# CORAL REEFS HARBOUR NEW SPECIES OF THE FAMILY VIBRIONACEAE

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## INTRODUCTION

The reef biome has a key role for the health of the oceans, furthermore it is important for the economy of several countries via fishing, ecotourism and as nursery species place. Studies of microbiota diversity associated with coral have revealed the importance of the microorganisms in the maintenance of the holobiont equilibrium (coral + microbiota + zooxanthella). Vibrios may represent a large proportion of the coral microbiota and it seems to establish different relationships with corals that vary from parasitism to mutualism. On the other hand much of this enormous bacterial diversity is represented by novel groups which are still waiting formal taxonomic characterization and may have an important ecological role in the holobiont survival.

In a survey on the diversity of heterotrophic bacteria associated with holobionts in Brazil, several isolates were taxonomically characterized by means of a polyphasic approach, comprising MLSA, DNA–DNA hybridizations (DDH), divergence between the thermal denaturation midpoint ($\Delta Tm$), and phenotypic characterization.
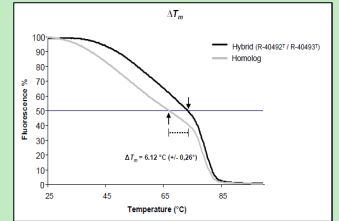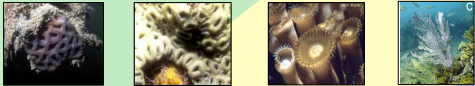
## MATERIAL & METHODS

### Coral sources

Mussismilia hispida   Palythoa caribaeorum   Palithoa variabilis   Phyllogorgia dilatata

### Study strategy

Strain isolation and purification

Taxonomic analysis by polyphasic approach

DNA fingerprint analysis (AFLP, rep-PCR, etc.)

Phenotypic Characterization

Fatty acids analysis (FAME)

Morphology, enzimatic activity, carbon source utilization (API and Biolog™ tests), etc.

% G+C Content

16S rRNA gene sequence

Multilocus Sequence Analysis (MLSA)

Blast analysis (GenBank)

Thermal denaturation midpoint ($\Delta Tm$)

DNA-DNA Hibridization (DDH)

## RESULTS



Photobacterium jeanii sp. nov., isolated from corals and zoanthids

Figure. Neighbour-joining phylogenetic tree based on concatenated 16S rRNA, ftsZ, mreB, recA, rpoA and topA gene sequences (4.949 nt) showing the position of P. jeanii sp. nov. The evolutionary distances were computed using the Jukes-Cantor method. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). Phylogenetic analyses were conducted in MEGA4. Bootstrap values (> 50 %) based on 1000 resamplings are shown. C. jejuni was used as outgroup. Bar, 5 % estimated sequence divergence.

Vibrio communis sp. nov., isolated from the marine animals Mussismilia hispida, Phyllogorgia dilatata, Palythoa caribaeorum, Palythoa variabilis and Litopenaeus vannamei

Figure. Neighbour-joining phylogenetic tree showing the phylogenetic position of V. communis sp. nov. based on concatenated 16S rRNA (1470 bp), rpoA (790 bp) and pyrH (531 bp) gene sequences. The evolutionary distances were computed using the Jukes-Cantor method. Phylogenetic analyses were conducted in MEGA4. Bootstrap values (> 70%) based on 1000 repetitions are shown. The numbers at nodes denote the level of bootstrap values derived from the neighbor-joining and maximum-parsimony methods. Escherichia coli was used as outgroup. Bar, 2 % estimated sequence divergence.

Vibrio variabilis sp. nov. and Vibrio maritimus sp. nov., isolated from Palythoa caribaeorum

Figure. Neighbour-joining phylogenetic tree showing the phylogenetic position of V. variabilis sp. nov. and V. marinus sp. nov. based on concatenated 16S rRNA, ftsZ, gyrB, recA, rpoA, topA, pyrH and mreB gene sequences (6035 bp). The evolutionary distances were computed using the Jukes-Cantor method. Phylogenetic analyses were conducted in MEGA4. Bootstrap values (> 50%) based on 1000 repetitions are shown. The numbers at nodes denote the level of bootstrap values derived from the neighbour-joining and maximum-parsimony methods. Escherichia coli was used as outgroup. Bar, 2 % estimated sequence divergence.



