Volume 5, Issue 8 — January — June — 2018

E
C
O
R
F
A
N

Journal-Bolivia

ISSN-On line: 2410-4191

ECORFAN®

-

ECORFAN-Bolivia

Indexing and database

- LATINDEX (Revistas Científicas de América Latina, España y Portugal)
- RESEARCH GATE (Alemania)
- GOOGLE SCHOLAR (Índices de citaciones-Google)
- REDIB (Red Iberoamericana de Innovación y Conocimiento Científico- CSIC)
- MENDELEY (Gestor de Referencias bibliográficas)

ECORFAN Journal-Bolivia, Volume 5, Issue 8, January - June 2018, is a journal edited four-monthly by ECORFAN-Bolivia. Loa 1179, Cd. Sucre. Chuquisaca, Bolivia. WEB: www.ecorfan.org, revista@ecorfan.org. Editora en Jefe: RAMOS-ESCAMILLA, María. PhD, Co-Editor: IGLESIAS-SUAREZ, Fernando. BsC. ISSN-On line: 2410-4191. Responsible for the latest update of this number ECORFAN Computer Unit. ESCAMILLA-BOUCHÁN, Imelda. PhD, LUNA SOTO-Vladimir. PhD. Loa 1179, Cd. Sucre. Chuquisaca, Bolivia, last updated June 30, 2018.

Editorial Board

GALICIA-PALACIOS, Alexander. PhD
Instituto Politécnico Nacional, México

NAVARRO-FRÓMETA, Enrique. PhD
Instituto Azerbaidzhan de Petróleo y Química Azizbekov, Russia

BARDEY, David. PhD
University of Besançon, France

IBARRA-ZAVALA, Darío. PhD
New School for Social Research, U.S.

COBOS-CAMPOS, Amalia. PhD
Universidad de Salamanca, Spain

ALVAREZ-ECHEVERRÍA, Francisco. PhD
University José Matías Delgado, El Salvador

BELTRÁN-MORALES, Luis Felipe. PhD
Universidad de Concepción, Chile, Chile

BELTRÁN-MIRANDA, Claudia. PhD
Universidad Industrial de Santander- Colombia, Colombia

ROCHA-RANGEL, Enrique. PhD
Oak Ridge National Laboratory, U.S.

RUIZ-AGUILAR, Graciela. PhD
University of Iowa, U.S.

TUTOR-SÁNCHEZ, Joaquín. PhD
Universidad de la Habana, Cuba.

VERDEGAY-GALDEANO, José. PhD
Universidad de Granada, Spain.

SOLIS-SOTO, María. PhD
Universidad San Francisco Xavier de Chuquisaca, Bolivia

GOMEZ-MONGE, Rodrigo. PhD
Universidad de Santiago de Compostela, Spain

ORDÓÑEZ-GUTIÉRREZ, Sergio. PhD
Université Paris Diderot-Paris, France

ARAUJO-BURGOS, Tania. PhD
Universita Degli Studi Di Napoli Federico II, Italy.

SORIA-FREIRE, Vladimir. PhD
Universidad de Guayaquil, Ecuador

FRANZONI-VELAZQUEZ, Ana. PhD
Instituto Tecnológico Autónomo de México, Mexico

OROZCO-GUILLÉN, Eber. PhD
Instituto Nacional de Astrofísica Óptica y Electrónica, Mexico

QUIROZ-MUÑOZ, Enriqueta. PhD
El Colegio de México, Mexico

SALAMANCA-COTS, María. PhD
Universidad Anáhuac, Mexico

Arbitration Committee

SCE, PhD
Universidad Latina, Mexico.

SMR, PhD
Universidad Autónoma Metropolitana, Mexico

VIR, PhD
Instituto Mexicano del Transporte, Mexico

WVA, PhD
Universidad Politécnica Metropolitana de Hidalgo, Mexico

YCD, PhD
Centro Eleia, Mexico

ZCN, MsC
Universidad Politécnica de Altamira, Mexico

Presentation

ECORFAN Journal-Bolivia is a research journal that publishes articles in the areas of:

Engineering Chemestry Optical Resources Food Technology Anatomy and Nutrition.

In Pro-Research, Teaching and Training of human resources committed to Science. The content of the articles and reviews that appear in each issue are those of the authors and does not necessarily the opinion of the editor in chief.

In Number 8 th presented the article *Bdellovibrio bacteriovorus as a possible biological control agent* by ARANDA-VIVAS, Leonardo Iván, SÁNCHEZ-VARELA, Alejandro, RODRÍGUEZ-LUNA, Isabel Cristina, & ELUFISAN, Temidayo with adscription in the Instituto Politécnico Nacional, in next Section the article *Driving training and evaluation using the Big-Rig HD1 driving simulator* by ÁGUILA-FLORES, Arturo, CORTÉS-MALDONADO, Ismael, CORTES-GARCÍA, Franco & PÉREZ-MENDOZA, Viridiana with adscription in the Universidad Politécnica de Tlaxcala, in the next Section the article *Molecular identification of Aeromonas spp. isolated from water in northeastern México* by SANCHEZ-VARELA, A., ORTEGA-BALLEZA, J. L, RODRIGUEZ- LUNA, I. C. & GUO, X., with adscription in the Instituto Politécnico Nacional in the next Section the article *Interaction between Aedes aegypti CPB1 and viral proteins* by LÓPEZ, Edgar & BARRIENTOS, Carolina with adscription in the Universidad Veracruzana, As a following article we present, *Description of microplastics on the coast of La Guancha in Ponce, Puerto Rico*, by COLÓN-ORTIZ, Abner J. & FEBLES-MORENO, Karelys, with ascription in the Pontificia Universidad Católica de Puerto Rico, as following article we present *The versatile biological and chemical reactivity of quinoline derivatives, to source of innovation for the chemist*, by GARZA-ORTIZ, Ariadna, RAMIREZ-DE LA CRUZ, Flor de María, LÓPEZ-GUTIÉRREZ, Tomás J. and MEX-ÁLVAREZ, Rafael M , with adscription at the Universidad Autónoma de Campeche, as follows article, *Mitigation of greenhouse gases in ruminants through acetic acid as a food additive*, by CRUZ-HERNÁNDEZ, Ángel Raúl, MALFAVÓN-MEJÍA, Janett, LUCIO-DOMÍNGUEZ Rodolfo, BEDOLLA- CEDEÑO, José Luis Carlos and GONZALES-RONQUILLO, Manuel, with adscription at the Universidad Michoacana de San Nicolás de Hidalgo and Universidad Autónoma del Estado de México , as the last article we present, *Preval ence de dental caries in children of 6 to 15 years of age with disorder due to deficit of attention and hyperactivity of the Center of Multiple Attention # 3 and the USAER # 55 of the city of San Francisco de Campeche*, by SANSORES-AMBROSIO, Fátima , ROSADO-VILA, Graciella, SÁNCHEZ-ALVAREZ, Lucía, and LLANES-CHIQUINI, Charlotte, with adscription at the Universidad Autónoma de Campeche.

Contenido

Article **ECORFAN-Bolivia Journal**
June 2018 Vol.5 No.8 1-8

Bdellovibrio bacteriovorus as a possible biological control agent

Bdellovibrio bacteriovorus como posible agente de control biológico

ARANDA-VIVAS, Leonardo Iván[1]†, SÁNCHEZ-VARELA, Alejandro[1]*, RODRÍGUEZ-LUNA, Isabel Cristina[2], & ELUFISAN, Temidayo[3]

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

ID 1° Autor: *Leonardo Iván Aranda- Vivas* /**ORC ID**: 0000-0002-9399-220X, **Researcher ID Thomson**: D-6349-2018, **arXiv ID**: Leonardo Ar, **PubMed ID**: LeonardoAr, **CVU CONACYT-ID**: 856718

ID 1° Coautor: *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 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: *Temidayo Oluyomi, Elufisan* /**ORC ID**: 0000-0001-9445-0229, **Researcher ID Thomson**: D-5854-2018, **arXiv ID**: ptemidayo, **PubMed ID**: ptemidayo, **CVU CONACYT-ID**: 668951

Received January 28, 2018; Accepted May 30, 2018

Abstract

Within ecosystems there is a hierarchical form of survival, where the predator hunts its prey for that purpose. From aquatic environments, to terrestrial ones, but we rarely turn to see the microscopic world of bacteria where in the same way these living beings look for a way of survival and some of these microorganisms develop in a predatory way. In this review we focus on Bdellovibrio bacteriovorus HD100, which belongs to the Bdellovibrio group and organisms similar to BALOs (Bdellovibrio and like organisms), which have different forms of attack and depend on a prey for their survival, growth and reproduction. These Bdellovibrio spp can be used as possible biological control agents against pathogenic bacteria resistant to antibiotics.

Prey, Predator, Bdellovibrio

Resumen

Dentro de los ecosistemas hay una forma jerarquizada de supervivencia, en donde el depredador caza a su presa con dicho fin. Desde ambientes acuáticos, hasta los terrestres, pero pocas veces volteamos a ver el microscópico mundo de las bacterias en donde de la misma manera estos seres vivos buscan una manera de supervivencia y algunos de estos microorganismos se desarrollan de manera depredadora. En esta revisión nos enfocamos a Bdellovibrio bacteriovorus HD100, el cual pertene al grupo Bdellovibrio y organismos similares a BALOs (Bdellovibrio and like organisms), los cuales tienen diversas formas de ataque y dependen de una presa para su supervivencia, crecimiento y reproducción. Estos Bdellovibrio spp pueden ser empleados como posibles agentes de control biológico contra bacterias patogenas resistentes a antibióticos.

Presa, Depredador, Bdellovibrio

Citation: ARANDA-VIVAS, Leonardo Iván[1]†, SÁNCHEZ-VARELA, Alejandro[1]*, RODRÍGUEZ-LUNA, Isabel Cristina[2], & ELUFISAN, Temidayo[3]. *Bdellovibrio bacteriovorus* as a possible biological control agent. ECORFAN Journal-Bolivia. 2018. 5-8: 1-8.

---

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

Introduction

In a pluribiótico planet where the alimentary chain forces to form a symbiosis, sometimes, this ends up forming an environment of predation.

From large mammals to microorganisms, which seek to survive. To understand this, let's situate ourselves in a hostile environment, where the hunter or predator needs food to get the necessary nutrients, this food is only obtained by another living being that is known as prey. Thus, sharks are the marine predators par excellence, the big cats are in their ecosystem and humans are the biggest predators in the world. However, in the microscopic world, there are also predatory bacteria, some need a host to survive. The first bacteria classified in this way were the myxobacteria, since then, several studies and reports have been made about the predatory bacteria, with what has been learned about their environmental consequences, their ways of hunting and even possible applications. Clearly, each predatory bacterium has its own way of hunting, as well as a different lifestyle.

Methodology

- Sample collections were obtained preferably from soil near water tanks.
- To isolate the bacteria it is necessary to perform a pre-enrichment stage, preparing a 1: 2 mixture of 10 g of soil with sterile water or 50 ml of aquatic sample, and centrifuge 5 min at 500 xg, discarding the pellet formed, the supernatant is centrifuged 20 min at 27,000 xg, but at this point, the supernatant is decanted and the resulting pellet is dissolved in a few drops of HM cold buffer, finally it is passed through a 1.2 µm filter keeping at 4 ° C for a few days.
- If necessary due to contamination, it should be filtered again on a 0.45 µm filter, although this could reduce the performance of BALOs.
- The agar with the prey is prepared separately in a medium containing the HM buffer. Allowing this layer to solidify and finally, mix and incubate at 30 ° C for 3 to 8 days, continuously checking the medium, until approximately 5 days when lysis is observed.
- It is cut around 1 millimeter, in the lysis area and aspirated and then resuspended in 0.5 ml of HM buffer. Let it rest for a few minutes and then shake.
- Moving cells can be observed by means of a phase contrast microscope.
- Repeat steps 3 through 6 twice to obtain pure cultures.
- To obtain the predator, 10 ml of the culture is centrifuged at 3000 RPM for 20 min and filtered through a 0.45 or 0.25 µm pore.
- Centrifuge to obtain a pellet with the predator.
- After this the DNA can be extracted, amplified by PCR and sequenced by means of 16S rRNA.

