

Heterotrophic microorganism's identification in Actopan River mouth in Chachalacas, Veracruz, Mexico.

Monroy-Dosta MC*, Castro-Mejía G, Ciprés-Chávez, Hernández-Sánchez A, Reyes-Ruiz AN, Castro-Mejía J and Ramirez-Tórres JA.

Universidad Autónoma Metropolitana Unidad Xochimilco. Licenciatura en Biología. Módulo Plagas y Enfermedades de un Recurso natural Renovable. Departamento El Hombre y su Ambiente. Laboratorio de Análisis Químico de Alimento Vivo. Calzada del Hueso 1100, Col. Villa Quietud, Coyoacán, México, D.F. C.P. 04960.

*Corresponding author: monroydosta@hotmail.com

ABSTRACT

A water quality study was made in Rio Actopan mouth in Chachalacas, Veracruz, by detecting heterotrophic microorganisms in water samples collected in three different zones (Puente, La Loma and La Bocana). For determination of heterotrophic bacterial groups, 0.1 mL was seeded in growing culture media for amylolytic, chitinolytic, cellulolytic and nitrifying microorganisms. Isolated strains belong to *Bacillus luteus*, *Serratia sp.*, *Vibrio sp.*, *Pseudomonas cepacia*, *Photobacterium damsela*, *Cytophaga sp.* and *Candida utilis* species.

Key words: Heterotrophic microorganisms, Rio Actopan,

RESUMEN

Se realizó un estudio de la calidad del agua en la desembocadura del Río Actopan en Chachalacas, Veracruz, mediante la detección de microorganismos heterótrofos, para lo cual se recolectaron muestras de agua en tres zonas distintas (Puente, la Loma y la Bocana). Para la determinación de los grupos heterótrofos bacterianos se sembró 0.1 mL en medios para el crecimiento de microorganismos amonolíticos, quitinolíticos, celulolíticos, nitrificantes. Las cepas aisladas pertenecieron a las especies *Bacillus luteus*, *Serratia sp.*, *Vibrio sp.*, *Pseudomonas cepacia*, *Photobacterium damsela*, *Cytophaga sp.* y *Candida utilis*.

Palabras clave: Microorganismos heterótrofos, Río Actopan.

INTRODUCTION

Mexican aquatic ecosystems such as estuaries, rivers and coastal lakes, represent an important source of energetic, mineral and high fishery productivity resources (Cisneros 2010). For several years, these biomes have been under pressure by direct or indirect deposit of urban and industrial man wastes, producing a damage to aquatic organisms who lives there. These ecosystems are extremely fragile, and with the passage of time they have been suffering a significant transformation, such as close of communication between lakes and sea, river dams and mainly by pollutants discharged in these (Villanueva y Botello 1992, Cisneros 2010). That is why the study of heterotrophic microorganisms in aquatic environments is important, due to the relation between this organisms and their ecosystem, being able to adapt to a diversity of environments, they are more efficient in the use of substrates, resulting in a large physiological diversity (Miravet 2003). Hence its importance as presence bioindicators of polluted materials or substances. Therefore, the goal of this work was to evaluate the heterotrophic microorganism's population as bioindicators of water quality in Rio Actopan mouth.

MATERIAL AND METHODS

Study zone

Heterotrophic microorganisms at Actopan River

Monroy-Dosta MC, Castro-Mejía G, Ciprés-Chávez, Hernández-Sánchez A, Reyes-Ruiz AN, Castro-Mejía J and Ramirez-Tórres JA..

Rio Actopan is located in Chachalacas beach in Ursulo Galvan town, Veracruz between coordinates 19°24'36.46" N – 96°20'16.31" O (Fig. 1).

After incubation period, reactive Nit1 and Nit2 (Biomerieux) were added to nitrogen media, taking as positive the change of color of the tube from pink

