International Food Research Journal 19(4): 1723-1727 (2012)
Journal homepage: *http://www.ifrj.upm.edu.my*

The curative action of *Lactobacillus plantarum* FNCC 226 to *Saprolegnia parasitica* A3 on catfish (*Pangasius hypophthalamus* Sauvage)

[1*]Nurhajati, J., [3]Atira, [2]Aryantha, I. N. P., [1]Kadek Indah, D. G.

[1]Department of Biologi, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Indonesia,
[2]Life and Sciences Institute of Technology Bandung, Bandung, Indonesia
[3]Department of Biologi, Faculty of Mathematics and Natural Sciences, Tadulaco University, Palu, Indonesia

Article history

Received: 26 January 2012
Received in revised form: 11 April 2012
Accepted:12 April 2012

Keywords

Saprolegnia parasitica
Lactobacillus plantarum
Pangasius hypophthalmus
infectious concentration
maximal nir lethal concentration

Abstract

The inhibition of the growth of the parasitic *S. parasitica* A3 on catfish using *L. plantarum* FNCC 226 *in vivo* and *in vitro*, were examined in this study. To prevent fungal proliferation, different concentration of *S. parasitica* A3 were employed in a two step approach: to determine the concentration of zoospore suspension count that caused a 50% infection on catfish and concentration of *L. plantarum* FNCC 226 that inhibits the growth of the *S. parasitica* A3 on catfish. The design use was the Completely Randomized Design with 4 x4 factorial pattern consisting of 4 infection concentration of *S. parasitica* A3 suspensions, i.e. 0xIC_{50}, IC_{50} (Infectious Concentration 50%), 2xIC_{50}, and 4xIC_{50} and 4 concentrations of *L. plantarum* FNCC 226 inoculums, i.e. MaxNLC (Maximal Nir Lethal Concentration), ½xMaxNLC, ¼xMaxNLC, and 0xMaxNLC, with 4 times replication. The results obtained showed that the IC_{50} count for a 50% infectious was 1 x 10^7 zoospore/mL and were dose dependent. *L. plantarum* FNCC 226 inhibit mycelium of *S. parasitica* A3 were found to be 7.7 x 10^5 cfu/mL (MaxNLC), 4.0 x 10^5 cfu/mL (½xMaxNLC) and 2.3 x 105 cfu/mL (¼xMaxNLC) and were dependent on the initial number of the infectious *S. parasitica* A3. The decrement of *S. parasitica* A3 infection coincide with the increment of *L. plantarum* FNCC 226 blocked. Thus, this study indicated that *L. plantarum* has the capacity to inhibit *S. parasitica* and it would be possible to design new biocontrol of this pathogen in catfish.

Introduction

Saprolegnia parasitica causes "Saprolegniasis", a disease marked by the presence of white or grey mycelium, which adheres to the body or fin of freshwater fish. *S. parasitica* will attach to the injured part of a fish body, and then spread to other healthy tissues (Suriawiria, 2003). An *S. parastitica* attack will cause losses in the form of decrease in catfish production in freshwater fish farming. Mass production of catfish seeds still faces some constraints, among others, the fact that catfish are often attacked by *S. parasitica*, resulting in the death of a large number of catfish seeds, in particular the 1-2 month-aged seeds (Susanto and Amri, 2002).

West (2006) reported that *S. parasitica* infection thus far has been prevented using malachite green, which is an antifungal compound to kill pathogenic fungi. The use of malachite green is hazardous to the environment. Prevention and cure of this fish disease may be implemented by various natural methods, among others, by the *Lactobacillus plantarum* microbe. *L. plantarum* is a member of the lactic acid bacteria group, already known to be environmental friendly because it is not pathogenic and may benefit other organisms. *L. plantarum* can be found in animal and human digestive system, in the mouth and vagina cavity, as well as in fermented foodstuff (yoghurt and salted vegetables) (Pelczar and Chan, 1986). *L. plantarum*, also known as probiotic, produce MSA (*Mannose Specific* Adhesin) extracellular secondary metabolite, a substance that enhance the attachment of pathogenic bacteria to the *L. plantarum* probiotic cell wall.

The catfish is a fresh water fish, with high potential for development, widely consumed by consumers in Asia because its flesh is tender, savory and delicious, with large individual dimension, resulting in high selling price. These characteristics have drawn the interest and attention of restaurant owners to farm catfish (Susanto and Amri, 2002). In fresh water farming, fish are exposed to an environment

*Corresponding author.
Email: *jettynurhajati@yahoo.com*

containing various pests and diseases, like virus, bacteria, fungi, and parasites (Wahyuningsih, 2002).

