Chapter – III: Investigation on virulence dose and selection of probiotics against 
*Aphanomyces invadans* and *Aeromonas hydrophila*

**Introduction**

The aquaculture industry is affected by many disease problems. Epizootic Ulcerative Syndrome (EUS) is one of the most important problems in aquaculture. Hatai *et al.*, (1977) and Willoughby *et al.*, (1995) reported *A. invadans* as the primary causative agent of EUS and the pathogenicity of *A. hydrophila* consistently associating with EUS affected fish (Lio Po *et al.*, 1992, Boonyaratpalin 1989). Dykstra *et al.*, (1986) have isolated *Aphanomyces sp* from ulcerative mycosis affected fish in the Eastern USA. Hatai (1994) noticed fish mortality and reported the susceptibility and resistance of 11 species of fish against *Aphanomyces* infection, while Khan *et al*. (1998) used progressive histopathological changes in tilapia (*Oreochromis niloticus*), rosy barb (*Puntius schwanenfeldi*), rainbow trout (*Oncorhynchus mykiss*), roach (*Rutilus rutilus*) and stickleback (*Gasterosteus aculeatus*) to demonstrate differential host susceptibility to the fungus.
Byers et al., (1986) have shown that *A. hydrophila* can produce siderophores that confer resistance against the ability of serum transferrin to inhibit bacterial growth. Many studies have been attempted further to describe the virulence mechanisms of motile aeromonads. Kou (1973) found that many of the virulent, avirulent, and attenuated aeromonads possessed hemorrhagic factors and lethal toxins. The virulent bacteria had quantitatively more toxic potential than the avirulent or attenuated counterparts. Oliver et al., (1981) indicated that both *A. hydrophila* and *A. sobria* produced enterotoxins, dermonecrotic factors, and hemolysins. Lio-po et al., (1998) have studied the biochemical characteristics of the EUS associated bacteria and tested their virulence on healthy snakeheads *C. striatus* and catfish *C. batrachus*.

Prevention and control of these diseases have been concentrated on good husbandry practices (Austin and Austin, 1993). The use of antibiotics to prevent and control bacterial diseases in aquaculture, have led to an increase in antibiotic-resistant bacteria (Alderman and Hastings, 1998; Teuber, 2001). The aquaculture industry worldwide faces issues related to antimicrobial use and the major issues deal with antimicrobial use in aquaculture leading to drug resistance. Therefore, alternative strategies to the use of antimicrobials, such as the use of probiotics have been proposed as biological control agents. The discovery and development of antimicrobial agents to treat systemic bacterial infections is one of the most fascinating stories in the history of microbiology (Sokatch and Ferretti, 1976). There have been many studies aimed at developing effective prophylactic methods for use in aquaculture as alternatives to chemotherapy (Smith et al., 1994, Writte et al., 1999).
Several testing methods, including disc diffusion, broth micro dilution, agar dilution and the E-test (AB Biodisk, Solna, Sweden) has been used to determine the *in vitro* susceptibilities of pathogenic bacteria to antimicrobial agents (Huang *et al*., 1992). Smith *et al*., (1994) reviewed the results of several studies performed all over the world on the sensitivity and resistance of bacterial pathogens of aquatic species to a variety of antibacterial agents. In fish, as in other aquatic organisms, the whole microorganisms have been mainly administered bacterial species in the form of feed additives. It has shown improvement in the intestinal microbial balance and the health status of fish by colonizing the gut and acting as antagonists to pathogens and so increasing resistance to pathogens (Gatesoupe, 1999; Fuller, 1989; Tannock, 1997). Plumb (1999) reported vaccines can not completely eliminate disease organisms or prevent the target organisms from being present in vaccinated populations. Thus, in order to treat the pathogen, several antimicrobial agents such as amoxacillin, ampicillin, chloramphenicol, erythromycin, flumequine, oxolinic acid, oxytetracycline, nitrofurazone, sulphadiazine-trimethoprim and tetracycline (Toranzo *et al*., 1991; Bakopoulos *et al*., 1995; Sano, 1998) have been used. However, over the last decade, drug-resistant strains carrying a transferable R-plasmid have developed (Takashima *et al*., 1985; Kim *et al*., 1993) making treatment with antimicrobial chemotherapeutics less successful. For a treatment to be effective, antimicrobial susceptibility experiments should be carried out to evaluate the susceptibility and resistance development to antimicrobial agents.

