MOLECULAR AND BIOCHEMICAL CHARACTERIZATION OF BATERIA ISOLATED FROM YUCATAN SEA WATER

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Introduction. The genus *Vibrio* is a highly diverse group of Gram-negative bacteria composed of approximately 72 species. The group includes symbioses and commensals found in or on marine animals, as well as many animal pathogenic species. *Vibrio* species routinely isolated from human clinical samples are implicated in diarrheal diseases, septicemia, and wound infections. Advances in molecular biology technology, particularly the polymerase chain reaction (PCR), have allowed more reliable microbial identification and surveillance. PCR techniques have increased the sensitivity, allowed more rapid detection process, and enhanced the likelihood of bacterial identification. In addition, the use of molecular and biochemical methods combined has been successfully applied to the detection and identification of organisms [1]. The present work describes the molecular and biochemical characterization of bacteria isolated from Yucatan sea water. .

Methods.
Bacterial strains.-Microorganisms used in this study were isolated from Yucatan (Mexico) seawater and grown on TBS and LB media at 37° C. The isolates were tentatively identified based on the morphological and physiological characteristics using biochemical tests according to Bergey's Manual of Systematic Bacteriology and Alsina and Blanch's (1994).[2] The 16S rDNA sequence was amplified with the primers V.16S-700F CGG TGA AAT GCG TAG AGA T 0.05 663 , V.16S-1325R TTA CTA GCG ATT CCG AGT TC and sequenced.[1]

Results
The isolate V17 was a Gram-negative, facultative anaerobe, oxidase-, catalase- and gelatinase positive motile rod that forms green mucoid colonies on the selective Thiosulfate-citrate-bile salts-sucrose (TCBS) agar. In addition, the microorganism was negative for inositol, sorbitol, and sucrose, positive for lysine and ornithine decarboxylases, and sensitive to the *Vibrio* static agent 0/29. The isolate was tentatively identified as *V. cincinnatiensis*.
The isolate V17 growth kinetics on TCBS medium is shown in Figure 1. As can be observed the microorganisms grew exponentially between 6 and 24 h.

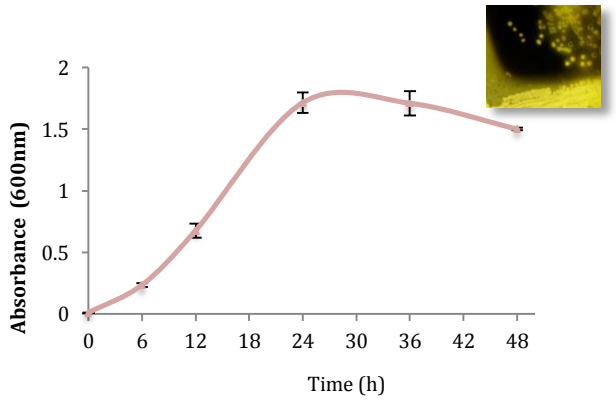

Figure 1 Isolate V-17 growth kinetics on TCBS broth + 1.5% NaCl at 35°C, pH 7 and 160rpm.

The 1200 nucleotides 16S rDNA sequences were aligned using Clustal W program, version 1.5 and then manually adjusted. The reference sequences were obtained from the Ribosomal Database Project and GenBank database. [3]

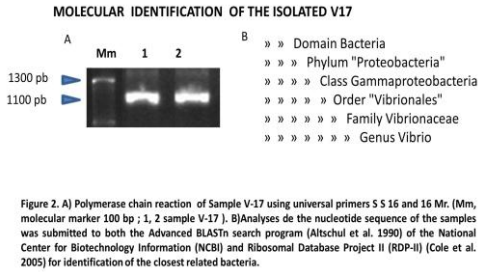

Figure 2. A) Polymerase chain reaction of Sample V-17 using universal primers S S 16 and 16 Mr. (Mm, molecular marker 100 bp ; 1, 2 sample V-17). B)Analyses de the nucleotide sequence of the samples was submitted to both the Advanced BLASTn search program (Altschul et al. 1990) of the National Center for Biotechnology Information (NCBI) and Ribosomal Database Project II (RDP-II) (Cole et al. 2005) for identification of the closest related bacteria.

Conclusions. Isolate V17 was identified as a *V. cincinnatiensis* strain. The molecular and biochemical characterization of this microorganism isolated from the coast of the Yucatan Peninsula, represents a scientific, epidemiological and ecological contribution to our region.

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References.

1. Tarr, C. L. Patel J. S. Puhr N. D, G. Sowers E, Bopp C. A.and Strockbine N. A. (2006) Identification of Vibrio isolates by a multiplex PCR assay and rpoB sequence determination J. Clin. Micro, 45: 134–140
2. Alsina, M. and Blanch, A.R. A set of keys for biochemical identification of environmental Vibrio species. (1994) J Appl Bacteriol 76:79–85.
3.Thompson, J.D., Higgings, D.G. and Gibson, T.J. CLUSTAL W: improving the sensitivity of progressive multiplesequence alignment through sequence weighting, position- specificgap penalties and weight matrix choice (1994) Nucleic. Acids. Res. 22, 4673–4680.