Research need to be conducted on the negative impact caused by the *S. parasitica* parasite and natural curative measure with *L. plantarum* need to be implented to decrease the losses due to S. parasitica. Therefore this research was conducted on the infectious dose of the *S. parasitica* on catfish (*P. hypophthalmus*) and its biocontrol method with *L. plantarum*.

Materials and Methods

Fungal and bacterial cultures

The *S. parasitica* A3 fungus was, isolated from the A3 infected catfish was obtained from the Center for Life Sciences of ITB (Bandung Institute of Technology), Bandung, whereas the *L. plantarum* FNCC 226 bacteria was isolated from the fermented food product was obtained from the Life Sciences Microbiology Laboratory of ITB. A 2-month old catfish (*P. hypophthalmus* Sauvage) of about ± 6 cm long and weighing ± 3 gram was obtained from the Fresh Water Fish Farming Agency, Subang, West Java.

Determining the 50% (IC_{50}) infectious dose of S. parasitica suspension in catfish (P. hypophthalmus)

In this study, the Completely Randomized Design and the REED-MUENCH method were used to calculate IC_{50}. The treatment used *S. parasitica* zoospora suspension with concentration levels of 4 x 10^6 zoospore/mL, 6.5 x 10^6 zoospore/mL, 8.5 x 10^6 zoospore/mL, 1 x 10^7 zoospore/mL, 1.25 x 10^7 zoospore/mL and 1.5 x 10^7 zoospore/mL. All treatments were carried out with four replications and controls were included (treatments without zoospore). Observation was carried out daily for five days to determine the number of *S. parasitica* infected catfish of every test unit and the infection diameter was measured. The water physical condition and chemistry (pH 6.75 – 6.90, temperature 23-26^{o}C, OD (oxygen demand) 6.16 – 6.18 mg/L, ammonia 0.0 - 1.0 mg/L, and nitrite 0.0 - 0.2 mg/L. The data obtained were analyzed using the Variant Analysis Statistical Test and, in case of a significant difference, was followed by the Duncan Multiple Distance Test. (Gomez and Gomez, 1995).

The Completely Randomized Design was used with the 4 x 4 factorial patterns to determine the effects of *L. plantarum* on the *S. parasitica* infected catfish. The concentrations of *S. parasitica* suspension used were 0xIC_{50}, IC_{50}, 2xIC_{50}, dan 4xIC_{50} whereas the concentrations of Maximal Nir-Lethal (MaxNLC) *L. plantarum* inoculums used were 0 x MaxNLC, ¼ x MaxNLC, ½ x MaxNLC and MaxNLC (7.7 x 105 cfu/ml). All treatments were carried out with four replications and the ratio of *S. parasitica* infection diameters on catfish before and after administration of *L. plantarum* inoculums curative concentrations in each treatment were observed daily for five days. The data obtained were analyzed using the Variant Analysis Statistical analysis and, in case of significant differences, was followed by the Duncan Multiple Distance Test.

Determination of the infection rate of S. parasitica on Catfish

Infection *S. parasitica* on catfish was carried out as follows: 28 aquaria (15 x1 5 x 25 cm) were prepared for *S. parasitica* infecting places and four other aquaria were prepared as control. Six catfish were put into each aquarium and they were infected with a 2 liter *S. parasitica* zoospore suspension calculated using a haemocytometer) as follows: aquarium A1 = 4 x 10^6 zoospore/mL, A2 = 6.5 x 10^6 zoospore/mL, A3 = 8.5 x 106 zoospore/mL, A4 = 1 x 10^7 zoospore/mL, A5 = 1.25 x 10^7 zoospore/mL, A6 = 1.5 x 10^7 zoospore/mL. All the catfish incised with a scalpel on one side of its body with an incision length of ± 0.5 cm and incision depth of ± 0.2 cm. *S. parasitica* infection rate on the catfish was observed daily for five days.

Effects of L. plantarum on the S. parasitica *infected catfish*

The catfish infected with *S. parasitica* at various concentrations were exposed to *L. plantarum* starting with the MaxNLC concentration (Maximal Nir Lethal Concentration of 7.7 x 10^5 cfu/mL concentration (which is the highest initial inoculums concentration which does not result in death for the catfish), followed by lower concentrations of two-fold dilutions (¼ x MaxNLC (2.3 x 10^5 cfu/mL) and ½ x MaxNLC (4.0 x 10^5 cfu/mL), prepared according to the National Committee for Clinical Laboratory Standard (Murray *et al*. 1995). A control of 0xMaxNLC was included. Observation was carried out daily for five days.

