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Amino Acids Composition in Skin Epidermal Mucus from Fresh Water Fish Channa Striatus

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Abstract

In fish, the epidermal mucus is the external barrier between the environment and fish which is considered as a key component of innate immunity. The present study identification of amino acids composition from freshwater fish mucus Channa striatus has huge variety about 16 amino acids like, aspartic acid (0.1045%), glutamic acid (0.1195%), asparagine (0.2045%), serine (0.3914%), glutamine (0.4046%), glycine (0.2964%), tyrosine (0.3315%), histidine (0.4022%), valine (0.3193%), methonine (0.1035%), iso leucine (0.2135%), phenylanine (0.3054%), leucine (0.1936%), lysine (0.1036%), proline (0.1365%) and tryptophan (0.2193) were isolated by the method of HPLC. The results of amino acids glugamine, histidine and phenylanine are more quantity other then the amino acids. This is responsible for the pathogenic infection from the aquatic environment. In conclusions, the amino acids play a important role of fish immunity. Father studies we carry out identification of bioactive protein in the fish mucus.

Key words: Channa striatus, HPLC, amino acids, fish mucus

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INTRODUCTION

Fish lives in aqueous environment which itself is a source of microbial pathogens invading aquatic organisms. Even with a close contact with high concentrations of such pathogens, fish can still preserve a fit and vigorous system under normal condition. The fish produces mucus substances composing of biochemically diverse secretions from epidermal and epithelial cells which are key components of innate immunity [1, 2]. Fish live in a microbe-rich environment and are vulnerable to invasion by pathogenic or opportunistic micro-organisms. Over the past years, it has also been shown that mucus plays a role in the prevention of colonization by parasites, bacteria and fungi [3, 4].

During the past few years, fish have been proven having good sources of monounsaturated, polyunsaturated fatty acids and amino acid constituents. Together with vitamins and minerals compositions, the mucus is providing potential sources in alleviating health diseases and disorders such as arthritis and inflammatory disorder [5].

Certain basic amino acids (histidine, lysine and arginine) are known to produce effective anti-inflammatory and anti bactericidal products [6]. A polypeptide was formed by the other essential amino acids such as proline, alamine, arginine, isoleucine, phenylalanine and serine which repairs the tissue and heals the wound [7]. Edema and pain was suppressed by lipoamino acid called arachdonoglycine [8].

Curiously, several studies have proved that preparations from fish skin secretions can enhance the rate of wound healing and antimicrobial activity in animals and the healing of diabetic foot ulcers in humans [9, 10].

Amino acid is sub-components of a complex protein and very important in the mechanical pathway in defense mechanism of organism. An inadequate supply of even one of the essential amino acid can hinder the synthesis and reduce body levels, of necessary proteins. Further, all of the essential amino acids must be present simultaneously in diet in order for the other amino acids to be utilized. It is important to realize that amino acid plays a different metabolic or biochemical role in the human body and that deficiency of one amino acid may also affect the functioning or the production of another.

MATERIALS AND METHODS

Collection of animals

The healthy fish *Channa striatus* weight approximately of 300 - 500g were purchased from Denkanikottai fish market, Krishnagiri, Tamilnadu, India. The collected fish were acclimatized in laboratory condition about 15 days. After 15 days these fish were used for mucus collection. Mucus was carefully scraped from the dorsal body using a sterile spatula. Mucus was not collected in the ventral side to avoid intestinal and urinogenital contamination. The collected fish mucus was stored separately at 4 °C for further use.

Analysis of Amino Acid Compositions in *Channa striatus* skin mucus

The amino acid analysis of skin mucus sample was performed according to the methods described by [11]. The mucus sample 20 μl was hydrolysed with 15 ml of 6 Molar hydrochloric acid in a closed test tube, shaken for 15 min and then flushed with nitrogen for 1 min prior to being put in an oven for 24 hours at 110 °C. After cooling, 10 ml of the internal standard -aminobutyric acid (AABA) was added to the sample prior to the addition of 20 μl redrying solution (methanol: water: triethylamine, 2: 2: 1, v/v/v) and 20 μL derivatization reagent (methanol: triethylamine: water: phenylisocyanate, 7 : 1 : 1 : 1, v/v/v/v). The mixture was then poured into volumetric flasks and deionizer water was added to a final volume of 50 ml. Five to 15 ml of the upper layer was discarded; the rest of the upper layer was filtered through Whatman No. 1 filter paper. The hydrolysed sample obtained after filtration was kept as such for upto 4 weeks at -20°C until use.

Before injection into HPLC, the hydrolysed samples were filtered using a nylon 0.2 μm cellulose nitrate membrane filter. Then, 10 μl of filtered sample was put into a vial and the same volume of internal standard was added. Then the sample was dried under vacuum for 30 min. The re-drying solution (20 μL) was then added to the dried sample and the mixture was shaken vigorously for 15 minutes. The sample was dried again under vacuum for another 30 minutes, followed by the addition of 20 μl derivatization reagent. The mixture was again shaken vigorously for 15 min and then left at room temperature for 20 min and dried again under vacuum for 30 min. The dried sample was kept at -20°C until analysis by HPLC.

