FATTY ACIDS AND AMINO ACIDS COMPOSITION IN SKIN EPIDERMAL MUCUS OF SELECTED FRESH WATER FISH

MUGIL CEPHALUS

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ABSTRACT

The epidermal mucus layer on the skin of fish consists of several antimicrobial agents that provide a first line of defense against invading pathogens from the surrounding environment. During the past few years, fish have been proven having good sources of fatty 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 disorders. The present study was performed to identify the amino acid and fatty acid profile of epidermal mucus of Mugil cephalus. Among 18 amino acids, lysine, phenyl alanine, glysine, proline were found at maximum percentage, moderate amount of leucine, arginine, tyrosine, iso-leucine, threonine, valine, alanine were observed. Amino acidssuch as aspartic acid, tryptophan, serine, glutamic acid, histidine, asparagine, cystine were found in lesser quantity. The fatty acids content of mucus the monounsaturated fatty acid-oleic acid was found more. Other fatty acids are the poly-unsaturated fatty acids-morocitic acid, linolenic acid and alpha linolenic acid; the saturated fatty acids-palmitic acid and stearic acid also found. The amino acid and fatty acid profile from the epidermal mucus of Mugil cephalus contains most of the essential components required to play a possible role in its defense mechanism.

KEYWORDS: Saturated (SFA) – monounsaturated (MUFA) - polyunsaturated fatty acids (PUFA)- Fatty acids (FAs).
INDRODUCTION

The surfaces of fish gill, skin, and gastrointestinal tract with mucus form a thin physical barrier between the external environment and the internal milieu and they are exposed sites of microbial attack. Host defence mechanism was provided by their epithelia (with living cells) and the mucus (Shephard, 1994; Iger and Abraham, 1994).

Skin mucus has robust mechanisms that can trap and immobilize pathogens before they can contact epithelial surfaces, because it is impairment to most bacteria and many pathogens (Cone, 2005). This occurs because the mucus layer, entrap particles, bacteria or viruses and remove them with the water current (Mayer, 2003).

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 (Ghosh and Dua, 1997).

Fish is known to induce wound healing by having polyunsaturated fatty acids that can regulate prostaglandin synthesis (Bowman and Rand, 1980). x-3 and x-6 polyunsaturated fatty acids (PUFA) have been shown to have positive effects on cardiovascular diseases and cancers (Conner, 1997). PUFA composition may vary among species of fish, even among freshwater and marine fish (Abdul Rahman et al., 1995; Osman et al., 2001).

Certain basic amino acids (histidine, lysine and arginine) are known to produce effective anti-inflammatory and anti bactricidal products (Frankel, 1998). 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 (Willett and Morse, 1966). Edema and pain was suppressed by lipoamino acid called arachdonoglycine (Huang et al., 2001).

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 (Al-Hassan et al., 1986; Al-Hassan et al., 1990).

Mat Jais et al. (1998) have reported preliminary analysis of fatty acids and amino acids in Channa striatus. Zuraini et al. (2006) reported the amino acids and fatty acids of Channa striatus, Channa micropeltes and Channa lucius. Manivasagan et al. (2009) studied the
amino acids of cat fish *Arius maculates*. Venkatachalam Uthayakumar *et al.* (2012) reported the profiles of amino acids and fatty acids in the mucus of *Mastacembelus armatus*.

However, in the recent reports it was established that the freshwater fish contain relatively large amounts of EPA (Eicosapentaenoic acid) and DHA (Decosahexaenoic acid) (Wang and Johnson, 1992). Ghosh and Dua, (1997) stated that Indian fresh water fish have high concentration of amino acids. *Mugil cephalus* is a coastal species that often enters estuaries and freshwater environments spend most of their lives in freshwater. In addition there are no previous reports on amino acids (AAs) and fatty acids (FAs) of the epidermal mucus of *Mugil cephalus*.

