PROBIOTIC EFFECTS OF BACTERIA ON THE GROWTH OF THE ROTIFER BRACHIONUS Plicatilis IN CULTURE.

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ABSTRACT

Thirty bacterial strains isolated from culture tanks of the rotifer Brachionus plicatilis were screened for probiotic effects on rotifer growth. The bacterial inoculum supplied was 10⁶ cells/ml. Consecutive experiments demonstrate that some bacterial strains repeatedly increase the rotifer growth rate. Significant growth effects were obtained with Enterococcus faecium, a probiotic for terrestrial organisms. Upscaling tests in 90 l tanks demonstrate that rotifer growth promotion can be induced with E. faecium under operational rotifer culture conditions.

INTRODUCTION

Yeast are commonly used as food for rotifer mass culture. It is hypothesized that a diet of yeast cells only meets the nutritional requirements of the rotifers in the presence of the accompanying microflora. Such a complementary nutritive effect has been demonstrated with vitamin B₉₉-producing bacteria, isolated from rotifer culture tanks (Yu et al., 1988). Positive growth effects in rotifer culture have also been reported by Gatesoupe (1991), using Lactobacillus spp. from commercial probiotics.

In this study, several bacterial strains isolated from rotifer culture tanks as well as Enterococcus faecium, a probiotic for terrestrial organisms, were tested for positive growth effects on Brachionus plicatilis. The aim of this work is to evaluate the possible probiotic effect of these selected bacteria in commercial rotifer culture.

MATERIAL & METHODS

Thirty bacterial strains isolated from rotifer culture tanks were screened for growth effects on rotifers by means of 0.5-liter cone tests, using Culture Selco (CS, Artemia Systems NV, Belgium) as a rotifer diet. The bacterial density used for inoculation was 10⁶ CFU per ml. Four growth promoting strains, three strains with no effect and three strains causing a population crash were submitted to five consecutive growth tests to confirm the observed effects. E. faecium was tested and yielded a positive growth effect on rotifers as well. E. faecium was further tested out as a monodiet and compared to CS under different feeding regimes. Supplementation of CS with increasing concentration of E. faecium was studied for two feeding regimes. Finally, growth experiments were repeated in 90-liter culture tanks under operational rotifer culture conditions.

In all experiments, initial rotifer density was 100 rot.ml⁻¹. After three days, the rotifer growth rates were calculated using the following formula:

\[ r = \frac{(\ln N_t - \ln N_0)}{\Delta t} \]

(\( r \) = intrinsic growth rate, \( N_0 \) = initial rotifer density, \( N_t \) = rotifer density at day \( t \), \( \Delta t \) = culture period in days)

The number of replicates was three per treatment. The controls were set up without addition of bacteria.
RESULTS

Of the 30 bacterial strains initially tested, 15 strains were able to promote rotifer growth, 11 strains gave no growth effect and three strains were found to cause a population crash. All effects initially observed in the 10 selected strains (growth promotion, no effect or crash) could be confirmed (Table I). Of the four strains that promoted rotifer growth, one strain was identified as Vibrio alginolyticus. All strains causing crashes were identified as V. anguillarum.

When cone cultures of rotifers were provided with E. faecium as a single food source, growth rates were inferior as compared to cultures fed on CS (Fig.1). The growth of the rotifers fed CS was improved when the diet was supplemented with E. faecium (Fig.2). The growth promotion was very significant when the bacterial density was $10^7$-$10^9$ cells/ml. The highest growth rate ($r = 0.69$) was obtained when a feeding regime of 0.5g CS/10$^5$ rotifers.day was supplemented with 10$^5$ cells/ml: rotifer densities increased from 100/ml up to 800/ml in a 3-days period. Upscaling tests gave confirmation that growth promotion is possible under operational culture conditions (Fig.3). Highest growth rates were obtained with an inoculum of E. faecium at 10$^5$ cells/ml. For a culture period of three days, the amount of E. faecium accounted for 25% of the total amount of food administered.

Table I. Reliability of growth effects during 5 consecutive cone tests: average rotifer growth rate ($r$) of ten selected strains with positive growth effect (+), no effect (0) or negative effect (crash) on rotifer culture. Levels of significance according to the results obtained with Student’s T-test on the paired observations.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Identification</th>
<th>r</th>
<th>stdv</th>
<th>growth effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.22</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td>TR 28</td>
<td>V. alginolyticus</td>
<td>0.29</td>
<td>0.05</td>
<td>+</td>
</tr>
<tr>
<td>TR 23</td>
<td>V. alginolyticus</td>
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<td>0.09</td>
<td>0</td>
</tr>
<tr>
<td>TR 8</td>
<td>Vibrio sp.</td>
<td>0.24</td>
<td>0.08</td>
<td>0</td>
</tr>
<tr>
<td>TR 29</td>
<td>Vibrio sp.</td>
<td>0.28</td>
<td>0.07</td>
<td>+</td>
</tr>
<tr>
<td>TR 16</td>
<td>n.i.</td>
<td>0.28</td>
<td>0.08</td>
<td>+</td>
</tr>
<tr>
<td>TR 17</td>
<td>n.i.</td>
<td>0.22</td>
<td>0.06</td>
<td>0</td>
</tr>
<tr>
<td>TR 13</td>
<td>V. anguillaran</td>
<td>crash**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR 25</td>
<td>V. anguillaran</td>
<td>crash**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TR 27</td>
<td>V. anguillaran</td>
<td>crash**</td>
<td></td>
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</tr>
</tbody>
</table>

(n.i. = not identified; stdv = standard deviation; * = p<0.05; ** = p<0.01)
Fig. 1. Comparison of *E. faecium* with CS as sole food source for rotifers. Relationship between rotifer growth rate after 3 days cone culture and 4 different feeding regimes.

Fig. 2. Complementarity of *E. faecium* to CS. Relationship between rotifer growth rate after 3 days cone culture and inoculation density of *E. faecium*, for two feeding regimes.

Fig. 3. Growth promotion of *E. faecium* in 90 liter tanks. Relationship between rotifer growth rate after 3 days culture and inoculation density of *E. faecium*. 
DISCUSSION

50% of the bacterial strains tested in our experiments had potential to promote rotifer growth. Some of the strains were identified as Vibrio spp.. Yu et al. (1988, 1989) demonstrated positive growth effects in 19 out of 100 bacterial strains, and characterised 8 strains as Pseudomonas spp.. It can be concluded that rotifer growth promotion is not restricted to a few bacterial species. Moreover, Lactobacillus spp., alien to the rotifer environment, have been reported to provoke similar growth effects (Gatesoupe, 1991). The experiments with E. faecium demonstrate that this probiotic can be readily applied in operational rotifer culture, especially when fast development of rotifers is required.

REFERENCES

