**Table S1.** Oligonucleotide probes used to detect *Vibrio* spp. The table contains only probes that, according to the published literature, were used for detection of different *Vibrio* species over the last 20 years. The sequence of each probe, its specificity and citing literature are indicated.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **PROBE** | **SEQUENCE (5’→3’)** | **HYBRIDIZATION** | **REFERENCE** | | |
| GV841 | AGGCCACAACCTCCAAGTAG | *Vibrio* genus (except *V. cholerae* and *V. mimicus*) | | 1-5 |
| MS6 | AGTTTTACATTTGCGACC | *V. shiloi* | | 6 |
| Vchomin 1276 | ACTTTGTGAGATTCGCTCCACCTCG | *V. cholerae, V. mimicus* | | 7-9 |
| VIB | ACAGTACTCTAGTCTGCCAG | *Vibrio* genus | | 10,11 |
| Vib 1 | GTGGTAGTGTTAATAGCACT | *V. vulnificus* | | 12 |
| Vib 2 | TCTAGCGGAGACGCTGGA | *V. vulnificus* | | 12 |
| Vib 3R | GCTCACTTTCGCAAGTTGGCC | *V. vulnificus* | | 12 |
| Vib-16S-1 | AGGAGCTTCGCTTGC | *Vibrio* genus | | 13 |
| VIB572a | ACCACCTGCATGCGCTTT | *Vibrio* genus (occasionally *Photobacterium* and *Listonella)* | | 5,14-16 |
| VIB572b | ACCGCCTGCATGCGCTTT | *Vibrio, Photobacterium* | | 14 |
| Vib749 | TCGCATCTGAGTGTCAGT | *Vibrio, Aliivibrio* | | 5 |
| Vvul3 | TCCTCACGACTGAAAG | *V. vulnificus* | | 7 |

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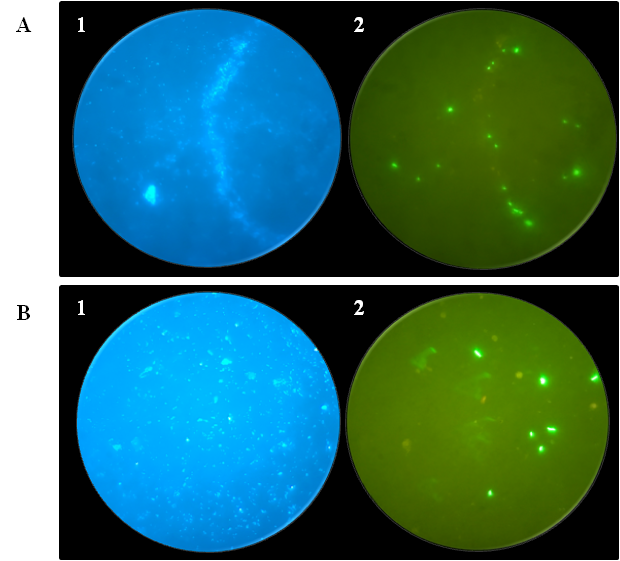
**Table S2.** The main characteristics of pre-selected CARD-FISH probes according to SILVA database. The percentage of coverage and specificity of three probes (i.e. GV841, Vib-16S-1 and VIB572a) are indicated. The taxa potentially detected by these probes are included only if the percentage of coverage and specificity is higher than 75%.

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| --- | --- | --- | --- | --- | --- | --- |
| **TAXA** | **PROBES** | | | | | |
| **GV 841** | | **Vib-16S-1** | | **VIB572a** | |
| **Coverage**  **(%)** | **Specificity (%)** | **Coverage (%)** | **Specificity (%)** | **Coverage (%)** | **Specificity (%)** |
| Alteromonadales; Alteromonadaceae; *Gayadomonas* | 0 | 0 | 100 | 99.3 | 0 | 0 |
| Alteromonadales; Gallaecimonadaceae; *Gallaecimonas* | 0 | 0 | 100 | 99.3 | 0 | 0 |
| Enterobacteriales; Enterobacteriaceae; endosymbionts10 | 0 | 0 | 100 | 99.3 | 0 | 0 |
| Vibrionales; Vibrionaceae; *Candidatus Photodesmus* | 50 | 99.3 | 100 | 99.3 | 25 | 99.3 |
| Aeromonadales; Aeromonadaceae; *Oceanisphaera* | 0 | 0 | 94.4 | 99.3 | 0 | 0 |
| Pasteurellales; Pasteurellaceae; *Avibacterium* | 0 | 0 | 94.1 | 99.3 | 0 | 0 |
| Alteromonadales; Alteromonadaceae; *Catenovulum* | 0 | 0 | 92.3 | 99.3 | 0 | 0 |
| Vibrionales; Vibrionaceae; *Vibrio* | 89.9 | 99.9 | 92.1 | 99.9 | 94 | 100 |
| Vibrionales | 78 | 100 | 76.1 | 99.9 | 78.9 | 100 |
| Vibrionales; Vibrionaceae | 78 | 100 | 76.1 | 99.9 | 78.9 | 100 |
| Vibrionales; Vibrionaceae; *Aliivibrio* | 60.2 | 99.4 | 1.4 | 99.3 | 95.6 | 99.4 |
| Vibrionales; Vibrionaceae; *Catenococcus* | 93.8 | 99.3 | 56.3 | 99.3 | 93.8 | 99.3 |

