A Survey of Some Bacterial Fish Pathogens on Whiting (*Merlangius merlangus euxinus*) in Eastern Black Sea Coast, Turkey

Mustafa Ture1,*, D. Selim Misi1, Cemil Altuntas1, Ilyas Kutlu1

1 Central Fisheries Research Institute, 61250 Yomra, Trabzon, Turkey.

* Corresponding Author: Tel.: +90.462 3411053; Fax: +90.462 3411152 ; E-mail: mustafa.ture@tarim.gov.tr

Received 21 September 2017 Accepted 16 January 2018

Abstract

In the present study, a total of 180 whiting (*Merlangius merlangus euxinus*) were caught in the Eastern Black Sea coast of Turkey and investigated through for six month period for their bacteria. The bacterial agents isolated from fish were identified by Analytical Profile Index (API 20NE, 20E and rapid ID 32 Strep). Fifty-two bacteria were further confirmed by 16S rRNA gene sequencing. Nine bacterial species, *Pseudomonas luteola*, *Staphylococcus equorum*, *Vibrio anguillarum*, *Pseudomonas putida*, *Acinetobacter johnsonii*, *Pseudomonas protegens*, *Oceanisphaera profunda*, *Pseudomonas fluorescens*, and *Serratia fonticola* were identified. This study is the first report on the bacteria of whiting in Turkey and provides significant information in terms of bacterial fish pathogens of this area.

Keywords: Wildfish, whiting, bacteria.

Introduction

Akcaabat, which is located in the Southeastern of Black Sea, is one of the important places in terms of aquaculture activities and fisheries. Whiting (*Merlangius merlangus euxinus*) as well as Anchovy (*Engraulis encrasicoicus*), Sprat (*Sprattus sprattus*) and Horse mackerel (*Trachurus trachurus*) are among the most important fish species economically in Turkey. It is approximately obtained 14000 tons via fisheries per year (TUİK, 2015; Kasapoğlu & Düzgün, 2017).

In recent years, there has started been an important awareness regarding the transmission of the diseases between the cultured and wild fish stocks. Some scientific studies indicate that several diseases were affecting wild fish populations before the aquaculture industry existed (Olivier, 2012). The studies related to fish diseases such as pseudomoniasis, pasteurellosis, motile Aeromonas septicemia and lactococcosis generally focused on the reared fish species in Turkey (Korun & Timur, 2005; Onuk et al. 2015; Ture & Alp, 2016). On the other hand, Nishizawa et al. (2006) investigated to detection of viral hemorrhagic septicemia virus from wild turbot (*Psetta maxima*) in a Turkish coastal area. Fish pathogenic bacteria described in cultured fish are also present in wild fish populations. However, they seldom cause mortality due to the lack of stressful conditions and stock density in natural environments (Toranzo, Magarinos & Romalde, 2005). In spite of efforts to improve culture techniques, bacterial diseases are still one of the most crucial problems in marine aquaculture, Therefore the success of sustainable aquaculture depends on efficient fish health management (Kusuda & Kawai, 1998).

The aim of this study was to investigate the bacterial fauna and infection prevalence of the whiting collected near from the Akcaabat port, Turkey. This study is first to provide data on bacterial fauna in whiting collected from the Eastern Black Sea coast. Our results can be beneficial for determining the suitability of the area for aquaculture activity.

Material and Methods

Sampling and Microbiological Examination

The study area is located near to Akcaabat port, Turkey. A total of 180 fish (*Merlangius merlangus euxinus*) were monthly sampled at the
period December 2016 and May 2017. All fish samples were caught by using different types of gill nets. The length and weight of fish varied between 14.3-17.7cm and 24.28-40.64g respectively. The temperature of water varied between 7.11°C and 11.55°C during sample collection were measured by CTD profile. Fish were examined for bacterial pathogens (Austin & Austin, 2012). For this purpose, fish samples were aseptically collected and transported to the laboratory (Central Fisheries Research Institute, Turkey, Laboratory of Fish Diseases) per month. 30 fish were sampled each sampling. The body surfaces of the fish were cleaned with ethyl alcohol before accessing internal organs. After that, liver and head-kidney of fish were streaked onto Nutrient agar and Tryptic soy agar (NA and TSA, Merck) and incubated at 25°C for 3 days. Following incubation, typical colonies were selected from the plate and streaked onto same media to check the purity of bacteria. Pure colonies were biochemically characterized by following biochemical tests: Gram staining, cytochrome oxidase, catalase, and motility. Analytical Profile Index (API 20NE, 20E and rapid ID 32 Strep) were performed to identify for bacteria species (Capkin, Terzi & Altinok, 2015). Isolates were stored in Nutrient broth (NB, Merck) supplemented with 15-20% glycerol at −80°C.

