

# Bioactivities of Loach (*Misgurnus anguillicaudatus*) Skin Mucus Protein (Purification and characterization).

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**Abstract:** Loach (*Misgurnus anguillicaudatus*), is a freshwater fish found in Asia, it is having a skin which is covered by a mucous layer that has a mucus structure formed by Mucin-type glycoproteins. Therefore, it is used as a traditional medicinal food to cure some human diseases. Due to its diverse biological activities, the products of fish compete to become the research demand of scholars from various countries. Therefore, the experiment was conducted to identify the availability of proteins in loach skin which is a by-product of loach processing. Three kg of live loaches with approximately the same size was placed in a basin with 300 ml of distilled water at room temperature for 10 minutes. It was allowed to produce plenty of mucus on loach skin, loaches were removed. The extracted mucus was centrifuged at a  $4,200 \times g$  for 15 minutes at  $4^{\circ}\text{C}$ . The supernatant solution was freeze-dried to obtain fine mucus powder. The powder was stored at  $-20^{\circ}\text{C}$  until use. The mucus extract was purified by ion exchange chromatography and gel filtration chromatography techniques. Mucus powder was dissolved in 20 mL of 50 mMol/L Tris-HCl (pH 7.8, containing 5 mMol/L of EDTA). Two grams of mucus powder was dissolved with distilled water (200 mL) at  $4^{\circ}\text{C}$  for 2.5 hours, and then the extracts were centrifuged at  $4,200 \times g$  for 20 minutes ( $4^{\circ}\text{C}$ ). Then supernatant was precipitated by ethanol (supernatant: ethanol 1:4, v/v) for 24 h at  $4^{\circ}\text{C}$ . After centrifugation at  $4,200 \times g$  for 20 min ( $4^{\circ}\text{C}$ ), the sediment was obtained and lyophilized as crude extract. Then, the sample was loaded in DEAE-Cellulose (DE-52) column (2.6 cm $\times$ 20 cm). The elution was performed at a flow rate of 30 mL/h with a linear NaCl gradient. The result showed that there are two peaks on the elutions when observed through UV spectroscopic absorbance level at 220 nm and 280 nm. Therefore, it can be concluded that the polypeptide available at the mucus could be the factor for bioactivities such as antimicrobial properties, antitumor properties.

**Keywords:** Loach skin mucus, bioactivity, polypeptides

## Introduction

Mucus is thin gel layer secreted from the mucous gland cells available in the inner layer of skin covering the scales. Loach is a fish species belongs to mud fish lives in the bottom sludge of ponds and lakes, but their skins remain clean and undamaged. The excellent functions such as lubricating and antifouling are closely associated with the rheological features of the skin mucous layer (Wang et al., 2015). Loach fish (*Misgurnus anguillicaudatus*), is a freshwater fish distributed in Asia, the skin is covered by a mucous layer that is having a gel structure formed by Mucin-type glycoproteins available in the fish. It is mostly used traditionally as a medicine to treat some major diseases. Most of the Fish have an inherent viscous mucous layer which can be acted as a defense mechanism for externally induced stresses, such as scratching, frictions, touch, environmental pollutants and water toxins.

Mucus produced by Loach fish serves as an important component of innate immune. Arasu et al., (2013) stated that the mucus which is produced by some of the fish is having immunity by producing

continuously and being sloughed off regularly this prevents the adherence of pathogens and stable colonization of potential infectious microbes and invasion of parasites. Also, Arockiaraj et al., (2012), mentioned that the mucus produced by fish contains a number of factors of innate immunity like proteins and enzymes such as lysozyme, immunoglobulin, complement proteins, lectins and various other antibacterial proteins and peptides.

Major constituents of the mucus were analyzed by the researchers and found that mucus contains protein and carbohydrates. The carbohydrate compounds of the skin mucus of several species of fish were examined and it was found that the sialic acids are also available there. Also, protein compounds were also analyzed and still need some improvement on it. Therefore, this study is focusing on mucus protein. Also study which is focusing on external fish mucus becomes more popular because it provides nonlethal alternatives for both detecting fish infections and monitoring the environmental related pollutants too. The protein isolation, characterization, and purification as well as bioactivity analysis on mucus of loach studies are having limited number of papers. Therefore, the study will focus on isolation,

characterization and purification of protein from the mucus of loach and the bioactivity (immunity regulation) of this mucus protein.

