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Detection of *Catabacter hongkongensis* in polluted European water samples*

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Abstract: The Catabacteriaceae is a new bacterial family with a unique member: *Catabacter hongkongensis* is a strictly anaerobic, non-sporulating, Gram-positive coccobacillus that is phylogenetically related to some clostridial clusters. Little is known of its epidemiology and environmental distribution, but the inclusion of its 16S rRNA gene sequence in GenBank has allowed it to be detected qualitatively. As a first approach for prospective surveys, a real-time polymerase chain reaction (PCR) procedure to identify *C. hongkongensis* has been developed. The presence of Catabacteriaceae in 29 water bodies subjected to possible human or animal impact has been investigated. Four of them were positive. The results confirm that highly polluted water can contain *C. hongkongensis*.

Key words: *Catabacter hongkongensis*, Real-time polymerase chain reaction (PCR), Environmental water samples
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INTRODUCTION

In 2007, Lau *et al*.(2007) proposed Catabacteriaceae as a new bacterial family and assigned to it the unique family member *Catabacter hongkongensis*, which is a strictly anaerobic, non-sporulating, Gram-positive coccobacillus that is phylogenetically related to some clostridial clusters (Lau *et al*., 2007). The first complete description was made during a study of four human cases of sepsis resulting from gastrointestinal disease. In this study, the authors suggested that the sepsis-causing bacteria originated in the intestinal tract (Lau *et al*., 2007). Little is known of the epidemiology and environmental distribution of *C. hongkongensis*. Nevertheless, the inclusion of its 16S rRNA gene sequence in GenBank has allowed it to be detected qualitatively in several studies. *C. hongkongensis* 16S rRNA has been detected in Manzala lake in Egypt (El Saied, 2007), in urban aerosols in cities in the United States (Brodie *et al*., 2007), among the fecal microbiota of a captive marine mammal (*Dugong dugon*) (Tsukinowa *et al*., 2008), in an anaerobic wastewater treatment system (Fernández *et al*., 2008), in a subtropical mangrove (Liang *et al*., 2007), and in microbial fuel cells (Ishii *et al*., 2008). This strongly suggests that *C. hongkongensis* is environmentally associated with water.

To learn more about *C. hongkongensis* and the risk it poses to human health, analytical tools are necessary to detect and quantify it in environmental samples. To date, there is no microbiological procedure to isolate and culture this bacterium; moreover, its identity must be confirmed by 16S rRNA gene

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sequence analysis. Therefore, as a first approach for prospective surveys, we developed a real-time polymerase chain reaction (PCR) procedure to identify *C. hongkongensis*.

MATERIALS AND METHODS

Samples selection

We investigated the presence of Catabacteriaceae in 29 water bodies subjected to possible human or animal impact. All of the samples were collected from the county of Barcelona in northeast Spain: (1) Ten different points along 50 km of coastline at points corresponding to urban bathing areas. These bathing areas are frequently monitored by the local public health authorities and were of good quality (data not shown) in compliance with the European directive (Official Journal of the European Union, 2006). Nevertheless, they were sampled because of their proximity to wastewater facilities. (2) Twelve samples were collected from urban wastewater treatment facilities: six from three different facilities (inlets and outlets), and six from effluent from different treatment facilities. (3) Finally, we collected seven continental water samples: four from rivers (the Llobregat, Besos, Tena, and Tordera) at points where they pass through urban areas, and three from groundwater in urban wells.

Real-time PCR quantification

One hundred milliliters of water from each source was concentrated by membrane filtration using a nylon membrane (0.45 μm porous diameter; Millipore, MA, USA). Cells were resuspended in 5 ml of sterile saline solution by vigorous vortexing with 15 glass beads (5 mm diameter) for 60 s and sonicated for 3 min (6 L, 150 W; Selecta, Barcelona, Spain). The resulting suspension (4 ml) was concentrated to 200 μl by centrifugation (10000×*g*, 5 min) and DNA was extracted with the DNeasy Tissue Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

To design *Catabacter*-specific PCR primers we used as a reference the 16S rRNA sequence deposited in the GenBank database (accession No. AY574991). Primers to amplify a 153 bp fragment were designed and analyzed for real-time quantitative PCR using LightCycler probe design software (version 2.0; Roche Applied Science, Mannheim, Germany). The sequences of the output regions were searched against GenBank sequences with the BLAST family program package (Altschul *et al*., 1997) to ensure the specificity of the primers.

Quantification was performed using real-time PCR with the LightCycler 1.5 PCR system (Roche Applied Science). The reaction mixture, 20 μl in total, consisted of 10 μl of Quantifast SYBR Green Mix (Qiagen, Valencia, CA, USA) and 10 μl of sample, giving a final concentration of 0.5 μmol/L of reverse (CAT GCG GTT TCG TGG TC) and forward primers (GTC GAA CGA AGT TGC TCT TT), and 0.2 U of uracil-DNA-glycosylase (New England BioLabs, Ipswich, MA, USA). The experimental LightCycler protocol was one 2-min step at 50 °C to allow uracil-DNA-glycosylase to break down the possible contaminating amplicons, one 5-min step at 95 °C for *Taq* polymerase activation, 45 cycles of PCR amplification (95 °C for 10 s, and 60 °C for 30 s), and a final melting temperature ramp from 65 °C to 95 °C at 0.1 °C per second.