Classification of BALOs and BALO Diagnostics by PCR

- Filter the BALO suspension twice by a 0.45 µm micropore, centrifuge for 10 minutes at 10,000 x g at 4 ° C and resuspend in sterile water at 1/10 volume.
- Extract the DNA according to the standard protocol of Moore and Dowhan, 2002, or with an extraction kit.
- Cool on ice, and add DMSO to obtain a final concentration of 10%.
- On the other hand, prepare the PCR mixture in the following manner in a 50 µL tube.

I. DNA, 10 ng on 1 o 2 µL.
II. 3 Mm $MgCl_2$
III. 20 µM de dNTPs
IV. Buffer 1X
V. 1.25 u Taq DNA polymerase
VI. 1 µM of each first. (63 F 5´ CAGGCCTAACACATGCAAGT C 3´ and 1278R 5´CGGTGTGTAC AAGGCCCGGGAACG 3´)
VII. 12.5 µg of BSA
VIII. Water mili-Q degree PCR cbp. 50 µL.

- Amplify using the following protocol for the Thermal Cycler.

I. 4 min at 94 ° C for initial denaturation.
II. 35 ciclos de:
III. 1 min at 94 ° C denaturation
IV. 1 min at 50 ° C alignment
V. 1 min at 72 ° C extension
VI. 5 min at 72 ° C for the final extension

- Verify the presence of the PCR product running 5 μL in 1% agarose gel in TAE.
- Sequence the PCR product

Results

Predatory bacteria

Due to the behavior of bacteria there is controversy regarding the classification of this type of cells as predators or parasitic, we must put in context that the term predator should be used for that which has a prey and hunting for various purposes, mainly to get nutrients while parasitic cells are those that form symbiosis with its host without kill (Perez et al., 2016).

These bacteria have a wide distribution ranging from aquatic environments, to extreme habitats with high concentrations of sulfates that are reduced to methane by some bacteria such as Desulfovibrio, Desulfobacter and Desulfobacterium (Sahaniuk et al., 2004) (Kevorkian et al., 2018) . These have co-evolved with prey bacteria, however we can see that within the applications of these predatory cells do not spread beyond the environment where they are, that is, they are not pathogenic for it (Tyson and Sockett, 2017).

Predation forms

There are several forms of predation, which can be classified into three main groups, considering previous knowledge of this type of bacteria (Perez et al., 2016).

Epibiotic depredation

In this strategy the predator contacting the cell without an invasion of the host, the predatory bacteria remain bound prey from the outer membrane and from there degrade cell wall to consume. Some examples of predators are Vampirococcus, Micavibrio and Bdellovibrio exovorus (Martin, 2002).

Endobiotic predation

It is also known as direct invasion, since in this form of predation, the bacterium penetrates its prey and invades the periplasm or cytoplasm in order to feed, grow and reproduce until it lyses the host cell to begin its cycle again, a The main example is B. bacteriovorus (Pérez et al., 2016).

Predation in group

As the name implies, are those bacteria that have the strategy of preying on their prey in groups.

In this tactic predatory cells secrete compounds which kill and decompose nearby prey, these products can bind to the surface of the predator or, they are embedded in the extracellular matrix in this category is found to Mixococcus xanthus and some bacteria of the Lysobacter genus spp. (Velicer & Mendes-Soares, 2009) (Livingstone et al., 2018). There are other groups of unicellular or multicellular organisms such as marine algae that produce enzymes capable of attacking some human pathogens and also to combat some pathogens of plants such as the potato blight P. infestans (Bartulos et al., 2018).

BALOs

The group of Gram-negative bacteria predators of other Gram-negative bacteria, are known as BALOs or bacteria similar to Bdellovibrio by its acronym in English "Bdellovibrio and Like Organisms" (Chen et al., 2011).

These organisms typically have a double lifestyle, one external to the prey cell, usually the recognition phase and another within the host, where it carries out its feeding, growth, development and reproduction, by multiple fission (Jurkevitch and Jacquet , 2017).These organisms include the genus Micavibrio and the families known as: Bdellovibrionaceae (Stolp and Starr, 1963). Bacteriovoracaceae (Baer et al., 2000), Peredibacteraceae (Piñeiro et al., 2008) and Halobacteriovoraceae (Koval et al., 2015). Thanks to the nature of these organisms and their hosts, as well as to biotechnology, an application approach can be given to the BALOs to study them from the environmental impact to their applications in agriculture, industry, as well as clinical issues (Jurkevitch and Jacquet, 2017) (Kevorkian et al., 2018).

Bdellovibrio bacteriovorus

In an effort to meet a species of BALOs, we focused on B. bacteriovorus, which comes from the Bdellovibrionaceae family, which was first described by Starr and Stolp as a predatory, ectoparasitic and bacteriolytic bacterium, since it lysed Pseudomonas in soil samples (Stolp and Starr, .1963).

In later studies they demonstrate the second phase of their life cycle, which consists of penetrating the prey cell in order to grow parasitically (Starr and Baigent, 1966).

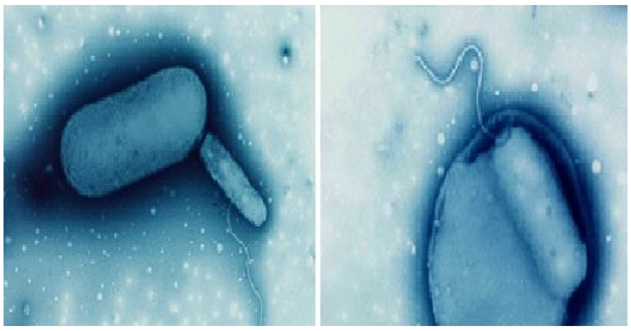

A B

Figure 1 Electron micrograph of B. bacteriovorus predating E. Coli, in figure A. we observe the prey binding phase, while in figure B we can observe the intracellular phase, (Lamber et al., 2006)

It is a Gram negative bacterium, obligatory aerobic, uniflagelada whose flagellum measures near 50 μm, which gives motility to the cell, of the class Deltaproteobacteria, this flagellum is lost when entering its prey (Sockett, 2009), without However, in strains such as HD100, it has been shown that the flagellum sometimes fails (Lamber et al., 2006).

To exemplify in more detail, strains for this organism have been identified as strain HD100, which is described as dependent hosts (HD), where their dimensions were discovered, which are approximately between 0.2-0.5 μm wide and 0.5-2.5 μm long, its genome is 37.82950 bp, and it codified for 3584 proteins (Rendulic, 2004), Figure 2.

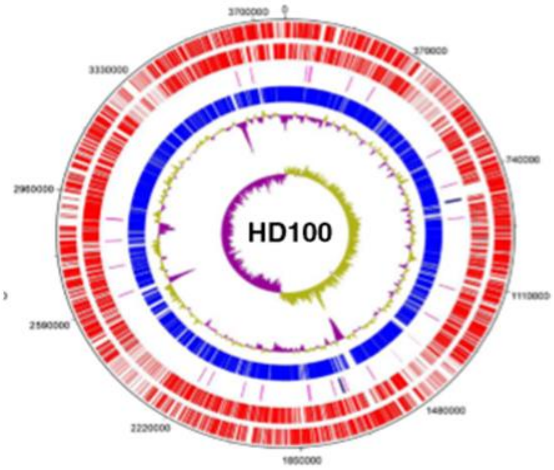


Figure 2 This image shows the circular genome of B. bacteriovorus HD100. Image taken from (Hobley et al., 2012)

It is ubiquitous and has been isolated from various habitats such as freshwater, wastewater, soil and mammalian intestines (Feng et al., 2016) proteins (Rendulic, 2004) (Atterbury et al., 2011). It is a host-dependent bacterium, because it needs a prey cell or host for its growth, it seeks to obtain a place in the periplasmic space of the bacteria Gram-negative prey forming a bdelloplasto, which allows a balance to be able to take the nutrients from its host (Willis et al., 2016). It has also been possible to develop an independent growth phase of the host achieved in laboratory conditions (Sockett, 2009).

Thus, we can say that its life cycle consists of eight stages; 1) B. bacteriovorus swims freely with its flagellum in the middle, 2) later when the bacterium finds its prey, collides and joins it and remains in a "recognition" stage, 3) in stage 3, Bdellovibrio generates an opening between the outer membrane of the prey cell, and the peptidoglycan layer performing mechanical movements on its own axis, which is resealed when the predator enters, in this stage B. bacteriovorus detaches from its flagellum upon entering to the periplasm of the dam, 4) DNA replication and synthesis of biopolymerases begins, 5) Once it is in the periplasm, Bdellovibrio forms a structure called bdelloplasto, 6) The filaments of the predator have grown several times their size and begin to form a wall, 7) Bdellovibrio becomes a flagellated cell and lists again, at this point, 8) Finally, the predator produces hydrolytic enzymes in order to dissolve the peptide layer Doglicane and the outer membrane of the cell prey to release progeny proteins (Rendulic, 2004) (Kuru et al., 2018), Figure 3.

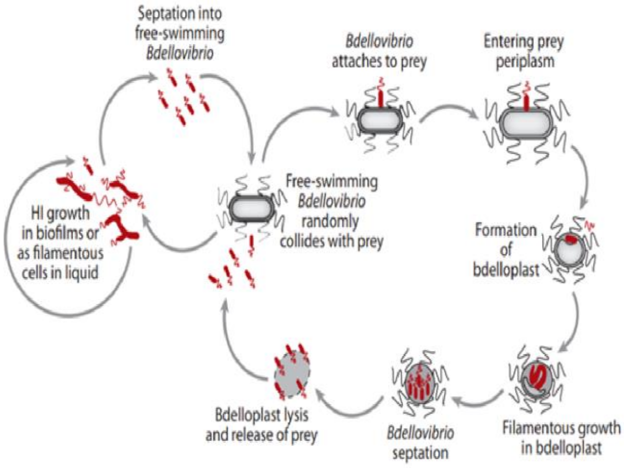


Figure 3 Image taken from the Sockett 2009. doi: 10.1146 / annurev.micro.091208.073346 where the life cycle of Bdellovibrio bacteriovorus is illustrated (Sockett, 2009).

Likewise, through the study of the genome of Bdellovibrio bacteriovorus it has been possible to understand part of the process of interaction with its host, from knowing the genes that encode certain enzymes and the function they perform, Table 1.

Family	Bdellovibrionaceae
Species	*B. bacteriovorus*
Type of first isolated sample	Oil
Dam of choice	Gram negative
Genome size	3,782,950 pb
Content of G + C in DNA	50.6 %
Content of G + C that encode	50.4 %
OrFs	3584
RNAr operons	2
RNAr genes	36
Transposons	4
Regions encoding hydrolytic enzymes:	150 proteases
	20 DNAsas
	9 RNAsas
	10 Glicanasas
	15 Lipasas
	Otras 89

Table 1 Diagram of Bdellovibrio Bacteriovorus HD100

* Base pairs
** Open reading frames
*** Ribosomal RNA
**** Transfer RNA

It is thought that its high content of GC over the other BALOs is due to the microbiota of the environment where it is located, so the first isolate obtained from soil samples, where the genomes of the prey bacteria have rich regions in GC content in their DNA, so the transfer of predator-prey genes probably provided that result (Rendulic, 2004).

