THE OCCURRENCE OF PSEUDOMONAS SP IN GROUPER FISH FILETS (PLECTROPOMA LEOPARDUS) AT BONE PANTE

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*Universitas Muhammadiyah Gorontalo

ABSTRACT

Study on the occurrence of Pseudomonas sp in grouper fish fillets was done. The research aimed to know the concentration and total plate count (TPC) of Pseudomonas sp in fresh grouper fish fillets. The fish samples were taken from two sampling area located at Bone-Pante Village. The result showed that TPC of Pseudomonas sp was lower in samples from sampling area II. Sensory values from sampling area I were 2.1 with average value of 2.4, while from sampling area II the value was 3.2 with average value of 3.0. Sensory values were lower than the required value of 4.

The number of Pseudomonas sp in fresh grouper fish fillets ranged between 1.1 to 10^6 CFU/g. Pseudomonas sp was isolated from fresh grouper fish fillets. The concentration of Pseudomonas sp ranged between 3.5 to 10^5 CFU/g. P. aeruginosa (11.1%) and P. fluorescens (11.8%) were the dominant in the samples. The number of Pseudomonas sp was higher than the required number as suggested by Indonesian National Standard (SNI) for the quality of fresh fish fillets.

Using the Bergey's Manual of Microbiological Identification, the isolated strain were identified as Pseudomonas aeruginosa (11.1%), P. fluorescens (11.8%), and P. putida (2.5%). They have demonstrated the characteristics of Pseudomonas sp, which produce a number of enzymes such as halozone, gelatinase, and caseinase. While P. aeruginosa produced a number of enzymes such as lecithinase, lipase, and trypsinase.

The result showed that both of total plate count (TPC) and total viable count (TVC) of Pseudomonas sp was lower in samples from sampling area II. Sensory values from sampling area I were 3.2 with average value of 4.6, while from sampling area II the value was 2.4 with average value of 4.7. Sensory values were lower than the required value of 5.
INTRODUCTION

Background

Grouper fish (*Plectropoma leopardus*) is a potential export commodity and can contribute to national foreign exchange. The fish is also a source of protein, lipid and minerals essential for human. However, grouper fish fillet is a perishable product. Currently some studies focused on the occurrence of bacteria in grouper fish are conducted. From the studies, it was known that bacteria commonly found in the fish are Pseudomonas sp., *Salmonella, Vibrio, Staphylococcus*, and some more. *Pseudomonas* sp is one of fish contaminating bacterium that may cause diseases. Based on these findings, the writer want to research more about the occurrence of *Pseudomonas* sp. in the grouper fish fillet (*Plectropoma leopardus*). It is expected that the dominant species of *Pseudomonas* sp. associated with the fish can be identified.

The objectives of research

The research aims are as follows.

1. To understand the occurrence of *Pseudomonas* sp. in freshly chilled fillet of grouper fish taken from Bone Pante area 1 and II.
2. To identify the species of *Pseudomonas* sp. dominantly associated with grouper fish fish fillet.

THE METHODOLOGY OF RESEARCH

Place and Time of Research

This research was conducted in Microbiology laboratory at Faculty of Agriculture National University of Gorontalo. The research was completed in three months from October to December 2008.

Material and Methods

The equipment utilized were autoclave, incubators, petridisc, ovens, blenders, pipette, analytical balances, volumetric glasses, beaker glass, reaction tubes, magnetic stirrers, microscopes and object glasses, ose needles, pH meters, and durharn tubes. The material used were chilled grouper fish fillets, aquadest, Natrium medium, *Cetrimide Agar* and NaCl, *APW (enrichment)*, alcohol.

Sampling

Grouper fish fillets were sampled from processing unit at Pante I and Bone Pante II. The fish fillets samples were 250 ± 5 weight x 2 pieces. Sampling was taken triplicate in each location. Frequency of sampling was every one month after previous sampling. Live grouper fish was used as test controls. The controls were from water each time the fish fillet and sea water was sampled.

Microbiology analysis

Fish fillet was analyzed for its *Total Plate Count (TPC)* and *Pseudomonas (TP)*.

