THE OCCURRENCE OF *Pseudomonas sp* IN GROUPER FISH FILLETS (*Plectropoma leopordus)* AT BONE PANTE

Rieny Sulistijowati* and Royhan Alhasen**

* Universitas **Muhamrnadiyah** Gorontalo
**Universitas Negeri Gorontalo
rinysulistijowati@ymail.com

ABSTRACT

Study on the occurrence of *Pseudomonas* sp., associated **with grouper** fish (*Plectropoma **leopardus)*** **was** done. The **research** aimed to know the occurrence of *Pseudomonas* **sp** in fresh grouper fish (*P.* leopardus) fillets chilled in **ice.** The fish **samples** were taken from grouper **fish** farm located **at** Bone Pante village (separated into two sampling area I and II).

The result showed that both of total **plate** count [TPC) and total Pseudomonas (TP) of grouper fish fillet were **lower** in samples taken from sampling area I than those taken from **sampling** area II. The TPC of sample from sampling area 1 ranged between 2,1 x 10^4 CFU/g to *2,8* x 10^4, while from sampling area II it ranged between 1,9 x 10^4 CFU/g to 5,Z x 10^4 CFU/g. Total *Pseudomonas* **sp.** of sample from **sampling** area I ranged between 1.8 x10^3 CFU/g to 4,4 x10^3 CFU/g, while from sampling area II, it **ranged** between 8,0 x10^2 CFU/g to 3,7 x 10^3 CFU/g. The number of TPC and TP varied among samples and the number **was** lower than the required number as **suggested** by ***Standirr Nasional** Indonesia* (SNI) for the quality of microbiological of frozen grouper fish.

Using the Bergey's **Manual** of Microbiological Identification, the isolated strain were identified as *Pseudomonas caryophylli (6,9%)* , *P. fluorescens* (11,1 %), *P. delafiedii* (23,6%), **and** *P. aeruginosa* (12,5 %). They have physiological characteristic as follows : grow well at temperature between 0^0 - 37^0 C with optimum temperature for growth at 37°C: **pH** range between **4** - *9*, **with** optimum **pH** of 7, tolerate salinity with NaCl concentration between 3-8 %. *P. caryophylli* isolated from grouper fish fillet demonstrated 13- haemolysis and a- haernolysis for *P. aeruginosa*. While *P. caryophylli* **and** P. *flourecens* were agglutinase positive. In general, samples **taken** From farm at sampling area 1 were better as compared to that of sampling area II. Sensory values from **sampling** area I were 24,9 with averages **value** of more than 8,f while from sampling area II, the value **were** between 21- 23,06 with **averages** sensory of Iess **than** 7,6.

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 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, Sthaphylococcus,* and some more. *Pseudomonas sp* is one of fish contaminating bacterium that may cause diseases. Base on these findings, the writer want to research more abou "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 I 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 ***outaclave, 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 Agar **medium,** *Cetrimide Agar* and NaCl, **APW** (enrichment), alcohol.

Sampling

Grouper fish **fillets** were sampled from processing unit at Bone Pante I and **Bone Pante** 11. **The** fish fillets samples were 250 - 500 gr weight x 2 pieces. Sampling was taken triplicate in each location. The **frequency** of sampling **was** every one month after previous sampling. Live grouper fish **was** used as **test** controls. The controls were taken from water each time the fish fillet and sea water was sampled.

Microbiology analysis

Fish fillet was analyzed for its *Total Place Count* (TPC) **arid** Total *Pseudomonas* **(TP).**

Isolation and Identification of Pseudomonas

Selection and isolation steps **of** *Pseudomonas* consist of several physiology **and biochemical** *tests* **such** as: Cram coloration test, motility test, oxidase enzyme test, catalase enzyme test, fermentation test, indol test, *methyl* red *test, voges-proskauer* **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; 0^0 C, 2^0 **C,** 4^0 C, 6^0 C, 25^0 **C,** 37^0 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

Location	Replicates			The average of TPC values (CFU/gr)
	I	II	III	
I	2,1 x 10^4	2,6 x 10^4	2,8 x 10^4	2,5 x 10^4
II	1,9 x 10^4	-	5,2 x 10^4	1,8 x 10^4

CFU: *Colony Forming Unit*

Tabel 2. TPC of control fish and sea water

Control	Replication		Average of TPC values (CFU/gr)
	I	II	
Live Grouper fish	8,2 x 10^3	7,9 X10^3	8,1 X 10^3
Sea water	1,6 x 10^4	1,8 X 10^4	1,7 X 10^3

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 5x10^5 colonies / gr (Anonymous, 1992).