Discussions

As we can see, this predator focuses on its prey and at the moment has not been shown to have a negative influence on the environment around it. From this we can take advantage in various fields of study as we will analyze below. On the one hand, (Feng et al., 2016), they found B. bacteriovorus strain UP in a wastewater treatment plant in Singapore, where this bacterium was found to be predatory of planktonic species and species with biofilms, an interesting result it was the decrease of prey cells, which showed a decrease in predatory cells, a result that they had previously tested (Chu and Zhu 2010), by placing B. bacteriovorus C-1 in the presence of Aeromonas hydrophila, this result although it would seem Obviously, it shows us that different strains of Bdellovibrio are specific for a given prey (Feng et al., 2016).

In a study conducted by (Willis et al., 2016), focused on the veterinary-preclinical biomedical area injected Bdellovibrio in larvae of zebrafish (Danio rerio) infected with a strain of Shigella flexneri resistant to antibiotic and pathogenic to humans, where showed that Bdellovibrio can remain in vivo inside Danio rerio, without causing pathogenic damage in the larva, but it was observed predation in Shigella, when injecting a lethal dose, which denoted survival of the larvae of Danio rerio. The immune system of this fish, properly constituted by neutrophils and macrophages, gets rid of the predator in a sufficient time, once fulfilled its function.

However, a better effect of Bdellovibrio with immunosuppressed fish was observed, for obvious reasons, the predator had more time to fulfill its function (Willis et al., 2016).

From this we can rescue two things, the study argues that the immune system of the fish achieves, end with Bdellovibrio allowing time for the bacteria to deprecate Shigella.

However, in immunosuppressed fish this bacterium has more efficient predation, which may give rise to to more studies prior to a clinical study, however, the fact that bdellovibrio is a dependent predator, should die when there are no more prey, so if bdellovibrio persists inside the primary host, in this case Danio rerio, it should be look for, what is the underlying source of prey for which this bacterium remains.

In zootechnical veterinary medicine several studies have been carried out as they are in chickens and cattle, and even in rats, where Bdellovibrio has been exposed to pathogenic agents such as E. coli and Salmonella (Atterbury et al., 2011) (Boileau et al., 2011) (Shatzkes et al., 2017) .

For the chickens, when feeding the predatory cell, it was shown that Salmonella was not expressed, since it served as a host for B. bacteriovorus, there was no contamination of the chickens' environment with the bacteria and the life expectancy of the birds was improved. cattle were studied to study Bdellovibrio strain 109J as a predator of Moraxella bovis bacterium that causes bovine infectious keratoconjunctivitis, to reach a conclusion in vitro studies and subsequently in vivo were made to determine that B. bacteriovorus has potential as an effective bacterial control against M. Bovis (Boileau et al., 2011).

Likewise, several investigations have been made to know the effect of B. bacteriovorus in combination with some substances, such as (Im et al., 2017), in their study with violacein, a Gram-positive antibiotic where they showed synergy when used with the predatory bacteria of Gram negative, obtaining results of 19% and 68%, of effectiveness being used separately for each type of bacteria, however, when this combination was used, they gave up to 99.98% effectiveness in an in vitro medium (Im et al., 2017).

Among other applications, preclinical tests have been carried out (Baker et al., 2017) and clinical studies as reviewed by (Lebba et al., 2013), where the first focuses on an in vitro environment to know the viability of our predator in question, demonstrating in a buffer at body temperature the development of a pathogenic bacterium, in this case Klebsiella pneumoniae, same as when using B. bacteriovorus HD100, a remarkable decrease of the prey cell was observed.

In the study conducted by (Lebba et al., 2013), faecal samples from pediatrics found that our predator was being studied in healthy patients, while in patients with celiac disease the presence of Bdellovibrio was considerably reduced, which appeared to be associated with mucosa.

Therefore, in some studies, this bacterium is proposed as a bactericidal agent or "live antibiotic" (Harini et al., 2013) (Negus et al., 2017). However, we must learn to direct it, since the strains for each predator have not been detected, as Baker who talks about the strain HD100 preying on K. pneumoniae, when it has been found that this strain is also predator of E. coli, or the strains are multi-predators, which would lead to new studies to check if there are collateral damages due to the use of this bacterium because it could possibly be preying on other bacteria of the human microbiota, since as mentioned in the study by Lebba et al. ., 2013, the predator has been found in the microbiota of healthy pediatrics (Baker et al., 2017).

It is important the role of Bdellovibrio in eliminating certain pathogens and that could probably act as potential prophylactic or therapeutic agents to fight infections, however many studies are still needed (Tester And Al-Ghazzewi, 2018).

Conclusions

Bdellovibrio bacteriovorus is an antimicrobial agent that can be isolated from diverse aquatic environments, this can have different applications, in the medical or clinical area, as probiotic in humans or combating pathogens resistant to multi-drugs, as an environmental bioremediating agent, as an agent of biological control of insect pests, in veterinary, among other applications. The role of Bdellovibrio in eliminating certain pathogens and that could probably act as prophylactic or therapeutic agents to fight infections is important, however, many studies are still needed.

Finally, studies could be carried out using biotechnological tools, on the interactions between the predator and its prey, tracking genes of specific interaction and looking for associations that do not affect the human microbiota of healthy individuals.

Acknowledgments

To the Instituto Politécnico Nacional, to the Secretary of Research and Postgraduate of Instituto Politécnico Nacional and to the Commission of Operation and Promotion of Academic Activities, as well as for the BEIFI Scholarship granted.

References

Atterbury, R.J., Hobley, L., Till, R., Lambert, C., Capeness, M.J., Lerner, T.R. (2011). Effects of orally administered Bdellovibrio bacteriovorus on the well-being and Salmonella colonization of young chicks. Appl. Environ. Microbiol;77:5, pp. 794–803.

Baer, M.L., Ravel, J., Chun, J., Hill, R.T., Williams, H.N. (2000). A proposal for the reclassification of Bdellovibrio stolpii and Bdellovibrio starrii into a new genus, Bacteriovorax gen. nov. as Bacteriovorax stolpii comb. nov. and Bacteriovorax starrii comb. nov., respectively. Int. J. Syst. Evol. Microbiol;50:2, pp. 19–24.

Baker, M., Negus, D., Raghunathan, D., Radford, P., Moore, C., Clark, G. (2017). Measuring and modelling the response of Klebsiella pneumoniae KPC prey to Bdellovibrio bacteriovorus predation, in human serum and defined buffer. Sci. Rep;7.

Bartulos, C. R., Rogers, M. B., Williams, T. A., Gentekaki, E., Brinkmann, H., Cerff, R., Liaud, M. F., Hehl, A. B., Yarlett, N. R., Gruber, A. and Kroth, P. G. (2018). Mitochondrial targeting of glycolysis in a major linage of eukaryotes doi:https://doi.org/10.1101/257790

Boileau, M.J., Clinkenbeard, K.D., Iandolo, J.J. (2011). Assessment of Bdellovibrio bacteriovorus 109J killing of Moraxella bovis in an in vitro model of infectious bovine keratoconjunctivitis. Can. J. Vet. Res;75:2, pp. 85–91.

Chen, H., Athar, R., Zheng, G., Williams, H.N. (2011). Prey bacteria shape the community structure of their predators. ISME J; 5:13, pp. 14–22.

Feng, S., Tan, C.H., Cohen, Y., Rice, S.A. (2016). Isolation of Bdellovibrio bacteriovorus from a tropical wastewater treatment plant and predation of mixed species biofilms assembled by the native community members. Environ Microbiol;18:39, pp. 23–31.

Harini, K., Ajila, V., Hegde, S. (2013). Bdellovibrio bacteriovorus : A future antimicrobial agent?. J. Indian. Soc. Periodontol;17:823.

Hobley, L., Lerner, T.R., Williams, L.E., Lambert, C., Till, R., Milner, D.S. (2012). Genome analysis of a simultaneously predatory and prey-independent, novel Bdellovibrio bacteriovorus from the River Tiber, supports in silico predictions of both ancient and recent lateral gene transfer from diverse bacteria. BMC Genomics;13.

Im, H., Choi, S.Y., Son, S., Mitchell, R.J. (2017). Combined Application of Bacterial Predation and Violacein to Kill Polymicrobial Pathogenic Communities. Sci. Rep;7.

Jurkevitch, É., Jacquet, S. (2017). Bdellovibrio et organismes apparentés. Médecine/sciences; 33:5, pp. 19–27.

Kevorkian, R., Bird, J. T., Shumaker, A. and Lloyd, K. G. (2018). Estimating population turnover rates by relative quantification methods reveals microbial dynamics in marine sediment.Applied and environmental microbial, 84(1)17.

Koval, S.F., Williams, H.N., Stine, O.C. (2015). Reclassification of bacteriovorax marinus as Halobacteriovorax marinus gen. Nov., comb. nov. and bacteriovorax litoralis as halobacteriovorax litoralis comb. nov.; description of halobacteriovoraceae fam. nov. in the class Deltaproteobacteria. Int. J. Syst. Evol. Microbiol; 65:59, pp. 3–7.

Kuru, E., Lambert, C., Rittichier, J., Till, R., Ducret, A., Derouaux, A., Gray, J., Biboy, J., Vollmer, W., VanNieuwenhze, M., Brun, Y. V. And Sockett, R. E. (2018). Author correction: Fluorescent D-amino acids reveal bi-cellular cell wall modifications important for *Bdellovibrio bacteriovorus* predation. Nat. Microbiol. 3(2):254.

Lambert, C., Evans, K.J., Till, R., Hobley, L., Capeness, M., Rendulic, S. (2006). Characterizing the flagellar filament and the role of motility in bacterial prey-penetration by Bdellovibrio bacteriovorus. Mol. Microbiol;60:2, pp. 74–86.

Lebba, V., Santangelo, F., Totino, V., Nicoletti, M., Gagliardi, A., De Biase, R.V. (2013). Higher Prevalence and Abundance of Bdellovibrio bacteriovorus in the Human Gut of Healthy Subjects. PLoS One;8.

Livingstone, P. G., Millard, A.D., Swain, M. T. And Whitworth, D. E. (2018). Transcriptional changes when Myxococcus Xanthus preys on Escherichia coli suggest myxobacterial predators are constitutively toxic but regulate their feeding. Microb Genom.

Martin, M.O. (2002). Predatory Prokaryotes: An Emerging Research Opportunity. J Mol Microb Biotechnol; 4:4, pp. 67–77.

Negus, D., Moore, C., Baker, M., Raghunathan, D., Tyson, J., Sockett, R.E. (2017). Predator Versus Pathogen: How Does Predatory Bdellovibrio bacteriovorus Interface with the Challenges of Killing Gram-Negative Pathogens in a Host Setting? Annu. Rev. Microbiol;71:441–57.

Pérez, J., Moraleda-Muñoz, A., Marcos-Torres, F.J., Muñoz-Dorado, J. (2016). Bacterial predation: 75 years and counting! Environ Microbiol; 18:7, pp. 66–79.

Piñeiro, S.A., Williams, H.N., Stine, O.C. (2008). Phylogenetic relationships amongst the saltwater members of the genus Bacteriovorax using rpoB sequences and reclassification of Bacteriovorax stolpii as Bacteriolyticum stolpii gen. nov., comb. nov. Int. J. Syst. Evol. Microbiol;58:120, pp. 3–9.

Rendulic, S. (2004). A Predator Unmasked: Life Cycle of Bdellovibrio bacteriovorus from a Genomic Perspective. Science (80);303:6, pp. 89–92.

Sahaniuk, G.E., Romberg, E., Williams, H.N., Pineiro, S.A. (2004). Predation Pattern and Phylogenetic Analysis of Bdellovibrionaceae from the Great Salt Lake, Utah. Curr Microbiol; 48:11, pp. 3–7.