### PCR Amplification and Sequencing of Bacteria

Genomic DNA of Gram-positive bacteria was extracted for the PCR assay by QIAamp DNA kit (Qiagen), according to the manufacturer’s instructions. DNA was extracted from Gram-negative bacteria using a boiling technique with minor modifications described by Capkin et al. (2015). RNA/DNA calculator (Spectrophotometer, Biorad) was used to measure optical density at 260 and 280 nm. All strains were also identified by DNA sequencing. The purity of bacteria. Pure colonies were extracted for the PCR assay by QIAamp DNA kit (Qiagen) in a thermocycler (Applied Biosystems) as described by Weisburg et al. (1991). The PCR products were subjected to electrophoresis in 1.5% (w/v) agarose gel prepared with 1×TBE (Tris-Borate-EDTA) buffer and run at 100 V for 30 min. The stained DNA bands were viewed by UV transillumination. The sizes of the PCR products were determined with the migration of 100-bp DNA ladder (Bio Basic).

Sequencing reaction was done on a 10 μl scale using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), according to the manufacturer's instructions. 1 μl of 125 mM of EDTA and 30 μl of 100% ethanol were added to each sequencing reaction, and the mixture was vortexed. After centrifugation, the supernatant was aspirated and discarded. Next, after drying the precipitate, 10 μl of Hi-Di Formamide was added to each reaction. ABI PRISM 3500 Genetic analyzer and POP-7 polymer were used as the separation machine and matrices. The sequence data were analyzed with ABI Prism DNA Sequencing Analysis Software v5.1. The quality value score 20 (QV20) was used as an indicator of sequencing quality. The derived nucleotide sequences were analyzed and aligned with the BioEdit Sequence Alignment Editor (NCBI). The 16S rRNA gene sequences of isolates have been submitted to the GenBank databases under relevant accession numbers.

### Results

Different bacterial species were isolated during the screening of 180 whiting for six months. There were no significant clinical signs of the collected fish. A total of 52 bacterial isolates (9 species) were identified to species level (Table 1). Two bacteria species were only identified by molecularly (sequencing). These bacteria species are *Vibrio*.

### Table 1. Bacteria isolated from whiting and their molecular and biochemical identification rates

<table>
<thead>
<tr>
<th>Bacteria species</th>
<th>N</th>
<th>Molecular Similarity %</th>
<th>Accession number</th>
<th>API Profiles (NE/E/ID32)</th>
<th>According to API</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas luteola</em></td>
<td>13</td>
<td>97</td>
<td>MG779444</td>
<td>NE/1563655</td>
<td><em>P. luteola</em></td>
</tr>
<tr>
<td><em>Staphylococcus equorum</em></td>
<td>10</td>
<td>98</td>
<td>MG779447</td>
<td>ID 32/34135301151</td>
<td><em>Enterococcus hirae</em></td>
</tr>
<tr>
<td><em>Vibrio anguillarum</em></td>
<td>7</td>
<td>98</td>
<td>MG779441</td>
<td>indefinable</td>
<td>indefinable</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>6</td>
<td>97</td>
<td>MG779446</td>
<td>NE/0540457</td>
<td><em>P. putida</em></td>
</tr>
<tr>
<td><em>Acinetobacter johnsonii</em></td>
<td>5</td>
<td>96</td>
<td>MG779440</td>
<td>NE/0040071</td>
<td><em>Acinetobacter baumannii</em></td>
</tr>
<tr>
<td><em>Pseudomonas protogens</em></td>
<td>5</td>
<td>96</td>
<td>MG779445</td>
<td>NE/0146555</td>
<td><em>P. fluorescens</em></td>
</tr>
<tr>
<td><em>Oceanisphaera profunda</em></td>
<td>3</td>
<td>97</td>
<td>MG779442</td>
<td>indefinable</td>
<td>indefinable</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>2</td>
<td>97</td>
<td>MG779443</td>
<td>NE/1146515</td>
<td><em>P. fluorescens</em></td>
</tr>
<tr>
<td><em>Serratia fonticola</em></td>
<td>1</td>
<td>96</td>
<td>MG779448</td>
<td>E/5304776</td>
<td><em>S. fonticola</em></td>
</tr>
</tbody>
</table>

N: number of bacteria isolated from fish.
bacterial fauna of the whiting captured from the Eastern Black Sea coast of Turkey. This study provides new information about its bacterial fauna and determining the compatibility of this area for mariculture.

In this study, *Pseudomonas luteola* found to be the most prevalent bacteria in whiting, followed by *Staphylococcus equorum*, *Vibrio anguillarum*, and *Pseudomonas putida*. Pseudomoniasis is the main disease caused by *Pseudomonas* species in fish. It is found in microbial flora of both freshwater and saltwater fish. These bacteria are generally considered as a possible opportunistic pathogen and may lead to secondary infections. Species of *Pseudomonas* causes numerous diseases including hemorrhagic septicemia, ulcerative disease, red spot disease and gill diseases (Austin & Austin, 2012).