Several bacterial strains which are common members of the non-pathogenic microflora of fish are capable of inhibiting fish pathogenic bacteria and fungi *in vitro* assay and this has been demonstrated for lactic acid bacteria by Gatesoupe, (1994) and
Joborn et al., (1997). Smith and Davey (1993); Austin et al., (1995); Moriarty, (1998) and Gram et al., (1999) have found that the addition of antagonistic bacteria to the water results in reduction of number of fish pathogenic bacteria in water. Gram et al., (1999) reported that *P. fluorescens* AH2 was strongly inhibitory against *Vibrio anguillarum* in model systems and this effect could be transferred to an *in vivo* situation. Further they observed significantly reduced mortality in the experimental fish infected with *V. anguillarum* following the addition of probiotics to the tank water. Indeed there has already been intensive research on probiotics for use in aquaculture (Moriarty, 1998; Gatesoupe, 1994). Probiotics for human and terrestrial animals are mainly lactic acid bacteria (LAB) of different species and *Bacillus* sp (Gatesoupe, 1991, Gildberg et al., 1995, Gildberg and Mikkelsen, 1998; Irianto and Austin, 2002).

The present study deals with the determination of virulence dose (LD$_{50}$) of *A. hydrophila* and *A. invadans* on healthy catfish *H. fossilis* and to evaluate and compare the *in vitro* antimicrobial susceptibility using probiotics against *A. invadans* and *A. hydrophila* isolates.

**Materials and methods**

*Aphanomyces invadans*

**Taxonomical position**

- **Kingdom:** Chromista
- **Phylum:** Oomycota
- **Class:** Oomycetes
- **Subclass:** Incertae sedis
- **Order:** Saprolegniales
- **Family:** Leptolegniaceae
- **Genus:** Aphanomyces
- **Species:** Invadans
A. invadans is an oomycete (order Saprolegniales) which can be maintained readily in laboratory culture in its hyphal stage (Lilley et al., 1998). However this species lacks the usual sexual reproductive structures and is assigned to Aphanomyces on the basis of its pattern of asexual spore morphogenesis (Lilley et al., 1998). When exposed to freshwater or water with low salinity, the hyphae produce sporangia and diplanetic zoospores are formed. The secondary zoospores eventually form secondary cysts, which germinate to form new hyphae. Cyst stages are infectious because they attach to the intact skin and produce germination tubes that penetrate the skin (Kiryu et al., 2003). However inoculated zoospores also can result in disease (Blazer et al., 2002, Kiryu et al., 2002), although they likely have to form cysts before penetrating tissue.

Isolation of A. invadans

Moderate, pale, raised, dermal lesions are most suitable for fungal isolation attempts. The scales around the peripheral portion of the lesion were removed and the underlying skin was seared using a red-hot spatula for surface sterilization. Using a sterile scalpel blade and sterile fine-pointed forceps, a piece of muscle (2 mm³) was cut underlying the seared area and placed on a petridish containing Czapek Dox agar with penicillin G (100 units/ml) and oxolinic acid (100 µg/ml). The plates were sealed, incubated at room temperature and examined daily. The emerging hyphal tips were repeatedly transferred to fresh plates of Czapek Dox agar until cultures are free of contamination. The mother culture was examined daily with microscope for atleast 5 days and subculture was maintained (plate. 4 C). Recovered fungi were identified by sporulation features, hyphal diameter, growth rate at 22°C and failure to grow at 37°C.
**Aeromonas hydrophila**

**Taxonomical position**
- **Domain:** Bacteria
- **Kingdom:** Proteobacteria
- **Phylum:** Gammaproteobacteria
- **Class:** Aeromonadales
- **Genus:** Aeromonas
- **Species:** hydrophila

*A. hydrophila* is a ubiquitous, free-living, heterotrophic, Gram-negative bacterium mainly found in areas with a warm climate (plate. 4 D). It is motile bacilli with single flagellum that ferments glucose with or without the production of gas. It is prevalent in aquatic habitats with cosmopolitan distribution; it is an opportunistic pathogen that has resulted in heavy mortalities in farmed and wild fishes. This bacterium can also be found in fresh, salt, marine, estuarine, chlorinated and un-chlorinated water. *A. hydrophila* can survive in aerobic and anaerobic environments. This bacterium can digest materials such as gelatin, and hemoglobin.