Shatzkes, K., Tang, C., Singleton, E., Shukla, S., Zuena, M., Gupta, S. (2017). Effect of predatory bacteria on the gut bacterial microbiota in rats. Sci Rep;7.

Sockett, R.E. (2009). Predatory Lifestyle of Bdellovibrio bacteriovorus. Annu Rev Microbiol;63:5, pp. 23–39.

Starr, M.P., Baigent, N.L. (1966). Parasitic interaction of Bdellovibrio bacteriovorus with other bacteria. J. Bacteriol; 91:200, pp. 6–17.

Stolp, H., Petzold, H. (1962). Untersuchungen über einen obligat parasitischen Mikroorganismus mit lytischer Aktivität für Pseudomonas-Bakterien. J. Phytopathol; 45:3, pp. 64–90.

Stolp, H., Starr, M.P. (1963). Bdellovibrio bacteriovorus gen. et sp. n., a predatory, ectoparasitic, and bacteriolytic microorganism. Antonie Van Leeuwenhoek; 29:2, pp. 17–48.

Tester, R. F. And Al-Ghazzewi, F. H. (2018). Role of prebiotics and probiotics in oral health, Nutrition and Food Sci., doi:https://doi.org/10.1108/NFS-03-2017-0056

Tyson, J., Sockett, R.E. (2017). Predatory Bacteria: Moving from Curiosity Towards Curative. Trends Microbiol; 25: 9, pp. 0–1.

Velicer, G.J., Mendes-Soares, H. (2009). Bacterial predators. Curr Biol; 19.

Willis, A.R., Moore, C., Mazon-Moya, M., Krokowski, S., Lambert, C., Till, R. (2016). Injections of Predatory Bacteria Work Alongside Host Immune Cells to Treat Shigella Infection in Zebrafish Larvae. Curr Biol;26:33, pp. 43–51.

Wurtzel, O., Dori-Bachash, M., Pietrokovski, S., Jurkevitch, E., Sorek, R. (2010). Mutation detection with next-generation resequencing through a mediator genome. PLoS One;5.

Article ECORFAN-Bolivia Journal
June 2018 Vol.5 No.8 9-13

Driving training and evaluation using the Big-Rig HD1 driving simulator

Capacitación y evaluación de conducción a través del simulador de manejo Big-Rig HD1

ÁGUILA-FLORES, Arturo[1]*†, CORTÉS-MALDONADO, Ismael[1], CORTES-GARCÍA, Franco[2] & PÉREZ-MENDOZA, Viridiana[3]

Universidad Politécnica de Tlaxcala Región Poniente, Carretera Federal libre a cuatro carriles México-Veracruz km 85, C.P. 90240, San Ildefonso Hueyotlipan, Tlaxcala, México

ID 1° Autor: Arturo Águila-Flores / **ORC ID**: 0000-0002-3775-5525, **Researcher ID Thomson**: F-8900-2018, **CVU CONACYT-ID**: 876498

ID 1° Coautor: Ismael Cortés-Maldonado/ **ORC ID**: 0000-0002-4408-5101, **Researcher ID Thomson**: F-8872-2018, **CVU CONACYT-ID**: 161976

ID 2° Coautor: Franco Cortes-García/ **ORC ID**: 0000-0002-6747-6287, **Researcher ID Thomson**: F-8747-2018, **CVU CONACYT-ID**: 899376

ID 3° Coautor: Viridiana Pérez-Mendoza**/ ORC ID** 0000-0002-7143-9323, **Researcher ID Thomson**: F-8758-2018, **CVU CONACYT-ID**: 899370

Received: February 25, 2018; Accepted: May 20, 2018

Abstract

In this research project drivers' driving skills are evaluated using the BIG-RIG HD1 simulator. This evaluation is focused on two levels, which are: driver training through the BIG-RIG HD1 simulator and field evaluation using a standard two-axle motorized vehicle, and with this check the skills acquired in the simulator during the training. To facilitate the development and follow-up of the project, we chose to work with students from the Polytechnic University of Tlaxcala, West Region of Engineering in Logistics and Transportation who do not have the skills to drive a vehicle. A total of 30 students were trained from Monday to Friday for four months, of which three month consisted of training using the simulator and the last month the evaluation was carried out in a motor vehicle. The results within the positive factors presented by the driver are: 1) Familiarization with the functions of the vehicle. 2) Identification of pedals and vehicle components. 3) Vehicle running without stoppages due to the change of speeds. 4) Gear changes to the appropriate revolutions. Regarding the negative aspects, the following were observed: 1) The driver has anxiety before starting the vehicle. 2) Excessive pressure. 3) The maneuvers in reverse still turn out to be complex. 4) The maximum driving speed ranges between 35 and 40 km / hr.

Train, Evaluate, Simulator, Vehicle

Resumen

En este proyecto de investigación se evalúan las habilidades de manejo de conductores empleando el simulador BIG-RIG HD1. Esta evaluación está enfocada en dos niveles los cuales son: la capacitación de conductores mediante el simulador BIG-RIG HD1 y la evaluación en campo mediante un vehículo motorizado de dos ejes de transmisión estándar, y así comprobar las habilidades adquiridas en el simulador durante la capacitación. Para facilitar el desarrollo y seguimiento del proyecto, se optó por trabajar con alumnos de la Universidad Politécnica de Tlaxcala Región Poniente de la Ingeniería en Logística y Transporte que no cuentan con las habilidades para conducir un vehículo. En total se capacitaron a 30 alumnos de lunes a viernes durante cuatro meses, de los cuales, tres meses consistieron en capacitación mediante el simulador y el último mes se realizó la evaluación en un vehículo motorizado. Los resultados dentro de los factores positivos que presenta el conductor son: 1) Familiarización con las funciones del vehículo. 2) Identificación de pedales y componentes del vehículo. 3) Marcha del vehículo sin paros a causa del cambio de velocidades. 4) Cambios de marcha a las revoluciones apropiadas. En cuanto a los aspectos negativos se observaron los siguientes: 1) El conductor presenta ansiedad antes de encender el vehículo. 2) Presión excesiva. 3) Las maniobras en reversa aun resultan ser complejas. 4) La velocidad máxima de conducción oscila entre los 35 y 40 km/hr.

Capacitar, Evaluar, Simulador, Vehículo

Citation: ÁGUILA-FLORES, Arturo[1]*†, CORTÉS-MALDONADO, Ismael[1], CORTES-GARCÍA, Franco[2] & PÉREZ-MENDOZA, Viridiana[3]. Driving training and evaluation using the Big-Rig HD1 driving simulator. ECORFAN Journal-Bolivia. 2018. 5-8: 9-13.

---

* Correspondence to Author (email: arturoaguila@uptlaxponiente.edu.mx).
† Researcher contributing as first author

Introduction

Automobiles are a technology that facilitates the movement of people, provides access to markets, jobs and even is used for public services such as taxis, ambulances, fire trucks or police vehicles. Each car trip ends in an economic transaction or some other benefit that improves the quality of life, which is why to this day it has remained one of the technologies with the greatest impact on the world economy.

In Mexico there are more than 38 million vehicles; 43.5% of households have a car (INEGI 2015), however, for most people it is difficult to acquire driving experience, due to the fact that they present behaviors such as anxiety and stress when maneuvering a car, which produces a lack of concentration to become familiar with the different components of the vehicle. The use of simulators in any discipline, allows to obtain knowledge of the behavior of a certain system, for example, the operation of a vehicle, where the learning of the different actions is monitored by the instructor. This type of tools can be of great value in the performance of new drivers, reducing risks, streamlining tasks typical of the field (Aranzazu, Villada, Ocampo & Reyes, 2018).

For this reason, the training and training of drivers through simulators is necessary because they allow acquiring knowledge and driving skills, thus avoiding risks that threaten physical integrity and traffic accidents, which is achieved by reproducing real sensations that stimulate senses such as sight and hearing.

For the training of students with no driving experience should take into account a virtual model of vehicular dynamics so that through this and the use of technology are generated scenarios with virtual content that simulates acceleration, braking and maneuvers made by the driver, to guarantee fidelity with the actual driving experience (Schwartz, 2011; Nuñez, 2018).

For this training should be considered the three types of simulators (desktop, panoramic without cabin movement, panoramic with cabin movement) in which, according to Allen (2003), a computer is mainly used to which a steering wheel is incorporated and Acceleration and braking pedals.

The aim of the driving simulators is to represent the driving practices of the driver, basic skills such as the operation of the vehicle, its system of handling and traffic control, as well as representations of traffic flow conditions and the environment (Figueira, 2012 Vlakveld, 2005; Yamada, 2002) and, in turn, as mentioned by Rimini-Doering, (2001); Ranney (1999); Gillberg (1996); Lee (2003), evaluate the effectiveness of the signs and infrastructures for its design and configuration, incorporating other factors such as driver behavior and fatigue studies without involving potential risks.

In this project we are especially interested in the process of training and evaluation of young drivers with no driving experience, taking into account basically two levels: the training of drivers using the Big-Rig HD1 simulator and the evaluation in the field by means of a motorized vehicle of two. Standard axes to check the skills acquired in the simulator.

The development of this research is divided into the following sections, we describe the technical characteristics of the simulator HD1, operation information is provided and. The methodology addresses the different stages that were considered for the training and evaluation process. Finally, a section of conclusions will offer a discussion about the results obtained.

Big-Rig HD1 driving simulator

For the research, the Big-Rig HD1 simulator was used, which complements the theoretical aspects of the training with the firm purpose of identifying the principles of road driving.

It consists of the following elements:

– Panoramic visual system that virtually reproduces the environment around the driver, including different driving scenarios through three monitors with a 180 degree range of vision. The operation is through a Silicon Graphics computer that receives the signals from the PC computer and builds a graphic simulation of vehicles traveling in a virtual road scenario.

– Audio system to recreate real driving situations, both environmental and those of the vehicle. Which has stereo type speakers with subwoofer and 50W audio translator with amplifier.

- Modeling system for the dynamic response of vehicles, which produces, through the motorized force system of the steering wheel, the different disturbances and movements applied by the operator and the interaction of the unit with the pavement. These controls are connected to sensors that, when activated, send signals to a computer.

- System of internal components of the user, those components such as clutch, brake pedal, steering wheel and switches that are responsible for the drive and sensitive reproduction of all controls driven by the driver; by means of a computer PC that sends the signals of the vehicle to a central computer Silicon Graphics model ONYX 2 Infinity Reality.

- Interaction system and menu with sequence of tasks. During the simulation the user is informed in real time of the mistakes he makes during the training by means of sound warnings and on-screen messages, in order to warn the trainers of the errors that they make, including the inappropriate change of speeds, ignoring the preventive signals , do not adjust the safety belt and exceed speed limits. (See figure 1)

Figure 1 Big-Rig HD1 driving simulator

Methodology

For the development of this research, five processes are defined: initiation, planning, execution, monitoring, and control and closure.

In the initiation of the project, the project formalization act was created in which the people involved in the project were defined, tools to be used, and the scope of the project.

In planning, the schedule of activities and the responsibilities matrix were created.

For the execution process, 30 students were trained from Monday to Friday for four months, of which three months would be in the laboratory and one month in the field. To facilitate the development and follow-up of the project, it was decided to work with the students of the Universidad Politécnica de Tlaxcala West Region of the eighth semester of the Logistics and Transportation Career that do not know how to drive a vehicular unit.

To monitor and control, it was supervised that the activities were carried out within the dates that were established and a change control bin was carried out. Finally, in the closing process, the Project was finalized in a first stage, completing all the activities.

