Article **ECORFAN-Bolivia Journal**
June 2018 Vol.5 No.8 14-18

Molecular identification of *Aeromonas* spp. isolated from water in northeastern México

Identificación molecular de Aeromonas spp. aisladas de agua en el noreste de México

SANCHEZ-VARELA, A.[1], ORTEGA-BALLEZA, J. L.[1], RODRIGUEZ- LUNA, I. C.[2] & GUO, X.[3]

Instituto Politécnico Nacional, Centro de Biotecnología Genómica, boulevard del Maestro, con Elías Piña, Col. Narciso Mendoza, s/n, CP. 88710, Reynosa Tamaulipas, México. Laboratorio de Biotecnología Genómica[1].

ID 1° Autor: *Alejandro Sánchez-Varela/***ORC ID**: 0000-0002-3801-0162, **Researcher ID Thomson**: D-5397-2018, **arXiv ID**: asanvare, **PubMed ID**: sanchvas, **CVU CONACYT-ID**: 90836

ID 1° Coautor: *Jessica Lizbeth Ortega-Balleza /***ORC ID**: 0000-0002-9564-097X, **Researcher ID Thomson**: D-6372-2018, **arXiv ID**: Jessica_Ortega7, **PubMed ID**: jessica_ortega, **CVU CONACYT-ID**: 704038

ID 2° Coautor: *Isabel Cristina Rodríguez-Luna /***ORC ID**: 0000-0001-5999-8715, **Researcher ID Thomson**: D-6561-2018, **arXiv ID**: isacri77, **PubMed ID**: isacricbg, **CVU CONACYT-ID**: 463015

ID 3° Coautor: *Xianwu Guo /***ORC ID**: 0000-0003-2013-5853, **Researcher ID Thomson**: D-6709-2018, **arXiv ID**: xianwu, **PubMed ID**: xianwuguo, **CVU CONACYT-ID**: 26051

Received: February 05, 2018; Accepted: May 10, 2018

Abstract

Aeromonas are ubiquitous, predominantly in aquatic environments, in foods including meats, fish and vegetables, and in the intestine of healthy humans with diarrhea, there are strains circulating between humans and the environment. Some species are pathogenic in humans and poikilotherms. *Aeromonas* have been isolated since the 80s and have been associated with various pathologies in humans, especially in pediatric and immunocompromised patients. To date, more than 25 species have been identified through the use of molecular methods, including maintenance genes, including *gyr*B. Due to the above, the importance of an accurate and specific diagnosis for an adequate treatment. The objective of this work was to characterize molecularly isolated *Aeromonas* spp. from water from the environment of northeastern Mexico by PCR and sequencing of *gyr*B. 123 strains were identified as *Aeromonas* spp. using the *gyr*B gene. When sequencing, three species were detected: *A. veronii* (43.75%), *A. hydrophila* (43.75%) and *A. caviae* (12.50%). These results show that, the presence of this bacterium in different sources of water from the environment could be a latent risk for human health.

Molecular, Aeromonas, Patógeno

Resumen

Las *Aeromonas* son ubicuas, predominantemente en ambientes acuáticos, en alimentos incluyendo carnes, pescado y vegetales, y en el intestino de humanos sanos y con diarrea, existen cepas circulando entre el humano y el ambiente. Algunas especies son patógenas en humanos y poiquilotermos. Se han aislado *Aeromonas* desde los 80s y se han asociado a diversas patologías en humanos, sobre todo en pacientes pediátricos e inmunocomprometidos. A la fecha se han identificado más de 25 especies mediante el empleo de métodos moleculares incluyendo los genes de mantenimiento, entre ellos el *gyr*B. Debido a lo anterior la importancia de un diagnostico preciso y especifico para un tratamiento adecuado. El objetivo de este trabajo fue caracterizar molecularmente aislados de *Aeromonas* spp. a partir de agua del ambiente del noreste de Mexico mediante PCR y secuenciación de *gyrB.* Fueron identificadas 123 cepas como *Aeromonas* spp. mediante el gen *gyrB.* Al secuenciar fueron detectadas principalmente tres especies: *A. veronii* (43.75%), *A. hydrophila* (43.75%) y *A. caviae* (12.50%). Estos resultados muestran que, la presencia de esta bacteria en distintas fuentes de agua del medio ambiente, podría ser un riesgo latente para la salud humana.