**A. hydrophila growth studies**

The growth pattern of selected *A. hydrophila* was studied in detail. *A. hydrophila* is easily cultured using Aeromonas isolation agar. Growth of the bacterial cells was measured by direct count using Haemocytometer and Total plate count method (Lakshmanan *et al.*, 1971). The number of cells was calculated after measuring the sample intensity or cell count at intervals of 0, 3, 6, 9, 12, 15, 18, 24, 48 and 72 hrs after inoculation of the cells in the fresh medium and the cells were harvested by centrifugation at 5000rpm for 15 min. The pellet was serially diluted and total count was
taken using neubaur counting chamber. For viable count 0.1 ml from the dilution, was spread plated on agar plates, incubated at 37°C for 24 hrs and the colonies were counted.

**Determination of LD$_{50}$ Value of A. invadans and A. hydrophila on H. fossilis**

The *H. fossilis* of average length 20 ± 3 cm and average weight 65 ± 2.5g were randomly selected and distributed into 3m x 1.5m x 1m cement tank filled with well water at the stocking rate of 10 fingerlings per tank separately for *A. invadans* and *A. hydrophila* treatments. Triplicates were maintained for each treatment for a period of 10 days and mortalities were recorded. To find out the LD$_{50}$ value of *A. invadans* (viable spores) and *A. hydrophila*, 18 hrs old broth culture (logarithmic phase) containing different loads of bacteria in physiological saline (0.85% Nacl; pH 7.2) were inoculated intraperitoneally. Ten fishes were administered with the dose of *A. invadans* ($10^2$ to $10^8$) and *A. hydrophila* ($10^3$ to $10^9$) cells per 0.2 ml. The LD$_{50}$ value was calculated following Reed and Muench (1938). The fish were observed carefully for visible external symptoms and behavioral changes. Time taken to lose the balance and the individual death were noted. The fish were considered to be dead when there was no opercular movement. The number of dead fish was noted and their individual length and weight were recorded. The mortality of the challenged fish was recorded and death due to *A. invadans* and *A. hydrophila* was confirmed by re isolation of organism from the liver, spleen, body fluids and intestine.

**Antagonistic activity of probiotic bacteria against A. invadans and A. hydrophila**

Antagonistic activity of *B. subtilis*, *B. coagulans*, *B. licheniformis*, *Saccharomyces cerevisiae*, *P. fluorescens* and *Lactobacillus acidophilus* against target fungi *A. invadans* and bacteria *A. hydrophila* were assessed by well diffusion assay. For
A. **hydrophila** antagonistic activity was performed using the plates containing solidified Muller Hinton agar (20 ml) and inoculated with 0.5 ml of overnight culture of *A. hydrophila* (10⁶ cfu/ml). A well having six mm diameter was made in the agar using cork borer and 50 µl of culture supernatant of *B. subtilis, B. coagulans, B. licheniformis, S. cerevisiae, P. fluorescens* and *L. acidophilus* were transferred into each well. The bacterial plates were incubated for 18 hrs at 37⁰C in aerobic environment and width of the zone of incubation (mm) was measured (Jin *et al.*, 1996). In case of *A. invadans* similar method was followed using Czapek Dox Agar and the inoculated plates were incubated at 25⁰C for 72hrs. The MIC were observed and recorded.

**Results**

*A. invadans* and *A. hydrophila* injected test fishes showed slight to severe dermomuscular lesions. *A. invadans* concentrations of 10⁸ cfu/ml and 10⁷ cfu/ml injected fish showed 100% mortality. They produced severe necrotic lesions in infected tissues and at end of the trial they lost the layer of skin and all the individuals died. *A. hydrophila* (10⁶ cfu/ml) injected fishes showed 89.47 % mortality in this concentration and severe lesions and wound were noticed in the infected portions. The injured tail showed reddish and loss of skin layer was observed. 10⁵cfu/ml dose injected fish showed 56.25 % cumulative mortality. They showed slight lesions and swelling on the infected portion. No mortality was found in 10²cfu/ml and 10³cfu/ml concentration injected fishes. The determined LD₅₀ was 7.9 x 10⁵cfu/ml for *A. invadans* (Table 3.1).