In relation to the methodology of the research, a pre-experimental design with a quantitative approach of correlational type is used, which will consist of taking a sample of the group of people who do not know how to drive a car; train them through the simulator and evaluate them with a motor unit.

Characteristics of the scope of the investigation:

1) Space: Universidad Politécnica de Tlaxcala West Region.
2) Temporality: September - December 2017
3) Universe: 30 students who do not know how to drive vehicles.
4) Characteristics of the participants: Male and female gender older than 20 years.
5) Sample size: 30

In this case, the sample is not probabilistic and directed, in which the total of 30 people who do not know how to drive were taken.

It is considered non-probabilistic because students are not taken at random, but it is aimed at those students of eighth semesters of engineering in Logistics and Transport who do not know how to drive a vehicle.

To obtain a minimum margin of error and a higher confidence level and for the size of the universe, it is considered taking the universe total as a sample.

Results

Theoretical training

Figure 2 shows the initial training that consisted of a theoretical course in the first week, with a daily module of 90 minutes in which the main topics to be pointed out were: circulation, speed, road signs and vehicle functions.

Figure 2 Vehicular theoretical training

Training in the Big-Rig HD1 Simulator

It begins with training people through the simulator in a standard transmission vehicle (See Figure 3). In this stage, the training was conducted in groups of six people per day with modules of 60 minutes each, where the instructor performed sixteen practical tests with different environmental conditions, among which:

- Maneuver equipment.
- Labyrinth with obstacles.
- Reaction time test.
- Change lane and pass.
- Parallel parking.
- Driving on highway and highway.
- Driving in urban areas with traffic and pedestrians.

Figure 3 Training in the Big-Rig HD1 simulator

Motorized vehicle evaluation

After the eleven weeks of laboratory training, the apprentice drivers carried out the field tests with a motorized vehicle with two standard transmission shafts; on the free Federal Highway to four lanes Mexico-Veracruz. In this stage, the evaluation was given to groups of six people per day with modules of 60 minutes each, in which the instructor and the evaluators analyzed the behavior in the vehicle and the real environment through different driving behaviors. (See figure 4)

Figure 4 Motorized vehicle evaluation

Conclusions

One of the key issues in relation to training with driving simulators is the amount of realism that is necessary to make the system real. Obviously, achieving absolute reality is practically impossible because, even if the simulation were perfect, there would always be subjective knowledge on the part of the user of this virtual reality that can qualify what, on the other hand, his senses may be experiencing. In the case of the driving simulator, the amount of realism required is that which leads the subjects who drive through it to maintain a realistic behavior, in accordance with the usual parameters in the real situation. The objective of this work is to perform a validation of the driving simulator to train and evaluate drivers

In general, the results in the evaluation in the field by means of a motor vehicle can be presented in positive factors and negative factors. Among the positive factors that the novel driver presents are: 1) Familiarization with the functions of the vehicle. 2) Identification of pedals and components of the vehicle, 3) Running the vehicle without stops due to the change of speeds. 4) Gear changes to the appropriate revolutions. 5) Correct use of rear-view mirrors. 6) Identification of signs on the road.

In the negative aspects presented by the drivers were the following: 1) The novice driver has nerves before starting the vehicle. 2) Excess brake pressure. 3) Do not use directional. 4) Does not stop the vehicle completely before turning on the streets. 5) Reverse maneuvers are complex. 6) The maximum driving speed ranges between 35 and 40 km / hr.

References

Allen R., Park G., Cook M., Fiorentino D., y Viirre E., (2003). Novice Driver Training Results and Experience with a PC Based Simulator. Second International Driving Symposium on Human Factors in Driver Assessment. Training and Vehicle Design. pp 165-170.

Aranzazu M., L., Villada A., Ocampo T., B. y Reyes P., G. (2018). Simulador hemodinámico cardiac “HEMOSIM-1”. ÁNFORA, 4(7), 53-67. Berger J., Schwartz E. (2011). What Drives Immediate and Ongoing Word of Mouth?. Journal of Marketing Research: October 2011. Vol. 48. No. 5. pp. 869-880.

Figueira A. (2012). O Uso de Ferramentas de Visualização Tridimensional na Detecção de Deficiências em Projetos Geométricos de Vias. Dissertação de Mestrado. Departamento de Engenharia de Transportes da Escola Politécnica da USP, 2012.

Gillberg M., Kecklund G., y Aakerstedt T. (1996). Sleepiness and Performance of Professional Drivers in a Truck Simulator -- Comparisons between Day and Night Driving. Karolinska Institutet. Stockholm (Sweden) 4 p. Journal of Sleep Research. Vol 5. No 1. pp. 12-15.

INEGI (2015). Encuesta intercensal 2015 principales resultados. Recuperado el 28 de enero de 2018 de http://internet.contenidos.inegi.org.mx/contenidos/productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/promo/eic_2015_presentacion.pdf

Lee H., Lee A., Cameron D., y Li-Tsang C. (2003). Using a Driving Simulator to Identify older Drivers at Inflated Risk of Motor Vehicle Crashes. Journal of Safety Research. Vol 34. No 4.

Nuñez F. (2018). Uso de los simuladores de realidad virtual en la enseñanza de ciencias básicas en pregrado de medicina. (Trabajo de grado). Universidad Militar Nueva Granada. Bogota Colombia.

Ranney T., Simmons L., y Masalonis A (1999). Prolonged Exposure to Glare and Driving Time: Effects on Performance in a Driving Simulator. Accident Analysis and Prevention Vol: 31, No: 6.

Rimini M., Manstetten D., Altmueller T., Ladstaetter U. y Mahler M. (2001). Monitoring Driver Drowsiness and Stress in a Driving Simulator. Driving Assessment 2001: The First International Driving Symposium on Human Factors in Driver Assessment. Training and Vehicle Design.

Vlakveld W. (2005). The Use of Simulators in Basic Driver Training, documento presentado a un taller en el contexto del proyecto HUMANIST de la UE, en Brno, Czech Republic. The Netherlands' Institute for Road Safety Research (SWOV). Leidschendam, Holanda.

Yamada, K. (2002). Evaluation of Forward Obstacles Collision Avoidance Support System using Driving Simulator. 9th World Congress on Intelligent Transport Systems.

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.

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.

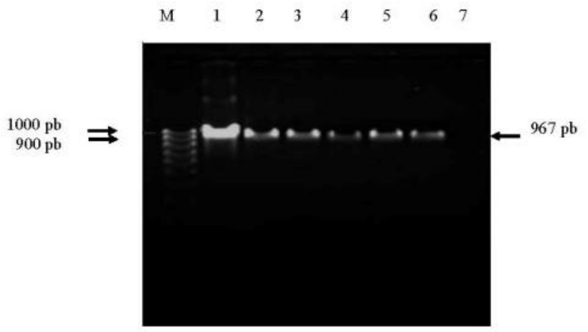


Figure 1 PCR product of the gyrB gene. 1.5% agarose gel with SYBR Gold, 80 V for 1 h. (M), HyperLadder ™ 100bp molecular weight marker; (1), Aeromonas hydrophila subsp. hydrophila ATCC 7966; (2), 027-05a; (3), 027-05b; (4), 028-05a; (5), 028-05b; (6), 028-05c; (7), Negative control.

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.

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.

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.

Article
ECORFAN-Bolivia Journal
June 2018 Vol.5 No.8 19-23

Interaction between Aedes aegypti CPB1 and viral proteins

Interacción entre Aedes aegypti CPB1 y proteínas virales

LÓPEZ-LÓPEZ, Edgar[1]*† & BARRIENTOS-SALCEDO, Carolina[2]

Universidad Veracruzana, Facultad de Bioanálisis-Veracruz, Agustín de Iturbide Esq. Carmen Serdan, 91700, Veracruz, Ver. Lab. de Química Médica y Quimiogenómica

ID 1° Autor: *Edgar López-López* / **ORC ID:** 0000-0002-7422-6059, **Researcher ID Thomson:** D-4905-2018**, arXiv ID:** edgar_lo, **PubMed ID**: el2, **CVU CONACYT ID:** 894234

ID 2° Coautor: *Carolina Barrientos-Salcedo* / **ORC ID:** 0000-0003-4023-8980, **PubMed Autor ID**: cabarrientos , **CVU CONACYT ID:** 252581

Received: February 09, 2018; Accepted: May 30, 2018

Abstract

The viruses chikingunya (CHIKV), zika (ZIKV) and mayaro (MAYV) are etiological agents of tropical diseases that represent a public health problem. The mechanisms of infection are produced through the Aedes aegypti vector, where a series of viral proteins interact, such as the protein of dengue virus E pro-tein (DENV). Therefore, the identification of surface proteins similar to this allows a better understanding of the internalization mechanisms in their respective vector; that in the future it will facilitate the generation of new therapeutic agents.

The aim of the present work is to analyze, by means of silica methods, proteins similar to the DENV E protein, where the organisms of interest are CHIKV (protein E1), ZIKV (protein E) and MAYV (protein P130) also to propose sites of Interaction between the proteins of the capsid and the intestinal protein CPB1 of the vector. Clustal Omega, I-TASSER, ClusPro online servers and the Protein Data Bank (PDB) database were used for this purpose. In addition to the bioinformatics tools TMPRED and BLAST.

The binding sites, the possible interacting attributes, the types of existing links and their type of environment were elucidated. This study received a history of molecular interaction in silica, with which it can be approached in a guided way, with in vitro or in vivo studies, the problem of diseases such as dengue and zika.

Molecular Coupling (Docking), Aedes Aegypti, Dengue, Zika, Chikungunya, Mayaro, CPB1

Resumen

Los virus chikingunya (CHIKV), zika (ZIKV) y mayaro (MAYV) son agentes etiológicos de enfermedades tropicales emergentes que representan un problema de salud pública. Los mecanismos de infección se producen a través del vector Aedes aegypti, donde interactúan una serie de proteínas virales, como la proteína del virus del dengue E pro-teína (DENV). Por lo tanto, la identificación de proteínas de superficie similares a esta, permite una mejor comprensión de los mecanismos de internalización en su vector respectivo; que en el futuro facilitará la generación de nuevos agentes terapéuticos.

El objetivo del presente trabajo es analizar, mediante métodos in silico, proteínas similares a la proteína E de DENV, donde los organismos de interés son CHIKV (proteína E1), ZIKV (proteína E) y MAYV (proteína P130) también para proponer sitios de interacción entre las proteínas de la capside y la proteína intestinal CPB1 del vector. Clustal Omega, I-TASSER, servidores en línea ClusPro y la base de datos Protein Data Bank (PDB) fueron utilizados para este propósito. Además de las herramientas bioinformáticas TMPRED y BLAST.

Se elucidaron los sitios de unión, los posibles aminoácidos que interactúan, los tipos de enlaces existentes y su tipo de entorno. Este estudio permitió determinar un antecedente de la interaccción molecular in silico, con el que se puede abordar de forma guiada, con estudios in vitro o in vivo, el problema de las enfermedades como el dengue y el zika.

Acoplamiento Molecular (Atraque), Aedes Aegypti, Dengue, Zika, Chikungunya, Mayaro, CPB1

Citation: LÓPEZ-LÓPEZ, Edgar[1]*† & BARRIENTOS-SALCEDO[2], Carolina. Interaction between Aedes aegypti CPB1 and viral proteins. ECORFAN Journal-Bolivia. 2018. 5-8: 19-18.

---

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

Introduction

Diseases that are transmitted by vectors, especially mosquitoes, are among the main causes of morbidity and mortality in humans. Aedes aegypti and Aedes albopictus are vectors that can infect human viruses, generating (to name a few diseases) dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), emerging diseases worldwide transmitted by insects that threaten a third of the human population.