Prior to injection into the HPLC, the sample and standard were mixed with 100 μl sample diluents [11], shaken for 15 min and injected onto the HPLC in volumes of 20 μl, respectively. The free amino acids were separated using the (DEALI C18 5MICROMM 4.6 x150mm) by reversed phase HPLC, with the flow rate of 1.0 ml/min and detected using a UV detector at 254 nm. The eluent A (50 mM sodium acetate trihydrate buffer, pH 5.7) and eluent B (60 % acetonitrile in water) were used as transporters/mobiles phase. Gradient conditions were used as shown in the Table:1.

RESULTS

Amino acids analysis by HPLC

The quantitative analysis of the freshwater fish mucus *Channa striatus* showed the presence of 16 amino acids and is tabulated in Table-1. The amino acids present in fish mucus are aspartic acid (0.1045%), glutamic acid (0.1195%), asparagine (0.2045%), serine (0.3914%), glutamine (0.4046%), glycine (0.2964%), tyrosine (0.3315%), histidine (0.4022%), valine (0.3193%), methonine (0.1035%), iso leucine (0.2135%), phenylanine (0.3054%), leucine (0.1936%), lysine (0.1036%), proline (0.1365%) and tryptophan (0.2193).

Among the above mentioned amino acids glutamine present in the highest quantity. This is followed by the histidine, serine and phenylanine. The moderate percentage of asparagines, glycine, valine, iso leucine and tryptophan are observed.

The least percentage of the amino acids is aspartic acid, glutamine, methionine, leucine, lysine and proline. The result reveals that the fish mucus of *Channa striatus* home good source of essential and non-essential amino acid and their graphical representation are given in Fig-1.

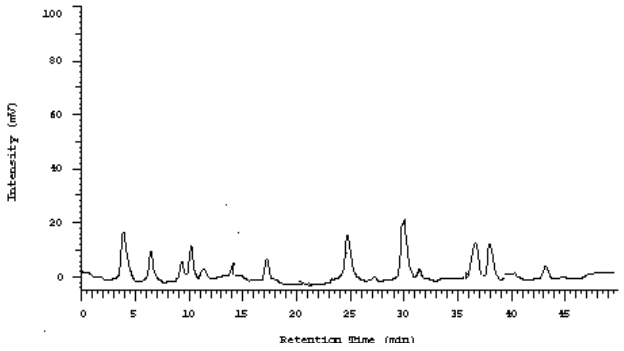

Fig.1: Chromatogram showing the amino acids of fish mucus Channa striatus

Table-1: Amino acid composition of fish mucus Channa striatus

S.No.	Name of amino acids	% of amino acids
1	Aspartic acid	0.1045
2	Glutamic acid	0.1195
3	Asparagine	0.1195
4	Serine	0.2045
5	Glutamine	0.4046
6	Glycine	0.2964
7	Tyrosine	0.3315
8	Histidine	0.4022
9	Valine	0.3193
10	Methonine	0.1035
11	Iso Leucine	0.2135
12	Phenylanine	0.3054
13	Leucine	0.1936
14	Lysine	0.1036
15	Proline	0.1365
16	Tryptophan	0.2193

DISCUSSION

In every human civilization, natural fauna have been used as medicinal resources for the cure and relief of a multitude of disease and illness [12]. Utilization of fish biomass offers a wide range of attractive methods for including and building protection against diseases [13]. Fish are in constant interaction with their aquatic environment, which contains a wide range of pathogenic and nonpathogenic microorganisms. The epidermis and the epidermal mucus secretions act as biological barriers between fish and the potential pathogens of their environment [14].

Many organisms produce arginine and lysine rich polycationic peptides to protect themselves from pathogenic microbes [20]. It has been established that the peptides with lysine from higher animals and plants exhibit antimicrobial activity [21]. The present study higher content of lysine is in agreement with the above mentioned studies. [15] have also studied the mucus shows amino acids component to highest content of lysine. Above findings support our results of amino acids analysis of *Channa striatus*.

Commonly, amino acids like histidine, lysine and arginine are known to produce the antioxidant and antimicrobial products with sugars or glicoamino acid [6, 16, 17]. [18] who have reported that the marine cat fish also has the approximately similar amino acids. In the present study *Channa striatus* contains moderate quantity of the few amino acids such as lysine, phenyl alanine, glycine, proline and leucine. Administration of moderate quantity of phenyl alanine induces antibacterial

activity along with cholorompheniсol at the same time larger dose of phenyl alanine reduces antibacterial activity [19].

Glutamine acids with larger quantity present in the mucus of *Channa striatus* may have important role on antimicrobial activity. In that, further study to be isolating and identification of bio active compound in the particular fish mucus.

CONCLUSION

The results suggest that fish mucus have several amino acids compound as play important role in the protection of fish against the invasion of pathogens. Thus this work has been an excellent evidence to prove the medicinal value of the mucus collected from selected fish mucus from *Channa striatus*. Further studies will conformation and identification of the bioactive protein in their fish mucus.

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