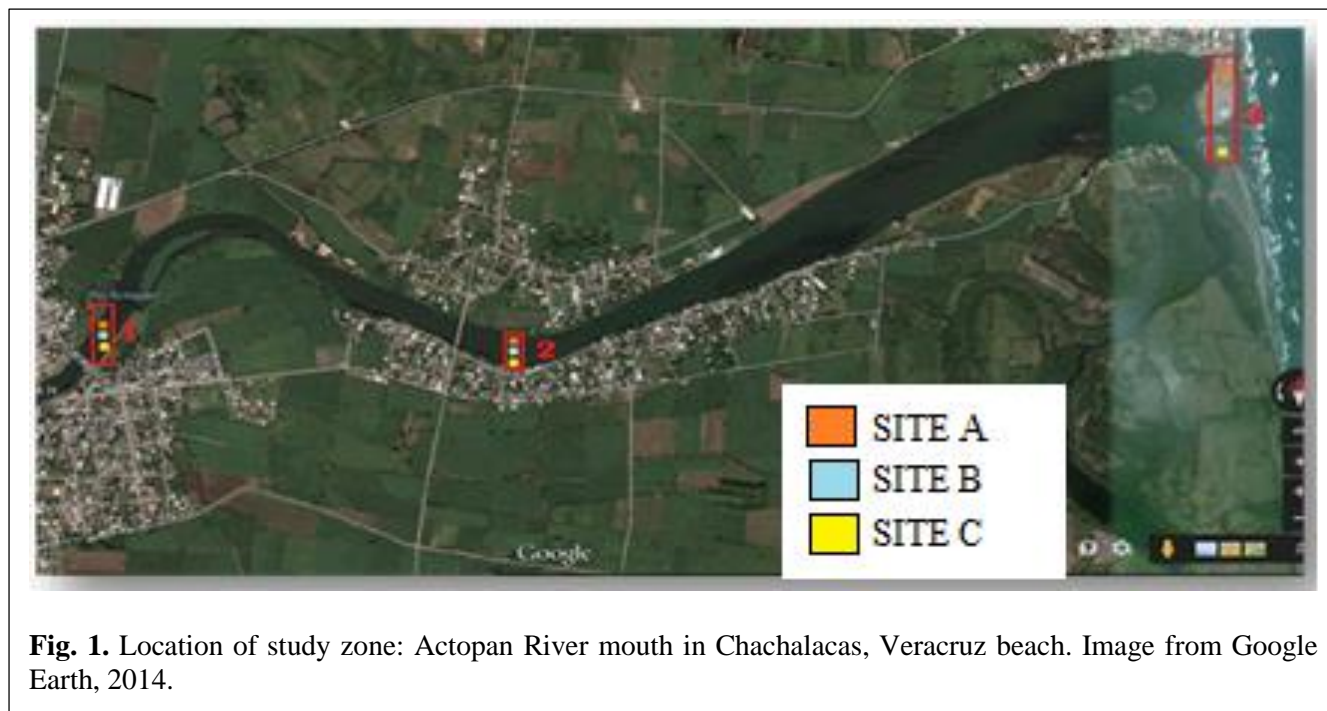


Fig. 1. Location of study zone: Actopan River mouth in Chachalacas, Veracruz beach. Image from Google Earth, 2014.

Fieldwork

Sample collection

Three sample zones were identified as vulnerable to contaminants, mainly by anthropogenic activity: Zone A: Ursulo Galvan bridge, Zone B: La Loma de San Rafael and Zone C: La Bocana. Using sterile beakers of 100 mL, three samples of water from each zone were taken.

Microbiological analysis

For detection of heterotrophic microorganisms that degrade chitin, cellulose, starch, keratin and nitrogen compounds, 1 mL of the samples was inoculated in liquid media of cellulose and nitrogen, and seeded in agar plates of chitin, starch and keratin. Once inoculated, were incubated during 72 hours at 30°C.

to purple. From all used media, microorganisms were isolated through consecutive seeding in Brain Heart Infusion (BHI) media, to perform the Gram stain in order to observe the cell morphology with a microscope Olympus BZ600.

Bacterial abundance

For the determination of bacterial abundance a direct counting in the plates was made, using a colony counter SOL-BAT brand, Q-20 model.

Bacterial identification

Conventional microbiological tests were performed on isolated strains for their identification such as: catalase, oxidase, fermentation oxide, mobility and indole (Bergey & Holt 994). The strains identification was confirmed with API20E, API20NE, APICHL, API CHL 50 system and Apiweb™ Biomerieux program.

Heterotrophic microorganisms at Actopan River

Monroy-Dosta MC, Castro-Mejía G, Ciprés-Chávez, Hernández-Sánchez A, Reyes-Ruiz AN, Castro-Mejía J and Ramirez-Tórres JA..

Physical and chemical analysis

Analysis considered in this study were of temperature, dissolved oxygen, turbidity, ammonium, nitrites and nitrates, taking 5 mL of the sample and were analyzed with the multi parameter device to determine the water quality HANNA brand.

Data analysis

To determine if there were differences between bacterial abundance and association between abiotic variables, a variance analysis and a discriminant analysis were made with the help of a statistical package Systat 10.2.

RESULTS

The results showed that cellulite bacteria were the predominant heterotrophic group with 43% frequency. The nitrifying and chitinolytic bacteria were less abundant with only 14% (Fig. 2).