**Table S3.** The main characteristics of pre-selected CARD-FISH probes determined by BLAST. Indicated are coverage (QC, query cover, %), identity (Ident, %), matches (Match) and E value (i.e. the probability of finding the sequence among all organisms within this database). The taxa potentially detected by these probes are included only if the percentage of coverage and specificity is higher than 75%.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **TAXA** | **PROBES** | | | | | | | | | | | | |
| **GV 841** | | | | **Vib-16S-1** | | | | **VIB572a** | | | | |
| **QC** | **Ident** | **Match** | **E** | **QC** | **Ident** | **Match** | **E** | | **QC** | **Ident** | **Match** | **E** | |
| Alteromonadales; Alteromonadaceae; *Gayadomonas* | 75 | 100 | 8/20 | 0.53 | 43 | 100 | 7/16 | 2.7 | | 94 | 88.24 | 15/18 | 0.11 | |
| Alteromonadales; Gallaecimonadaceae; *Gallaecimonas* | 70 | 92.86 | 13/20 | 25 | 43 | 100 | 7/16 | 774\* | | 77 | 92.86 | 13/18 | 19\* | |
| Enterobacteriales; Enterobacteriaceae; endosymbionts10 | 100-90 | 100 | 20/20-18/20 | 0.025-0.40 | 87 | 100 | 14/16 | 100\* | | 77 | 100 | 14/18 | 99\* | |
| Vibrionales; Vibrionaceae; *Candidatus Photodesmus* | 100 | 100 | 20/20 | 4 10-7\* | 62 | 100 | 7/16 | 13 | | 100 | 100 | 18/18 | 5 10-6\* | |
| Aeromonadales; Aeromonadaceae; *Oceanisphaera* | 45 | 100 | 9/20 | 138 | 100 | 100 | 12/16 | 0.029\* | | 77 | 92.86 | 13/18 | 30\* | |
| Pasteurellales; Pasteurellaceae; *Avibacterium* | 40 | 100 | 8/20 | 449\* | 100 | 100 | 12/16 | 1\* | | 44 | 100 | 10/18 | 385\* | |
| Alteromonadales; Alteromonadaceae; *Catenovulum* | 40 | 100 | 8/20 | 497\* | 100 | 100 | 8/16 | 0.3\* | | 94 | 88.24 | 15/18 | 108\* | |
| Vibrionales; Vibrionaceae; *Vibrio* | 100 | 100 | 20/20 | 0,004 | 100 | 100 | 16/16 | 0.49\* | | 100 | 100 | 18/18 | 0.031 | |
| Vibrionales | 100 | 100 | 20/20 | 0.004 | 100 | 100 | 16/16 | 0.51\* | | 100 | 100 | 18/18 | 0.033 | |
| Vibrionales; Vibrionaceae | 100 | 100 | 20/20 | 0,004 | 100 | 100 | 16/16 | 0,51\* | | 100 | 100 | 18/18 | 0.033 | |
| Vibrionales; Vibrionaceae; *Aliivibrio* | 100 | 100 | 20/20 | 1 10-4 | 100 | 100 | 13/16 | 0,92\* | | 100 | 100 | 18/18 | 0.002 | |
| Vibrionales; Vibrionaceae; *Catenococcus* | 100 | 100 | 20/20 | 2 10-7 | 50 | 100 | 7/16 | 6.7 | | 100 | 100 | 18/18 | 2 10-6 | |

\* The probe is complementary to non-16S rRNA genome regions.



**Figure S1.** CARD-FISH detection of *Vibrio* spp. in environmental samples. Water samples from coastal (panel A) or estuarine (panel B) areas were stained with DAPI (image 1) or were either subjected to CARD-FISH detection using VIB572a (image 2) and further examined by epifluorescence microscopy as described in Materials and Methods.