During the bite of the female mosquito to a person infected by the DENV, CHIKV, MAYV or ZIKV, it usually contracts, becoming a carrier. Once the virus enters the mosquito system in the blood, the virus spreads from the intestine to the salivary glands of the mosquito and can then transmit the virus to another person while feeding. (Kabra SK, 1999)

That said, it was thought that the internalization method should be shared among different viruses, so the following research question was posed: is there structural convergence between viral proteins whose vector is Aedes aegypti?

Over the years, bioinformatics has generated great theoretical contributions that have subsequently been tested both in vitro and in vivo. That is why it is currently considered an important antecedent for any biological research. Our work reflects the possibility of interaction of the selected proteins with the protein carboxypeptidase 1 (CPB1) in Aedes aegypti. CPB1 is located in the intestinal cells of the dipterid, which is experimentally defined as participating in the internalization of DENV, where the E protein of this pathogen is anchored to domain II of it. (Hong-Wai Tham 1, 2014).

This work postulates molecular interactions between proteins that are structurally similar to the E protein of DENV, so, we believe that its mechanism of internalization can be carried out by the same receptor protein in Aedes, we speak of CPB1. Proteins of pathogens such as DENV and ZIKV (Flavovirus), CHIKV and MAYV (Togavirus) were evaluated. Where the inclusion criteria were: to be superficial, structurally conserved domains and sequential homology with the E protein of DENV.

Methodology

Homology of sequences by the algorithm of close neighbors

Initially, the possibilities of analyzing and structurally comparing proteins from different viral organisms were considered, with the purpose of identifying if in addition to "homology" there was functional similarity. However, the point of convergence selected for the analysis of the mentioned viral proteins was the fact of owning the same vector (Aedes aegypty). It is known that these viruses use the aforementioned dipterous as a transport, for which reason they considered the possibility that they use similar internalization mechanisms.

Once the organisms of interest were defined, they were analyzed using the technique of "homology of sequences by algorithms of close neighbors". The product obtained was a phylogenetic tree. Which is composed of nodes and branches; These nodes can represent either an individual, a species, or a higher grouping and are therefore widely termed taxonomical units. In this case, the terminal nodes represent the species of analysis and are the Operational Taxonomic Units (OTUS). The ordering of the nodes determines the topology of the tree and describes how the lineages have diverged in the course of evolution. The branches of the tree represent the amount of evolutionary divergence between two nodes in the tree and can be based on different measurements.

A tree is completely specified by its topology and the set of all the lengths of the edges. (Woese C., 2000)

Sequential analysis of the proteins of interest with Clustal Omega

Once the proteins of interest for each of the organisms were identified, the similarity of their sequences was analyzed. This was done through the "Clustal Omega" server (http://www.ebi.ac.uk/Tools/msa/clustalo/).

When the homologies were obtained respectively for each protein, the next step was to individually analyze different characteristics such as the existence of transmembrane domains, using the TMPRED tool (http://embnet.vitalit.ch/software/TMPRED_form.html)

A BLAST of the E1 protein of DENV was made in the PDB and NCBI database obtaining no crystallizable version. Therefore, we proceeded to use bioinformatics tools such as the I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) which allowed us to make a structural prediction of the mentioned protein.

Molecular docking and search for interacting sites

Once the generated model was obtained and selected, we performed a molecular Docking using the Clus-Pro server (https://cluspro.bu.edu/home.php). At the end of this process we observe and analyze the molecular couplings, selecting the models generated with structurally more stable scores (Balanced, Electrostatic, Hydrophobic and VdW + Elec).

Following the process, the software Swiss-PDBViewer v. 4.1.0 in each one of the proteins of interest were introduced, in order to observe their supposed interacting sites.

Organism	Protein	Localization
DENV	E	None / I-TASSER
MAYV	P130	Q8QZ72 – UniProt
CHIKV	E1	3N42 - PDB
ZIKV	E	None / I-TASSER
Aedes a.	CPB1	None / I-TASSER

Table 1 Location of viral proteins. It shows the IDs in different databases respectively for each protein of interest. "None" refers that there is no crystallographic structure of the protein, for which models were generated by homology.

Results

Homology of sequences by the algorithm of close neighbors

The diagram shows the relationship between the capsid protein sequences of some viruses, where the grouping of the proteins coming from different serotypes of DENV is clearly observed, immediately to this it bifurcates having on the one hand ZIKV with a value of 0.429 of its most immediate node and the CHIKV and MAYV proteins that are part of another branch with a distance of 0.064 and 0.495, respectively.

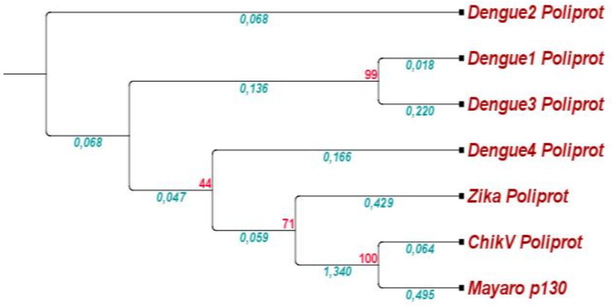
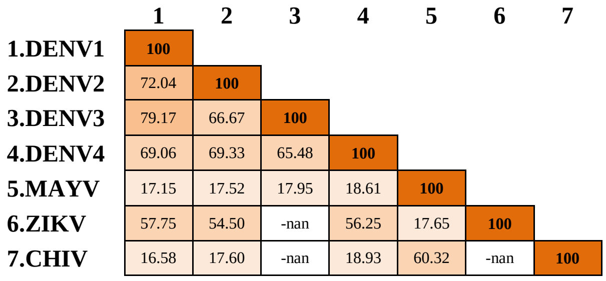


Figure 1 Phylogenetic tree of viral structural proteins. In blue refers phylogenetic distance between the sequences of the proteins to be studied and in red the percentage of times that the calculation generated this same results.

Sequential analysis of the proteins of interest with Clustal Omega

The server was used introducing the sequence of each of the proteins of interest, being the E1 proteins of CHIKV, p130 of MAYV (Togaviridae family), E of DENV and ZIKV (Family Flavoviridae). With what was obtained the following table shows the percentage of homology among all these.



	1	2	3	4	5	6	7
1.DENV1	100						
2.DENV2	72.04	100					
3.DENV3	79.17	66.67	100				
4.DENV4	69.06	69.33	65.48	100			
5.MAYV	17.15	17.52	17.95	18.61	100		
6.ZIKV	57.75	54.50	-nan	56.25	17.65	100	
7.CHIV	16.58	17.60	-nan	18.93	60.32	-nan	100

Figure 2 Homologous-sequential analysis of the proteins of interest. Alignments of biologically significant multiple sequences of amino acid sequence were produced. Evolutionary relationships are seen in percentage.

Molecular docking and search for interacting sites

Complementing the results, we used the TMPRED server (http://www.ch.embnet.org/software/TMPRED_form.html), with the idea of splicing the possible transmembrane sites with the interacting assumptions obtained from the previous study. Figure 3.

Obtaining significantly positive sites in the first 25 aa (being able to be a signal peptide), as well as approximate amino acids at 150, 225, 320 and 400. And sites statistically less significant at 150-200 and 350.

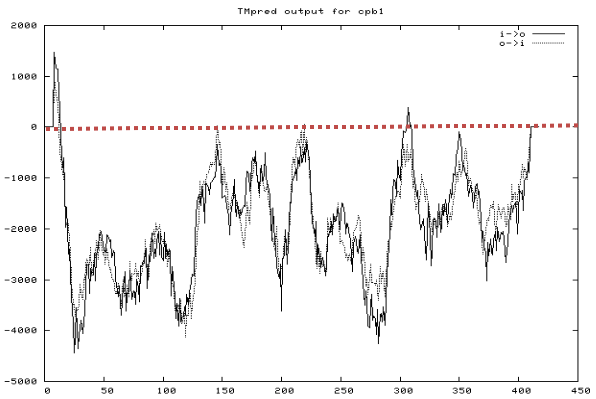


Figure 3 Possible transmembrane sites of CPB1. The algorithm is based on statistical base analysis, a database of natural transmembrane proteins. Sequences of amino acids close to zero (dotted line) will be the statistically most likely to be transmembrane sites.

We used standard E protein in DENV, which Hong-Wai T. and Vinod R.M.T in 2014 reported their interacting amino acids with CPB1 protein in *Aedes aegypti*.

We managed to reproduce these same results in our work, apart from the fact that in parallel we obtained new ones with the different proteins mentioned.

The joining energies being the following: DENV (-203.3), MAYV (-157.3), ZIKV (-210.4) and CHIKV (-247.0).

The results of homology of sequences by means of the algorithm of close neighbors revealed the similarity between the analyzed proteins, existing between each one of the sequences "possible common ancestors", which explains the possibility of having similar functions, such as the inter-nalization to the Aedes enterocyte in the same way, that is, through CPB1.

We were able to reproduce the previously published results on the interaction between the E protein of DENV and CPB1 in Aedes, which confirms the good manipulation of the bioinformatic data.

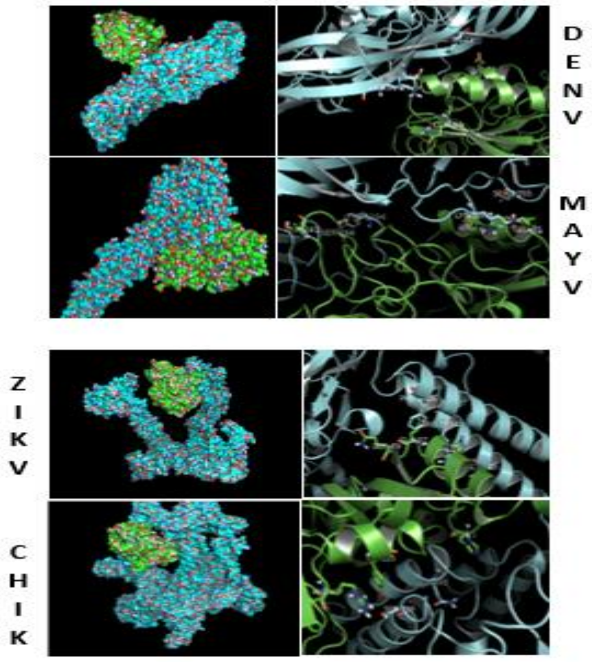


Figure 4 Molecular docking of viral proteins vs CPB1 of Aedes aegypti. In green CPB1 is shown and in blue the respective proteins of the different viruses.

Viral Protein	aa CPB1		aa Viral Protein	
E (DENV)	GLN	89	THR, ASN, THR	66,67,68
	GLU	43	LYS	122
P130 (MAYV)	GLU, ARG	35,42	LYS	1060
	ASN	39	SER	1055
	SER	244	TYR	1194
E1 (CHIKV)	GLN	89	ARG	2162
	GLU	87	PHE	2436
	GLN	85	ARG, VAL	2894, 2890
E (ZIKV)	HIS	21	---	---
	TYR	225	GLN	465

Table 2 Proposal of interacting amino acids. List of interacting aa in CPB1 and in the viral proteins evaluated.

Discussion

We analyzed the interacting sites of CPB1 with the different proteins, obtaining that between the E protein of DENV and the E1 protein of CHIKV there are very similar couplings, as well as the p130 protein of MAYV and the E protein in ZIKV.Therefore, the importance of the prediction of transmembrane helices of CPB1, which demonstrates the possibility of multiple binding sites is noteworthy. In addition, the existing interactions between the viral proteins and CPB1 conserve in all cases hydrophobic and polar characters, which are indispensable in the stability and viability of the proposed models.

Conclusions

The E protein in DENV is coupled to CPB1 in a similar way in terms of interaction type compared to the other protein models reported here. However, the binding sites were not homogenous, suggesting multiple binding sites in CPB1. The next step will be to evaluate the dimeric or trimeric conformations (depending on the case) of the different viral proteins, with the idea of simulating in a more realistic way the existing interactions in nature.