In relation with microorganisms abundance (CFU mL⁻¹) found for each study zone, it was determined that Zone A (El Puente) reached counts up to 628 x10⁶ CFU mL⁻¹, in comparison with Zone C (Bocana) that presented only 33.667 x10⁶ CFU mL⁻¹ (Fig. 3).

Bacterial identification indicates a higher diversity in Zone B, where dominant species are from *Bacillus* genus. In Zone C representatives of *Vibrio* genus were mostly identified (Table 1).

By making the variance analysis between obtained values of bacterial abundance with each of abiotic variables, it is observed that temperature, salinity, dissolved oxygen, nitrites, ammonium, and phosphates make significant differences (P<0.05) (Table 2.).

By applying discriminant analysis, it was observed that variables of dissolved oxygen and temperature discriminate them in a positive way and ammonium and salinity variables in a negative way. As shown in figure 4, salinity and ammonium brings together the stations that are upstream; oxygen, salinity and ammonium

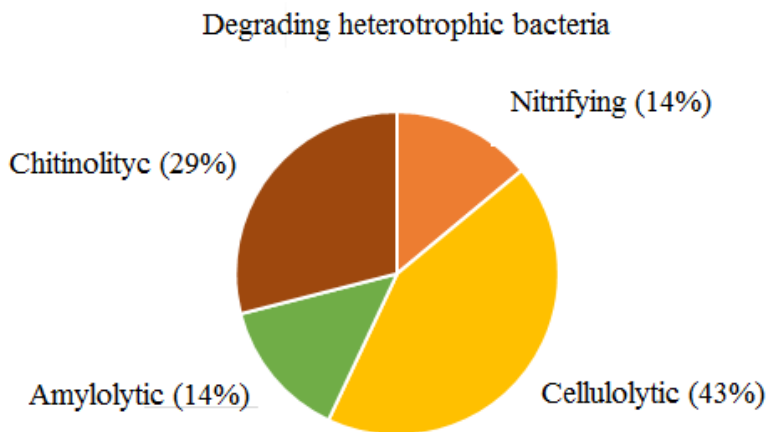


Fig. 2. Heterotrophic functional groups in Actopan River mouth.

Heterotrophic microorganisms at Actopan River

Monroy-Dosta MC, Castro-Mejía G, Ciprés-Chávez, Hernández-Sánchez A, Reyes-Ruiz AN, Castro-Mejía J and Ramirez-Tórres JA..

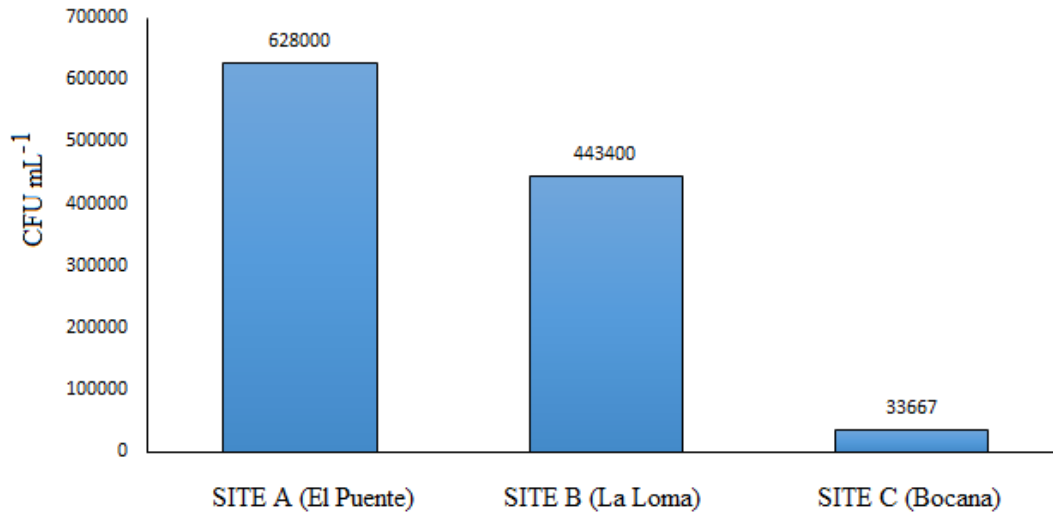


Fig.3. Microorganisms abundance (CFU mL⁻¹) for each study zone.

