Analysis of the evolution of microbial communities associated with different cultures of rotifer strains belonging to different cryptic species of the *Brachionus plicatilis* species complex

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**Abstract**

The evolution of the composition of microbial communities associated with cultures of 3 different strains belonging to different cryptic species of the rotifer *Brachionus plicatilis* was monitored during four subsequent cycles of batch cultivation using denaturing gradient gel electrophoresis, cluster analysis, multidimensional scaling and principal component analysis. The data suggest that the evolving microbial communities are different with different *B. plicatilis* strain cultures. Moreover, large changes in rotifer growth rate were found to be associated with large changes in the microbial community composition, suggesting that there might be a causal link. Finally, Lorenz curves and Gini-coefficient analysis revealed that good performing *B. plicatilis* cultures showed a more even microbial community structure.

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**1. Introduction**

Rotifers (*Brachionus* spp.) have been used as a live food for feeding larval marine fishes for over 30 years (Yúfera, 2001). Today, more than 60 marine fish species and 18 crustacean species require adequate and reliable production of high-quality, nutritious rotifers. The success of rotifer mass cultures is determined not only by reproduction rate and density, but also by their nutritional composition and their associated microbiota (Dhert, 1996; Dhert et al., 2001).

Batch cultivation, due to its simplicity is probably the most common type of rotifer production in marine fish hatcheries (Lubzens et al., 2001). Initially, rotifers are introduced at low density into tanks or ponds. The culture strategy consists of either the maintenance of a constant culture volume with an increasing rotifer density or the maintenance of a constant rotifer density by increasing the culture volume. Rotifers are fed with microalgae, bakers’ yeast or an artificial diet. A total harvest of the rotifers is applied with part of the rotifers used as food for fish larvae and part used as inoculum for the next culture (Lubzens et al., 1989). Using an artificial diet (e.g. Culture Selco®), the density at harvest time is about 600 rotifers ml\(^{-1}\) after four days of culture starting from 200 to 250 rotifers ml\(^{-1}\) (Suantika et al., 2000).

Although they are frequently used, batch culture systems generate highly variable conditions that can have an influence on growth performance that also affects the composition of associated microbial communities (Rombaut et al., 2001). Bacteria are always associated with mass production of rotifers and may cause unexpected mortality or suppressed growth of rotifers. Moreover, since they are used as the first food of larvae, rotifers are often suspected as vectors of potential harmful bacteria to the cultured animals (Dhert et al., 2001). Using conventional culture-based methods, the dominant bacterial groups in rotifer cultures were classified as *Pseudomonas*, *Vibrio* and *Aeromonas* spp. (Nicolas et al., 1989; Skjermo and Vadstein, 1993; Verdonck et al., 1997). Large variations in the number of rotifer-associated \((1.8–7.6 \times 10^3\) CFU rotifer\(^{-1}\) and free-living bacteria \((0.6–25 \times 10^{7}\) CFU ml\(^{-1}\) have been observed (Skjermo and Vadstein, 1993). Rombaut et al. (2001) described the evolving microbial community present in rotifer batch and recirculation systems by means of the molecular biological technique DGGE. The authors found that in a recirculation system, subsequent to a high-variable period, a climax community was established, which remained more or less stable and was characterized by the dominance of one bacterial genus, *Marinomonas*. In contrast to the recirculation system, the profiles of the microbial community present in the batch system were more susceptible to variation.

Especially in relation to microbial communities associated with live food, it is important to study microbial communities associated with closely related hosts. A recent study found that different cryptic *B. plicatilis* strains exist within aquaculture hatcheries (Papakostas...
et al., 2006). It is likely that many previous studies were done with unknown and different strains of the *B. plicatilis* complex or even mixtures of species. Therefore, the characterization of microbial communities of rotifer cultures needs to be done with clear knowledge of the rotifer species.

To address the questions whether cultures of different cyanobacteria *B. plicatilis* strains have different associated microbial communities, and how the microbial communities are evolving in consecutive batch cultures, DGGE fingerprints of PCR-amplified 16S rRNA gene fragments were made and analyzed with a series of ecological tools.