*A. hydrophila* injected fishes showed reddening and swelling at the site of infection and immediate changes was noticed at 7hr and twelve hrs with 10⁹ cfu/ml, 10⁸ cfu/ml and 10⁷ cfu/ml concentrations and cumulative mortalities were 100 %, 96.66 %
and 83.33 % respectively. Initially slight lesion was produced and it developed as a blanched area with slight swelling and then deep lesion was observed (plate. 3 A to F). In 10^6 cfu/ml dose injected fishes 59.09 % mortality was observed. The determined LD_{50} was 2.4 x 10^6 cfu/ml for *A. hydrophila* (Table. 3.2). No mortality was found in 10^3 cfu/ml injected fishes but swelling and mild lesion were observed (plate. 4 A and B).

The present study was attempted to find out the antagonistic activity of selected probiotics against EUS causative pathogens *A. invadans* and *A. hydrophila*. After the incubation period zones of inhibition were recorded. *B. subtilis*, *B. coagulans*, *S. cerevisiae*, *B. licheniformis* *P. fluorescens* and *L. acidophilus* exhibited zones of inhibition against *A. hydrophila* (10^6 cfu/ml) and *B. subtilis*, *B. coagulans* and *L. acidophilus* exhibited zones of inhibition against *A. invadans* (10^5 cfu/ml). The highest zone of inhibition was recorded by *B. subtilis* (12 ± 0.2 mm) followed by *B. coagulans* (10 ± 0.7 mm), *L. acidophilus* (9 ± 0.3 mm) , *S. cerevisiae* (4 ± 0.7mm), *P. fluorescens* (2 ± 0.5 mm) and *B. licheniformis* (2 ± 0.2 mm) against *A. hydrophila* (plate. 4 F). In case of *A. invadans*, the highest zone of inhibition was recorded by *B. subtilis* (7 ± 0.6 mm) followed by *B. coagulans* (6 ± 0.5 mm) and *L. acidophilus* (5 ± 0.8 mm) (Table 3.3) (plate. 4 E). *B. licheniformis*, *S. cerevisiae* and *P. fluorescens* didn’t produce zones of inhibition against *A. invadans*. In the case of *B. subtilis*, *B. coagulans* and *L. acidophilus* zones of inhibition were observed against both pathogens *A. invadans* and *A. hydrophila*.

**Discussion**

The present study showed differences in susceptibility of *H. fossilis* to *A. invadans* and *A. hydrophila*. *A. invadans* and *A. hydrophila* have been consistently associated with EUS and the pathogenicity of EUS susceptible fish has already been
reported (Lilley et al., 1998; Lio-Po et al., 1992). In our study *A. invadans* concentrations $10^8$ cfu/ml and $10^7$ cfu/ml injected test fish showed 100% mortality. The LD$_{50}$ was $7.9 \times 10^5$ cfu/ml for *A. invadans*. Hatai (1994) injected goldfish with 5000 spores/fish while Wada et al., (1996) injected common scarp, *C. carpio*, with 3000 spores. However, initial natural challenges are unlikely to be of such magnitude under field conditions and therefore, they developed a method of reproducing EUS which uses more realistic numbers of infective zoospores of *A. invadans* given by intramuscular injection at approximately the same and they found mortality at higher level. *A. invadans* ($10^5$ cfu/ml) administered fishes showed moderate, pale, raised, dermal lesions. Similarly Kiryu et al., (2003) reported the cyst stages are infectious and they attach to the intact skin and produce germination tubes that penetrate the skin and produce lesions and their findings supported our study.