The binding sites reported here may be used in the future for the development of CPB1 blockers, thus avoiding the excessive propagation of said viruses, and in turn improving public health strategies.

In silico methods provide several advantages for the resolution of biological problems, because through them it is possible to observe molecurlarly a macrophenomeno, in addition to reducing costs (with the use of free servers and academic sofwares) and time in basic science.

References

Hong-Wai Tham 1, V. R. (2014). CPB1 of Aedes aegypti Interacts with DENV2 E Protein and Regulates Intracellular Viral Accumulation and Release from Midgut Cell. Viruses. 6(12): 5028–5046.

Izabela A. Rodenhuis-Zybert, J. W. (2010). Dengue virus life cycle: viral and host factors modulating infectivity. Cellular and Molecular Life Sciences. 67(16):2773-86.

Kabra SK, J. Y. (1999). Dengue hemorrhagic fever: clinical manifestations and management. Pediatr, 66: 93-101.

Maher A. Noureddine, P. Y.-J. (2005). Genomic Convergence to Identify Candidate Genes for Parkinson Disease: SAGE Analysis of the Substantia Nigra. Movement Disorders. v.40(8). Óscar Coltell, M. A. (2008). Integration of clinical and biological data in clinical practice using bioinformatics. Rev Méd Chile. 136(5):645-52.

Woese, C. R. (2000). Interpreting the universal phylogenetic tree. Proceedings of the National Academy of Sciences of the United States of America, 97(15), 8392-8396.

K. Hofmann & W. Stoffel (1993). TMbase - A database of membrane spanning proteins segments.Biol. Chem. Hoppe-Seyler 374,166.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990) "Basic local alignment search tool." J. Mol. Biol. 215:403-410.

Kozakov D, Hall DR, Xia B, Porter KA, Padhorny D, Yueh C, Beglov D, Vajda S. (2017) The ClusPro web server for protein-protein docking. Nature Protocols. 12(2):255-278.

Kozakov D, Beglov D, Bohnuud T, Mottarella S, Xia B, Hall DR, Vajda, S. (2013). How good is automated protein docking? Proteins: Structure, Function, and Bioinformatics. 12: 255–278

Kozakov D., Beglov D., Bohnuud T., Mottarella, S. E., Xia B., Hall D. R. and Vajda S. (2013), How good is automated protein docking?. Proteins. 81: 2159-2166.

Comeau SR, Gatchell DW, Vajda S, Camacho CJ. (2004). ClusPro: an automated docking and discrimination method for the prediction of protein complexes. Bioinformatics. 20; 45-50.

Comeau SR, Gatchell DW, Vajda S, Camacho CJ. ClusPro: a fully automated algorithm for protein-protein docking. Nucleic Acids Research. 32; 96-99.

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Molecular Systems Biology (2011) 7, 539.

Y Zhang. (2008). I-TASSER server for protein 3D structure prediction. BMC Bioinformatics, 9: 40.

Description of microplastics on the coast of La Guancha in Ponce, Puerto Rico

Descripción de microplásticos en la costa de La Guancha en Ponce, Puerto Rico

COLÓN-ORTIZ, Abner J. † & FEBLES-MORENO, Karelys*

Pontificia Universidad Católica de Puerto Rico 2250 Boulevard Luis A. Ferré Aguayo Ponce, P.R. 00717-9997

ID 1° Autor: *Abner J, Colón-Ortiz* / **ORC ID:** 0000-0002-2852-0038

ID 1° Coautor: *Karelys, Febles-Moreno* / **ORC ID:** 0000-0003-4166-0155

Received: February 19, 2018; Accepted: June 30, 2018

Abstract

La costa de La Guancha en Ponce, Puerto Rico, se localiza en una zona turística y zona portuaria. El propósito de esta investigación fue conocer y analizar qué tipo y qué cantidad de microplásticos se encuentran en esta área. Para la investigación de campo, se mide 200 metros a lo largo de la costa. Por cada 50 metros se escoge un cuadrante aleatoriamente, totalizando con 4 muestras de arena. Una vez seca la arena, se cierne y se identifican microplásticos. Las partículas de plástico encontradas se pesan en una balanza analítica y luego se colocan en bolsitas de muestras. Luego de muestrear la costa durante 5 meses, se clasificaron los microplásticos en categorías tales como fragmentos, pellets, fibras y filamentos. La categoría más abundante fueron los pellets, luego fragmentos, filamentos y por último fibras. Además, se realizó la prueba de *Kruskal-Wallis*, la cual reflejó que no existe diferencia significativa en la cantidad de micro plásticos en la playa de la Guancha de Ponce Puerto Rico y los cuadrantes de muestreo (p >0.05). La realización de esta investigación es de utilidad, dado que los microplásticos plantean una de las amenazas más severas para la sostenibilidad de los hábitats sensibles, la vida silvestre, la ecología y los océanos.

Pellet, Fibra, Fragmentos

Resumen

Microplastics were first recorded in North America in marine plankton along the New England coast in the 1970s (Masura, 2015). Since then, they have been found in most large bodies of water such as oceans, seas, lakes, and rivers (Masura, 2015). This research aimed to describe which type and amount of microplastics are found on the coast of La Guancha a tourist and port area, in Ponce, Puerto Rico. 200 meters were measured along the coast for field research. A quadrant was selected randomly for each 50 meters. Plastic particles were found, weighed on an analytical balance and then placed in sachets. After sampling the coast for 5 months, microplastics were classified into categories such as fragments, pellets, fibers and filaments. The most abundant category were the pellets, followed by fragments, filaments and finally fibers. In addition, the Kruskal-Wallis test was carried out, which showed that there is no significant difference between the amount of microplastics in the coast of La Guancha in Ponce, Puerto Rico and the sampling quadrants (p> 0.05). Therefore, microplastics were described in all the quadrants during the research period. The research findings are useful, given that microplastics pose as one of the most severe threats to the sustainability of sensitive habitats, ecology and oceans.

Fragments, Pellets, Fibers And Filaments

Citation: COLÓN-ORTIZ, Abner J. & FEBLES-MORENO, Karelys. Description of microplastics on the coast of La Guancha in Ponce, Puerto Rico. ECORFAN Journal-Bolivia. 2018. 5-8: 24-32.

---

* Correspondence to Author (email: kfeblesmoreno@pucpr.edu)
† Researcher contributing as first author.

Introduction

Microplastics were first recorded in North America in marine plankton along the New England coast in the 1970s (Masura, 2015). Since then, they have been found in most large bodies of water such as oceans, seas, lakes, and rivers (Masura, 2015). The microplastics are located in cleaning products, hygiene either toothpaste and cosmetics such as exfoliants (Greenpeace Organization, 2016). According to Masura (2015), the fibers that come off synthetic clothing and rope are microplastics that also end up in bodies of water.

According to the Greenpeace Organization (2016), microplastics consist of a diameter of less than 5mm on any of its sides composed of polyethylene, polypropylene or polystyrene material; They also have the ability to attract chemicals and release them. The micro pearls, fibers and many of these microplastics are small enough to pass through wastewater treatment plants and enter a watershed (Masura, 2015). According to PhMj (2014) the coast of Guancha in Ponce, Puerto Rico is a beach where sand particles vary from 0.0625 mm to 2 mm in diameter. The coasts of Puerto Rico vary in three categories: rocky cliffs and promontories; mangrove coasts; and sandy or gravel beaches (PhMj, 2014).

The beach of La Guancha is a combination of mangrove coasts and sand or gravel, since it is located in a water transition zone (PhMj, 2014). The level of the sea is mostly low so the depth of the sea is estimated far from the coast.

Sabaté (2016), published the topic of plastic microspheres in hygiene products such as exfoliators or granular toothpaste that have a composition based on synthetic developments such as polypropylene (PP), polyethylene terephthalate (PET), and polymethyl methacrylate (PMMA). This author commented that these particles are capable of forming associations with pollutants such as pesticides (Sabaté, 2016).

The microplastics filter in the subsoil, then they are released in the sea, they pass to the fish and then to the human food (Sabaté, 2016). The organization Greenpeace (2016), published that 83% of Norwegian lobsters have microplastics in their stomach.

Galloway & Lewis have stated that oysters exposed to microplastics during gametogenesis lead to a reduction in their energy absorption and reproductive performance (Galloway & Lewis, 2016). However, Greenpeace (2016) pointed out the lack of data and lack of knowledge about the toxicity of micro plastics in humans. Likewise, it points out the potential of the surface of these particles to transport and disseminate pathogens that are relevant to diseases humanities (Greenpeace Scientific Department, 2016). Ogunola & Palanisami (2016), estimated that the world fishing fleet threw approximately 135,400 tons of plastic fishing tools and 23,600 tons of packaging materials into the ocean. The abandoned, lost or discarded fishing utensils is a source of plastic waste for the fishing and aquaculture sectors, but its contribution is little known at regional and global level.

The production of plastics has increased exponentially since the beginning of the 1950s, reaching 322 million tons in 2015 (Lusher, A.L., Hollman, P.C.H., Mendoza-Hill, J.J., 2017). This figure does not include synthetic fibers, which accounted for an additional 61 million tons in 2015 (Lusher, A.L., Hollman, P.C.H, Mendoza-Hill, J.J., 2017). It is expected that plastics production will continue to increase in the foreseeable future and it is likely that production levels will double by 2025 (Lusher, A.L.; Hollman, P.C.H .; Mendoza-Hill, J.J., 2017). It has even been estimated that in 2010 between 4.8 million and 12.7 million tons of plastic waste entered the oceans (Lusher, A.L., Hollman, P.C.H., Mendoza-Hill, J.J., 2017).

As a consequence, the human being is prone to the exposure of microplastics when consuming seafood or fish, since the animal carries those chemical properties in its digestive tract; as well as the chemical substances that are degraded by the high temperatures in cooking them. Therefore, this leads to this research to describe the micro plastics found in the coast of La Guancha in Ponce, Puerto Rico.

Marine debris such as microplastics poses one of the most severe threats to the sustainability of sensitive habitats, wildlife, ecology and oceans (Sheavly, 2010). It also threatens the culture, the economy, people around the world and the inhabitants of coastal and riverine communities (Sheavly, 2010).

A recent report from the National Academy of Sciences on marine debris concluded that there was still relatively little information available on the quantities and sources of marine debris, encouraging the implementation of coordinated monitoring efforts to assess marine debris (Sheavly, 2010).

The performance of monitoring activities to evaluate the types and quantities of microplastics, help to have a description of what is and in what quantities. According to GESAMP (2015) more information on the fragmentation of PE, PP and EPS plastics should be collected; design sampling strategies to establish temporal trends and spatial trends in selected marine areas; evaluate the possible risk of microplastics for the health of humans and examine the accumulations of microplastics.

Investigation questions

1. What kind of microplastics are found on the beach of Guancha de Ponce Puerto Rico?
2. What is the difference of the amount of microplastics in the beach of Guancha de Ponce Puerto Rico and the sampling quadrants?

Literature review

The research conducted by McDermid & McMullen (2004), showed the presence of small plastics waste on the beaches of the Hawaiian archipelago. Nine remote locations along the Hawaiian Islands were sampled between September 2001 and February 2003. At each beach a sediment sample was taken from the high tide line. Nested sieves of 4.75, 2.8 and 1 mm were used. Only particles of 1 - 15 mm in size were retained.

The classification consisted of dry type, where each sample was placed on white paper. It was classified as plastic, plants and "others" (ie, shell, paper, and ceramic matter). They were placed in separate containers, then labeled with the size, location and type of sieve. The second step is a wet type where each sub-sample of plastic is rinsed to remove sand. Then, small amounts of sample are poured into a container of fresh water. The container was swirled for 1 minute and the floating particles (plastic) were sieved.