Isolation and Identification of Pseudomonas

Selection and isolation steps of *Pseudomonas* consist of several physiological and biochemical tests such as: Gram coloration motility test, oxidase enzyme test, catalase enzyme test, ferment test, indol test, methyl red test, *Voges-Proskauer* test, and citric test.

To examine factors influences the growth of *Pseudomonas* several growth conditions were tested, i.e. growth at six different temperatures: 0°C, 20°C, 4°C, 6°C, 25°C, 37°C for 24 hours; at 5, 6, 7, 8, 9, and 10; in NaCl 0.3, 5, 7, 9%.

Assay of pathogenic characteristics of *Pseudomonas* sp. in agglutination and haemolysis tests.

Sensory test

Sensory test was performed according to SNI...
INTRODUCTION

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Fish fillet was analyzed for its Total Plate Count (TPC and Total Pseudomonas (TP).

Isolation and Identification of Pseudomonas

Selection and isolation steps of Pseudomonas consist of several physiology and biochemical tests such as: Cram coloeration test, motility test, oxidase enzyme test, catalase enzyme test, fermentation test, indol test, methyl red test, voges-proshauer test, and citric acid test.

To examine factors influences the growth of Pseudomonas sp., several growth conditions were tested, i.e. growth at six different temperatures; 0°C, 2°C, 4°C, 6°C, 25°C, 37°C for 24 hours; at pH 4, 5, 6, 7, 8, 9, and 10; in NaCl 0, 3, 5, 7, 9%.

Assay of pathogenic characteristics of Pseudomonas sp. include agglutination and haemolysis tests.

Sensory test

Sensory test was performed according to SNI...
RESULT AND DISCUSSION

The Occurrence of Bacteria in Grouper fish fillets
The average of TPC value are shown in Table 1 and 2.

Table 1. TPC of samples

<table>
<thead>
<tr>
<th>Location</th>
<th>Replicates</th>
<th>The average of TPC values [CFU/gr]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>I</td>
<td>2,1 x 10^4</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>10^4</td>
</tr>
<tr>
<td>II</td>
<td>I</td>
<td>1,9 x 10^4</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>10^4</td>
</tr>
</tbody>
</table>

CFU: Colony Forming Unit

Table 2. TPC of control fish and sea water

<table>
<thead>
<tr>
<th>Control</th>
<th>Replication</th>
<th>Average of TPC values [CFU/gr]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live Grouper fish</td>
<td>I</td>
<td>8,2 x 10^3</td>
</tr>
<tr>
<td>Sea water</td>
<td>II</td>
<td>1,6 x 10^4</td>
</tr>
</tbody>
</table>

CFU: Colony Forming Unit

TPC values varied between 1.8 x 10^4 CFU/gr and 2.5 x 10^4 CFU/gr with average were 2.2 x 10^4 CFU/gr. The average of TPC value of sample from location I were 2.5 x 10^4 CFU/gr. This value was higher as compared to the sample from location II with average was 1.8 x 10^4 CFU/gr.

TPC values of sample from both location were at the range of 1.8 x 10^4 CFU/gr to 2.5 x 10^4 CFU/gr. Whereas TPC values of control of live grouper fish and sea water ranged from 1.7 x 10^3 to 8.1 x 10^3 CFU/gr. The TPC values of control were higher than TPC values of control. Based on quality requirements from Standar Nasional Indonesia (SNI 01-2696-1992) the maximum number for bacteria in snapper fish fillets were 5x10^3 colonies / gr (Anonymous, 1992).

According to Huss (1995), the number of total bacteria in fish seafood products was 10^3 - 10^7.

The average of total Pseudomonas value in samples is shown in Table 3 and in control can be seen at Table 4.