Result and Discussion

The results for the isolation of *S. parasitica* from catfish infected with aquatic fungi by using benny seeds feed in sterilized distilled water for 2 days are as shown in Fig. 1A,1B and 1C. They were confirmed and idenitified by microscopic method according to Ainsworth *et al*. (1973) and Coker (1923).

Fig. 1C clearly shows the internal proliferation

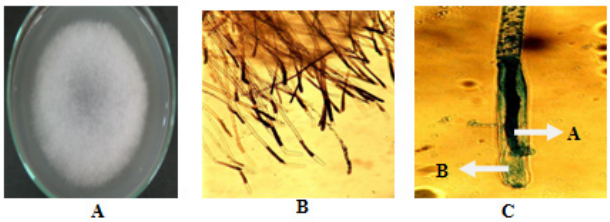

Figure 1. A: *S. parasitica* Mycelium Growth Shape in PDA, 1B = *S. parasitica* Mycelium Growth Shape in Aquadest at 1000x magnification and 1C =Internal Proliferation Shape of *S. parasitica* Zoosporangium (A = new Zoosporangium, B: old Zoosporangium) 1000x Magnification Pattern

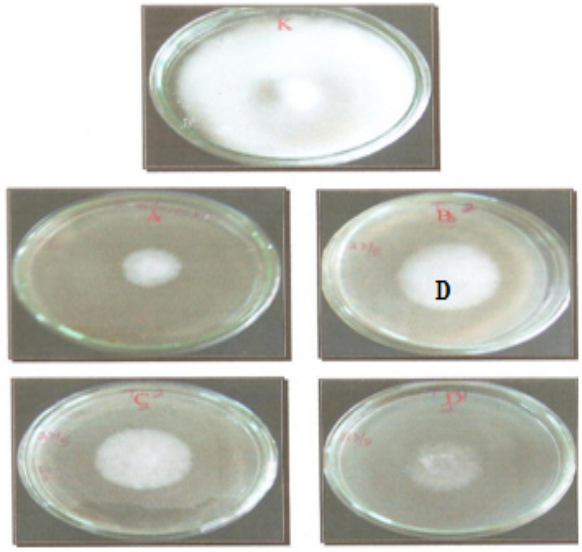

Figure 2. Antagonism *Lactobacillus plantarum* to the *Saprolegnia parasitica*. Picture information: K (*S. parasitica* coloni without *Lactobacillus*), A (*S. parasitica* coloni with *L. acidophilus*), B (*S. parasitica* coloni with *L. casei*), C (*S. parasitica* coloni with *L. delbrueckii*), and D (*S. parasitica* coloni with *L. plantarum*).

shape, namely, new zoosporangium was formed in the old or empty zoosporangium, a general characteristic possessed by *S. parasitica* (Coker, 1923). In addition, no oogonium formation were observed in test samples, a characteristic that differentiate it from other species. Antagonism between *Lactobacillus plantarum* and *Saprolegnia parasitica* were shown in Figure 2.

Results showed that there is significant difference in each of the treatment containing different *S. parasitica* suspension concentration. Water quality at every testing during the research was still within tolerance limits of fish life, that is, average pH 5.9 – 6.95; average temperature 26 - 28°C; average DO 6.06 – 6.175 mg/L; average ammonia 0.0 – 1.0 mg/L and average nitrite 0.0 – 1.0 mg/L.

According to Gay (2005), infection could come about because of the absence of equilibrium between agent, host, and environment, and the greater the infection concentration the greater the number of infected hosts. On the basis of calculations using the REED-MUENCH method, the *S. parasitica* spores

Table 1. The effect of interaction between the administration of various *L. plantarum* concentrations and various *S. parasitica* concentrations according to two administration methods on *S. parasitica* mycelium growth diameter (mm)