### 2. Materials and methods

#### 2.1. Rotifer strains

Experiments were performed with 3 different cryptic *B. plicatilis* strains, namely *B. plicatilis sensu strictu*, *B. plicatilis* Cayman and *B. plicatilis* Nevada. Before the start of the experiment, rotifer strains were kept in non-sterile cultures at the Laboratory of Aquaculture and Artemia Reference Center (Gent, Belgium). The rotifer stocks were regular examined by microscopy for the presence of ciliates and other protists and maintained at controlled culture conditions: 28 °C, light intensity 2000 lx, salinity 25 g l$^{-1}$, and fed with yeast-based Culture Selco® following the culture procedure described by Dhert (1996).

#### 2.2. Preparation of microbial communities (MCs)

MCs were prepared following the methods described by Tinh et al. (2006). Briefly, MCs were isolated from normal performing rotifer *B. plicatilis sensu strictu* cultures. For isolation of MCs, the culture water collected from rotifer culture was filtered through 250 μm and 60 μm meshes to remove big food particles and all rotifers, respectively, and was subsequently centrifuged at 1600 g for 5 min to remove the algal cells, thus only retaining the MCs in the supernatant. These MCs were preserved for further experiments in 1 ml eppendorfs containing 20% glycerol and 80% bacterial suspension and kept at −80 °C. Before starting each experiment, the Eppendorf tubes were thawed. Subsequently, 50 μl of the MC suspension was spread plated on Marine Agar (MA). After 24 h of incubation at 28 °C, the bacteria were harvested by swabbing the MA plate and suspending in autoclaved Nine Salt Solution (NSS). Cell density of the suspensions was calculated according to the McFarland standard (BioMerieux, Marcy L’Etoile, France), based on optical density measurements (OD$_{550}$ = 1.000 corresponds to 1.2 × 10$^6$ cells ml$^{-1}$). Subsequently, the appropriate volume to be added to each treatment was calculated in order to have a density of 10$^6$ cells ml$^{-1}$ in the rotifer culture water at the start of each experiment independent of the *Brachionus* strains.

#### 2.3. Experimental set-up of rotifer batch cultures

The separate runs of experiments of each rotifer strain were performed in 50 ml sterile Falcon tubes (TRP®, γ-irradiated) with four replicates for rotifer *B. plicatilis* Nevada and three replicates for *B. plicatilis sensu strictu* and *B. plicatilis* Cayman. Rotifers were harvested by filtration from stock cultures, rinsed three times with 0.22 μm-filtered and autoclaved natural seawater (FASW) to remove most of the bacterial load, and distributed into falcon tubes containing 32.5 ml of 25 g l$^{-1}$ FASW, to have a density of 30 rotifers ml$^{-1}$ at the start of each experiment. The MCs were added once to each tube to have a density of 10$^6$ cells ml$^{-1}$ at the start of experiment. The Falcon tubes were put on a rotor (4 rpm) which was placed inside a temperature-controlled room (28 °C, light intensity 2000 lx). Each experiment was run for 4 batch cycles and each cycle consisted of a 3-day culture period. At the end of each batch, rotifers were harvested, rinsed and re-distributed at a density of around 30 rotifers ml$^{-1}$ in fresh FASW before starting the next batch culture.

#### 2.4. Rotifer diet

The rotifer diet consisted of a commercial diet Culture Selco 3000®, CS 3000 (INVE), Belgium. The rotifers were fed daily following a standard feeding regime for Culture Selco 3000® according to manufacturer’s instructions below:

\[
CS \, 3000 = 0.0168D^{0.415}V
\]

where: CS 3000 = the weight of experimental diet (g); $D =$ rotifer density (individuals ml$^{-1}$); $V =$ culture water volume (L).

#### 2.5. Sampling, counting and growth data analysis

Three samples of 0.5 ml were taken from the rotifer cultures. The rotifers in each sample were killed by adding three drops of lugol, and were counted. Empty and transparent lorica belonging to dead rotifers were not taken into consideration.