Table 3. Total Pseudomonas in samples

<table>
<thead>
<tr>
<th>Location</th>
<th>Replication</th>
<th>Average of Total Pseudomonas value [TVC/gr]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>I</td>
<td>1.8 x 10^4</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>8.0 x 10^2</td>
</tr>
</tbody>
</table>

TVC: Total Viable Count

Table 4. Total Pseudomonas in control fish and seawater

<table>
<thead>
<tr>
<th>Control</th>
<th>Replication</th>
<th>Average of Total Pseudomonas value [TVC/gr]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live Grouper fish</td>
<td>I</td>
<td>1.6 x 10^3</td>
</tr>
<tr>
<td>Sea water</td>
<td>II</td>
<td>1.2 x 10^4</td>
</tr>
</tbody>
</table>

TVC: Total Viable Count

From table above it can be seen that the total number pseudomonas in samples from location I was 3.3 x 10^4 TVC/gr. number was higher than those found in samples from location II which was 2.3 x 10^4 TVC/gr. The results also showed that the average of total pseudomonas in samples from location I and II was higher as compared to controls of live-grouper fish. However, average of total Pseudomonas in sea water was higher. There possibility that the bacteria were washed away during processing of grouper fish fillets.
ULT AND DISCUSSION

Occurrence of Bacteria in Grouper fish fillets average of TPC value are shown in Table 1 and 2.

1. TPC of samples

<table>
<thead>
<tr>
<th>Location</th>
<th>Replicates</th>
<th>The average of TPC values (CFU/gr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.1 x 10^4</td>
<td>7.3 x 10^4</td>
</tr>
<tr>
<td>II</td>
<td>2.3 x 10^4</td>
<td>5.3 x 10^4</td>
</tr>
</tbody>
</table>

Colony Forming Unit

12. TPC of control fish and sea water

<table>
<thead>
<tr>
<th>Control</th>
<th>Replication</th>
<th>Average of TPC values (CFU/gr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grouper fish</td>
<td>I</td>
<td>1.6 x 10^4</td>
</tr>
<tr>
<td>II</td>
<td>1.8 x 10^4</td>
<td>1.7 x 10^4</td>
</tr>
<tr>
<td>Sea water</td>
<td></td>
<td>2.5 x 10^4</td>
</tr>
</tbody>
</table>

Colony Forming Unit

TPC values varied between 1.8 x 10^4 CFU/gr and 2.5 x 10^4 CFU/gr with average were 2.2 x 10^4 CFU/gr. The average of TPC value from location I was 1.9 x 10^4 CFU/gr. This value was closer compared to the sample from location II with average was 1.7 x 10^4 CFU/gr.

TPC values of sample from both location were at the range of 1.7 x 10^4 to 8.1 x 10^4 CFU/gr. The TPC values of bacteria control grouper fish and sea water ranged from 1.7 x 10^4 to 8.1 x 10^4 CFU/gr. The TPC values of control from location II were higher compared to controls of live-grouper fish. However the average of total Pseudomonas in sea water was higher. There was possibility that the bacteria were washed away during the processing of grouper fish fillets.
Physiological and biochemical characteristics of the isolated Pseudomonas sp.

**Physiological and biochemical characteristics**

The result of gram coloration into 72 bacteria strains isolated from *Cetrimide Agar*, showed gram negative characteristic with red color rod. The result of motility test showed that there were 48 bacteria strains gave positive results and the other 24 bacteria gave negative results. Positive result means that tested bacteria had ability to make movement or had flagella to move.

The result of oxidase test showed that there are 61 bacteria strains had positive result, while 11 other bacteria strains had negative result. Positive reaction means that these bacteria had *stachrometer oxidase* enzyme that take apart in aerobic respiration.

Catalase test showed that 57 bacteria strains gave positive result, while 15 bacteria strains were negative. Positive result means that the bacteria produce catalase enzyme.

Fermentation tests were done on several sugar medium such as sucrose, glucose, maltose and mannitol. The result from the tests was varied. There were 14 bacteria strains fermented glucose and produced air bubble. Whereas 17 bacteria strains fermented glucose without produced air bubble, and another 14 strains gave uncertain results.

Indol tests of 19 strains showed positive result. This means the isolates could produce *triptophanase* enzyme as catalyst in separating indol moiety from triptophan.

The result of methyl red-tests was varied. There were 11 strains showed positive result and another 11 strains showed negative result.

*Vages* Proskauer tests revealed that 10 strains had positive result. This test was conduct to show the ability of bacteria to ferment carbohydrate and produce acetylmethylcarbinol.

