ECRONICON OPEN ACCESS

MICROBIOLOGY
Research Article

Transport DNA by Outer Membrane Vesicles of *Aeromonas hydrophila*

Andrea Guerrero-Mandujano, Ingrid Palma-Martínez and Graciela Castro-Escarpulli*

Laboratorio de Bacteriología Médica, Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico

***Corresponding Author:** Graciela Castro-Escarpulli, Laboratorio de Bacteriología Médica, Departamento de Microbiología, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Prolongación de Carpio y Plan de Ayala s/n Colonia Santo Tomás, Delegación Miguel Hidalgo, C.P. 011340, Distrito Federal, Mexico.

Received: November 16, 2015; **Published:** December 17, 2015

Abstract

Aeromonas is a bacterial genus of medical, environmental and veterinarian relevance. It presents a multifactor pathogenicity mechanism, attributed to different putative factors of virulence among which there are types II, III, IV, VI secretion systems, and the recently termed type 0 secretion systems. The latter transports biomolecules through the outer membrane vesicles; until now, it exists evidence indicating that *Aeromonas* produces outer membrane vesicles while growing *in vivo* and *in vitro,* but no data is known about the molecules that it transports. Consequently, the aim of this study was to determine if those particles transport DNA. After isolating and purifying the outer membrane vesicles, the DNA extraction was carried out; where a concentration superior to 100 mg/mL was obtained. The present study describes the first evidence about the particles produced by the *Aeromonas* genus transport DNA.

Keywords: *Outer membrane vesicle; Bacteria; Secretion systems; Aeromonas; DNA*

Abbreviations: OMV: Outer membrane vesicle; TEM: Transmission Electron Microscopy

Introduction

Aeromonas is a bacterial genus that groups Gram-negative bacilli, positive oxidase and catalase; it is autochthone from the aquatic environment, it presents a global distribution and the various species have been isolated from different ecological niches as free bacteria or as etiological agents of bacterial infections. Its pathogenic capacity is of veterinarian relevance; however, it can cause intestinal or extraintestinal infections in humans. Numerous studies have demonstrated that the pathogenicity mechanism of *Aeromonas* is multifactor, attributed to different putative factors of virulence [1].

Among the putative factors of virulence of the *Aeromonas* genus there are types II, III, IV, and VI secretion systems, through which they secrete enzymes and toxins from the cytoplasm outside the bacterium or inside a target cell [2]. In the last few years, a new secretion system termed type 0 has been described in Gram-negative bacteria, this system releases molecules inside the particles derived from the outer membrane, called the outer membrane vesicles (OMVs) [3].

The OMVs are spherical particles with a 50-250 nm diameter; they are formed by a lipid bilayer, phospholipids and proteins of the outer membrane. The OMVs are generated when a protuberance is formed in the membrane that eventually will strangle itself and will be release as a vesicle, the functions described for the OMVs are numerous, and among them is the DNA transportation [4].

Even though, there are no reports of non-productive strains of outer membrane vesicles, there are bacterial genus in which this phenomenon has not been studied, such is the case of the *Aeromonas*, in which the microscopic evidences have demonstrated the production

of OMVs during *in vivo* and *in vitro* growth, but it has not yet been established its structure and the possible functions it performs [5,6]. The present study demonstrated that the OMVs produced during *in vitro* culture of of *Aeromonas hydrophila* F-0050 transport DNA.

Materials and Methods

Biological material and growth conditions

A. *hydrophila* F-0050 was isolated from diarrhoeal stool of a two-year-old child and identified by RFLP-PCR. First the strain was grown in tryptone soy agar (TSA) incubated at 37°C for 24h; then to obtain sufficient biomass for the isolation and purification of OMVs the strain was grown in one hundred plates with Craig agar (30 g/l of Cas-amino acids, 4 g/l yeast extract, 15 g/l bacteriological agar, 0.4 g/l of potassium phosphate dihydrate) incubated in the same conditions.