### Objectives

- To use mucus of the loach skin which is a by-product of loach processing.
- To use mucus as a raw material, and comprehensively use raw material for isolation, purification, characterization, and analyze the molecular structures, physicochemical properties and bioactivities of mucus of the loach.

### Methodology

#### Study on separation of mucus from loach

Ten kg of loach was purchased and 3 kg of live loaches with approximately the same size was placed in a basin like equipment with 300 ml of distilled water at room temperature for 10 minutes. External stimuli were applied by the researcher by wearing a disposable glove and imposed on loaches to make them act fast. When plentiful mucus is produced on loach skin, loaches was removed by the operator. Finally, and then skin mucus of loaches was obtained. The extracted mucus was centrifuged at a  $4,200 \times g$  for 15 minutes using centrifuge at the temperature  $4^{\circ}\text{C}$  to maintain the standard and minimize denaturation. The supernatant solution was freeze-dried to obtain fine mucus powder. The powder was stored at  $-20^{\circ}\text{C}$  until use it for

experiment.

#### Extraction technology of protein from mucus

The loach mucus extract was dissolved with and purified by ion exchange chromatography and gel filtration chromatography techniques. Mucus powder was dissolved in 20 mL of 50 mMol/L Tris-HCl buffer (pH 7.8, 5 mMol/L of EDTA). Two grams of mucus powder was dissolved with distilled water (200 mL) at  $4^{\circ}\text{C}$  for 2.5 h, and then the extracts were centrifuged at  $4,200 \times g$  for 20 min ( $4^{\circ}\text{C}$ ). Then supernatant was precipitated by ethanol (supernatant: ethanol 1:4, v/v) for 24 h at  $4^{\circ}\text{C}$ . After centrifugation at  $4,200 \times g$  for 20 min ( $4^{\circ}\text{C}$ ), the sediment was obtained and lyophilized as crude extract.

Then, the sample was loaded in DEAE-Cellulose (DE-52) column (2.6 cm $\times$ 20 cm column size). The elution was performed at a flow rate of 30 mL/h with a linear NaCl gradient. The elutions were observed through 220 nm and 280 nm absorbance level through UV spectroscopy.

### Results and Discussions

#### Structure of Loach Skin Mucus

Eunseok Seo (2019) illustrated that the morphological structures of the surfaces of the skin layer and scales of loach were observed with SEM. The sample for SEM imaging was taken from the lateral region of loach skin (figure a & b).