According to Huss (1995), the number of total bacteria in fresh 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 *Pseudomonos in samples*

Location	Replication			Average of Total *Pseudomonas value* (TVC/gr)
	I	II	III	
1	1.8 x 103	4.4 x 103	3.6 x 103	3.3×10^3
II	8.0×10^2	2.4 x 103	$3,7 \times 10^3$	2,3 x 103

TVC: Total *Viable Count*

Tabel 4. Total *Pseudomonas* in control fish and seawater

control	Ulangan		Average of Total *Pseudomonas value* (TVC/gr)
	I	II	
Live Grouper fish	$1,6 \times 10^3$	$1,9 \times 10^3$	$1,8 \times 10^3$
Sea water	-	$1,2 \times 10^4$	$1,2 \times 10^4$

TVC: *Total Viable Count*

From table above it can be seen that the total number of *pseudomonas* in samples from location I was 3.3×10^3 TVC/gr. This number was higher than those found in samples from location II which was $2,3 \times 10^3$ TVC/gr. The results also showed that t the average of total *pseudomonas* in samples from location I and II were higher as compared to controls of live-grouper fish. However the average of total *Pseudornonas* in sea water was higher. There was possibility that the bacteria were washed away during the processing of grouper fish fillets.

Physiological and biochemical characteristic of the isolated *Pseudornonas* 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 *sitochrom 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 moety **from** *triptophan.*

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

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

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.

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

Genus/species	Strains	Amount (%)
P. caryophylli	FK.Ia-1, FK.Ia-2, FK.Ia-3, FK.Ia-5, FK.Ia-6	6.9
P. fluorescens	FK.Ia-8, FK.Ia-9, FK.Ia-10, FK.Ia-12, FK.Ia-13, FK.Ib-3, FK.Ic-9, FK.Ic-10	11.1
P. dela fieldii	FK.Ib-1, FK.Ib-6, FK.Ib-7, FK.Ib-9, FK.Ib-12, FK.Ic-1, FK.Ic-2, FK.Ic-3, FK.IIa-1, FK.IIa-2, FK.IIa-3, FK.IIa-8, FK.IIa-9, FK.IIb-9, FK.IIc-4, FK.IIc-11, FK.IIc-12	23.6
P. aeruginosa	FK.Ic-5, FK.Ic-7, FK.Ic-14, FK.IIa-6, FK.IIb-1, FK.IIb-2, FK.IIb-3, FK.IIc-9, FK.IIc-10	**12.5**

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.Ia-8), *P. aeruginosa*, (FK.Ic-5), *P. delafieldii*, (FK.Iia-9) and *P. **caryophylli*** (FK.Ia-1) to represent 72 strains.

The growth temperature of the **4** isolates at 0º, 2º, 4º, 6ºC, 25ºC, and 37ºC **is** shown in **Table** 6.

Table 6. Growth Temperature of ***Pseudomonas*** **sp.**

Isolates	Time (hour)	Temperature											
		0°		2°		4°		6°		25°		37°	
		GI	%	GI	%	GI	%	GI	%	GI	%	GI	%
P. fluorescens FK.la-8	24	8	80	10	100	10	100	10	100	10	100	10	100
P. aeruginosa FK.lc-5	24	7	70	10	100	10	100	10	100	10	100	10	100
P. delafieldii FK.lia-9	24	9	90	10	100	10	100	10	100	10	100	10	100
P. caryophylli FK.la-1	24	8	80	10	100	10	100	10	100	10	100	10	100

GI = Growth Index

Table 6 and Figure 1 showed that ***Pseudornonas*** could grow **well** at temperature 37ºC when incubated for 24 hour. The optimum temperature for the growth of microbe **was** between 2- 37ºC.

The effect of various levels NaCI on the growth of Pseudornonas is presented in Table 7.