Isolation and purification of OMVs

After the incubation, the biomass was harvested in phosphate buffered saline (PBS), it was centrifuged and discarded; the supernatant was filtered with a 0.22 µm membrane and an aliquot was inoculated on TSA for sterility test. The filtered supernatant was ultracentrifuged at 100,000 × g for 1 h at 4°C. The pellet was re-suspended in 500 µl of PBS and the OMVs were purified by ultracentrifugation in an iodixanol concentration gradient [7].

Transmission Electron Microscopy (TEM)

The sample was analyzed by TEM, 20 µl of the OMV sample was taken and placed on a formvar-coated grid, then was negative stained with phosphotungstic acid, and it was analyzed with a JEOL Model 1010 transmission electron microscope at an accelerating voltage of 60 Kv.

DNA extraction

To eliminate the free and associated DNA at the OMVs surface, the OMV sample was treated with DNase for 10 min at 37°C, the enzyme was deactivated at 80°C 10 min. The DNA was extracted by phenol-chloroform technique and quantified by spectrometry; the integrity was verified by electrophoresis at 0.8 % agarose and stained with ethidium bromide concentration.

Results and Discussion

Recent studies have demonstrated that OMVs perform numerous functions in favour of bacterial survival. The most studied function is the protein transportation; nevertheless, recent reports indicate that those structures are also able to transport nucleic acids. Various techniques of isolation and purification have been developed to study OMVs' structure and functions [8].

To determine OMVs structure it is important to remove any other cellular component from the strain, consequently the purification phase is essential in this study [8]. After the purification in the density gradient, the micrographs obtained through TEM, there are only observed spherical structures delineated by one membrane with a diameter of 20 to 250 nm, this corresponds to the morphology described for the OMVs and it confirms that the purification technique was successful; thus, it can be ensured that the obtained sample is exempt from other cellular structures like scourges or cellular detritus (Figure 1).

Once the OMVs were purified, the DNA extraction was carried out, although the microscopic evidence the sample only contains OMVs. A prior treatment was carried out with DNase to remove DNA that might be associated through ionic interactions with the outer surface of the vesicles [9].

Some authors perform the DNA extraction by dissociating the vesicles with some type of detergent and then purify it with a purification kit of PCR products [9], this technique was not positive in the vesicles produced by *Aeromonas* (results not shown), thus, the DNA extraction was carried out based on the phenol-chloroform extraction. The DNA sample, presented a concentration higher than 100 mg/mL and after the agarose gel electrophoresis, a unique band of complete DNA was observed (Figure 2).

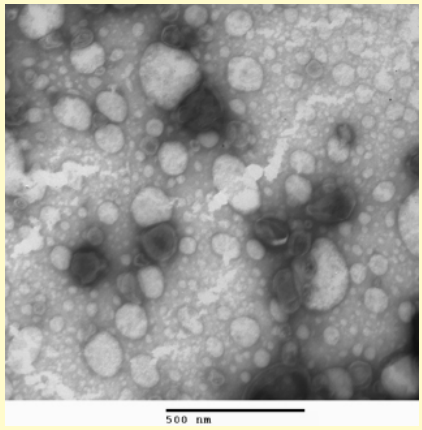

Figure 1: *Micrograph of purified OMVs from Aeromonas hydrophila. Particles delimited by a membrane with OMVs characteristic morphology are observed.*

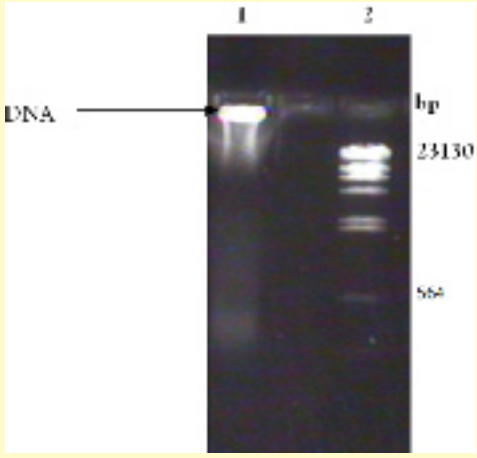

Figure 2: *Electropherogram of the DNA contained in Aeromonas hydrophila OMVs (lane 1); λ DNA/ HIND III fragments (lane 2).*

Based on the prior treatment carried out, it is possible to ensure that the DNA obtained was inside the OMVs, consequently it is now interesting to determine which is the codified data of that DNA and if the OMVs produced by the *Aeromonas* genus participate in a new mechanism of horizontal gene transfer.

