PROBIOTICS TECHNOLOGY: APPLICATION FOR FISH AND PRAWN

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AQUACULTURE

- Global fish production - more than 30% between 2006-2011 or 47.3 million tons to 63.6 million
- Shrimp farming - in the Asian countries 91% of the world food production.
Many fisheries have reached their maximum sustainable exploitation.

Concerns about security and safety of food.

High demand for high quality, low calorie, high protein aquatic products.

Minimum carbon dioxide emission.
Challenges in Aquaculture

Pollution

Diseases

Low productivity
PROBIOTICS represent one of the most promising alternatives to antimicrobials developed in recent years.
PROBIOTICS IN AQUACULTURE

- live microorganisms that have a beneficial effect on the host by modifying the microbial community associated with the host.

- ensure improved use of the feed or enhance its nutritional value

- enhance the host response towards disease

- improve the quality of its ambient environment
Microorganisms with Probiotic Properties

- Lactobacillus spp.
- Bacillus spp.
- Bifidobacterium spp.
- Saccharomyces spp.
- Streptococcus spp.
- Pediococcus spp.
- Leuconostoc spp.
- Streptomyces spp.
COMMERCIAL PREPARATIONS

(Available in liquid or powder form)

Global Market for Probiotics -
US $ 19,600 million in 2013
Annual Growth rate- 4.3%

BIOSTART
YIELD
ENVIRON-AC
AQUA-PS
OBJECTIVES

1. Develop a probiotic product using characterized and established probiotic strains.
2. Establish process technology
3. Evaluate product quality
Production of Probiotics
Batch Process of Powder Probiotic Production

Microbial Strain → Seed Medium → Propagation → Seed inoculant → Fermentation Medium → BIOREACTOR

- Cultivation
- Conditioning
- Formulation
- Drying
- Labeling
- Powder Probiotics
Production of Probiotics

Batch Process of Liquid Probiotic Production

- Microbial Strain → Seed Medium → Propagation → Seed inoculant
- Fermentation Medium → BIOREACTOR → Cultivation → Conditioning → Formulation → Bottling → Labeling → Liquid Probiotics
SELECTION OF PROBIOTIC STRAINS

Screening of Microorganisms

- Biomass Determination
- Lactic Acid Concentration
- Co-culture Screening Tests
- Test for Hemolysis
- Well Diffusion Assay

Sources

- As cited in literature
- Stocks from laboratory & local culture collections (PNCM)
PROBIOTIC PRODUCTS

Aquaculture probiotics in liquid and powder form produced using the five strains that passed the final screening procedures.
# PROPERTIES

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Powder Probiotics</th>
<th>Liquid Probiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live Microbial Cell count</td>
<td>Trillion cfu/g</td>
<td>$1.25 \times 10^{13}$</td>
</tr>
<tr>
<td>Appearance</td>
<td>Free flowing white powder</td>
<td>Viscous, beige-colored liquid</td>
</tr>
<tr>
<td>odor</td>
<td>Milky sweet</td>
<td>Milky sweet</td>
</tr>
<tr>
<td>Moisture</td>
<td>10%</td>
<td>NA</td>
</tr>
<tr>
<td>pH</td>
<td>Neutral</td>
<td>4.7</td>
</tr>
</tbody>
</table>
EVALUATION OF PERFORMANCE
Laboratory feeding trial: Red Tilapia (*Oreochromis* spp.)

- Control
- 2% Liquid
- 2% Powder
FEEDING TRIALS IN FISH AND PRAWN

AQUAPROBIOTICS

Batch # ______________  Date ______________
National Institute of Molecular Biology and Biotechnology (BIOTECH)
U.P. Los Baños
# Feeding Trial on Bangus

<table>
<thead>
<tr>
<th>PARAMETERS/ POND #</th>
<th>34 (CONTROL)</th>
<th>38 (2% PROBIOTICS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Stocked</td>
<td>08/13/13</td>
<td>08/22/13</td>
</tr>
<tr>
<td>Date Harvested</td>
<td>01/12/14</td>
<td>01/14/14</td>
</tr>
<tr>
<td>Area</td>
<td>4,258 m²</td>
<td>4,258 m²</td>
</tr>
<tr>
<td>Population</td>
<td>550,000 pcs</td>
<td>550,000 pcs</td>
</tr>
<tr>
<td>ABW</td>
<td>37.58 g</td>
<td>35 g</td>
</tr>
<tr>
<td>Biomass</td>
<td>2,261.10 kg</td>
<td>4,702.10 kg</td>
</tr>
<tr>
<td>D.O.C.</td>
<td>134 days</td>
<td>156 days</td>
</tr>
<tr>
<td>FCR</td>
<td>3.58</td>
<td>2.49</td>
</tr>
<tr>
<td>Pieces Harvested</td>
<td>56,700 pcs</td>
<td>182,740 pcs</td>
</tr>
<tr>
<td>Survival</td>
<td>11.23%</td>
<td>33.23%</td>
</tr>
</tbody>
</table>
Feeding trial on Bangus (pond)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>2% Probiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCR</td>
<td>3.58</td>
<td>2.49</td>
</tr>
<tr>
<td>ABW, g</td>
<td>37.58</td>
<td>35.00</td>
</tr>
<tr>
<td>% Survival</td>
<td>11.23</td>
<td>33.23</td>
</tr>
<tr>
<td>Biomass, kg</td>
<td>4702</td>
<td>2261</td>
</tr>
</tbody>
</table>
Feeding Trial on *Penaeus vannamei* (Intensive Culture)