**MATERIALS AND METHODS**

**Collection of animals**
The fish *Mugil cephalus* weight approximately of 750 - 1000g were purchased from lower Anaikkat in Kollidam River, Thanjavur district. 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 Fatty acids composition in *Mugil cephalus* skin mucus**
The fatty acid present in the crude epidermal mucus were assayed by following the method of Mat Jais *et al.* (1998) with slight modification. 20 µl of the *Mugil cephalus* skin mucus was taken into a 10 ml volumetric flask, dissolved in hexane containing 50 mg of butylhydroxytoluene per litre and diluted to 10 ml with the same solvent. Transferred 2.0 ml of the solution into quartz tube and evaporated the solvent with a gentle current of nitrogen, closed tightly with a polytetrafluoroethylene lined cap, mixed and heated in a water bath for 7 minutes. After cooling in running water bath, added 2 ml of boron trichloride-methanol solution, covered with nitrogen cap tightly, mixed and heated in water bath for 30 minutes. Cooled to 40-50°C added 1ml of trimethylpentane and shaken vigorously for at least 30 seconds. Immediately 5 ml of saturated sodium chloride solution was added, closed it and or shaken thoroughly for 15 minutes. Allowed as such till the upper layer become clear and transferred to a separate tube. The methanol layer was shaken once more with 1 ml of trimethylpentane. Washed the combined extracts with 2 quantities, each of 1 ml of water and
dried over anhydrous sodium sulphate. Then the solution was taken into the Gas-
chromatography for fatty acid analysis.

**Gas Chromatographic Instrument Specification.**

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Gas chromatography, (Fison instrument Italy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detector</td>
<td>Flame ionization</td>
</tr>
<tr>
<td>Injector</td>
<td>Split ratio: 1:200 (Temp. at 50°C ).</td>
</tr>
<tr>
<td>Column</td>
<td>Fused silica, l= 30m, Ø= 0.25mm, Macrogo 120,000R  (film thickness 0.25µm).</td>
</tr>
<tr>
<td>Carrier gas</td>
<td>Hydrogen.</td>
</tr>
<tr>
<td>Operating Condition</td>
<td>Temperature 170°C for 4 minutes. 225°C, 0°C, 5°C/min for 65 minutes.</td>
</tr>
</tbody>
</table>

The peak integration was interpreted and calculated with the chromcard software version (Fisoninstrument-Italy).

**Analysis of Amino Acid Compositions in *Mugil cephalus* skin mucus**

The amino acid analysis of skin mucus sample was performed according to the methods described by Khan *et al.* (1994). 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 α-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/v). The mixture was then poured into volumetric flasks and deionized 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 (Khan et al., 1994), 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 2.

RESULTS
The gas chromatographic profiling of fatty acids in the mucus of *Mugil cephalus* is shown in Fig. 1 and the fatty acid composition percentage are presented in Table 1

![Fig 1. Fatty acids composition of skin mucus from Mugil cephalus](image)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Component Name</th>
<th>% of fatty acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palamitic acid (Saturated)</td>
<td>0.33</td>
</tr>
<tr>
<td>2</td>
<td>Stearic acid(Saturated)</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>Oleic acid ( Mono- Unsaturated)</td>
<td>0.52</td>
</tr>
<tr>
<td>4</td>
<td>linolenic acid (Poly- unsaturated)</td>
<td>0.22</td>
</tr>
<tr>
<td>5</td>
<td>Alpha linolenic acid (Poly- unsaturated)</td>
<td>0.21</td>
</tr>
<tr>
<td>6</td>
<td>Moroctic acid (Poly- unsaturated)</td>
<td>0.48</td>
</tr>
</tbody>
</table>

When we are analysing the fatty acids content, the percentage of unsaturated fatty acids (1.43%) are more than the saturated fatty acids (0.53%). The highest percentage of fatty acids present in the sample is oleic acid – mono-unsaturated fatty acid (0.52%). Other major fatty acids are moroctic acid - poly-unsaturated fatty acid (0.48%), palmitic acid- saturated fatty
acid (0.33%), linolenic acid - poly-unsaturated fatty acid (0.22%), alpha linolenic acid - poly-unsaturated fatty acid (0.21%), stearic acid - saturated fatty acid (0.20%) were found.

Amino acids composition

The HPLC profiling of amino acids of *Mugil cephalus* skin mucus sample is shown in Fig. 2 and the composition of each amino acid is depicted in Table 2.