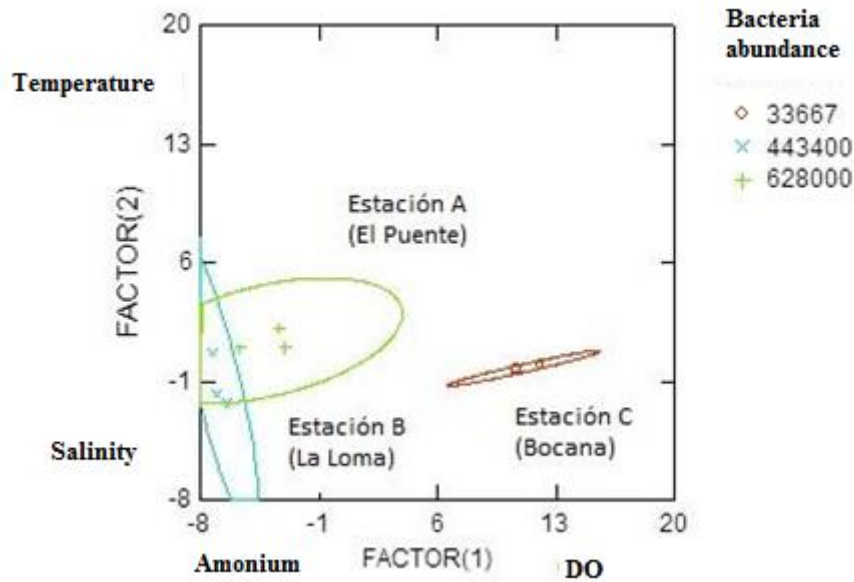


Fig. 4. Discriminant analysis of abiotic variables regarding to bacterial abundance between sampling stations of Actopan River.

Heterotrophic microorganisms at Actopan River

Monroy-Dosta MC, Castro-Mejía G, Ciprés-Chávez, Hernández-Sánchez A, Reyes-Ruiz AN, Castro-Mejía J and Ramirez-Tórres JA..

levels separate Bocana station with respect to the other two.

Table 1. Identified heterotrophic species in study zones.

Zone	Heterotrophic microorganisms	Functional group
A	<i>Nitrospira sp</i> <i>Pseudomonas cepacea</i> <i>Cytophaga spp.</i> <i>Bacillus.luteus</i>	Nitrifying Chitinolytic Cellulolytic Nitrifying
B	<i>Pseudomona cepacea</i> <i>Cytophaga spp.</i> <i>Candida utilis</i> <i>Bacillus brevis</i> <i>Bacillus pumillus</i>	Nitrifying Cellulolytic Amyolytic Nitrifying Chitinolytic
C	<i>Bacillus luteus</i> <i>Vibrio spp</i> <i>Vibrio harveyi</i>	Nitrifying Cellulolytic Chitinolytic

DISCUSSION

As a result of this study it is established that Ursulo Galvan bridge and La Loma de San Rafael are the zones with higher input of pollutants since higher abundances of heterotrophic bacteria were obtained and the five

functional groups were identified: cellulolytic, gelatinases, amyolytic, nitrifying and chitinolytic; being the degrader group of cellulose, gelatinase and nitrogen the ones with more presence, this is related with the presence of *Pseudomonas* genus bacteria as mentioned by Alvarado (2012), his bacterial genus has the capacity to degrade a large variety of toxic compounds (cellulose, starch, chlorinated phenols, phenoxyacetate, pesticides contents) so they are commonly used for bioremediation. In study zones, sugar mill landfill increases the presence of carbohydrates and cellulose in the environment which directly influences the increase of amyolytic bacteria, like *Vibrio sp.* *Cytophaga spp.* and *C. utilis* yeast, which were detected in this study. According to Jiménez *et al.* (1994) these bacteria species have a great capacity to degrade cellulosic organic compounds, using it as a sole carbon source. On the other hand Palmerín *et al.* 2011, suggest that the presence of these bacteria is mainly due to fluctuating plant material presence, and also paper wastes and industrial derivatives that increase heterotrophic microbial mass. *P. damsela* and *Serratia sp.* presence suggest a higher input of detritus, because these bacteria have the capacity to degrade chitin formed by filamentous fungi or arthropod exoskeletons

Table 2. Variance analysis between bacterial abundance and abiotic variables in the three sampling zones.

	Turbidity	Temperature	Salinity	DO	Nitrates	Nitrites	Ammonium	Phosphates
Bacterial abundance	0.288	0.017*	0.0001*	0.0001*	0.312	0.0001*	0.001*	0.0001*

*Significant values (P<0.05)

Heterotrophic microorganisms at Actopan River

Monroy-Dosta MC, Castro-Mejía G, Ciprés-Chávez, Hernández-Sánchez A, Reyes-Ruiz AN, Castro-Mejía J and Ramirez-Tórres JA..

