

**Diversity of bacterial endosymbionts
of environmental *Acanthamoeba* isolates**

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Supplemental Material

Supplementary Table 1: Primers and oligonucleotide probes used in this study.

Short name	Sequence (5'-3')	Specificity	Target site	Annealing temperature / Formamide concentration	Reference
16S and 18S rDNA targeted primers used for PCR					
616F	AGAGTTTGATYMTGGCTC	most <i>Bacteria</i>	8-25 ^a	52°C	(9)
1492R	GGYTACCTTGTTACGACTT	most <i>Bacteria</i>	1492-1510 ^a	52°C	(11)
16S1 ^c	CGGATCCTGAGAATTGATC	<i>Chlamydiae</i>	1-18 ^a	44°C	(12)
16S2 ^c	TGTCGACAAAGGAGGTGATCCA	<i>Chlamydiae</i>	1528-1549 ^a	44°C	
SSU1	AACCTGGTTGATCCTGCCAG	Eukarya	1-17 ^b	48°C	(6)
SSU2	GATCCTTCTGCAGTTCACCTAT	Eukarya	2273-2295 ^b	48°C	
18SF	GTAGTCATATGCTTGTCTC	Amoebozoa	17-35 ^b	48°C	This study
18SR	CGRARACCTTGTTACGAC	Amoebozoa	2256-2273 ^b	48°C	
S12.2	GATYAGATACCGTCGTAGTC	Amoebozoa	1220-1239 ^b	48°C	(5)
Aas79F	ACACTTCGGTGTGTCTGG	' <i>Cand. Amoebophilus asiaticus</i> '	79-98 ^a	50°C	This study
Aas1467R	GTCGCTGATCTAACCTA	' <i>Cand. Amoebophilus asiaticus</i> '	1467-1484 ^a	50°C	
16S and 18S rRNA targeted oligonucleotides used for FISH^d					
EUB338-I ^e	GCTGCCTCCCCTAGGAGT	Most <i>Bacteria</i>	338-355 ^a	0-70%	(1)
EUB338-II ^e	GCAGCCACCCGTAGGTGT	Bacteria not covered by probe EUB338, e.g., many <i>Planctomycetes</i>	338-355 ^a	0-70%	(4)
EUB338-III ^e	GCTGCCACCCGTAGGTGT	Bacteria not covered by probe EUB338, e.g., many <i>Verrucomicrobia</i>	338-355 ^a	0-70%	
EUK516	ACCAGACTTGCCCTCC	Most Eukarya	502-517 ^a	0-70%	(1)
Bn9-658	TCCGTTTTCTCCGCCTAC	Subgroup of the <i>Parachlamydiaceae</i>	658-675 ^a	10%	(2)
Aph1180	CTGACCTCATCCCCTCCT	' <i>Cand. Amoebophilus asiaticus</i> '	1180-1197 ^a	20%	(8)
Proca438	CGATTTCTCCCRGACAA	' <i>Cand. Procabacter acanthamoebae</i> '	438-455 ^a	20%	(7)
CC23a	TTC CAC TTT CCT CTC TCG	<i>Caedibacter caryophilus</i> and other <i>Caedibacter</i> -related endosymbionts of <i>Acanthamoeba</i> spp.	658-675 ^a	20%	(13)

^a 16S rRNA position, *E. coli* numbering according to (3)

^b 18S rRNA position in *A. castellanii* Neff ATCC50373 (U07416)

^c Primer sequences contain restriction enzyme site for BamH1 and Sall

^d Further details on the probes used for FISH are available at probeBase (www.microbial-ecology.net/probebase; 10)

^e EUB338, EUB338-II, and EUB338-III were applied simultaneously to target most *Bacteria*

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