The specific growth rate was calculated using the following equation described by Rombaut et al. (2001): \[ \mu = (\ln N_t - \ln N_0) / t \]

where: $\mu =$ specific growth rate; $N_t =$ rotifer density after culture period t (individuals ml$^{-1}$); $N_0 =$ initial rotifer density (individuals ml$^{-1}$); t = culture period (day).

Data of the growth rates on day 3 were evaluated using Levene's test for homogeneity of variances and Shapiro–Wilk's test for normality. As data were normal-distributed and homoscedastic, the growth rates on day 3 for batch 1 and batch 4 were compared between experiments using one-way ANOVA, followed by Tukey test. All the tests were performed using the computer program SPSS release 12.0 (SPSS, USA).

### 2.6. Microbial analyses

#### 2.6.1. Sampling procedure

One-milliliter culture water with rotifers was collected from the rotifer culture after inoculation at the start of experiment. Samples were centrifuged at 5000 g for 5 min and stored at −20 °C.

#### 2.6.2. DNA extraction

Total DNA from the samples was obtained by a modified DNA extraction method as described previously by Rombaut et al. (2001). To obtain bacterial DNA, the samples were centrifuged for 30 min at 5000 g. The pellet was dissolved in 0.2 ml Milli-Q water, transferred to 0.4 ml of 10 mM Tris–HCl (pH 9) and 0.3 g of glass beads (0.10–0.11 mm diameter) was added. This mixture was homogenised three times for 30 s using a bead beater at 2000 rpm (B. Braun Biotech International, Melsungen, Germany). After this, 16 μl of 50 mg ml$^{-1}$ lysozyme was added, and then the suspension was incubated at 37 °C for 15 min on a shaker (200 rpm). Chemical lysis of the bacterial cells was achieved by adding 30 μl of 20% SDS after which the suspension was slowly mixed for 5–10 min. Subsequently, 0.1 ml of 8 M ammonium acetate was added. DNA was obtained from the lysates using standard phenol–chloroform extraction and isopropanol precipitation procedures (Boon et al., 2000). The total DNA extracted was quantified by a spectrophotometer ND-1000 at 280 nm (NanoDrop Technologies, Wilmington, USA).

#### 2.6.3. Amplification of 16S rRNA genes

DNA extracted from samples was amplified with primers gc338f and 518r spanning the V3 region of the 16S rRNA gene (Muyzer et al., 1993) using a 9600 thermal cycler (Perkin-Elmer, Norwalk, CT, USA). PCR amplification was carried out in 24 μl reaction volumes to which 1 μl of DNA extract was added. The PCR master mix contained 0.5 μM of each primer, 200 μM of each deoxynucleoside triphosphate, 1.5 mM MgCl$_2$, 10 μl of thermophilic DNA polymerase 10× reaction buffer (MgCl$_2$-free), 2.5 U of Taq
DNA polymerase (Promega, Madison, WI, USA), 400 ng μl⁻¹ of bovine serum albumin (Boehringer) and sterile water, to a final volume of 100 μl. PCR was performed in a 9600 thermal cycler as follows: 95 °C for 10 min, followed by 30 cycles at 95 °C for 1 min, 53 °C for 1 min, and 72 °C for 2 min.

2.6.4. Analysis of PCR products by DGGE

DGGE was performed as described previously (Boon et al., 2002), using the Bio-Rad D Gene System (Hercules, CA, USA) with 8% (w/v) polyacrylamide gels in 1× TAE (20 mM Tris, 10 mM acetate, 0.5 mM EDTA, pH 7.4). The gels contained a linear gradient ranging from 40% to 60% denaturant (where 100% denaturant contains 7 M urea and 40% formamide). PCR products (10 μl) obtained from total DNA of samples were used for separation in denaturing gradient gels. The electrophoresis was run for 16 h at 60 °C, at 38 V. After completion of electrophoresis, the gels were stained for 20 min in SYBR Green I nucleic acid gel stain solution (1:10,000 dilution in 1× TAE; FMC BioProducts, Rockland, ME, USA). The stained gel was immediately photographed on a UV transillumination table with a video camera module (Vilbert Lourmat, Marne-la Vallé, France).