In the present study severe lesions were observed following *A. hydrophila* administration in $10^9$ cfu/ml during the LD$_{50}$ assay. Similarly Lio-po et al., (1998) stated *A. hydrophila* injected intramuscularly at a concentration of $10^9$ cfu/ml induced severe dermomuscular necrotic lesions in both catfish and snakeheads. Khail and Mansour (1997) found that *A. hydrophila* was found to produce haemolytic and proteolytic exotoxin, that are lethal to tilapia and the LD$_{50}$ value was $2.1 \times 10^4$ cells/fish. The lethal effect was also attributed to the unknown virulent factors that were responsible for 20% mortality. Lipton (1987) observed that *P. aeruginosa* had a lethal dose of $1.5 \times 10^5$ cfu/ml for *C. carpio* and $4.2 \times 10^5$ for *O. mossambicus* and *A. hydrophila* had $2.1 \times 10^6$ cfu/ml and $3.2 \times 10^6$ cfu/ml for *C. carpio* and *O. mossambicus*. 
In the antagonistic study, \textit{B. subtilis} (7 ± 0.6 mm) and \textit{B. coagulans} (6 ± 0.5 mm) showed maximum zones of inhibition against \textit{A. invadans}. Similarly Matsumo \textit{et al.}, (1990) and Shigeru \textit{et al.}, (1996) reported that many species of \textit{Bacillus} are capable of producing biologically active substances able to disintegrate fungal cell walls. Podile and Parkash (1996) examined \textit{B. subtilis} strain AF1 which produced extracellular protein that disintegrates fungal cell walls by lysis of chitin. Similar results were obtained by Gulewicz and Trojanowska (1995), who observed the susceptibility of \textit{Aspergillus niger} when \textit{B. subtilis} AF1 was added within 12 h of the growth of fungi. Itami \textit{et al.}, (1998) reported that \textit{Bacillus} sp surface antigens or their metabolites act as immunogens for shrimp by stimulating phagocytic activity of granulocytes. Gulewicz and Trojanowska (1995) isolated active lupine from strains of the \textit{Bacillus} sp, the majority of which showed antifungal properties.

\textit{L. acidophilus} (5 ± 0.8 mm) showed minimum inhibition against \textit{A. invadans} when compared with \textit{B. subtilis} and \textit{B. coagulans}. Among the probiotics \textit{B. licheniformis}, \textit{S. cerevisiae} and \textit{P. fluorescens} did not produce zone of inhibition against \textit{A. invadans}. In the present investigation \textit{B. subtilis}, \textit{B. coagulans}, \textit{L. acidophilus}, \textit{S. cerevisiae}, \textit{B. licheniformis} and \textit{P. fluorescens} produced zones of inhibition against \textit{A. hydrophila}. Among the probiotics \textit{B. subtilis} (12 ± 0.2 mm) and \textit{B. coagulans} (10 ± 0.7 mm) produced maximum zone of inhibition against \textit{A. hydrophila}. \textit{Bacillus sp} has produced secondary metabolite of extracellular compounds such as bacteriocin, hydrogen peroxidase and other organic acids (Klaenhammer, 1988; Daeschel, 1989) and also produced inhibited pathogenic bacteria in fish and shellfish by successful colonization in the gut of the host (Gatesoupe, 1999; Irianto and Austin, 2002). Skjermo and Vadstein
(1999), Rengipipat et al., (2000) also reported that Bacillus spores have been used as biocontrol agents to reduce Vibrio sp in shrimp culture practices. Vaseeharan and Ramasamy (2003) investigated the inhibitory activity of B. subtilis BT23, isolated from shrimp culture ponds, against pathogenic Vibrio harveyi under in vitro and in vivo conditions.

L. acidophilus showed maximum zone of inhibition (9 ±0.3mm) against A. hydrophila. Similarly Manohar (2005) has found the maximum antagonistic activity of L. bulgaricus (5.5 ± 0.6 mm) and L. acidophilus (5.3 ± 0.5 mm) against A. hydrophila. Griffith (1995) reported the effect of Lactic Acid Bacteria in increasing disease resistance to Vibrio pathogens in shrimps. Mishra and Lambert, (1996) also reported the maximum antagonistic activity of Lactobacillus sp against different pathogenic organisms like Escherichia coli, Staphylococcus aureus and Enterococcus faecalis. Among the probiotics, B. subtilis, B. coagulans and L. acidophilus only produced zone of inhibition against A. invadans and A. hydrophila. Hence these three probiotics were chosen for the growth performance and disease challenge of H. fossilis.
Table 3.1 Determination of LD$_{50}$ for virulent *Aphanomyces invadans* in *H. fossilis* by intraperitonial route (Read and Muench, 1938).