*P. putida*, *P. luteola*, *P. aeruginosa*, *P. fluorescens*, *P. chlororaphis* and *P. anguillaricida* found to be the pathogenic species of *Pseudomonas* genus in Turkey (Ozturk & Altinok, 2014). In the present study, determination of a high number of strains belonging to *Pseudomonas* genus may indicate a considerable risk for aquaculture activities.

Vibriosis is a term for a group of well-known fish diseases and has a wide distribution and host range all over the world. Vibriosis, red-pest, and salt-water furunculosis are diseases caused by *Vibrio anguillarum* (*Listonella anguillarum*). The bacteria are Gram-negative, motile, rod-shaped and grow between 5-40°C. They may behave as opportunistic agents or be primer pathogens (Austin & Austin, 2012). *V. anguillarum* was first isolated from sea bream (*Sparus aurata*) in Turkey (Candan, 1991). It has subsequently been reported in many fish species including sea bass (*Dicentrarchus labrax*), salmonid spp. (*Salmo salar* and *Oncorhynchus mykiss*) and red porgy (*Pagrus pagrus*) in Turkey due to the uncontrolled fish transfer (Ozturk & Altinok, 2014). In this study,
Table 2. Number of isolated bacteria at different sampling times and sampling organs

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>N</th>
<th>Dec</th>
<th>Jun</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>K</th>
<th>L</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas luteola</em></td>
<td>13</td>
<td>13</td>
<td>7</td>
<td>2</td>
<td>4</td>
<td>-</td>
<td>2</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td><em>Staphylococcus equorum</em></td>
<td>10</td>
<td>10</td>
<td>3</td>
<td>1</td>
<td>5</td>
<td>2</td>
<td>8</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><em>Vibrio anguillarum</em></td>
<td>7</td>
<td>-</td>
<td>2</td>
<td>5</td>
<td>-</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>6</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>1</td>
<td>-</td>
<td>3</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>Acinetobacter johnsonii</em></td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>Pseudomonas protegens</em></td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>Oceanisphaera profunda</em></td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Serratia fonticola</em></td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

N: number of bacterial isolates, K: kidney, L: liver

Vibrio anguillarum strains were isolated from seven whiting especially in spring samplings. Consequently, this bacterial agent is still posing a risk for aquaculture facilities in Eastern Black Sea.

*Acinetobacter johnsonii* is a fish pathogenic bacteria belonging to *Acinetobacter* genus which are widely distributed in the environment. This bacteria from this genus are Gram-negative, non-motile, rods. The bacteria are precisely aerobic and grow at a temperature from 20°C to 37°C. These bacteria are also considered as a possible opportunistic pathogen (Kozinska, Pazdzior, Pekala& Niemcezuk, 2014). *Oceanisphaera profunda* a bacteria isolated from marine bottom samples. The bacteria is Gram-negative, aerobic, halophilic and grow between at 4-42°C (Romanenko et al. 2003). *Staphylococcus equorum* has frequently been isolated from fermented food products especially cheeses and sausages. They may be as exceptional opportunistic pathogens in some clinical situations. Moreover, they have emerged as causative agents of nosocomial infection (Irlinger et al. 2012). According to the available literature, *Oceanisphaera profunda* and *Staphylococcus equorum* have not been isolated from any fish species. In this study, *Staphylococcus equorum*, *Acinetobacter johnsonii* and *Oceanisphaera profunda* strains were isolated from ten, five and three fish samples respectively.

In the present study, all bacterial isolates were tried to be identified by both API and 16S rRNA gene sequencing methods. However, there is a poor relationship between the phenotypic and genotypic identification methods. According to both identification methods, bacteria of *P. luteola*, *P. putida*, *P. fluorescens* and *S. fonticola* were identified correctly. It is believed that DNA sequencing method was more discriminative than the other methods.

In conclusion, the current study was to identify bacteria isolated from whiting which found in the mentioned area. In terms of bacterial fish pathogens, current case of this area has been revealed by the present study. Before performing aquaculture activities, the presence of these pathogens should be taken into consideration. Bacteria belonging to *Pseudomonas* genus were noted as the most common bacteria. To our knowledge, in fish, the presence of *Staphylococcus equorum* and *Oceanisphaera profunda* is the first report for Turkey.

Acknowledgement

This study was funded by Central Fisheries Research Institute. Samples were collected while conducting field studies for a project named “Investigation of Gillnet and Effect in Black Sea Fisheries” TAGRİ /HAYSUD/2015/A11/P-09/02.

References


Olivier, G. (2012). Disease interactions between wild and cultured fish- Perspectives from the American