The citric acid tests showed variation in results. There were positive and negative results. The result of the test can be seen in Table 5. The positive result showed that the bacteria could use citric acid as carbon source for its cell to produce energy.
logical and biochemical characteristic of the isolated Pseudomonas sp.

**logical and biochemical characteristics**
- The result of gram coloration into 72 bacteria strains isolated from 'Tetramide Agar', showed gram negative characteristic with red.
- The result of motility test showed that there were 48 bacteria gave positive results and the other 24 bacteria gave negative results. Positive result means that tested bacteria had ability to make motility or had flagella to move.
- The result of oxidase test showed that there are 61 bacteria strains gave positive result, while 11 other bacteria strains had negative result. Positive reaction means that these bacteria had oxidase enzyme that take apart in aerobic respiration.
- The result of Zymase test showed that 57 bacteria strains gave positive result, 15 bacteria strains were negative. Positive result means that bacteria produce catalase enzyme.

**Table 5.** Composition of *Pseudomonas* sp. In Grouper fish Fillet

<table>
<thead>
<tr>
<th>Genus/species</th>
<th>Strains</th>
<th>Amount (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. caryophylli</em></td>
<td>FK.la-1, FK.la-2, FK.la-3, FK.la-5, FK.la-6</td>
<td>6.9</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>FK.la-8, FK.la-9, FK.la-10, FK.la-12, FK.la-13, FK.lb-3, FK.lc-9, FK.lc-10</td>
<td>11.1</td>
</tr>
<tr>
<td><em>P. delafeldii</em></td>
<td>FK.lb-1, FK.lb-6, FK.lb-7, FK.lb-9, FK.lb-12, FK.lc-1, FK.lc-2, FK.lc-3, FK.la-1, FK.la-2, FK.la-3, FK.la-8, FK.la-9, FK.lb-9, FK.lc-4, FK.lc-11, FK.lc-12</td>
<td>23.6</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>FK.lc-5, FK.lc-7, FK.lc-14, FK.la-6, FK.lb-1, FK.lb-2, FK.lb-3, FK.lc-9, FK.lc-10</td>
<td>12.5</td>
</tr>
</tbody>
</table>

The growth characteristic of *Pseudomonas* sp.

Todar, (2004) stated that the optimum growth of *Pseudomonas* were influenced by temperature, salinity and pH. Base on biochemical tests, 4 strains that had been identified were selected. They were *P. fluorescens* (FK.la-8), *P. aeruginosa* (FK.lc-5), *P. delafeldii* (FK.la-9) and *P. caryophylli* (FK.la-1) to represent 72 strains.

The growth temperature of the 4 isolates at 0°, 2°, 4°, 6°C, 25°C, and 37°C is shown in Table 6.
Table 6. Growth Temperature of *Pseudomonas* sp.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Time (h)</th>
<th>0°C</th>
<th>5°C</th>
<th>10°C</th>
<th>15°C</th>
<th>20°C</th>
<th>25°C</th>
<th>30°C</th>
<th>35°C</th>
<th>40°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em></td>
<td>24</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>24</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>P. delactifieldii</em></td>
<td>24</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>P. caryophylii</em></td>
<td>24</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 6 and Figure 1 showed that *Pseudomonas* could grow well at temperature of 37°C when incubated for 24 hour. The optimum temperature for the growth of microbe was between 27°C.

The effect of various levels of NaCl on the growth of *Pseudomonas* is presented in Table 7.

### Table 7. Growth of *Pseudomonas* sp. at different NaCl concentration

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Time (h)</th>
<th>0%</th>
<th>2%</th>
<th>4%</th>
<th>6%</th>
<th>8%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em></td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. delactifieldii</em></td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. caryophylii</em></td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All *Pseudomonas* isolates could grow well in the absence of salt. *P. delactifieldii*, *P. caryophylii* grew at NaCl concentration of 0-7%, *P. fluorescens*, grew at NaCl concentration of 0-3%, and inhibited at concentration 5-9%. At level 9% of NaCl all isolates were inhibited. Therefore it can be concluded that *Pseudomonas* need 0-7% salt for their growth.

The growth of *Pseudomonas* at pH range 5-9 can be seen in Table 8. No growth was observed at pH 4 dan 10.