There are studies indicating that the OMVs produced by other bacterial genres like *Acinetobacter*, where it was observed that OMVs transfer carbapenem resistance genes or *Escherichia coli* where the OMVs transfer genes of the Shiga toxins [10,11]. Whereas the species of the *Aeromonas* genus present intrinsic resistance to some antimicrobials and those species can be found in multiple environments polymicrobials, the results presented in this study are highly relevant to research if the DNA presented in the OMVs produced by *Aeromonas*, has microbial resistance genes or virulence putative factors that might be transferred interspecies or intraspecies.

Conclusion

The first function described for the OMVs produced during the *in vitro* culture of *Aeromonas hydrophila* is the DNA transportation.

Acknowledgements

This study was funded by *Instituto Politécnico Nacional* (SIP- IPN code 20150750). The SIP-IPN was not involved in the development of the study design, collection, analysis, and interpretation of the data, the writing of the report nor the decision to submit the paper for publication. Graciela Castro-Escarpulli received support from of *Estímulos al Desempeño en Investigación* and *Comisión y Fomento de Actividades Académicas (Instituto Politécnico Nacional)* and *Sistema Nacional de Investigadores* (SNI, CONACyT) and Andrea Guerrero Mandujano and Ingrid Palma Martínez held a scholarship from CONACyT and BIFI. We thank Patricia Arzate Barbosa for providing strain isolates. We would like to thank Sofia Marteli Mulia for kindly proofreading this manuscript.

Conflict of interests

The author(s) declare that they have no competing interests.

Bibliography

1. Figueras M J and Baez-Hidalgo R. "*Aeromonas* infections in humans." *J. Graf. Aeromonas*. E.U.A.: Caister Academic Press (2015). 65-108.
2. Beaz-Hidalgo R and MJ Figueras. "*Aeromonas* spp. whole genomes and virulence factors implicated in fish disease". *Journal of fish diseases* 36.4 (2013): 371-388.
3. Kieselbach T., *et al*. "Proteomics of *Aggregatibacter actinomycetemcomitans* Outer Membrane Vesicles". *PloS one* 10.9 (2015): 1-21.
4. Schwechheimer C and Meta JK. "Outer-membrane vesicles from Gram-negative bacteria: biogenesis and functions". *Nature Reviews Microbiology* 13.10 (2015): 605-619.
5. Longa-Briceño A., *et al*. "Experimental toxicity of *Aeromonas* spp. in mouse´ s small intestine: ultrastructural aspects". *Interciencia: Revista de ciencia y tecnología de América* 33.6 (2008): 457-460.
6. Guerrero-Mandujano A., *et al*. "Production of Outer Membrane Vesicles in a Clinical Strain of *Aeromonas hydrophila*". *EC Microbiology* (2015): 113-117.
7. Kyoung-Soon J., *et al*. "Comprehensive proteomic profiling of outer membrane vesicles from *Campylobacter jejuni*". *Journal of proteomics* 98.8 (2014): 90-98.
8. Chatterjee SN and Chaudhuri K. "Gram-negative bacteria: The cell membranes." Outer Membrane Vesicles of Bacteria". *Springer Berlin Heidelberg* 64.2 (2012): 15-34.
9. Ramón-Rocha MO., *et al*. "Membrane vesicles released by *Avibacterium paragallinarum* contain putative virulence factors". *FEMS microbiology letters* 257.1 (2006): 63-68.
10. Rumbo C., *et al*. "Horizontal transfer of the OXA-24 carbapenemase gene via outer membrane vesicles: a new mechanism of dissemination of carbapenem resistance genes in *Acinetobacter baumannii*". *Antimicrobial agents and chemotherapy* 55.7 (2011): 3084-3090.
11. Kolling GL and Matthews KR. "Export of virulence genes and Shiga toxin by membrane vesicles of *Escherichia coli* O157: H7". *Applied and environmental microbiology* 65.5 (1999): 1843-1848.

Volume 2 Issue 4 December 2015