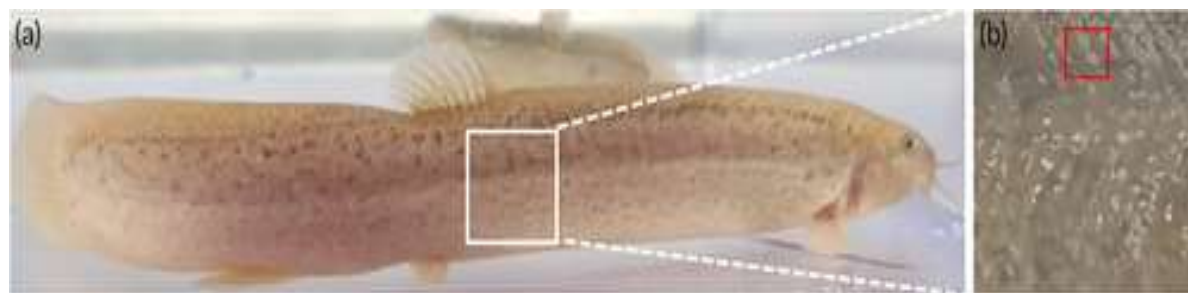
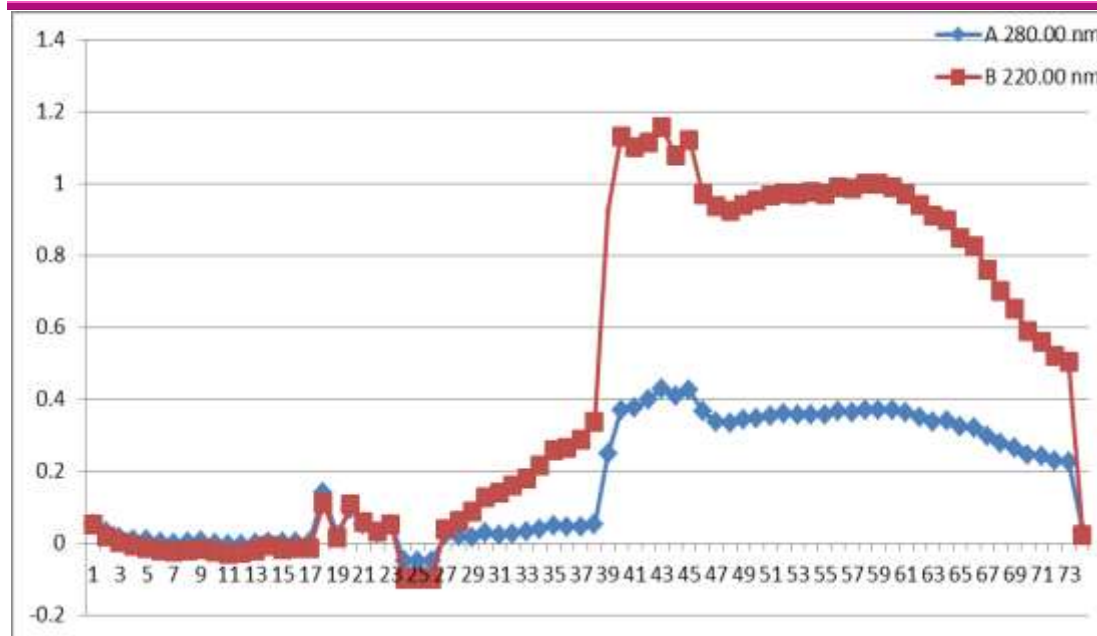


Figure a & b: SEM imaging - lateral region of loach skin (Eunseok Seo 2019).

#### Identification of Protein

Mucus sample obtained from loach skin mucus was loaded in DEAE-Cellulose (DE-52) column (2.6 cm $\times$ 20 cm column size). The elution was performed

at a flow rate of 30 mL/h with a linear NaCl gradient. The elutions were observed through 220 nm and 280 nm absorbance level through UV spectroscopy and the graph 1 showed the peak levels of this absorbance levels.



Graph 1: Elution and UV spectroscopic absorbance level at 220 nm and 280 nm

The result showed that there were two peaks on the elutions when observed through UV spectroscopic absorbance level at 220 nm and 280 nm. Therefore, it

can be concluded that the polypeptide available at the mucus could be the factor for bioactivities such as antimicrobial properties, antitumor properties.

**Table 1 : Antimicrobial peptides and specificity**

Peptide	Specificity	References
Pluerocidin	Act against Gram-positive and Gram-negative bacteria	Douglas <i>et al.</i> (2001)
Piscidins	Bacteria	Fernandes <i>et al.</i> (2010);
Epinecidins	Bacteria, virus	Yin <i>et al.</i> (2006); Peng <i>et al.</i> (2010)
Gaduscidins	Bacteria, virus	Browne <i>et al.</i> (2011)

Foreign studies also showed that the Isolation and characterization of a novel polysaccharide from the mucus of the loach and were focused on antimicrobial properties of mucus proteins. In recent years, with the development, separation and purification and structural identification technology have been increasingly improved, so that the research on protein has developed rapidly. There is no in-depth research on its structure analysis; while foreign scholars have systematically studied the chemical structure of active fragments available in the mucus produced by loach fish.

**Conclusions**

The result showed that there were two peaks on the elutions when observed through UV spectroscopic

absorbance level at 220 nm and 280 nm. Therefore, it can be concluded that the polypeptide available at the mucus could be the factor for bioactivities such as antimicrobial properties, antitumor properties.

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