Monitoring of the bioencapsulation of a probiotic *Phaeobacter* strain in the rotifer *Brachionus plicatilis* using denaturing gradient gel electrophoresis

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The bioencapsulation of the probiotic bacteria *Phaeobacter* 27-4 in the rotifer *Brachionus plicatilis* was monitored by culture methods and denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16 S rDNA.
In a first experiment, the permanence of the probiotic bacteria in clear water and green water was studied. *Phaeobacter* 27-4 added to the water of the tanks (107 CFU ml-1) remained at levels around 106 CFU ml-1 for 72 h and was not affected by the presence of the algae added (*Isochrysis galbana*, 105 cells ml^{-1}). The DGGE fingerprints showed a temporal predominance of the probiont in the water and the presence of bacteria belonging to the *Flavobacteria*, γ-proteobacteria, and *Sphingobacteria* groups. A *Tenacibaculum* strain bécame predominant when *Phaeobacter* 27-4 decline, and at the end of the experiment, bacterial profiles bécame similar to the initial ones with predominance of bacteria belonging to the *Oceanospirillaceae* family. Three different ways of bioencapsulation of the probiont in the rotifer were assayed: E24, addition of *Phaeobacter* 27-4 for 24 h during the enrichment with *I. galbana*; E3, addition of *Phaeobacter* 27-4 during the last 3 h of the enrichment with *I. galbana* and E3+, with the bioencapsulation done in a separated step, after the 24 h enrichment with I. galbana, being the rotifers filtered, washed and transferred into tanks containing *Phaeobacter* 27-4 in seawater, and maintained for 3 h. The result showed that the presence of the algae was not determinant in the effectiveness of the bioencapsulation and the probiont was bioencapsulated in all cases in the first 3 h to a level of 102 cfu $rotifer^{-1}$. When the rotifers with the bacteria bioencapsulated were transferred to green-water tanks and kept in the conditions used in turbot larvae rearing, *Phaeobacter* 27-4 maintained levels close to 102 CFU $rotifer^{-1}$ for 48 h in the case of E24 and E3, and for 24 h in the case of E3+, a period of time sufficient to the larvae to graze on themand to incorporate the probiotic. The E24 protocol was selected for the simplicity of the procedure. DGGE fingerprints showed the incorporation of the probiotic and a temporal colonization of the rotifers. Predominant bands identified in the rotifers correspond to γ-proteobacteria as *Pseudoalteromonas*.