<table>
<thead>
<tr>
<th>PARAMETERS/ POND #</th>
<th>8 (CONTROL)</th>
<th>9 (2% PROBIOTICS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Stocked</td>
<td>11/20/13</td>
<td>11/18/13</td>
</tr>
<tr>
<td>Date Harvested</td>
<td>12/22/13</td>
<td>12/22/13</td>
</tr>
<tr>
<td>Population</td>
<td>418,176 pcs</td>
<td>456,190 pcs</td>
</tr>
<tr>
<td>ABW</td>
<td>15.06 g</td>
<td>9.48 g</td>
</tr>
<tr>
<td>Biomass</td>
<td>4,934.50 kg</td>
<td>861 kg</td>
</tr>
<tr>
<td>D.O.C.</td>
<td>94</td>
<td>96</td>
</tr>
<tr>
<td>FCR</td>
<td>1.528</td>
<td>6.13</td>
</tr>
<tr>
<td>Survival</td>
<td>78.30%</td>
<td>19.90%</td>
</tr>
<tr>
<td>Total Feeds</td>
<td>7,541.20 kg</td>
<td>5,282.80 kg</td>
</tr>
</tbody>
</table>
# Feeding Trial on *Penaeus monodon* (Extensive Culture)

<table>
<thead>
<tr>
<th>PARAMETERS/ POND #</th>
<th>CONTROL</th>
<th>ZOLETA’S FARM</th>
<th>AQUA PROBIOTICS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Stocked</td>
<td>03/15/14</td>
<td>03/15/14</td>
<td>03/15/14</td>
</tr>
<tr>
<td>ABW</td>
<td>18.20 g</td>
<td>22.30 g</td>
<td>23.90 g</td>
</tr>
<tr>
<td>ABL</td>
<td>13.60</td>
<td>14.40 cm</td>
<td>14.60 cm</td>
</tr>
<tr>
<td>Survival</td>
<td>41.8%</td>
<td>54.8%</td>
<td>59.40%</td>
</tr>
</tbody>
</table>
Average Body Weight

ABW, g

Days of Culture

Control
Zoleta's Farm Probiotics
Aqua Probiotics
Survival Rate (%)

- Aqua Probiotics: 59.4%
- Control: 41.8%
- Zoleta's Farm Probiotics: 54.8%
## Storage Studies – Powder Probiotics

<table>
<thead>
<tr>
<th>Months of Storage</th>
<th>Average Live Microbial Counts (CFU/mL)</th>
<th>Room</th>
<th>Refrigerator</th>
<th>Freezer</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td></td>
<td>4.00x10^{16}</td>
<td>1.87x10^{16}</td>
<td>1.91x10^{16}</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>3.62x10^{17}</td>
<td>1.66x10^{16}</td>
<td>8.06x10^{15}</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>7.80x10^{14}</td>
<td>5.70x10^{13}</td>
<td>4.64x10^{14}</td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>2.37x10^{15}</td>
<td>1.14x10^{15}</td>
<td>1.21x10^{15}</td>
</tr>
</tbody>
</table>
### Storage Studies – Liquid Probiotics

<table>
<thead>
<tr>
<th>Months of Storage</th>
<th>Average Live Microbial Counts (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Room</td>
</tr>
<tr>
<td>3</td>
<td>$9.00 \times 10^{16}$</td>
</tr>
<tr>
<td>6</td>
<td>$1.48 \times 10^{16}$</td>
</tr>
</tbody>
</table>
A probiotic product was developed for the aquaculture industry. This probiotic product offers attractive windows of opportunities to reduce the importation of feed additives such as antibiotics. The process technology is continuously improved for more competitive products that will meet the demands of continuous growth.
THANK YOU

PCIERD, DOST
OVERSEA FEEDS
ZOLETA’S FARM

THE PROBIOTICS TEAM