2.6.5. Analysis of DGGE patterns

The obtained DGGE patterns were analyzed using BioNumerics software version 5.0 (Applied Maths, Sint-Martens-Latem, Belgium). DGGE gels were normalized by the assigned markers. A matrix of similarities for the densitometric curves of the band patterns was calculated using the band-based Dice coefficient and dendrograms were created using on the Ward method (Ampe and Miambi, 2000). Multidimensional scaling (MDS) analysis was used for a three dimensional space view of clusters distribution (Boon et al., 2002). For further analysis of the bacterial community described by DGGE banding patterns, principal component analysis (PCA) was used to generate a few linear variables that served as indicators of the successive changes in the community composition (Hori et al., 2006; Alonso-Saez et al., 2007). For the PCA analysis, the bacterial DGGE fingerprints were converted to band match table and the quantitative values were used for PCA analysis by BioNumerics software version 5.0 (Applied Maths, Sint-Martens-Latem, Belgium). In order to evaluate the bacterial community stabilities of rotifer batch cultures, the correlation values from matrix of similarities between two consecutive PCR-DGGE analysis of 16S rRNA gene fragments. The analysis yielded fingerprints were converted to band match table and the quantitative values were used for PCA analysis by BioNumerics software version 5.0 (Applied Maths, Sint-Martens-Latem, Belgium). After the beginning of the experiments and at the end of each batch culture were subjected to PCR-DGGE analysis of 16S rRNA gene fragments. The analysis yielded highly reproducible profiles for different replicates of the same rotifer strain (Fig. 2). The DGGE patterns showed dominant bands that were apparently specific for the communities associated with B. plicatilis Nevada and sensu strictu (Fig. 2, arrows). In terms of presence or absence of dominant bands, large changes occurred during batch 1 for the Cayman strain and the sensu strictu strain (Fig. 2). After the first batch, the dominant bands of the mixed microbial culture that was used as inoculum were still present in the DGGE pattern of the MC associated with the Nevada strain, although extra bands appeared. The DGGE profile of the community associated with the Nevada culture underwent relatively large changes during the second batch, after which the pattern was relatively stable during further batches.

Cluster analysis of the DGGE profiles showed three major clusters (Fig. 2). Interestingly, each cluster corresponded to a different rotifer B. plicatilis cryptic species. MDS analysis showed a similar division in three major clusters (Fig. 3A). This observation shows that the microbial community associated with rotifers had evolved depending on the rotifer strains.

In order to reduce the number of variables of these profiles, principal component analysis (PCA) was applied. PCA simplified the DGGE patterns into three new linear variables that encompassed 58.8% of the variability present in the original data. Fig. 3B shows that the ordination of samples by PCA, based on the DGGE band patterns,

![Fig. 1. Culture performance during 4 cycles of sequential batch cultures of 3 strains belonging to 3 different cryptic species of the rotifer B. plicatilis fed with Culture Selco 3000®. The error bars represent the standard deviation (n = 3 for B. plicatilis sensu strictu and B. plicatilis Cayman; n = 4 for B. plicatilis Nevada).](image-url)

### Table 1

<table>
<thead>
<tr>
<th>Rotifer species</th>
<th>Batch</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. plicatilis sensu strictu</td>
<td></td>
<td>0.81±0.06</td>
<td>0.53±0.04</td>
<td>0.38±0.03</td>
<td>0.31±0.06</td>
<td>0.51±0.20</td>
</tr>
<tr>
<td>B. plicatilis Cayman</td>
<td></td>
<td>0.84±0.06</td>
<td>0.56±0.03</td>
<td>0.54±0.02</td>
<td>0.61±0.06</td>
<td>0.63±0.13</td>
</tr>
<tr>
<td>B. plicatilis Nevada</td>
<td></td>
<td>0.51±0.08</td>
<td>0.48±0.07</td>
<td>0.42±0.31</td>
<td>0.42±0.11</td>
<td>0.46±0.10</td>
</tr>
</tbody>
</table>

Treatments with different superscripts in each batch are significantly different from each other (Tukey test, *P* < 0.05).