### Table 8. Growth of *Pseudomonas* sp. at different pH values

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Time (h)</th>
<th>4°C</th>
<th>6°C</th>
<th>8°C</th>
<th>10°C</th>
<th>12°C</th>
<th>14°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em></td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. delactifieldii</em></td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. caryophylii</em></td>
<td>24</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Patogeny characteristics of *Pseudomonas* sp. In grouper fillets**

**Haemolysis Test**

The results from the test varied. There were beta haemolytic type (no blood surrounded colony), *alpha* haemolysis (several large colonies of the bacteria), and gamma haemolysis (no haemolysis).

**Agglutination test**

Agglutination test showed varied results. There were positive results and negative results.

**Sensory test of Grouper fish fillets**

Sensory tests on mucus, odors, and texture showed that grouper from location 1 showed that they had value of 24.9 and the average value was > 8. This meant that grouper fish samples from location I were categorized as class I (excellent). Their freshness scores were between 21 and 27 with no average values of less than 8. Meanwhile, grouper fish samples from location II had scores from 20 to 23, with average value was > 7.
The growth of *Pseudomonas* at pH range 5-9 can be seen in Table 8. Table 8 shows that *Pseudomonas* had optimum growth at pH range from 6 to 8. No growth was observed at pH 4 and 10.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Time (hour)</th>
<th>pH 5</th>
<th>pH 6</th>
<th>pH 7</th>
<th>pH 8</th>
<th>pH 9</th>
<th>pH 10</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em> M-15</td>
<td>24</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> NC-5</td>
<td>24</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>P. putida</em> M-21</td>
<td>24</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>P. caryophylli</em> NC-24</td>
<td>24</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Growth Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em> M-15</td>
<td>6</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> NC-5</td>
<td>6</td>
</tr>
<tr>
<td><em>P. putida</em> M-21</td>
<td>6</td>
</tr>
<tr>
<td><em>P. caryophylli</em> NC-24</td>
<td>6</td>
</tr>
</tbody>
</table>

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<table>
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<th>Table 6. Growth Temperature of <em>Pseudomonas</em> sp.</th>
</tr>
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<tbody>
<tr>
<td>Time (hour)</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>24</td>
</tr>
<tr>
<td>26</td>
</tr>
<tr>
<td>28</td>
</tr>
</tbody>
</table>

The growth of *Pseudomonas* at pH range 5-9 can be seen in Table 8. Table 8 shows that *Pseudomonas* had optimum growth at pH range from 6 to 8. No growth was observed at pH 4 and 10.

| Table 7. Growth of *Pseudomonas* sp. at different NaCl concentration |
|--------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Time (hour) | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % |
| 24          | 10    | 10    | 10    | 10    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| 26          | 10    | 10    | 10    | 10    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| 28          | 10    | 10    | 10    | 10    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |

The growth of *Pseudomonas* at pH range 5-9 can be seen in Table 8. Table 8 shows that *Pseudomonas* had optimum growth at pH range from 6 to 8. No growth was observed at pH 4 and 10.

Table 8. Growth of *Pseudomonas* sp. at different pH values.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Time (hour)</th>
<th>pH 5</th>
<th>pH 6</th>
<th>pH 7</th>
<th>pH 8</th>
<th>pH 9</th>
<th>pH 10</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em> M-15</td>
<td>24</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> NC-5</td>
<td>24</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>P. putida</em> M-21</td>
<td>24</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td><em>P. caryophylli</em> NC-24</td>
<td>24</td>
<td>6</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Growth Index</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em> M-15</td>
<td>6</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> NC-5</td>
<td>6</td>
</tr>
<tr>
<td><em>P. putida</em> M-21</td>
<td>6</td>
</tr>
<tr>
<td><em>P. caryophylli</em> NC-24</td>
<td>6</td>
</tr>
</tbody>
</table>

The growth of *Pseudomonas* at pH range 5-9 can be seen in Table 8. Table 8 shows that *Pseudomonas* had optimum growth at pH range from 6 to 8. No growth was observed at pH 4 and 10.

| Table 9. Growth of *Pseudomonas* sp. at different NaCl concentration |
|--------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Time (hour) | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % | IP % |
| 24          | 10    | 10    | 10    | 10    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| 26          | 10    | 10    | 10    | 10    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |
| 28          | 10    | 10    | 10    | 10    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    | 0    |

The growth of *Pseudomonas* at pH range 5-9 can be seen in Table 8. Table 8 shows that *Pseudomonas* had optimum growth at pH range from 6 to 8. No growth was observed at pH 4 and 10.