Lp / Sp	Without *L. plantarum*				With *L. plantarum*			
	0xMaxNLC (Control)	¼xMaxNLC (2.3×10^5)	½xMaxNLC (4.0×10^5)	MaxNLC (7.7×10^5)	0xMaxNLC (Control)	¼xMaxNLC (2.3×10^5)	½xMaxNLC (4.0×10^5)	MaxNLC (7.7×10^5)
0xIC_{50} (Control)	0 A	0 a	0 a	0 a	0 a	0 A	0 A	0 a
IC_{50} (1×10^7)	7.875 G	7.875 g	7.950 g	7.850 g	7.875 g	3.834 C	1.833 B	1.500 b
2xIC_{50} (1.25×10^7)	11.000 I	11.150 i	11.125 i	11.000 i	11.000 i	5.950 F	5.300 E	4.750 d
4xIC_{50} (1.5×10^7)	14.250 J	14.225 j	14.250 j	14.225 j	14.225 j	9.042 H	9.042 H	8.958 h

Note: Similar letters in all directions indicate a no-difference at 1% Level of Significance; IC_{50} Infectious Concentration; MaxNLC : Maximum Nir Lethal Concentration ;Sp = *S. parasitica* Supension Concentration ; Lp = *L. plantarum* inoculum Concentration

Table 2. The effect of time, various non lethal maximum concentrations and various concentrations of treatment *L. plantarum* against *S. parasitica* infection diameter dimensions (mm)

Time / Sp	2 Days post Infection *L. plantarum*				5 Days Post Infection *L. plantarum*			
	0xMaxNLC (Control)	¼xMaxNLC (2.3×10^5)	½xMaxNLC (4.0×10^5)	MaxNLC (7.7×10^5)	0xMaxNLC (Control)	¼xMaxNLC (2.3×10^5)	½xMaxNLC (4.0×10^5)	MaxNLC (7.7×10^5)
0xIC50 (Control)	0 A	0 a	0 a	0 a	0 a	0 a	0 A	0 a
IC_{50} (1×10^7)	4.000 C	4.000 c	4.000 c	4.000 c	7.875 g	3.834 c	1.833 B	1.500 b
2xIC_{50} (1.25×10^7)	6.000 F	6.000 f	6.000 f	6.000 f	11.000 i	5.950 f	5.300 E	4.750 d
4xIC_{50} (1.5×10^7)	9.042 H	9.042 h	9.042 h	9.042 h	14.225 j	9.042 h	9.042 H	8.958 h

Note: Similar letters in all directions indicate a no-difference at 1% Level of Significance; IC_{50}= Infection Concentration 50%; Sp = *S. parasitica* MaxNLC = Maximum Nir Lethal Concentration

suspension concentration causing the 50% (IC_{50}) for the catfish (*P. hypophthalmus*) within the five days was 1 x 10^7 zoospore/mL.

The difference in treatment of *S. parasitica* suspension concentration showed a very significant difference in the catfish (*P. hypophthalmus*) infection diameter, that is, the greater the *S. parasitica* suspension concentration, the greater its infection diameter. According to Av Hill (1996), suspension density is one of the most important factors affecting the coming about of infection. When suspension was administered in small amounts, more time was needed to reach infection accumulation; on the other hand, when suspension was increased in greater amounts, the resulting infection was more evenly spread within a short time.

The diameter (8.675 cm) of the *S. parasitica* colony in *in vitro* research in the non-administration of *L. plantarum* treatment, was longer than treatment with addition of 1 ml *L. plantarum* inoculums (3.475 cm). Livia (1998) reported that lactic acid bacteria produced antimicrobials that could impede some fungi growth. Impediment of *S. parasitica* mycelium growth was also caused by the fact that *L. plantarum* decreased the pH medium until pH 4.0. Klaenhammer (1993) reported that lactic acid bacteria possessed antimicrobial activity, in particular organic acid, which could decrease pH media to become pH 5.6 till pH 3.0, which are capable of impeding other

Table 3. The effect of interaction between the administration of various *L. plantarum* concentration, various *S. parasitica* concentration, and various concentrations of treatment *L. plantarum* against *S. parasitica* to percentage nir-infection

S. parasitica / *L. plantarum*	Without the administration *S. parasitica*				With the administration *S. parasitica*			
	0xIC$_{50}$	IC$_{50}$	2xIC$_{50}$	4xIC$_{50}$	0xIC$_{50}$	IC$_{50}$	2xIC$_{50}$	4xIC$_{50}$
0xMaxNLC	100% e	100% e	100% e	100% e	100% e	66.7% c	50% b	33.3% a
¼xMaxNLC	100% e	100% e	100% e	100% e	100% e	83.3% d	66.7% c	66.7% c
½xMaxNLC	100% e	100% e	100% e	100% e	100% e	100% e	100% e	100% e
MaxNLC	100% e	100% e	100% e	100% e	100% e	100% e	100% e	100% e