Molecular, Aeromonas, Patógeno

Citation: SANCHEZ-VARELA, A.[1], ORTEGA-BALLEZA, J. L.[1], RODRIGUEZ- LUNA, I. C.[2], GUO, X.[3]. Molecular identification of Aeromonas spp. isolated from water in northeastern México. ECORFAN Journal-Bolivia. 2018. 5-8: 14-18.

* Correspondence to Author (email: asanchezv@ipn.mx)
† Researcher contributing as first author.

Introduction

Aeromonas belong to the Aeromonadaceae family, and is constituted by Gram-negative bacteria, facultative anaerobes, oxidase and catalase positive, as well as resistant to the vibriostatic agent O / 129 (Janda and Abbott, 2010). This bacterium has been classified into two groups: mesophilic and psychrophilic; Aeromonas is indigenous to aquatic environments, some are producers of surfactants and grow in areas contaminated with hydrocarbons (Llori et al., 2005), others are isolated from food (meats, fish, seafood, vegetables and drinking water), as well as feces. of patients with diarrhea and wound infections who have been exposed to contaminated aquatic environments (Castro-Escarpulli et al., 2002), (Neyts et al., 2000), (Merino et al., 1995), (Parker and Shaw , 2011).

Some species are pathogenic in humans and poikilotherms (Merino et al., 1995). Aeromonas have been isolated since the 80s and have been associated with various pathologies in humans, especially in pediatric and immunocompromised patients (Castro-Escarpulli et al., 2002) (Batra et al., 2016). The main species of clinical importance associated with infections in humans are: A. caviae, A. hydrophila and A. veronii bv. sober (Janda and Abbott, 2010), in one study indicate that A. hydrophila was the most common (Figueras et al., 2009) (Figueras & Beaz-Hidalgo, 2015). By 2012, up to 25 species of the genus Aeromonas had already been identified (Roger et al., 2012). Aeromonas contains virulence genes with the potential to cause damage to a host (Graevenitz, 2007).

Although some studies have shown enterotoxicity in some strains of Aeromonas, it has not been established as an etiological agent, because there is no epidemiological information of outbreaks with infections associated with this bacterium and for not having an animal model established to reproduce the infections caused by this bacterium (Tomás, 2012).

In order to establish an effective diagnosis and treatment, it is essential to have methodologies that allow the correct identification of bacterial species from different types of samples (Castro-Escarpulli et al., 2003).

The 16S rRNA gene has several copies of the gene with intragenomic heterogeneity in Aeromonas strains and a high similarity (98.5%) in its nucleotide sequence (Janda and Abbott, 2010 (Persson et al., 2015), for the species of A. trota and A. caviae are distinguishable by a single nucleotide (Yanez et al., 2003), which is impractical for the identification of all species of the genus (Nhung et al., 2007). represent a good option, one of them is gyrB, which has been widely used in the identification of bacteria (Yamamoto and Harayama, 1995), because this gene has a greater synonymous substitution 16S rRNA and is important in the DNA replication (Yamamoto and Harayama, 1995).

Because of this, the aim of our study was to characterize molecularly isolated Aeromonas spp. from water samples from northeastern Mexico using the gyrB gene, and sequencing, which will allow to know their distribution and probable implications in the environment.

Methodology

We analyzed 136 strains of presumed Aeromonas collected from surface water (25), residual (25) and drinking water (25), Northeast region of Mexico. The isolates were analyzed by the following biochemical tests: Gram stain, oxidase, catalase, glucose fermentation and hemolysis. An aliquot of each isolate was stored at -70 ° C in 85% glycerol, until use.

The DNA was obtained using the Promega Wizard® Genomic Ref. A1120 commercial kit, following the instructions of it, it was analyzed by electrophoresis in 1% agarose gel for 1 h at 80 Volts, then visualized in a Kodak® photo-documenter with camera Gel Logic 112 using the Kodak® Ds 1D bioinformatics program. Additionally, the concentration and quality of the DNA samples were determined in a NanoDrop ™ 2000 from ThermoScientific.

