus and sample K4 obtained using the SDS-PAGE gel shows the protein composition difference, the range in the molecular weight, and the efficiency in the purification process. *Mytella strigata* proteins had a range of 17 kDa to 135 kDa, with functional proteins such as actin, heat shock proteins, and paramyosin detected. The presence of proteins, though low in concentration compared to other biological samples, was verified using the Bradford assay. Silver-stained SDS-PAGE gel analysis of sample K4, however, showed a distinct band in the range of 65–100 kDa, confirming the presence of a distinct protein or proteins. The presence of a distinct band in K4 suggests efficient extraction and separation of proteins, showing the success in the extraction process.

While the two analyses confirm the presence of proteins, the difference in the range in the molecular weight and detection method shows the difference in protein composition in the species and samples. This emphasizes the necessity for the use of multiple analytical techniques in a bid to gain a holistic understanding of protein profiles. The variety in the range of molecular weight and the detection techniques emphasizes the variety in protein make-up across species and sample types, even though the two analyses affirm the occurrence of proteins (Park & ha., 1985).

Silverman and Roberto (2007) examined the process of adhesion in marine mussels, *Mytilus edulis*, and the potential for the development of bio-inspired adhesives. The mussels secrete special proteins, mussel adhesive proteins (MAPs), that make it possible for the mussels to attach to underwater surfaces. The proteins are characterized by the presence of the amino acid 3,4-dihydroxyphenylalanine (DOPA), which plays a critical role in the process of adhesion through the coordination with metal ions and cross-linking. The study discusses the challenges in the synthesis of the natural adhesives and the potential for the application of recombinant MAPs in medical, industrial, and environmental applications. Scientists, despite having spent much research, are still to successfully imitate the mussels' complex adhesion process. The findings demonstrate the durability, strength, and water resistance of mussel adhesives, making them top contenders for bio-inspired adhesive technologies.

Lectins are carbohydrate-binding proteins that play a role in the immune defense mechanisms in marine animals, including bivalves like *Ruditapes philippinarum* and *Mytella strigata*. Bulgakov et al. (2004) purified a lectin from R. *philippinarum* successfully, which proved to be calcium-dependent and had a high affinity for N-Acetyl-D-galactosamine. The lectin with a molecular weight of 66 kDa specifically interacted with Perkinsus spp., a protozoan pathogen, suggesting that the lectin plays a role in pathogen recognition and immunity. In *Mytella strigata,* lectins with a range of 63–75 kDa have been found, which fall within the range of the known bivalve lectins' molecular weight. Structural and functional analysis of the lectins has been limited, but their molecular weight suggests that the lectins may share properties with those in R. *philippinarum.*

The heat shock protein 70 family has a crucial role in cellular stress responses, notably protein homeostasis during thermal and environmental stress conditions. Roberts, et al., (1997) demonstrated that in *Mytilus californianus*, HSP70 (approximately 70 kDa) expression varies with seasonal acclimatization and tidal-height exposure, with elevated levels during the

summer and in mussels at more elevated intertidal zones, suggesting that mussels in thermally stressful habitats upregulate HSP70 as a protective mechanism against protein denaturation and maintaining cellular function. Similarly, in *Mytella strigata*, HSP70 family proteins, with a range of 70 to 75 kDa, are involved in stress responses and protein folding, with functional conservation across bivalve species. One similarity that the two species, M. *californianus* and *Mytella strigata*, share is the likely role of HSP70 in thermal acclimation, as the two species are both subject to fluctuating environmental conditions in intertidal and estuarine environments, and their comparable molecular weight suggests that the two species share conserved structural and functional properties that make them resistant to thermal and osmotic stresses. However, the regulatory mechanisms governing HSP70 expression may differ, as *M. californianus* inhabits high-energy intertidal environments, while *Mytella strigata* is found in estuarine regions with variable salinity and pollution levels, meaning environmental factors such as salinity fluctuations and pollutants may influence HSP70 expression in *Mytella strigata* beyond temperature alone, suggesting a broader role in cellular stress mitigation.

