EFFECTIVE VACCINE DEVELOPMENT AGAINST STAPHYLOCOCCOSIS FROM STAPHYLOCOCCUS AUREUS

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ABSTRACT

Staphylococcus aureus causes Staphylococcosis in cultured and wild fish populations, implicated by exophthalmia and swollen lesion on the tail, often causing high mortality. Aquaculture has grown at an average rate of 8.9 percent per year since 1970 and has become the fastest growing sector of food production in the world. Fish vaccination with a safe and effective vaccine is a potential approach for prevention and control of fish disease. The main objective of the current study was to develop WC [Whole Cell-Formalin-Killed] and OMP [Outer membrane Protein] vaccines from Staphylococcus aureus against staphylococcosis. OMP and WC Vaccines were prepared from MTCC 96 strain of Staphylococcus aureus. Prepared Vaccines were resolved on a 10% SDS-PAGE, to generate protein profiles and it was quantified following the protocol of Lowry et al., SDS-PAGE results showed that the WC vaccines had ten polypeptide bands with molecular weight of 161.2, 137.1, 112.1, 85.8, 73.4, 63.1, 55.8, 46.2, 36.1 and 33.1 KDa and OMP vaccines had four Polypeptide band with molecular weight of 84.9, 72.2, 60.5, and 46.9 KDa. The Protein concentration for WC and OMP vaccines was estimated as 88µg/ml and 55 µg/ml. The immunoproteomic vaccines prepared might prove useful in the Aquaculture Industry due to its long lasting immunity and also it will be economically effective.

KEYWORDS: Staphylococcus aureus, WC, OMP.
INTRODUCTION
Aquaculture is very fast blooming industry because of need of its fish protein demand to meet the fast growing population and it also called ‘underwater agriculture’. Fish diseases are most important problems and challenges confronting fish culturing.

Aquaculture has grown at an average rate of 8.9 percent per year since 1970 and has become the fastest growing sector of food production in the world. Although global economic losses from aquaculture diseases have not been compiled, disease reports from many regions of the world have been increasing with advances in the live aquatic animal trade. Current methods for prevention and treatment of infectious aquatic diseases include a limited number of government-approved antibiotics and chemotherapeutics and limited vaccines that can be used to compliment environmental management. However, use of antibiotics has been seriously criticized for development of antibiotic-resistant bacterial strains.

Staphylococcosis caused by Staphylococcus spp. Staphylococcus bacteria are important opportunistic human pathogens and leading cause of a wide variety of diseases in humans and animals. Staphylococcus aureus is the aetiological agent responsible for a large extent of morbidity and mortality globally, in both hospital and community settings. Antibiotic resistance in S. aureus is a major clinical problem, in particular in infections caused by methicillin-resistant S. aureus (MRSA).

The typical symptoms of staphylococcosis are exophthalmia and swollen lesion on the tail. Currently no vaccine against Staphylococcus spp. is commercially available or experimentally tested in aquatic animals. Fish cultures are continuously affected by environmental fluctuations and management practices. All of these factors can impose considerable stress on the homeostatic mechanisms of fish, rendering them susceptible to a wide range of pathogens. The use of effective vaccines, combined with good health management techniques, may result in substantial disease prevention and production becomes more predictable.

OBJECTIVES
- The main objective of the present study was to develop the effective Whole Cell (WC) and Outer Membrane Protein (OMP) vaccines against staphylococcosis from Staphylococcus aureus as alternatives to antibiotics to protect aquatic animals from bacterial diseases.
Staphylococcus aureus

- Staphylococcosis caused by Gram positive bacterium *Staphylococcus aureus*. It is a gram-positive, small round shaped non-motile cocci.
- *Staphylococcus* bacteria are important opportunistic pathogens and leading to the cause of a wide variety of diseases in fishes, humans and animals.
- *Staphylococcus aureus* is the aetiological agent responsible for a large extent of morbidity and mortality globally, in both hospital and community settings.
- The typical symptoms of staphylococcosis are exophthalmia and swollen lesion on the tail.
- Currently no vaccine against Staphylococcus spp. is commercially available or experimentally tested in aquatic animals.
- Enterotoxins produced by *Staph. aureus* are another serious cause of gastroenteritis after consumption of fish and related products.

MATERIALS AND METHODS

Bacterial strain

MTCC 96 virulent strain of *Staphylococcus aureus* were used in this study.

Media and Culture

- *Cultures were routinely* grown in tryptic soy broth (TSB; Difco) or on tryptic soy agar (TSA; Difco) at 25°C. Stock
- Cultures were maintained at - 80°C as a suspension in TSB containing 25% (v/v) glycerol.

![Fig 1. Tryptic soy broth Medium before Bacterial inoculation](image1)

![Fig 2. Tryptic soy broth Medium after Bacterial inoculation](image2)
Types of Vaccine Prepared
1. Whole cell Vaccine [WC-Formalin Killed]
2. OMP Vaccine [Outer Membrane Protein]

1. Preparation of Whole cell Vaccine [Formalin-Killed]
In order to prepare the bacterins, bacterial isolate was inoculated separately into tryptic soy broth (TSB) and incubated for 24 h at 37°C. Formalin (40% w/v) was added to the broth culture at a final concentration of 0.5% (V/V) and left 48 hrs at room temperature.