The PCR of gyrB was performed at a final volume of 25 µl; 1µl DNA (average concentration 100-200 ng / µl), 2.5 µl 1X buffer, 0.75µl 1.5 mM MgCl 2, 0.5µl 0.05mM dNTPs, 0.5µl initiators 0.1µM gyrB-F and 1 µl DNA were placed in each reaction tube. gyrB-R and 1.25 U / µl of Taq polymerase and 19 µl of sterile milli-Q water.

ISSN-On line: 2410-4191

The reaction was performed in a Veriti® thermocycler from Applied Biosystems, under the following conditions: 95 ° C for 2 min, then 30 cycles at 95 ° C for 30 sec, 55 ° C for 30 sec, extension at 72 ° C for 1.50 min, and finish at 72 ° C for 10 min. The PCR products were analyzed by electrophoresis in 1.5% agarose gel for 1 h at 80 Volts.

The gel was then visualized in a Kodak® photodocument with a Gel Logic 112 camera. The PCR fragment was 967 bp, which was confirmed by means of a molecular weight marker HyperLadder ™ 100bp.

The ExoSAP-IT ™ commercial kit was used to remove impurities from the PCR products. Sequencing reactions were then performed using the BigDye® v.3.1 Ready Mix commercial kit of the 967 bp fragments from the gyrB gene, then the reactions were cleaned, using the BigDye® X-Terminator ™ commercial kit and SAM ™ solution from Applied Biosystems, to immediately read in an automated sequencer ® 3130 Genetic Analyzer from Applied Biosystems, for the subsequent identification of strains with the gyrB gene at the species level, in the NCBI.

Results and Discussion

Of the 136 strains identified biochemically only 123 were members of the genus Aeromonas, by means of PCR of the gyrB gene, some products are shown in Figure 1.

We obtained 65 strains of paritr wastewater (53%), 53 strains of surface water (43%) and 5 strains of drinking water (4%). In different studies that used maintenance genes, they show results co-occurring with Aeromonas, (Martínez-Murcia et al., 2011).

The gyrB gene was proposed for the identification of species of the genus Aeromonas, since it is universally distributed and capable of differentiating at the species level as well as at the intraspecific level, which is why it is a good option (Yáñez et al., 2003, Soler et al., 2004, Martínez-Murcia et al., 2011).

The specificity of the primers of the gyrB gene was corroborated, confirming with the genus Aeromonas, when sequencing the reference strain Aeromonas hydrophila subsp. hydrophila ATCC 7966 (Seshadri et al., 2006).

When comparing in the NCBI database, the sequence was analyzed and a 99% similarity and coverage was obtained.

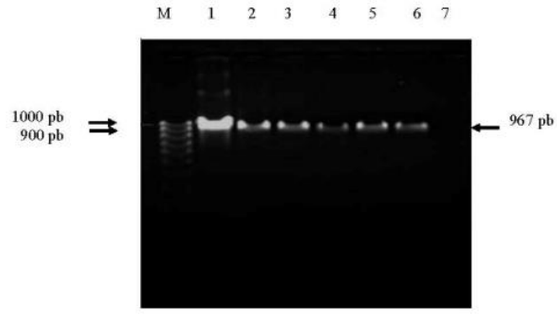

Figure 1 PCR product of the gyrB gene. 1.5% agarose gel with SYBR Gold, 80 V for 1 h. (M), HyperLadder ™ 100bp molecular weight marker; (1), Aeromonas hydrophila subsp. hydrophila ATCC 7966; (2), 027-05a; (3), 027-05b; (4), 028-05a; (5), 028-05b; (6), 028-05c; (7), Negative control.

Derived from the analysis and comparison of the obtained sequences, homologies of 95-99% were found and three species were identified: A. hydrophila, A. veronii both with the same proportion (43.75%) and A. caviae (12.50%).