# 3. Results

### 3.1. Rotifer performance

Starting from an initial density of around 30 rotifers ml⁻¹, the culture reached a maximum density of 400 rotifers ml⁻¹ after three days culture when fed with CS 3000. Fig. 1 shows the population density changes of three different rotifer strains during the four batch cycles. The maximum population densities were observed at the end of batch 1 for B. plicatilis sensu strictu and B. plicatilis Cayman, while for B. plicatilis Nevada the lowest population density was observed.

The rotifer growth rates of each batch culture are presented in Table 1. The highest growth rates were found for B. plicatilis Cayman and B. plicatilis sensu strictu in batch 1, being significantly different from the growth rate of B. plicatilis Nevada (P < 0.05). Considering the 4 batches together, the lowest growth rates were found for B. plicatilis Nevada (Table 1).

### 3.2. Evolution of the microbial community composition

To analyze the variability of the microbial community associated with the different rotifer cultures, samples collected at the beginning of experiments and at the end of each batch culture were subjected to PCR-DGGE analysis of 16S rRNA gene fragments. The analysis yielded highly reproducible profiles for different replicates of the same rotifer strain (Fig. 2). The DGGE patterns showed dominant bands that were apparently specific for the communities associated with B. plicatilis Nevada and sensu strictu (Fig. 2, arrows). In terms of presence or absence of dominant bands, large changes occurred during batch 1 for the Cayman strain and the sensu strictu strain (Fig. 2). After the first batch, the dominant bands of the mixed microbial culture that was used as inoculum were still present in the DGGE pattern of the MC associated with the Nevada strain, although extra bands appeared. The DGGE profile of the community associated with the Nevada culture underwent relatively large changes during the second batch, after which the pattern was relatively stable during further batches.

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agreed with the clustering results. Indeed, three different clusters were obtained, which correspond to the three different rotifer strains.

Finally, in order to track the successive dynamic changes within the MC of each species from batch 1 to batch 4, moving windows analysis was applied on the DGGE profiles. The analysis resulted in different curves for the three rotifer species (Fig. 4). For the species B. plicatilis sensu strictu and Cayman, relatively large changes of DGGE patterns occurred during batch 1, with the similarity between the patterns obtained before batch 1 and after batch 1 being lower than 50%. DGGE patterns of the Nevada strain in batch 1 also changed, but to a smaller extent (70% similarity between patterns obtained before and after batch 1). For all rotifer strains, the similarity between subsequent DGGE analyses increased with increasing batch cultures to 80% or more similarity between subsequent profiles. This suggests that the microbial communities associated with the rotifers became relatively stable after several subsequent batches and only minor shifts in the community composition occurred during the last batches.

3.3. Microbial species distribution evenness and variations

The microbial species distribution analysis was based on the method of Lorenz curves. Lorenz curves were plotted according to the numbers of bands and their intensities present in the DGGE pattern. Fig. 5 shows graphs of changing Lorenz curves of each culture unit in subsequent batch cultures. For MC in rotifer strain B. plicatilis sensu strictu cultures, it shows an overall tendency of less bending curves with increasing batch number compared to the curve of the initial MC. The flattest curve for B. plicatilis sensu strictu cultures indicates the nearly even distribution of microbial species at the end of batch 3 (Fig. 5 s.s end of batch 3 R1). This decreasing tendency in the bending of curves is also present for the rotifer strain B. plicatilis Cayman, but to a lesser extend, since the curves are closer and cross each other (Fig. 5 Cayman R1, R2 and R3). On the contrary, overall highly bended Lorenz curves are found in the rotifer strain B. plicatilis Nevada cultures, indicating an uneven distribution.
To assess changes in evenness of microbial species distribution, Gini coefficients were calculated (Fig. 6) and the results show the different change tendencies of microbial species distributions among the three rotifer strain cultures. In the first batch the increasing Gini coefficients indicated a more evenly distribution of microbial species. However during the consequent three batches, the three rotifer strain cultures were ending in different ways in terms of microbial species distribution as reflected by Gini coefficient changes. In general, high Gini coefficients were found in the cultures of rotifer strains Brachionus plicatilis sensu strictu and B. plicatilis Cayman, whereas low Gini coefficients were found for the B. plicatilis Nevada.

