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

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**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 x 4 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 10$^5$ 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.

**Keywords**

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

**Article history**

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**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. parasitica* 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...
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 implemted 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% (IC50) 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 IC50. The treatment used S. parasitica zoospore 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°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 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 x 1.5 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 10^6 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), 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 identified by microscopic method according to Ainsworth et al. (1973) and Coker (1923).

Fig. 1C clearly shows the internal proliferation of L. plantarum inoculums 0 x MaxNLC, ¼ x MaxNLC and MaxNLC (7.7 x 10^5 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.
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 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
against 5 concentration, and was very significant when concentration and.

infected infection inoculums concentration.

2009). prevent Saprolegnia parasitica administration of the results of the preventive and curative study, the against various concentrations of treatment L. plantarum against S. parasitica to percentage nir-infection.

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 10⁷ cfu/mL – 8,4 x 10⁷ cfu/mL which can inhibit dose with infection concentration until 4x10⁷ zoospora/ml.

Acknowledgement

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Bibliography


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 10⁷ ) 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 against Saprolegnia parasitica proved that L. plantarum could prevent S. parasitica infection and this contribute to increased catfish body endurance (Nurhajati et al., 2009).

Table 3.

<table>
<thead>
<tr>
<th>L. plantarum</th>
<th>Without S. parasitica</th>
<th>With S. parasitica</th>
</tr>
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<tbody>
<tr>
<td>0xMaxNLC</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>½xMaxNLC</td>
<td>100%</td>
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<tr>
<td>MaxNLC</td>
<td>100%</td>
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Note: Similar letters in all directions indicate a no-difference at 1%

MaxNLC = Maximum Nit-Lethal Concentration IC₅₀ = Infectious Concentration 50%

4xIC₅₀ = 2,1 x 10⁷ zoospora/ml
2xIC₅₀ = 8,4 x 10⁷ zoospora/ml
½xIC₅₀ = 4,2 x 10⁷ zoospora/ml
IC₅₀ = 2 x 10⁷ zoospora/ml
IC₀ = 1 x 10⁷ zoospora/ml

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