On the other hand, the strains from drinking water the most frequent species was A. veronii (66.66%). Surface water was detected mainly in A. hydrophila (50.0%), followed by A. veronii (33.33%) and A. caviae (16.66%). While in residual water A. hydrophila and A. veronii were presented with the same frequency (42.85%) and A. caviae in a lower percentage (14.28%);In previous studies, A. veronii was found as the most prevalent species from surface water (62.5%) and A. hydrophila only 5.0% (Hu et al., 2012), in another study high percentages were found (89.2). %) in this type of samples (Zhou et al., 2013). In other studies a great diversity of isolates from clinical isolates was found Aguilera-Arreola et al., 2007) (Puthucheary et al., 2012), as well as surface water in lakes (Khor et al., 2015).

Acknowledgments

To the Instituto Politécnico Nacional, to the Secretary of Research and Postgraduate of the Instituto Politécnico Nacional and to the Commission of Operation and Promotion of Academic Activities.

ISSN-On line: 2410-4191

Conclusions

The presence of Aeromonas in the environment is related to previously reported in different publications.

The use of the gyrB gene proved to be an efficient and rapid molecular tool for the identification of species of the genus Aeromonas. A higher frequency of Aeromonas was found in wastewater than in drinking water in the northeastern region of Mexico. Isolating Aeromonas, from environmental sources of water, considered a pathogen, indicates a latent risk to human health in the region.

Perspectives

As a future consideration, the determination of the microbiological processes of the genus of Aeromonas could be extended in the networks of ground, coastal and reservoir waters contemplating their presence and their impact on other ecosystems.

References

Aguilera-Arreola, M. G., Hernández-Rodríguez, C., Zúñiga, G., Figueras, M. J., Garduño, R. A, & Castro-Escarpulli, G. (2007). Virulence potential and genetic diversity of *Aeromonas caviae*, *Aeromonas veronii*, and *Aeromonas hydrophila* clinical isolates from Mexico and Spain: a comparative study. *Canadian Journal of Microbiology*, 53(7): 877–87.

Batra, P., Mathur, P and Misra, M. C. (2016). *Aeromonas* spp.: An Emerging Nosocomial Pathogen. *Journal of Laboratory Physicians*. 8 (1): 1-4.

Castro-Escarpulli, G., Aguilera-Arreola, M. G., Giono-Cerezo, S., Hernández-Rodríguez, C. H., Rodríguez-Chacón, M., Soler-Falgas, L., Aparicio-Ozores, G., Figueras-Salvat, M. J. (2002). El género *Aeromonas*. ¿Un patógeno importante en México? *Enfermedades Infecciosas y Microbiología*. 22 (4): 1-12.

Castro-Escarpulli, G., Figueras, M. J., Aguilera-Arreola, G., Soler, L., Fernández-Rendón, E., Aparicio, G. O., Guarro, J., Chacón, M. R. (2003). Characterisation of *Aeromonas* spp. isolated from frozen fish intended for human consumption in Mexico. *International Journal of Food Microbiology*. 84(1): 41–49.

Figueras, M. J., Alperi A., Saavedra, M.J., Ko, W.C., Gonzalo, N., Navarro, M., Martínez-Murcia, J. (2009). Clinical relevance of the recently described species *Aeromonas aquariorum*. *Journal of Clinical Microbiology*. 47 (11): 3742–3746.

Figueras, M. J. & Beaz-Hidalgo, R. (2015). *Aeromonas* infections in humans, in *Aeromonas*, Chap. 4. Chap. 4. Ed. Graf J. Caister Academic Press. EE. UU. 65–108.

Graevenitz, A. Von. (2007). The role of *Aeromonas* in diarrhea: a review. *Infection*. 2: 59–64.

Hu, M., Wang, N., Pan, Z. H., Lu, C. P. & Liu, Y. J. (2012). Identity and virulence properties of *Aeromonas* isolates from diseased fish, healthy controls and water environment in China. *Letters in Applied Microbiology.* 55(3): 224–233.

Janda, J. M. & Abbott, S. L. (2010). The genus *Aeromonas:* Taxonomy, pathogenicity, and infection. *Clinical Microbiology Reviews.* 23 (1): 35-73.

Khor, W. C., Puah, S. M., Tan, J. A. M. A., Puthucheary, S. D., & Chua, K. H. (2015). Phenotypic and genetic diversity of *Aeromonas* species isolated from fresh water lakes in Malaysia. *PLoS ONE*. 10(12). 1–13.