A positive PCR product from bacterial genomic DNA was used as the standard for DNA quantification. Serial 10-fold dilution was performed in duplicate. The first dilution without positive amplification was considered to have a concentration lower than 1 decimal log. All of the measurements were performed in duplicate. Controls consisting of a positive PCR product suspension and a negative control of PCR-grade double-distilled water (Eppendorf, Hamburg, Germany) were included with every determination to verify the proper functioning of the amplification-quantification system and the absence of cross-contamination, respectively. The PCR products from positive samples were then confirmed by DNA sequencing (Secugen, Madrid, Spain) after purification with the Cycle Pure Kit (Omega Bio-Tek, Norcross, GA, USA).

RESULTS

Of the 29 samples analyzed, we found that at least four contained sequences that may be compatible with Catabacteriaceae 16S rRNA genes: the three samples from wastewater inlets and one sample taken directly from a river. The levels of Catabacteriaceae

16S rRNA genes were 1.94×10^2, 2.7×10^4, 1.58×10^4, and 3.2×10^3 copies per 100 ml, respectively. The BLAST results indicated an identity of 100% (119/119), 98% (130/132), 96% (115/120), and 91% (120/130), respectively, and expectation values (*E*) of $4e^{-36}$~$4e^{-41}$. In all four cases the sequence was also compatible with two other GenBank accessions: an uncultured clostridial bacterium (accession No. AB264080) and a Ruminococcus-like organism from a human clinical source (accession No. AJ318864). However, in the first description of Catabacteriaceae in which the same homologies were detected, the authors suggested that these sequences are specific to Catabacteriaceae but were incorrectly classified in the past (Lau *et al*., 2007).

DISCUSSION

By consensus, it is considered that identities with percentages higher than or equal to 98% are necessary to be sure that two 16S RNAs correspond to the same species. Therefore, two of our positive samples may be considered to be *Catabacter-like* species. Regardless of this qualification, the results suggest that the presence of Catabacteriaceae or *Catabacter-like* organisms in the environment is associated with polluted continental water. This provides support for previously published evidence indicating that this is a bacterial group with a global distribution. The 16S rRNA sequences of all four of the positive samples were homologous to two additional GenBank accessions.

Because the 16S rRNA gene copy number is not known for *C. hongkongensis*, it is impossible to estimate accurately the concentration of cells in positive samples. For example, *Clostridium perfringens* and *Escherichia coli* have ten and seven copies of 16S rRNA in their respective genomes (Klappenbach *et al*., 2001). If we assume that the copy number/genome is similar for Catabacteriaceae, the cell levels in positive samples could range from 2×10^2 to 2×10^4 cells per 100 ml.

Our proof-of-concept should be replicated with water samples from additional sources; furthermore, other environmental sources such as soil should be considered. Nevertheless, our results confirm that highly polluted water can contain *C. hongkongensis*.

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References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.*, **25**(17):3389-3402. [doi:10.1093/nar/25.17.3389]

Brodie, E.L., DeSantis, T.Z., Parker, J.P., Zubietta, I.X., Piceno, Y.M., Andersen, G.L., 2007. Urban aerosols harbor diverse and dynamic bacterial populations. *Proc. Natl. Acad. Sci. USA*, **104**(1):299-304. [doi:10.1073/pnas.0608255104]

El Saied, H.E., 2007. Molecular genetic monitoring of bacterial communities in Manzala lake, Egypt, based on 16S rRNA gene analysis. *Egypt. J. Aquatic Res.*, **33**(3):179-194.

Fernández, N., Díaz, E.E., Amils, R., Sanz, J.L., 2008. Analysis of microbial community during biofilm development in an anaerobic wastewater treatment reactor. *Microb. Ecol.*, **56**(1):121-132. [doi:10.1007/s00248-007-9330-2]

Ishii, S., Hotta, Y., Watanabe, K., 2008. Methanogenesis versus electrogenesis: morphological comparisons of microbial communities. *Biosci. Biotechnol. Biochem.*, **72**(2):286-294. [doi:10.1271/bbb.70179]

Klappenbach, J.A., Saxman, P.R., Cole, J.R., Schmidt, T.M., 2001. rrndb: the ribosomal RNA operon copy number database. *Nucleic Acids Res.*, **29**(1):181-184. [doi:10.1093/nar/29.1.181]

Lau, S.K.P., McNabb, A., Woo, G.K.S., Hoang, L., Fung, A.M.Y., Chung, L.M.W., Woo, P.C.Y., Yuen, K.Y., 2007. *Catabacter hongkongensis* gen. nov., sp. nov., isolated from blood cultures of patients from Hong Kong and Canada. *J. Clin. Microbiol.*, **45**(2):395-401. [doi:10.1128/JCM.01831-06]

Liang, J.B., Chen, Y.Q., Lan, C.Y., Tam, N., Zan, Q.J., Huang, L.N., 2007. Recovery of novel bacterial diversity from mangrove sediment. *Mar. Biol.*, **150**(5):739-747. [doi:10.1007/s00227-006-0377-2]

Official Journal of the European Union, 2006. Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 Concerning the Management of Bathing Water Quality and Repealing Directive 76/160/EEC, L064:0037-0051. Avail from http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:064:0037:01:EN:HTML

Tsukinowa, E., Karita, S., Asano, S., Wakai, Y., Oka, Y., Furuta, M., Goto, M., 2008. Fecal microbiota of a dugong (*Dugong dugong*) in captivity at Toba Aquarium. *J. Gen. Appl. Microbiol.*, **54**(1):25-38. [doi:10.2323/jgam.54.25]