The skin mucus of *Mugil cephalus* were found to contain 18 amino acids. Lysine (7.61%), phenyl alanine (1.97%), glycine (1.51%), proline (1.39%) are found at maximum percentage. Moderate amount of leucine (0.93%), arginine (0.89%), tyrosine (0.87%), isoleucine (0.79%), threonine (0.78%), valine (0.75%), alanine (0.75%) are observed. Lesser amount of amino acids such as aspartic acid (0.29%), tryptophan (0.17%), serine (0.17%), glutamic acid (0.13%), histidine (0.13%), asparagine (0.08%) and cystine (0.79%) were found.

![Fig 2. Amino acid composition of skin mucus from *Mugil cephalus*](image)

Table 2. Amino acid composition of skin mucus from *Mugil cephalus*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Component Name</th>
<th>% of amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Aspartic acid</td>
<td>0.29</td>
</tr>
<tr>
<td>2.</td>
<td>Glutamic acid</td>
<td>0.13</td>
</tr>
<tr>
<td>3.</td>
<td>Asparagine</td>
<td>0.08</td>
</tr>
<tr>
<td>4.</td>
<td>Serine</td>
<td>0.17</td>
</tr>
<tr>
<td>5.</td>
<td>Glycine</td>
<td>1.51</td>
</tr>
<tr>
<td>6.</td>
<td>Threonine</td>
<td>0.78</td>
</tr>
<tr>
<td>7.</td>
<td>Arginine</td>
<td>0.89</td>
</tr>
<tr>
<td>8.</td>
<td>Alanine</td>
<td>0.75</td>
</tr>
<tr>
<td>9.</td>
<td>Cystine</td>
<td>0.79</td>
</tr>
<tr>
<td>10.</td>
<td>Tyrosine</td>
<td>0.87</td>
</tr>
<tr>
<td>11.</td>
<td>Histidine</td>
<td>0.13</td>
</tr>
<tr>
<td>12.</td>
<td>Valine</td>
<td>0.75</td>
</tr>
<tr>
<td>13.</td>
<td>Isoleucine</td>
<td>0.79</td>
</tr>
<tr>
<td>14.</td>
<td>Phenylalanine</td>
<td>1.97</td>
</tr>
<tr>
<td>15.</td>
<td>Leucine</td>
<td>0.93</td>
</tr>
<tr>
<td>16.</td>
<td>Lysine</td>
<td>7.61</td>
</tr>
<tr>
<td>17.</td>
<td>Proline</td>
<td>1.39</td>
</tr>
<tr>
<td>18.</td>
<td>Tryptophan</td>
<td>0.17</td>
</tr>
</tbody>
</table>
DISCUSSION

Fatty acids in the *Mugil cephalus* mucus extracts can be grouped to saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The SFA in the mucus are palmitic acid and stearic acids. MUFA is the oleic acid. PUFA acids are linoleic, alpha linolenic and moroctic acid. The higher level of oleic acid and palmitic acid has been described as a characteristic of freshwater fish (Ackman, 1967).

Thus, saturated and unsaturated FAs can prevent initial bacterial adhesion and subsequent bio-film formation (Won *et al.*, 2007; Stenz *et al.*, 2008; Davies and Marques, 2009). Certain toxins, haemolysins and enzymes exert their drug resistance which is regulated in the presence of various saturated and unsaturated FAs (Liaw *et al.*, 2004; Clarke *et al.*, 2007). Certain FFAs that are free from the cell membranes surrounding the photosynthetic plastids are mono and polyunsaturated AAs (Clarke *et al.*, 2007).

Unsaturated FAs are shown to have greater potency than saturated FAs with the same length carbon chain (Zheng *et al.*, 2005; Desbois *et al.*, 2008). Unsaturated FFAs get into the bacterial inner membrane, causes it to become more fluid and permeable (Chamberlain *et al.*, 1991). The increased permeability of the membrane by the insertion of unsaturated FAs, the long chain of FAs can allow internal contents to leak from the cell, which can cause the inhibition of growth or even death (Boyaval *et al.*, 1995; Shin *et al.*, 2007). If membrane fluidity increases excessively the membrane become unstable and the cell lysis will takes place (Carson and Daneo-Moore, 1980). Indeed, unsaturated FAs can lyse bacteria (Thompson *et al.*, 1994; Shin *et al.*, 2007), erythrocytes (Fu *et al.*, 2004), mammalian cells such as sheep fibroblasts (Thormar *et al.*, 1987) and vero cells (Thormar *et al.*, 1987), or even enveloped viruses (Thormar *et al.*, 1987).