Llori, M. O., Amobi, C. J. and Odocha, A. C. (2005). Factors affecting biosurfactant production by oil degrading Aeromonas spp. Isolated from a tropical environment. Elsevier, 61(7), pp. 985-992.

Martínez-Murcia, A. J., Monera, A., Saavedra, M. J., Oncina, R., Lopez-Alvarez, M., Lara, E., & Figueras, M. J. (2011). Multilocus phylogenetic analysis of the genus *Aeromonas. Systematic and Applied Microbiology*. 34(3), 189–199.

Merino, S., Rubires, X., Knochel, S., & Tomás, J. M. (1995). Emerging pathogens: *Aeromonas* spp. International journal of food microbiology, 28(2), 157-168.

Neyts, k., Huys, G., Uyttendaele, M., Swings, J., and Debevere, J. (2000). Incidence and Identification of mesophilic *Aeromonas* spp retail foods. Lett Appl Microbiol, 31(5), pp 359-363.

ISSN-On line: 2410-4191

Nhung, P. H., Hata, H., Ohkusu, K., Noda, M., Shah, M. M., Goto, K. & Ezaki, T. (2007). Use of the novel phylogenetic marker *dna*J and DNA – DNA hybridization to clarify interrelationships within the genus *Aeromonas. International Journal of Systematic and Evolutionary Microbiology*. 57 (5): 1232–1237.

Parker, J. L. and Shaw, J. G. (2011). *Aeromonas* spp. Clinical microbiology and disease. J. Infect. Vol. 62 (2), pp 109-118.

Persson, S., Al-Shuweli, S., Yapici, S., Jensen, J. N. & Olsen, K. E. P. (2015). Identification of clinical *Aeromonas* species by *rpo*B and sequencing and development of a multiplex PCR method for detection of *Aeromonas hydrophila*, *A. caviae*, *A. veronii*, and *A. media*. *Journal of Clinical Microbiology*. 53(2). 653–656.

Puthucheary, S. D., Puah, S. M. & Chua, K. H. (2012). Molecular characterization of clinical isolates of *Aeromonas* species from Malaysia. *PLoS ONE*. 7(2): 1-7.

Roger, F., Lamy B, Jumas-Bilak E & Kodjo A. (2012). Ribosomal multi-operon diversity: an original perspective on he genus *Aeromonas*. *PLoS One*. 7 (9): 1-12.

Seshadri, R., Joseph, S. W., Chopra, A. K., Sha, J., Shaw, J., Graf, J. & Heidelberg, J. F. (2006). Genome sequence of *Aeromonas hydrophila* ATCC 7966T: Jack of all trades. *Journal of Bacteriology*. 188(23). 8272–8282.

Soler, L., Yáñez, M. A., Chacon, M. R., Aguilera-Arreola, M. G., Catalán, V., Figueras, M. J., & Martínez-Murcia, A. J. (2004). Phylogenetic analysis of the genus *Aeromonas* based on two housekeeping genes. *International Journal of Systematic and Evolutionary Microbiology*. 54(5), 1511–1519.

Tomás, J. M. (2012). The main *Aeromonas* pathogenic factors. *ISRN Microbiology*. 2012: 1-22.

Yamamoto, S. & Harayama, S. (1995). PCR amplification and direct sequencing of *gyr*B genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Applied Environmental Microbiology*. 61(3): 1104–1109.

Yáñez, M. A., Catalán, V., Apráiz, D., Figueras, M. J. & Martínez-Murcia, A.J. (2003). Phylogenetic analysis of members of the genus *Aeromonas* based on *gyr*B gene sequences. *International Journal of Systematic and Evolutionary Microbiology*. 53(3): 875–883.

Zhou, Q. L., Wang, Y. J., Xie, J., Ge, X. P., Xi, B. W., & Liu, B. (2013). Distribution and virulence gene comparison of *Aeromonas* strains isolated from diseased fish and water environment. *Polish Journal of Microbiology*. 62(3), 299–302.

ISSN-On line: 2410-4191