OCCURRENCE OF CAMPYLOBACTER AND *ARCOBACTER* SPP. IN SEAWATER AND ZOOPLANKTON SPECIMENS

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Abstract

The presence of *Campylobacter* and *Arcobacter* spp., bacteria related to human and animal health, as free-living or associated with small (>64µm) and large plankton (>200µm) was monitored in a coastal zone. The occurrence was evaluated by cultural and molecular methods during an annual sampling cycle. The bacterial isolation was more frequent from water and large plankton than from small plankton. *Campylobacter concisus* was cultured from plankton and seawater samples in April, *C. coli* and *C. lari* only from plankton in May 2001. A multiplex PCR method was useful for the simultaneous detection and identification of *Arcobacter butzleri, A. cryaerophilus* and *A. skirrowii* on bacterial colonies and on samples without cultivation.

Keywords: free living bacteria, associated zooplankton bacteria.

Current taxonomic status of *Campylobacteraceae* includes three genera *Campylobacter*, *Arcobacter* and *Helicobacter* that constitute a phylogenetically distinct group referred as either ribosomal RNA superfamily or the epsilon division of the class *Proteobacteria.* All genera include human and no human pathogenic species and widespread forms. Arcobacters differ from other microaerophilic curved bacteria for their aerotolerance and ability to grow at temperature lower than 25°C. Four species, *A. butzleri*, *A. skirrowi*, *A. cryaerophilus* and *A. nitrofigilis*, have been described, differing for the ability to grow at 42°C and for the antibiotic sensitivity [1].

At present *Arcobacter*, as *Campylobacter*, are considered as emerging human foodborne pathogens. The survival of these bacteria in the environment is not well understood. Search on the campylobacters demonstrated the occurrence of thermophilic *Campylobacter* in fresh and marine water, and in the sewage [2]. The presence of *Campylobacter* outside warm-blooded animals, domestic and wild, is considered as sign of recent contamination because *Campylobacter* survive for a shorter time than the usual faecal indicators.

In the framework of a national survey we searched potentially pathogenic bacteria in water and plankton samples collected from a coastal station fixed in the Straits of Messina (Italy).

Seawater was monthly collected from April 2001 to March 2002 using sterilised bottles. To collect free-living bacteria, seawater samples were first filtered through 200 µm net and then through 64 µm net, and concentrated using 0.22 µm membrane filters (Millipore Corp., Bedford, MA). The filters were washed with filter-sterilised, phosphate-buffered saline (PBS) and used for cultural and molecular analyses. To collect small plankton (>64 µm), seawater samples passing through a 200 µm net were successively passed through a 64 µm net. The 64 µm net was washed and suspended in PBS. Sample containing small plankton and associated bacteria was divided in three aliquots for plankton, cultural and molecular analyses. Large plankton (>200µm) was collected with a 200 µm mesh plankton net. Retained large plankton and associated bacteria were suspended in 500 ml sterile seawater and divided in three aliquots for plankton, cultural and molecular analyses.

Seawater, small and large plankton were inoculated into tubes of *Campylobacter* Broth (BBL). After incubation for 2 days at 42°C a loop from positive cultures was streaked on Columbia Blood Agar Base (Karmali) (Oxoid) and incubated at 42°C in microaerobic atmosphere.

Samples were inoculated into *Arcobacter* Broth CM965 (AM) (Oxoid) supplemented with CAT (Cefoperazone, Amphotericin B, Teicoplanin) Selective Supplement SR 174E (AM174), selective for *Arcobacter* species, or with CCDA (Cefoperazone and Amphotericin B) Selective Supplement SR 155 for *Arcobacter butzleri* (AM155). After aerobic incubation at 30°C for 24 hours liquid cultures were streaked onto plates of the same enrichments media agarised with 1.5 % (AMA) [3]. In order to confirm the identification of the isolates as *A. butzleri*, *A. cryaerophilus* and *A. skirrowii* a multiplex PCR (m-PCR) assay was performed [4] including *Arcobacter butzleri* ATCC 49616, *A. cryaerophilus* ATCC 43157 and *A. skirrowii* ATCC 51132 as reference strains. Five PCR primers, named ARCO, BUTZ, SKIR, CRY1, and CRY2, based on the 16S rRNA and 23rRNA sequences [4] were used. The selected primers amplify a 257-bp fragment from *A. cryaerophilus*, a 401-bp fragment from *A. butzleri* and a 641-bp fragment from *A. skirrowii*. Amplified products were detected by electrophoresis in agarose gel. The m-PCR assay was also used for the detection and differentiation of *Arcobacter* spp. present in the samples without cultivation, to detect the "viable but not cultivable (VBNC)" state. Zooplankton was numerically largest in spring and in late autumn and mainly consisted of copepods.

Presumptive campylobacters were observed from all samples in April, but did not in summer when the level of UV radiation and changes in temperature influenced negatively their presence [5].

Campylobacter does not always correlate with faecal indicators given that they become non-culturable much faster than the indicators. This suggested that wild birds could represent the source of *Campylobacter* in our coastal waters rather than the sewage effluent. In April, species phenotypically identified as *C. concisus* were isolated from both seawater and large plankton samples. In May *C. coli* and *C. lari* were isolated only from large plankton but not from the other samples.

Presumptive Arcobacter strains obtained from both the enrichments used (AM174 and AM155 for the isolation of *Arcobacter* spp. and *A. butzleri*, respectively) were almost all identified as *A. butzleri*. This species was predominant in seawater samples but was also recovered from small and large plankton. The enrichment broth AM174 (permissive for *A. butzleri*) inoculated with seawater and plankton produced also isolates of *A. cryaerophilus*, confirmed by PCR.

Molecular assay was used to identify *A. butzleri*, *A. cryaerophilus* and *A. skirrowi* directly from samples without cultivation (Fig. 1). These results confirm that *Campylobacter* and *Arcobac ter* strains are widespread in the environment. They indicate recent contamination with animal (often avian) faeces or sewage. In the marine environment, plankton appear as potential reservoir of these bacteria.

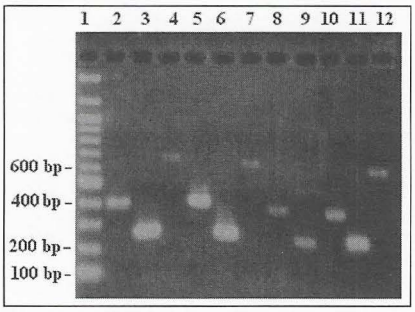

Fig. 1: m-PCR products amplified from marine samples and reference strains.
Lane 1: 100 bp ladder; lanes 2, 3 and 4: seawater samples; lanes 5, 6 and 7: large plankton; lanes 8 and 9: small plankton; 10: *A. butzleri* ATCC 49616; lane 11: *A. cryaerophilus* ATCC 43157; lane 12: *A. skirrowii* ATCC 51132.

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