Zuraini *et al.* (2006) found that the unsaturated fatty acids are more than the saturated fatty acids in the mucus of three channa species like *Channa striatus*, *Channa micropeltes* and *Channa lucius*. Mat Jais *et al.* (1998) also in their review reported that the mucus of *Channa striatus* is having more quantity of unsaturated fatty acid than the saturated fatty acids. Falling in line with the above observations in the present study also quantity of unsaturated fatty acids are 1.43% and saturated fatty acids are 0.53%. In support our findings Venkatachalam Udayakumar *et al.* (2012) stated that the UFA is more than SFA in the mucus of fresh water spiny eel *Mastacembelus armatus*. They added the quantity of PUFA is higher than MUFA similar to the present observation.
Many organisms produce arginine and lysine rich polycationic peptides to protect themselves from pathogenic microbes (Nishikawa and Ogawa, 2004). It has been established that the peptides with lysine from higher animals and plants exhibit antimicrobial activity (Berkowitz et al., 1990). The present result i.e., the higher content of lysine is in agreement with the above mentioned studies. Mor et al. (1994) studied the antifungal activity of amphibian frog Phyllomedusa sauvagii which was rich in lysine. Balasubramanian et al (2013) have also studied the mucus shows strong antifungal activity which might be due to highest content of lysine.

The present study has also supported by Manivasagan et al. (2009) who have reported higher quantity of lysine in the mucus of cat fish. Moderate quantity of phenyl alanine was reported in the three species of channa variety (Zuraini et al., 2006). Injured skin of cat fish Parasilurus asotus stimulated the high production or secretion of lysine in to the mucus layer (Park et al., 1998). It showed a strong antimicrobial activity towards Gram-positive bacteria, Gram-negative bacteria and fungi without any haemolytic activity. We observed that the mucus of the Mugil cephalus posses higher content of lysine. In the same way medium amount of phenyl alanine was found in the skin mucus of cat fish (Manivasagan et al., 2009). In the present observation also moderate quantity of phenyl alanine was noticed in the mucus of Mugil cephalus. Administration of moderate quantity of phenyl alanine induces antibacterial activity along with chloromphenicol at the same time larger dose of phenyl alanine reduces antibacterial activity (Esimone et al., 2007).

Glycine is the simplest amino acid, inhibits the growth of bacteria and is used as a nonspecific antiseptic agent due to low level of toxicity in animals. It was suggested that the glycine may be used as antimicrobial agent as it inhibit the synthesis of bacterial cell wall (Frankel, 1998; Esimone et al., 2007).

Members of proline rich antibacterial peptide, kill bacterial species by binding in the bacterial Dna k protein (Kragol et al., 2001). Arginine rich peptides with antimicrobial activity can enter into the cytoplasm of bacterial cells and affects the replication of bacterial cells (Frankel, 1998).

Basic amino acids such as histidine, lysine and arginine are known to produce the antioxidant and antimicrobial products with sugars or glicoamino acid (Frankel, 1998; Park et al., 1998; Wesley Alexander and Dorothy Supp, 2014). Manivasagan et al. (2009) who have reported
that the marine cat fish also has the approximately similar amino acids. In the present study
*Mugil cephalus* contains higher quantity of the few amino acids such as lysine, phenyl alanine, glycine, proline and leucine. These amino acids with larger quantity present in the mucus of *Mugil cephalus* may have important role on antimicrobial activity. Nevertheless, further analysis needs to be isolating and identification of new antimicrobial components.

**CONCLUSION**

In conclusion, the *Mugil cephalus* crude epidermal mucus contains 18 amino acids in which lysine, phenyl alanine, glycine, proline were found at maximum percentage. The fatty acids content of mucus the oleic acid was found more. All the important amino acids and fatty acids present in *Mugil cephalus* epidermal mucus are suggested to play an important role of defense against pathogens. Further study is necessary for the clinical applications and it their mode of action.

**ACKNOWLEDGMENTS**

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**REFERENCE**


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