Tabel 7. Growth of *Pseudomonas* sp. at different NaCl concentration

Isolates	Time (hour)	NaCl									
		0°		3°		5°		7°		9°	
		IP	%	IP	%	IP	%	IP	%	IP	%
P. fluorescens FK.la-8	24	10	100	10	100	8	80	0	0	0	0
P. aeruginosa FK.lc-5	24	10	100	10	100	9	90	10	100	0	0
P. delafieldii FK.lia-9	24	10	100	10	100	10	100	10	100	0	0
P. caryophylli FK.la-1	24	10	100	10	100	10	100	10	100	0	0

GI = Growth Index

Table 7 showed the effect of NaCl to the growth of ***Pseudomonas.*** **All** ***Pseudvmonos*** isolates **could** grow well in the absence of salt. *P.delafielddi, P.caryophylii* grew at **NaCl** concentration of **0-7%.** *P.fluorecens*, grew at NaCl concentration of 0-3%, and inhibited at concentration 5-9%. At level 9% NaCl all isolated **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. Table 8 shows that *Pseudomonas* had optimum growth at pH range from 5 to 8. No growth was observed at pH 4 dan **10**

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

Isolates	Time (hour)	PH													
		4		5		6		7		8		9		10	
		GI	%	GI	%	GI	%	GI	%	GI	%	GI	%	GI	%
P. fluorescens FK.Ia-8	24	0	0	10	100	10	100	10	100	10	100	8	80	0	0
P. aeruginosa FK.Ic-5	24	0	0	10	100	10	100	10	100	10	100	6	60	0	0
P. delafieldii FK.IIa-9	24	0	0	10	100	10	100	10	100	10	100	0	0	0	0
P. caryophylli FK Ia-1	24	0	0	10	100	10	100	10	100	10	100	0	0	0	0

GI = Growth Index

Patogenity characteristics of *Pseudomonas* sp. In grouper fish fillets

Haemolysis Test

The results from the test varied. There **were** beta haemolysis type (no blood surrounded colony), alpha hemolysis **(several** blood cells found at haernolysis zone or several **greenish** changing surrounded colony) 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, texture **showed** that grouper fish from location I 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 quality were between **21** and **27** with no average values of less than 8. Meanwhile, grouper fish samples from location II had scores from 21 **to** 23,06 with average value was of > *7.*

CONCLUSION AND RECOMMENDATION

Conclusion

The total Pseudomonas found in Grouper fish fillets from location I was higher than those from location II. 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. delafieldii* with percentage 23,6%.

Pseudornonas 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

Afrianto, E. dan E. Liviawaty., 1989. Pengawetan dan Pengolahan Ikan. Kanisius. Jogyakarta.

Anonimous, 1992. Standar Nasional Indonesia. 01-0729-1992: Ikan Segar. Pusat Standarisasi Industri. Departemen Perindustrian Jakarta.

_____.1998. Fact Sheeton *Pseudomonas* sp.. National Food Processors Associotion.http://www.nfa-food.Org/members/science/fact-vibrio.html

_____.2001a. Bacteriological Analytical Manual On Line, *Pseudornonas Cholerae, V. parahaemolyticus, V. Vulfunicus,* and Other *Pseudomonas* Sp.p. Chapter 9. Center For food Safety and Applied Nutrition. Washington, D.C, USA.

http://vm.cfsan.fda.gov/~ebam/bam-9.html

_____.2001b. Fish and Fishery Products Hazards and Controls Guide, 3rd ed. Department of Health and Human Services, Public Health Service, Food and Drug Administration, Center for Food

Safety and Applied Nutrition, **Office** of Seafood, Washington, **DC.**

http://seafood.ucdavis.edu/haccp/compendium/chapt20.htm#Vibrio%20sp.p.

_____,2002. *Cetrimide* Agar. EMD. Merck KgaA. Darmstadt, Germany.

http://www.emdchemicals.com/analytics/Micro Manual/TEDISdata/prods/1 10263 0500.html

_____,2003a. Food Safety And Shelfish Program.

http://www.doh.wa.gov/chp/sf/biotoxinprogram.html

_____,2003b. Bacteriological Analytical Manual , Pseudomonas *auruginosa*http://www.cfsan.fda.gov/~ebam-9.html

_____,2004. Bacteriological Analytical Manual On Line, *Pseudomonas.* Chap 9. CFSAN-Food And Drugs Administration. Washington DC, USA.

http://www.cfsan.fda.gov/~ebam/bam-9.html

_____ ,2005a. *Pseudomonas* Infection

http://www.microbiology.co.id/aurelia at.adu.washington

_____ ,2005b. Bio 203, General Microbiology & Infection Control http://www. microbio@iubio.bio.indiana.edu <microbi

_____ ,2005c. *Pseudomonas.*

http://www. Universtv af Texas/ Houston Medical School. DPALM MEDIC.