Figure. Melting curves of homologous and hybrid DNA (R-40492T / R-40493T). The $\Delta Tm$ is indicated (mean 6.12 °C +/- standard error 0.26; N = 8). The blue horizontal line delimits a reduction of 50% in the fluorescence, reflecting a 50% denaturation of the double strand DNAs.
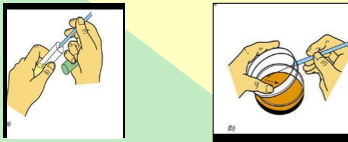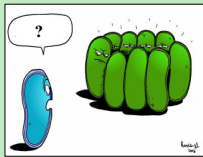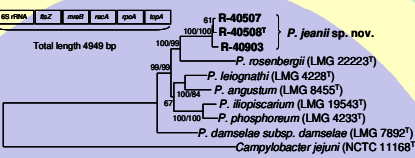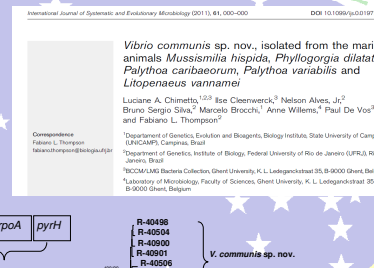
Vibrio alfacsensis sp. nov., isolated from marine organisms.
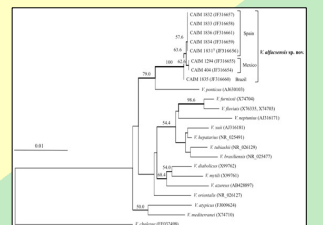
Figure. 16S rRNA dendrogram of Vibrio alfacsensis and its closest phylogenetic neighbours.
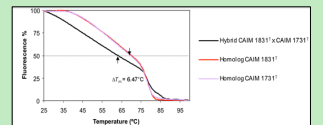
Figure. Melting curves generated with ABI 7500 for $\Delta T_m$ determination between homologous and hybrid DNA (CAIM 1831T x CAIM 1731T). Comparison between V. alfacsensis sp. nov. and V. ponticus (CAIM 1731T) provided mean $\Delta T_m$ 6.79 °C (+/- 0.32 Std. error). The $\Delta T_m$ between CAIM 1831T x CAIM 1731T (Hybrid) and CAIM 1831T (Homolog) was 7.1 °C and $\Delta T_m$ between CAIM 1831T x CAIM 1731T (Hybrid) and CAIM 1731T (Homolog) was 6.47 °C.
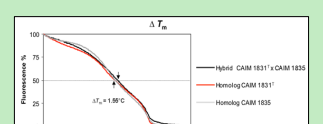
Figure. Melting curves generated with ABI 7500 for $\Delta T_m$ determination between homologous and hybrid DNA (CAIM 1831T x CAIM 1835). Comparison between V. alfacsensis sp. nov. strains provided mean $\Delta T_m$ 1.45 °C (+/- 0.1 Std. error). $\Delta T_m$ between CAIM 1831T x CAIM 1835 (Hybrid) and CAIM 1831T (Homolog) was 1.55 °C and $\Delta T_m$ between CAIM 1831T x CAIM 1835 (Hybrid) and CAIM 1835 (Homolog) was 1.35 °C.

## CONCLUSION

we have described 4 new Vibrio species (V. communis, V. maritimus, V. variabilis and V. alfacsensis) and one Photobacterium (P. jeanii). Some new species (e.g. V. communis) can fix nitrogen and may have a role in the health of marine animals.

FAPERJ   CNPq   CAPES