Table 9. Growth of *Pseudomonas* sp. at different NaCl concentration.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Time (hour)</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
<th>IP %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. fluorescens</em> M-15</td>
<td>24</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> NC-5</td>
<td>24</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. putida</em> M-21</td>
<td>24</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. caryophylli</em> NC-24</td>
<td>24</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Pseudomonas isolates could grow well in the absence of salt. *Stenotrophomonas* grew at NaCl concentration of 0-7%, *P. fluorescens* grew at NaCl concentration of 0-3%, and inhibited at NaCl concentration of 5-9%. At level 9% NaCl all isolated were inhibited. It can be concluded that *Pseudomonas* need 0-7% salt for growth.
CONCLUSION AND RECOMMENDATION

Conclusion

The total Pseudomonas found in Grouper fish fillets from location 1 was higher than those from location 2. The Pseudomonas content in Grouper fish fillets from both locations was higher than those in control-live grouper fish. The high content was caused by handling techniques in processing fillets that had not applied a correct cold chain system and low hygiene and sanitation process.

The dominant species of Pseudomonas associated with Grouper fish fillets was *P. delafeldii* with percentage 23.6%.

Pseudomonas was also found in control-live Grouper fish and in sea water samples taken from both locations. The existence of Pseudomonas in control and sea water can be used as early warning on the quality of the water in two locations because Pseudomonas had been proven as indicator of polluted water.

References


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http://www.University of Texas/ Houston Medical S

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LUSION AND RECOMMENDATION

The total Pseudomonas found in Grouper fillets from location I was higher than those from location II. The Pseudomonas in Grouper fillets from both locations was higher than in control-live grouper fish. The high content was caused by using techniques in processing fillets that had not applied a cold chain system and low hygiene and sanitation process.

The dominant species of Pseudomonas associated with Grouper fillets was P. delafeldii with percentage 23.6%. Pseudomonas was also found in control-live Grouper fish and in water samples taken from both locations. The existence of Pseudomonas in control and sea water can be used as early warning quality of the water in two locations because Pseudomonas had proven as indicator of polluted water.

References


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http://www.universityofTexas/ Houston Medical School. DPAIM MEDIC.
Proceeding of International Seminar Current Issues and Challenges in Food Safety Management


HACCP PLAN FOR GREEN TEA PRODUCT OF PT GT AND ITS IMPLEMENTATION

Raniayu and Hardoko
Department of Food Technology, Faculty of Industrial Technology, University of Pelita Harapan

ABSTRACT

HACCP plan for green tea product of PT GT is established in this research. The scope of the study covers the production process starting from receiving raw material at the factory until the packaging of the final product. Determination of Critical Control Point(s) (CCPs) refers to the decision in CAC (2003). Critical Control Points in green tea production in PT GT include on the final drying by drum tea and the sorting process based on density using section winnower. Critical limits in the first CCP are ball tea temperature ranging from 100 to 150°C, mass of tea leaves in a batch is 600 kg for grade A and 550 kg for grade B, and the drying time ranging from 8 to 10 hours. The critical limit in the second CCP is no metal component detected by the final metal detector at section winnower. An audit has been carried out to verify the implementation of the HACCP plan. Based on the audit, the corrective action form that has been arranged needed to correction so that the operator and supervisor will be able to fill the audit better. Key words: HACCP, green tea, Critical Control Point(s), critical control point, audit. Reference: 11 (1995-2009).

PREFACE

Background

Food industries are encountering many challenges, especially in the case of food safety. Some of the challenges are the increased number of new food pathogens; chemical contamination of food which can be caused by the usage of non permitted food additives; contamination comes from antibiotic residue, pesticide residue, and excessive use of disinfectant; and the remarkable growth of food products processing diversity, so the effort needed to ensure food safety increases. In addition to that, the international trade demand...