4. Discussion

Despite technological improvements of batch culture of Brachionus including turbidity regulation and optimization of diets (Dhert et al., 2001; Yoshimura et al., 2003), the problem of “bad performances” is still common in hatcheries. As a consequence, in practice, hatcheries have to set-up many tanks running batch cultures simultaneously in order to obtain sufficient amounts of live feed. Such practices increase overall cost and decrease efficiency in terms of labor and utilization of infrastructure (Dhert et al., 2001). Many factors have been linked to rotifer growth performance, such as nutrition, the presence of microalgae, cryptic rotifer species and different microorganisms. The bacterial communities associated with rotifers might be one of the most important factors. High rotifer densities demanding high feeding levels and producing high concentrations of waste products thus create a high load of organic material that is utilized as feed source by bacteria. These intensive rearing conditions allow heterotrophic bacteria to grow fast (Skjermo et al., 1997; Verschueren et al., 1997; Skjermo and Vadstein, 1999). Conventional microbial techniques have revealed high loads of potentially pathogenic bacteria, such as vibrios, in conventional rotifer batch cultures. Also highly variable microbial communities are found after rotifer disinfection (Rombaut et al., 1999). Consequently, there is currently an interest in manipulating the composition of the microbial community aiming at more reliable rotifer culture performances (Dhert et al., 2001). In this study, we analyzed the correlation between the culture performances of different Brachionus plicatilis strains and the microbial communities that were associated with the rotifers. Our results indicate that the microbial community composition shows large differences between different rotifer strains although all were inoculated with the same mixed bacterial community. Smaller differences in the microbial community composition were observed between replicates of the same rotifer strain.

From our results, we can conclude that shifts occur in the microbial communities associated with rotifers during batch cultivation, and the changes in the microbial community composition were found to be different for the different rotifer cryptic species. Interestingly, these changes were also associated with different culture performances. By comparing rotifer growth performances and stability of the microbial community, we found that large changes in growth rate were associated with large changes in the MC composition (Fig. 1, batches 1 to 2; Fig. 4 batch 1). The rotifer growth rate of the Cayman strain reached stability from batch 2 onwards and this was accompanied by the stabilization of the microbial community (Fig. 4A). This was also manifested in the Moving Window Analysis (Fig. 4A), where low similarities were observed in the patterns obtained before and after the first batch of the Cayman strain. Also, the smaller decrease in culture performances observed in later batches was accompanied by smaller changes in the microbial community composition. Culture performances of the Nevada strain, on the other hand, were more stable as was the composition of the microbial community stability. Hence, our results suggest that the performance of rotifer cultures is strongly influenced by the associated microbial community. This conclusion corroborates previous reports mentioning that the introduction of microorganisms can significantly change the performance of rotifer cultures (Rombaut et al., 1999).

Skjermo and Vadstein (1993) found that the use of enrichment diets caused a shift in the bacterial composition to a microbial community totally different from the initial community and reasoned that this was partly due to a bloom of fast-growing, opportunistic bacteria, which were favored by the high substrate levels in the culture. Also in our study, significant changes were observed in the microbial community associated with rotifers upon feeding them an artificial diet. However, the shifts we observed were unlikely determined solely by the diets. Indeed, although the microbial communities changed during cultivating of the rotifers, the dominant bands that appeared and/or disappeared were different for the different rotifer strains. This implies that the rotifer strain could have strong effects on the determination of the composition of the microbial community.