Austin, B., 1993. Marine Microbiology. Cambridge University Press.

Baumann, P. dan L.R.H.W . Schubert, 1984. Bergey's Manual Of Syaternatic Bacteriology. Vol. **1.** The Williams **and** Wilkins Co., Baltimore.

Berhirnpon, S., 1993. Mikrobiologi Pangan Ikani. Laboratorium Pengolahan Dan Pembinaan Mutu Hasil Perikanan. Fakultas Perikanan Dan Ilmu **Kelautan.** Unsrat. Manado

Boel, T. 2004, Infeksi Saluran Kemih Dan Kelamin, Fakultas Kedokteran Sumatera Utara. Medan.

Buckle,K. A., R. A. Edward,G. H.Fleet,M.Wooton.1987.Ilmu Pangan. Penerjemah Hari Purnomo dan Adiono.UI Press. Jakarta.

Cappucino and Sherman., 1992. Microbiology: A Laboratory Manual. 3rd ed. The Benjamin/Cummings Publishing, Inc. California.

Droop, MR dan H.W. Jannasch 1977, Advance In Aquatik Microbiologi,Vol 1. Academic Press,Inc New York

Feliatra, Irwan **Efendi,** Edwar Suryadi, 2004. Isolasi dan Identifikasi Bakteri Probiotik dari Ikan Grouper fish Macan (*Ephinephelus fuscogatus*) dalam **Upaya Efisiensi Pakan** Ikan. Jurnal Natur Indonesia 6(2): 75-80 (2004) **ISSN 1410-9379.** Jakarta.

Frazier , W.C. dan Westhof, D.C., 1998. Food Microbiology. 4th Edition. Mc Gram Hill Book co. New York.

Fix, D.F. 2004. *Pseudomonas. http://www.ches.siu.edu/fix/medmicro pseud.htm. 22 Juli 2004 Pukul 13.00 wita.*

Hadiwiyoto, S., 1993. Teknologi Refrigrasi Hasil Perikanan. Penerbit Liberti. **Yogyakarta.**

Huss, H., 1995. Quality and Change in Fresh Fish. FAO. Fisheries Technical Paper. Fisheries Library. Denmark.

Ijong, F.G., 2002. Mikrobiologi Dasar. Laboratorium Mikrobiologi Hasil Perikanan. Jurusan Pengolahan Hasil Perikanan. Fakultas Perikanan dan Ilmu Kelautan. UNSRAT. Manado.

Ilyas, S., 1983. Teknologi Pendinginan Ikan. Jilid I. Penerbit Yayasan Wijaya Kusuma. Jakarta.

Jawetz, E., Melnick, J.L., Adelberg, E.A. 1986. Mikrobiologi. Edisi 16. Peuerbit Buku Kedokteran. Jakarta.

Lay, B., 1994. Analisis Mikroba di Laboratorium. PT. Raja Grafindo Persada. Jakarta.

Matthysee,A.G, 1992. Ad. (Translated from japan) A.A. Balkema. Roterdam.Adhesion Bacterial. Encyclopedia of Microbiology Volume 1.A-C .Academic Press,Inc Harcourt Brace Jovanovich, Publishers, San Diego,California.

Moningka, M.R., 2005. Efek Pencucian Terhadap Daya Lekat *Escherichia coli* Pada Beberapa Produk Perikanan. Skripsi. FPIK UNSRAT. Manado.

Pritchard AE, Vasal ML. **1990.** Possible **insertion** sequences in a mosaic genorneorganization upstream of the exotoxin A gene in Pseudomonas aeruginosa. J Bacteriol 172:2020.

Singleton, P., dan D. Sainsbury, 1987. Dictionary of Microbiology and Molecular Biology. 2nd ed. JohnWiley and Song. New York, **USA.**

Todar K, 2004, Text Book **of M**ikrobiology, ***Pseudomonas** aeruginosa*, Department of Bacteriology. **University** of Wisconsin-Madison. http://www.textbookofmicrobiology

Varnam, A.H. **dan** Evans, **M.G.**, 1991. Food Born Patogens an Illustarted Text. Moosby Year Book, Inc. Toronto, Canada.

Zdzislaw, E.S., 1989. Seafood: Resources, Nutritional Composition And Preservation. Departement of Food Preservation and Technical Microbiologi. echnical University Plythenika Gdanska. Poland.