(Sastoque 2005), and as mentioned by Rheinheimer (1987), suspended or dissolved in water, organic matter has an important role as main bacterial nutrition source, specially heterotrophic, finding more chitin and cellulose disintegrating bacteria's attached to detritus than freely, being an enabling environment for the optimal development of these microorganisms.

In study zones A and C *Bacillus luteus* was present, but according to Subhash *et al.* (2014) bacteria of this genus normally inhabit in freshwater environments, they can live in hypersaline or brackish conditions, which suggest that *B. luteus* can develop and reproduce in estuarine zone. Microorganisms of the *Bacillus* genus, degrade organic matter, mainly carbohydrates, and participate in the nitrogen cycle, because they are capable of fix gaseous nitrogen (N₂). Castillo *et al.* (2007) mentions that bacteria of the genus *Bacillus* mainly use starch as energy source, suggesting that in the estuarine zone a small amount of vegetable material dragged down by the river existed, being the only zone where *B. luteus* was present.

BIBLIOGRAPHY

- Alvarado-Castro JA. 2012. Identificación de bacterias heterótrofas de género *Pseudomonas* en dos temporadas climáticas del Lago Catemaco y la Laguna Sontecomapan, Veracruz, México. Tesis de Licenciatura. Departamento El Hombre y su Ambiente. Universidad Autónoma Metropolitana. México.
- Castillo-Vázquez NP, M Ortiz-Caro, GV Nevárez-Moorillón, A Quintero-Ramos, Ma.L Ballinas-Casarrubias, T Siqueiros-Cendón, O Paredes-López y Q Calvillo, Rascón-Cruz. 2007. Caracterización de bacterias degradadoras de celulosa y almidón. Facultad de Ciencias Químicas. Universidad de Chihuahua. México.
- Chávez M. 2004. Análisis de la Composición de Bacterias Heterótrofas que Habitan los Sedimentos del Golfo de México. Informe final de servicio social. Departamento el Hombre y su Ambiente. Universidad Autónoma Metropolitana Xochimilco. México.
- Cisneros-Delgado ZF. 2010. Caracterización de bacterias heterótrofas en dos ecosistemas lagunares: Catemaco y Sontecomapan, Veracruz en dos temporadas climáticas invierno y primavera del año 2010. Informe final de servicio social. Departamento el Hombre y su Ambiente. Universidad Autónoma Metropolitana Unidad Xochimilco. México.
- Jiménez Madrazo CH, ALR Ramos y JL de la Escosura. 1994. Aislamiento ambiental de *Vibrio cholerae* en aguas continentales en la provincia de Sevilla. Rev. Higiénica Sanitaria Pública 1: 178-196.
- Kirchman DL. 2000. Microbial ecology of the oceans. Segunda edición. Editorial S.I willey. 542 pp.
- Ma.E Miravet, M Lugioyo, S Loza, D Enríquez, Y Delgado, M Carmenate y DMA. Pérez. 2009. Procedimientos para el monitoreo de la calidad ambiental en la zona marina costera a partir de microorganismos. Departamento de Microbiología-Necton. Instituto de Oceanología, CITMA. La Habana, Cuba.
- Miravet-Regalado Ma.E. 2003. Abundancia, actividad y diversidad de las bacterias heterótrofas en el Golfo de Batabanó y su uso como indicadores ambientales. Departamento de Biología Marina. Instituto de oceanografía. MCTMA. La Habana, Cuba.
- Rheinheimer G. 1987. Microbiología de las aguas. Editorial Acribia. 4ª edición. Zaragoza, España. 106-127 pp.
- Sastoque Cala SE. 2005. Aislamiento y selección de microorganismos productores de quitinasas a partir de residuos de concha de camarón con potencial biocontrolador. Facultad de Ciencias. Carrera de microbiología industrial, agrícola y veterinaria. Pontificia universidad javeriana. Bogotá, D.C.
- Torres Ma.deR y F Bernal. 1985. Caracterización de la Biota Microbiana en la Zona Pantanosa del Bajo Rio Coatzacoalcos, Veracruz, México. Universidad Autónoma Metropolitana Iztapalapa, México.
- Villanueva SF y AV Botello. 1992. Metales pesados en la zona costera del Golfo de México y Caribe Mexicano: Una revisión. Laboratorio de Contaminación Marina. Instituto de Ciencias del Mar y Limnología. UNAM. México.

Heterotrophic microorganisms at Actopan River

Monroy-Dosta MC, Castro-Mejía G, Ciprés-Chávez, Hernández-Sánchez A, Reyes-Ruiz AN, Castro-Mejía J and Ramirez-Tórres JA..