Another notable difference lies in the induction patterns of HSP70, as *M. californianus* mussels in laboratory acclimation showed different HSP70 expression profiles compared to field-acclimatized individuals, highlighting the influence of multiple environmental factors beyond temperature, and if a similar divergence occurs in *Mytella strigata*, it would emphasize the complexity of HSP70 regulation in natural versus controlled environments. Investigating how *Mytella strigata* modulates HSP70 expression under different environmental stressors, such as temperature shifts, salinity changes, and pollution exposure, could provide valuable insights into its adaptability, and overall, the comparison between *M. californianus* and *Mytella strigata* highlights the evolutionary conservation of HSP70 proteins in stress adaptation while underscoring species-specific regulatory differences shaped by habitat conditions, emphasizing the need for further research on *Mytella strigata* to elucidate the molecular mechanisms underlying HSP70 expression in response to environmental stressors, thereby contributing to a broader understanding of stress resilience in marine and estuarine bivalves.

Actin and paramyosin are structural proteins in marine bivalves that are essential for cell stability, muscle function, and adhesion. Actin (~42 kDa) governs cell form, intracellular movement, and muscle contraction, while paramyosin (90–100 kDa) stabilizes the thick filaments in mollusk muscle. Actin exists in the cytoplasm and the membranes, while paramyosin exists largely in muscle cells. Extracellular proteins involved in byssal thread

formation might include these components to ensure better adhesion. Their stability and function are regulated through post-translational modification, which might involve glycosylation, phosphorylation, and disulfide bonding.

CONCLUSION

This study contributes to the understanding of *Mytella strigata* mucus proteins, highlighting the relevance of these proteins to ecological adaptation and biomedical uses. A variety of physiological processes, including immunological defence, metabolic regulation, structural stability, and environmental stress responses, are highlighted by the variety of protein composition found.

By revealing the ecological function and prospective biological uses of *Mytella strigata* mucus, this study significantly adds to the knowledge of its protein profile. Bradford assay confirmed the presence of a significant protein content (27.23 µg/mL), indicating the involvement of mucus proteins in such physiological functions as adhesion, immunological protection, and adaptation to environmental stresses. Structural, enzymatic, and immune proteins were found via SDS-PAGE analysis, with a clear protein band in the 65–100 kDa range. Protein characterisation via UniProtKB analysis revealed the functional diversity of the proteins in ensuring bivalve viability in dynamic conditions and further confirmed their role in metabolic regulation, stress response, and cellular integrity.

The presence of heat shock proteins (HSPs) in 100 kDa highlights the importance of these proteins in cellular protection from temperature variations and environmental stresses. Furthermore, the occurrence of actin-binding proteins and enzymes involved in metabolism points toward involvement in energy regulation and cytoskeletal integrity. The occurrence of antimicrobial peptides, glycoproteins, and immune defense proteins reveals *Mytella strigata* mucus to be endowed with bioactive compounds that are antimicrobial and wound healing in nature. The observations concur with reports on other studies of bivalve mucus, highlighting the importance of bivalve mucus in biotechnology and ecological adaptation.

The study brings to light the potential biological uses of mucus-derived proteins and their ecological significance, specifically in the formulation of bioadhesives, antibacterial compounds, and regenerative medicine. Protein activity and stability are also facilitated through the presence of glycosylation and phosphorylation modifications, which render the proteins appropriate for use in industrial and medicinal settings. The Bradford assay, SDS-PAGE, and acetone precipitation were some of the methodological techniques that were effectively used to achieve protein extraction, quantitation, and characterisation, and proved to be a good foundation for further biochemical study.

Mytella strigata is a promising model organism to explore the mucus proteins and environmental adjustment role, in light of this study. For the realization of their potential in medicine, biotechnology, and environmental monitoring, future research should be directed towards large-scale protein extraction methods, functional verification of certain proteins, and molecular characterization. With the combination of marine biology, environmental science, and biomedical research, this study provides new avenues for the creative and responsible application of bivalve mucus proteins.

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