Note : Similar letters in all directions indicate a no-difference at 1%
MaxNLC = Maximum Nir-Lethal Concentration IC$_{50}$ = Infectious Concentration 50%
¼ xMaxNLC = 2,1x10^5 cfu/ml
IC$_{50}$= 1x107 zoospora/ml
½xMaxNLC = 4,2x10^5 cfu/ml
2xIC$_{50}$ = 2x10^7 zoospora/ml
MaxNLC = 8,4x10^5 cfu/ml
4xIC$_{50}$ = 4x10^7 zoospora/ml
100% = Nir infection/negative

microorganism growth (Moat and Foster, 1995).

The results in Table 1 shows a very significant difference on the effect of the *S. parasitica* infection diameter in catfish treated with *L. plantarum*. All treatments showed results that were different compared with the control treatment; the *S. parasitica* mycelium diameter with the addition of a smaller amount of *L. plantarum* was very significant when compared with that without *L. plantarum* and control. Greenwood (1992) reported that an antimicrobial material or substance might be fungicide or fungi static depending on several factors, among others, concentration, and generally the higher the concentration, the higher the fungicide nature, and in return, the lower the concentration the lower nature of the active substance and was only fungi static in nature.

Table 2 shows that the *S. parasitica* infection diameter after the addition of *L. plantarum* on the fifth day is smaller compared to that before *L. plantarum* addition on the second day. The smaller the *S. parasitica* infection, the greater the *L. plantarum* impeding capacity. Administration of ¼xMaxNLC (1.5 x 10^7) *L. plantarum* inoculums concentration did not show decrease in *S. parasitica* infection diameter of catfish from the first day of administering *L. plantarum* inoculum until the end of the five days.

Table 3 shows the effects of interaction between the administration of various *L. plantarum* concentration and *S .parasitica* concentration and various concentrations of treatment of *L. plantarum* against *S.parasitica* to percentage nir-infection. From the results of the preventive and curative study, the administration of *Lactobacillus plantarum* againts *Saprolegnia parasitica* proved that *L. plantarum* could prevent *S. parasitica* infection and this contribute to increased catfish body endurance (Nurhajati *et al*., 2009).

L. plantarum can produce bacteriocin, which is secondary metabolite compound which consists of lipocarbohydrate-protein which has 16 amino acids, 4 sugars, hexosamin and phosphor which can have the characteristics of impeding other microbe growth (Klaenhammer, 1993; Richard, 1996; Betty, 1999; Adams, 2000). Another peculiar characteristic of lactic acid bacteria, for instance *L. plantarum*, is its non-pathogenic characteristic and generally meets GRASS (Generally Recognized As Safe), that is, safe for other organisms (Wood, 1999).

Conclusion

Mycelium growth of *S. parasitica* infected catfish were impeded by administering *L. plantarum* in suitably adapted concentrations, indicating that *L. plantarum* could prevent *S. parasitica* infection and enhance the catfish body endurance.

The best concentration treatment of lactic acid bacteria with administration 4,2 x 10^5 cfu/mL – 8,4 x 10^5 cfu/mL which can inhibit dose with infection concentration until 4x10^7 zoospora/ml.

Acknowledgement

This research article could not have been finalized without the help of all related parties. It is therefore a great honor for the writer to extend thanks to the Head of the Center of Life Sciences, Bandung Institute of Technology, Dr. I Nyoman P. Aryantha who consented to the conducting of this research.

Bibliography

Adams, M. R. and Moss, M.O. 2000. Food Microbiology 2nd Edition. United Kingdom: The Royal Society of Chemistry (pp. 315-321).

Ainsworth, G. C., Sparrow, F.K. and Sussman, A.S. 1973. The Fungi: An Advanced Treatise Volume IVB, A Taxonomic Review with Keys: Basidiomycetes and Lower Fungi Academic Press, New York and London, pp. 61-72, 113-114.

Alexopoulos, C. J., Mims, C.W. and Blackwell, M. 1996. Introductory Mycology Four Editions New York: John Wiley and Sons, Inc. (pp. 696-706).

Betty, S. L. J. 1999. Potensi Bakteriosin dalam Pengawetan Pangan (The Potential of Bacteriocin in Food Preservation) IPB, Bogor, pp. 576-582.