The inactivated cells were harvested by centrifugation at 4000xg for 10 min., then washed twice in 0.3% formalized PBS and resuspended in PBS to the bacterial concentration of 1 x $10^8$ cells/ml. After that, the bacterins were tested for their sterility (free from the living cells) by streaking them onto trypticase soy agar which showed no growth.

2. Preparation of OMP Vaccine
Outer membrane protein (OMP) was extracted using the protocol of Austin & Rodgers (1981) with little modification. Briefly, S.aureus was grown in TSB broth at 37°C for 24h and harvested by centrifugation at 8500 rpm for 10 minutes.

After centrifuge supernatant was discarded and pellet was washed with 20mM Tris buffer at pH 7.2 and finally pellet was resuspended in 10mM EDTA buffer.

The bacterial cell suspensions were then subjected to sonication at 50hz for 10 mins in a sonicator to disrupt the cell wall. The unbroken cells were sedimented by centrifugation at 8500rpm and supernatant was collected. Ultracentrifugation of collected supernatant was carried out at a speed of 27400 rpm for 45 mins.

The supernatant was discarded and the sediment was resuspended in Tris buffer containing 0.5% sarkosyl. Further centrifugation at 27400 rpm for 45 mins was repeated. The sediment collected after ultracentrifugation was OMP and supernatant obtained was the CMP. OMP was kept in-20°C for further use.

Protein estimation was done by Lowery method (1951).
SDS – PAGE (sodium dodecyl sulphate - polyacrylamide gel electrophorosis) of Whole cell and OMP Proteins

Fig.3 Sodium dodecyl sulphate-Polyacrylamide Gel Electrophoresis Products of Flavobacterium columnare. Lane 1, 2, 3, 4, 5 and 6 were WC, before Sonic, before Sonic, UC supernatant, OMP (UC Pellet) and Marker respectively. Polypeptide bands and their Molecular weights (KDa) of all the proteins were tabulated in table 1.

Table 1 Molecular weight of WC and OMP Proteins

<table>
<thead>
<tr>
<th>Band No</th>
<th>WC Mol. Wt. (KDa)</th>
<th>Band No</th>
<th>OMP Mol. Wt. (KDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>161.2</td>
<td>1</td>
<td>84.9</td>
</tr>
<tr>
<td>2</td>
<td>137.1</td>
<td>2</td>
<td>72.2</td>
</tr>
<tr>
<td>3</td>
<td>112.1</td>
<td>3</td>
<td>60.5</td>
</tr>
<tr>
<td>4</td>
<td>85.8</td>
<td>4</td>
<td>46.9</td>
</tr>
<tr>
<td>5</td>
<td>73.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>63.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>55.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>46.2</td>
<td></td>
<td></td>
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<tr>
<td>9</td>
<td>36.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>33.1</td>
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</tbody>
</table>

ESTIMATION OF PROTEIN

The amount of protein present in the whole cell and OMP proteins were estimated by Lowry’s method.
Calculated Protein Concentrations

Protein Concentration of WC = **88 µg/ml**
Protein Concentration of OMP = **55 µg/ml**

RESULTS

SDS-PAGE results revealed that the WC vaccines had ten polypeptide bands with molecular weight of 161.2, 137.1, 112.1, 85.8, 73.4, 63.1, 55.8, 46.2, 36.1, and 33.1 KDa and OMP vaccines had four Polypeptide band with molecular weight of 84.9, 72.2, 60.5 and 46.9 KDa. Estimated Protein content of whole cell and OMP proteins were **88µg/ml** and **55 µg/ml**.

OMP and WC proteins may prove to be a more effective vaccines based on the observations made with regard to its number of polypeptide bands and its increased concentration of protein.

DISCUSSION

Prevention of disease is a more desired remedy to these threats to fish than intervention once the disease is in progress. Vaccination of fish is the only preventive method which may offer long term protection through immunity. *Staphylococcus aureus* has a wide range of potential virulence factors, either surface associated or secreted. The proteins present in the outer membrane of bacteria belong to one of two major classes, viz., lipoproteins, which are anchored to the outer membrane with N-terminal lipid tails, and integral proteins, which are referred to as outer membrane proteins (OMPs), containing membrane-spanning regions (Bos and Tommassen, 2004).
Sea food is one of the most essential nutritional needs of every community. Often, consumption of contaminated shrimp cause gastrointestinal diseases in human. Staphylococcal food poisoning causes vomiting, diarrhoea, and abdominal cramps within two to six hours after the ingestion of food contaminated with *S.aureus*.

To prevent bacterial diseases, using vaccines instead of antibiotics has been proven to be effective and beneficial. Although various vaccines have been developed to protect aquatic animals against various bacterial diseases, vaccines against multiple emerging diseases are still urgently needed for the aquaculture industry. In addition, majority of the vaccines available are bacterins which can only provide partial protection against certain strains for a limited time frame. Furthermore, most vaccines have to be delivered by injection, which is labour-intensive. Therefore, user-friendly (immersion or oral delivery) efficacious vaccines that can offer broader protection for a longer duration are urgently needed for the aquaculture industry.

CONCLUSION
OMP and WC proteins may prove to be a more effective vaccine based on the observations made with regard to its number of poly peptide bands and its increased concentration of protein.

The isolation of OMPs is a much more complex process, compared to whole-cell protein preparations, as separation from the inner membrane constituents, lipopolysaccharides and phospholipids is essential. (Benedí and Martínez- Martínez, 2001).

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