<table>
<thead>
<tr>
<th>No. of fungal spore (cfu/ml)</th>
<th>Initial number</th>
<th>Died</th>
<th>Survived</th>
<th>Dead ratio</th>
<th>Survival ratio</th>
<th>Mortality</th>
<th>Cumulative mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^8$</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>37</td>
<td>0</td>
<td>37/37</td>
<td>100.00</td>
</tr>
<tr>
<td>$10^7$</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>27</td>
<td>0</td>
<td>27/27</td>
<td>100.00</td>
</tr>
<tr>
<td>$10^6$</td>
<td>10</td>
<td>8</td>
<td>2</td>
<td>17</td>
<td>2</td>
<td>17/19</td>
<td>89.47</td>
</tr>
<tr>
<td>$10^5$</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>9</td>
<td>7</td>
<td>9/16</td>
<td>56.25</td>
</tr>
<tr>
<td>$10^4$</td>
<td>10</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>13</td>
<td>4/17</td>
<td>23.52</td>
</tr>
<tr>
<td>$10^3$</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>23</td>
<td>0/23</td>
<td>0.00</td>
</tr>
<tr>
<td>$10^2$</td>
<td>10</td>
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<td>10</td>
<td>0</td>
<td>33</td>
<td>0/33</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Proportionate distance = \[\frac{\text{Mortality above 50\%} - 50}{\text{Mortality above 50\%} - \text{Mortality below 50\%}}\]

\[
\frac{56.25 - 50.00}{56.25 - 23.52} = \frac{6.25}{32.73} = 0.19
\]

LD$_{50} =$ Dilution above 50\% - Proportionate distance

\[= 5 + 0.19 = 5.19\]

Antilog 5.19 = \[7.9 \times 10^5\]

LD$_{50} = 7.9 \times 10^5$
Table 3.2 Determination of LD$_{50}$ for virulent *Aeromonas hydrophila* in *H. fossilis* by intraperitonial route (Read and Muench, 1938).

<table>
<thead>
<tr>
<th>No. of bacterial cells (cfu/ml)</th>
<th>Initial number</th>
<th>Died</th>
<th>Survived</th>
<th>Dead ratio</th>
<th>Survival ratio</th>
<th>Mortality</th>
<th>Cumulative mortality (%)</th>
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<td>100.00</td>
</tr>
<tr>
<td>$10^8$</td>
<td>10</td>
<td>9</td>
<td>1</td>
<td>29</td>
<td>1</td>
<td>29/30</td>
<td>96.66</td>
</tr>
<tr>
<td>$10^7$</td>
<td>10</td>
<td>7</td>
<td>3</td>
<td>20</td>
<td>4</td>
<td>20/24</td>
<td>83.33</td>
</tr>
<tr>
<td>$10^6$</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>13</td>
<td>9</td>
<td>13/22</td>
<td>59.09</td>
</tr>
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<td>5</td>
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<td>7</td>
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<td>3/24</td>
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</tr>
<tr>
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<td>10</td>
<td>0</td>
<td>31</td>
<td>0/31</td>
<td>0.00</td>
</tr>
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</table>

Proportionate distance = \[
\frac{\text{Mortality above 50\%} - 50}{\text{Mortality above 50\%} - \text{Mortality below 50\%}}
\]

Mortality above 50\% - Mortality below 50\%

\[
59.09 - 50 / 59.09 - 36.36
\]

\[
9.09 / 22.73 = 0.39
\]

LD$_{50}$ = Dilution above 50\% - Proportionate distance

\[
= 6 + 0.39 = 6.39
\]

Antilog 6.39 = \[2.4 \times 10^6\]

LD$_{50}$ = \[2.4 \times 10^6\]
Table 3.3 Antimicrobial activities of probiotics against *A. hydrophila* and *A. invadans* by agar well diffusion method. Values are given as mean and ± indicates standard deviation.

<table>
<thead>
<tr>
<th>Probiotics</th>
<th>Zone of inhibition</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td><em>A. hydrophila</em></td>
<td><em>A. invadans</em></td>
<td></td>
</tr>
<tr>
<td><em>B. subtilis</em></td>
<td>12 ± 0.2mm</td>
<td>7 ± 0.6mm</td>
<td></td>
</tr>
<tr>
<td><em>B. lichniformis</em></td>
<td>2 ± 0.2mm</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>B. coagulans</em></td>
<td>10 ± 0.7mm</td>
<td>6 ± 0.5mm</td>
<td></td>
</tr>
<tr>
<td><em>L. acidophilus</em></td>
<td>9 ± 0.3mm</td>
<td>5 ± 0.8mm</td>
<td></td>
</tr>
<tr>
<td><em>S. cerevisiae</em></td>
<td>4 ± 0.7mm</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>2 ± 0.5 mm</td>
<td>-</td>
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