Bruno, D. W. and Wood, B.P. 1999. *Saprolegnia* And Other Oomycetes In Fish Diseases and Disorders Volume 3, Edited by P.T.K. Wood and D.W. Bruno CABI Publishing, Wallingford, Oxon, United Kingdom. pp. 599-659.

Coker, W. C. 1923. The Saprolegniaceae, With Notes on

Other Water Molds. Chapel Hill, N. C., USA: The University of North Carolina Press (pp. 17, 22-26, 57-58).

Gay, J.M. 2005. Epidemiology Concepts for Disease in Animal Groups College of Veterinary Medicine, Washington State University.

Gomez, K. A. and Gomez, A.A. 1995. Prosedur Statistik untuk Penelitian Pertanian, Edisi kedua (Statistical Procedure for Agricultural Research, Second Edition) Jakarta: UI Press. (pp. 7-20, 87-93).

Greenwood, R.B., Slack, J.F and Reuthree 1992. Medical Microbiology, 14th Edition ELBS, Churchill Living Stone, pp. 317-323.

Hill, A.1996. Effec of Inoculum Spore Load. Length of Infection Period, and Leaf Washing on Occurrence of *Perononspora tabacina* Adam (blue mould) of Tobacco Australian Journal of Agricultural Research 17 (2): 133-146.

Livia, A. 1998. Lactic Acid Bacteria as Probiotics for Prevention and Cure of Gastrointestinal Disease in Man and Animal Karolinska Institute, Stockholm, pp. 23.

Moat, A. G. and Foster, J.W. 1995. Medical Microbiology, 3rd Edition John Willey and Sons, Inc. Publication, New York 20, pp. 306-319.

Murray, P., Baron. E., Paller, M., Tenover, F. and Yolken, R. 1995. Manual of Clinical Microbiology Sixth Edition ASM Press, Washington, pp. 1327-1334.

Nurhajati, J. Murad. S. and Atira. 2009. Effect of *Lactobacillus plantarum* FNCC 226 to Catfish (*Pangasius hypophthalmus*) Body Endurance from *Saprolegnia parasitica* : Paper Research. Department of Biology Padjadjaran University. Bandung.

Nurhajati, J., Nyoman and Atira. 2010. Administration *Lactobacillus plantarum* FNCC 226 on Catfish (*Pangasius hypophthamus*) againts *Saprolegnia parasitica* A3 : Paper Research. Department of Biology Padjadjaran University. Bandung.

Pleczar, M. J. and Chan. E.S.C. 1986. Dasar-dasar Mikrobiologi 1 (Principles of Microbiology 1) Jakarta : Universitas Indonesia Press (pp: 177, 189-190, 193-194, 196-198).

Pretzer, G., Pretzer, G., Snel, J., Molenaar,D., Wiersma, A., Bron, P.A., Lambert, J., de Vos, W.M., van der Meer,R., Smits, M.A. and Kleerebezem, M. 2005. Biodiversity-based identification and functional characterization of the Mannose-Specific Adhesin of *Lactobacillus plantarum*. Journal of Bacteriology 187 (17): 6128–6136.

Richard, J. A. 1996. Use of Bacteriocin Producing Starters Advantageously in Dairy Industry. in Lactic Acid Bacteria in Metabolism, Genetic and Application Bozoglu dan Ray, B.S. Verlag Berlin Heidelberg, New York: pp. 137-142.

Suriawiria, U. 2003. Mikrobiologi Air (Water Microbiology) Bandung: P.T. Alumni IKAPI ITB (pp. 12)

Susanto, H. and Amri, K.. 2002. Budidaya Ikan Patin (Catfish Farming) Jakarta: PT Penebar Swadaya (Publisher) (pp. 1-7, & 83-86).

Swann, L. 2001. A Basic Overview of Aquaculture, Water Quality, Types of of Aquacultures and Production Methods, Illinois-Indiana

Wahyuningsih, S. P. A. 2002. Pengendalian Saprolegniasis Pada Telur Ikan Nila Merah (*Oreochromis* sp.) Saprolegniasis Control in Red Tilapia Eggs (*Oreochromis* sp.) With Malachite Green Research Report Surabaya: Faculty of Mathematics and Exact Sciences, Airlangga University

West, P. V. 2006. *Saprolegnia parasitica*, an Oomycete Pathogen With a Fishy Appetite: New Challenges For an Old Problem. Journal of. Mycologist 20(3): 99-104.

Wood, B. J. B. 1999. The Lactic Acid Bacteria in Health and Disease Blackie Academic and Professional, London, pp. 387-397.