Recently, axenically grown B. plicatilis sensu strictu were used by Tinh et al. (2006) as a test model to reveal the role of "endogenous microbiota" which were isolated from cultures of the same rotifer species. When yeast strains were given as food, rotifer growth...
performance was dependent on the origin of the MCs. When a MC isolated from a crashed rotifer culture was added, however, it did not have any negative effect in the rotifer growth test, suggesting that the MC associated with the crashed rotifer culture was not responsible for the crash. Hino (1993) suggested that changes in the composition of the microbial community, and not the microbiota that are present at that specific moment, are the cause of the collapse of rotifer cultures. In accordance to this, in this study, we found that the cultures showing the largest changes in microbial community composition also had the largest decreases in growth performance.

Most of the microbial community studies concentrate on comparing species richness (i.e. the number of distinct species). However, community diversity is more than richness. It also includes evenness and dynamics (Marzorati et al., 2008). Lorenz curves and Gini coefficients describe the distribution of the different species within a community and has shown to be a good estimator of microbial species evenness (Mertens et al., 2005; Halet et al., 2006; Wittebolle et al., 2008, 2009). In our study, differences in the shapes of Lorenz curves and Gini coefficients were found among three different strains of rotifer *B. plicatilis* batch cultures. The lower evenness (average Gini coefficient = 0.63) from batches 2 to 4 in *B. plicatilis* Nevada culture indicated that a smaller fraction of the different microbial species present was dominating the community. Interestingly, this few dominant microbial species corresponded to low rotifer growth rates (Table 1). On the contrary, an increasing trend of evenness of microbial species was found for the strain *B. plicatilis* Cayman when the rotifer growth rates were steady around 0.57 from batches 2 to 4. High rotifer densities were always associated with high Gini coefficients in all cultures at the end of batch 1 (Figs. 1 and 6). Thus it seems that for a *B. plicatilis* culture with high population densities, a high evenness is required.

The DGGE fingerprinting technique has shown to be an interesting tool for the study of microbial communities in aquaculture systems. This technique, combined with other physiological/chemical analyses, can be useful in unraveling the ecological functions of bacterial communities in rotifer culture systems. It has been shown that cryptic species differ in ecological preferences and life cycle parameters (Serra et al., 1998; Ortells et al., 2003) and the same might be true for the microbiota that are associated with the rotifers. In this study, we found that different cultures of rotifer strains belonging to three different cryptic species of the rotifer *B. plicatilis* carried different microbial communities. As microbial communities associated with the rotifers are transferred with the rotifers to the larval fish cultures, it would be interesting to verify if the larval fish performance (which is highly influenced by microorganisms) can be modulated by choosing the proper rotifer strain cultures.

![Fig. 4. Moving window analysis of the DGGE patterns of the bacterial communities associated with 3 different strains belonging to 3 different cryptic species of the rotifer *B. plicatilis*. The variability between two consecutive batches was calculated based on the Dice coefficients. For each batch, the similarity between the profiles of the batch of interest and the previous batch is plotted (for the first batch, this is the similarity between the profile after the batch and the initial community that was inoculated). Bar=SD.](image)

![Fig. 5. Lorenz curves of the microbial community associated with rotifer cultures. (s.s = *B. plicatilis* sensu strictu; Nevada = *B. plicatilis* Nevada; Cayman = *B. plicatilis* Cayman; R1, R2, R3 = replicates 1, 2 and 3; start of experiment = samples collected after inoculations.](image)

![Fig. 6. Gini coefficients of rotifer cultures samples at the end of each batch based on Lorenz curves (start = samples collected after inoculations, bar = SD).](image)
In our study, consecutive batch cultures displayed different dominant DGGE bands, indicating a dynamic evolution of the bacterial community after inoculation. It is likely that some bacterial species of the inoculum were favored in the rotifer batch cultures. It is also possible that rotifer-associated bacteria still present after washing, may have re-established themselves during the cultures. Therefore, the role of bacteria closely associated with rotifers should be evaluated carefully as drivers for the evolution of whole microbial community.

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