The samples were dried in an oven for 1 hour at 65 ° C. The third and last step was to classify and quantify, each size class was separated by type of plastic, counted and placed in separate containers.

As a result, a total of 22 samples were collected. Of the 736.47 g collected waste, 72% by weight were plastic particles, 22% vegetative material and 6% "other". Small plastics waste was found in each sample, totaling 19,100 pieces. After being classified, 87% of the plastic collected were fragments, while 11% were pre-production plastic granules. The total weight of the plastic for all the combined sites was 532.37 g. Significant amounts of small plastic debris (1-15mm in size) were found on all beaches from which samples were taken.

It should be noted that each beach had different current patterns, types of sand, wave action and exposure to the wind. This type of plastic pellet is abundant on beaches in areas close to plastic factories, loading docks and boarding lanes for raw plastic materials. The collections of the high tide line contained much more plastic than berm samples, perhaps because the particles suspended in the water will remain on land during each receding tide, while berm waste is deposited mainly during storms or as traces washed away the wind of the high tide line. The authors concluded that the effects of small plastic debris on marine animals, including the toxicity of granules and fragments that accumulate on beaches throughout the Hawaiian archipelago, remains unknown, but should be investigated.

In a study conducted by Yu, Peng, Wang, Wang and Bao in 2016, microplastics were investigated in the Bohai Sea sand in northeastern China. The objective of the research was to provide data on the abundance and chemical composition of micro plastics. Therefore, sand samples from three sites in the northern Bohai Sea were quantified and characterized to analyze micro plastics.

The study consisted of four steps: Collections of field sampling, isolation and quantification, verification and characterization of micro plastics; and finally, statistical analysis. Sand samples were collected from three locations north of the Bohai Sea from July 25-27, 2015.

At each site studied, samples were taken from two sites, the commercial and non-commercial beach. The beach for bathers where there is commercial activity and is visited by approximately 800 thousand tourists in summer; and the beach for non-bathers where there is no commercial activity.

All materials were collected by a metal ring with a diameter of 25 cm and 2 cm in height. In addition, the vertical distribution of the micro plastic was investigated so that 5 more samples were collected below the surface samples with a depth of 20 cm. In the second procedure, all the samples were dried at 50 ° C for at least 48 hours and 50 g of the dry sand was mixed with 250 mL of saturated saline for 2 minutes. After 2 hours of settling, the water solution on the sand was transferred to a 500 mL beaker for another settling hour. Then the solution was filtered through a 47 mm glass fiber filter with 1.0 mm pore size. The filters were dried for 24 hours and sealed in Petri dishes for quantitative and qualitative analyzes.

To quantify the number of fragments an AM3011 digital microscope with a magnification of 70x was used. All suspect microplastics were classified into fragment, sheet and fiber. The results of the abundance and composition of microplastics indicated that a certain portion of non-plastic material consisting of fibers was detected in the samples. These fibers include artificial and natural celluloses. The abundance of microplastics was 102.9 ± 39.9, 163.3 ± 37.7, and 117.5 ± 23.4 "items" / kg. Eight types of plastics were found in the sand samples. The three most abundant plastics were PEVA, LDPE and PS. PEVA plastic is found in bathing and waterproof curtains. In the plastic bags and dispensing bottles is the LDPE material. On the other hand, PS plastic is used for protective packages such as containers or peanut wraps.

The two possible reasons that the authors proposed for microplastics were the high density of industries and urbanizations in the region, or the geographic structure of the Bohai Sea that promotes the accumulation of microplastics. Even in the majority of the samples, the colored fibers that come from the wastewater of the washing machines predominated. Each garment produces> 1,900 fibers per wash.

These fibers are confused with microplastics because of their similar shape and the difficulty of identifying them without a microscope. Cellulose fibers are not classified as plastic despite having the capacity to absorb toxic contaminants; and they are found in dresses, coats, pants or any item of clothing. It should be noted that 90% of non-plastic particles were fibers and FTIR results indicated that most were cellulosic fibers.

The results showed that the verification of the FTIR combined with SEM was able to separate the artificial cellulose as cotton threads from the plastic fibers. Then, due to the high tourist activity in the studied sites, the bathers beach showed more abundance of micro plastics than the non-commercial beach. Yu, Peng, Wang, Wang and Bao (2016) demonstrated in their results that the 2 cm deep surface has a slightly higher abundance of microplastics compared to the 20 cm deep samples; it also contains more microplastics than the noncommercial beach evaluated (Yu, Peng, Wang, Wang, & Bao, 2016).

In another investigation of microplastics, carried out by Tanaka and Takada (2016), they evaluated microplastic fragments and microspheres in digestive tracts of planktivorous fishes of urban coastal waters. Plastics were captured in Tokyo Bay who receive water from the river, industrial wastewater, and surface runoff from the city.

In this research, plastics were studied in personal care products to determine the characteristics of micro plastics that are used. For this, they bought brands of facial cleansers where polyethylene is used. In addition, the Anchoa fish was examined or under its scientific name Engraulis Japonicus, since it is an important food for humans and even organisms. Scientists have observed physical impacts on the digestive system and deterioration of feeding capacity due to the ingestion of micro plastics in these fish. The intake of microplastics in this species is worrisome, since they contain dangerous chemicals to which humans are exposed.

The study method consisted of three steps. The first step was sampling and processing. Then classification and identification of plastics. Finally, the examination of micro spheres in cosmetics and personal care products.

To begin the sampling and processing study, they captured 64 anchovies in Tokyo Bay in a span of 7 hours. After measuring them, they removed their digestive tract and placed them in a 10 mL glass bottle that was baked at 550 ° C for 4 hours. To digest organic matter, a 10% solution of KOH is inserted from 7-8 mL (> 3x of the bowel volume). They were covered for 10 days until the digestion was completed in 3 to 4 days. The bottles were shaken in order to break the mass of non-digestible materials such as zooplankton shells and those that remained floating separated in another bottle. They were examined under a microscope and particles similar to natural prey were determined.

In the second step used was the classification and identification of microplastics, photographing the particles that remained floating in the intestinal tract of the fish. They analyzed those suspicious plastic pieces by infrared spectroscopy to identify polymers. Twelve particles were identified as being of natural origin, eleven of them were not identified and the rest was classified as synthetic polymers. Finally, in the third step they examined the plastics of the hygiene products that they bought to determine the properties of the micro spheres. The marks that were examined contained polyethylene. They were mixed with distilled water and floating solid particles were identified by FT-IR

The results showed that there were microplastics in the digestive tract in 49 of 64 anchovies. Of the 150 pieces of plastic, 129 were fragments, 11 balls, 8 filaments 2 "foams". The plastics consisted mostly of polyethylene and most were white or transparent. The results of the plastic particles of facial cleansers report that the 4 products contained micro spheres of polyethylene. To conclude, the researchers could not identify the origin of the fragments, but it can be part of a history of degradation in the environment. The results confirmed the presence of polyethylene particles in the four brands of facial cleansers in Japan and micro spheres in two of them. Polyethylene was found in fish and its appearance coincided with that of facial cleansers. The observations confirm that micro plastics have been introduced into the marine ecosystem. These small fragments absorb hydrophobic chemicals from salt water as well as contain compounds during their manufacture.

The human intake of micro plastics through anchovies can increase the body burden of hazardous chemicals, so, we are exposed to chemicals as well as anchovies.

In the studio of Reisser, Shaw, Hallegraeff, Proietti, Barnes, Thums, Wilcox, Hardesty, and Pattiaratchi; the types of organisms that inhabit the surface of 68 small floating plastics from coastal waters of Australia, whether tropical or temperate, were examined to characterize their biodiversity. The Scanning Electron Microscope SEM was used for the study. The variety of plastic forms and textures have interacted with environments and marine organisms that can physically affect the feeding deposits of the fauna; for example, blockages after ingestion. It can even alter pelagic biological life and modify the physical properties of its habitats. In addition, microplastics can transport species, changing their natural ranges to convert them into non-native species, including invasive pests.

To begin the study, 65 floating plastics were selected between> 1mm and <10mm to fit in the scanning electron microscope, and were preserved in 2.5% glutaraldehyde buffered in filtered seawater. They were dehydrated with increasing concentrations of ethanol, dried at critical points using CO2, mounted on aluminum pieces with carbon tape and then powdered with a layer of gold. The types of organisms detected in each piece of plastic were measured and classified into taxonomic and morphological groups; In addition, the frequency of occurrence for each type was calculated. Each piece of plastic was photographed with a magnification of 50x to measure the parameters of size and shape, observe the superficial fractures, the holes and the grooves. After the analyzes, the plastics were washed with distilled water and subjected to infrared spectrometry of the Fourier Transform to identify polymers.

As a result of the 65 hard plastic fragments, they had a diverse range of shapes and types of surface micro textures, including linear fractures, holes and scraping marks. The fragments were made of polyethylene (54) and polypropylene (11). Diatoms and bacteria were observed in all the marine regions sampled. However, the diatoms, specifically the genus Nitzschia, were the most abundant, widespread and diverse group of plastic colonizers.

Several unidentified organisms of morphotypes and sizes were found, mostly resembling bacteria, cyanobacteria and fungal cells. Frequently elongated cells were observed and were detected in 59% of the plastics examined. In addition, some invertebrates were observed in millimeter-sized plastics. Encrusting bryozoa colonies were the most common animals. Four of these colonies harbor diatom assemblages dominated by Licmophora Sp, Nitzschia Longissima, Amphora Sp and Nitzschia Sp. Even an unidentified sea worm was found in a 6mm plastic fragment.

Now there is ample evidence that millimeter-sized plastics are abundant and widespread in marine environments. This study showed that the number of known genera of diatoms that inhabit marine plastics doubled. These diatoms fixed firmly the plastic, they resisted the turbulence of the water and the action of the wave. This study showed that micro plastics provide a new niche for other types of microorganisms and invertebrates.

Methodology

The samples were analyzed on the beach according to the protocol of the 5 Gyres Institute (5 Gyres).

1. The 200 meter measuring tape was placed parallel to the ocean. To randomly evaluate microplastic contamination in the area, four random zones were selected between 0 and 50. Then, a random quadrant was placed on the middle beach or trail every 50 m of transept.

2. In each quadrant, pieces of natural debris such as seaweed, leaves and wood were removed.

3. Sieving of dry sand:
a. Half of the 5-gallon bucket was filled with sand, scraping the surface of the quadrant evenly, using a flat cup or dustpan. It was approximately 3 cm (in depth) of the entire surface within the quadrant. The sediment was scraped off the surface as evenly as possible.
b. Once the bucket was half full, the sediment was removed from the bucket and poured into the 1 mm sieve. All contamination of plastic and other non-natural waste was placed in a sample jar or bag.

4. Wet sand screening:
a. The wet sand was poured into a wide, flat container.
b. Once the sand was spread evenly, it was left to rest for a day until it was completely dry.
c. Return the sand to its respective bucket and start sifting.

5. The magnifying glass was used to identify the plastic. To differentiate the plastic material from shells was used the technique of squeezing with the intention of observing if the shell breaks.

6. All the plastic pieces were placed on graph paper (if possible, let it dry somewhere without disturbance). The rule or graph paper was used to separate the plastic pieces in size and categories:
a. Size categories - (particles> 5mm), (particles <5mm)
b. Type of categories - (Fragment, "Film", "Foam", Pellet, "Line")

7. The number of plastics for each category was quantified and recorded in the data sheet.
a. The sample was photographed for the posterior shape, size, color and type of identification.
b. b. The information was sent to citizenscience@5gyres.org and Marine DebrisTracker.

Location

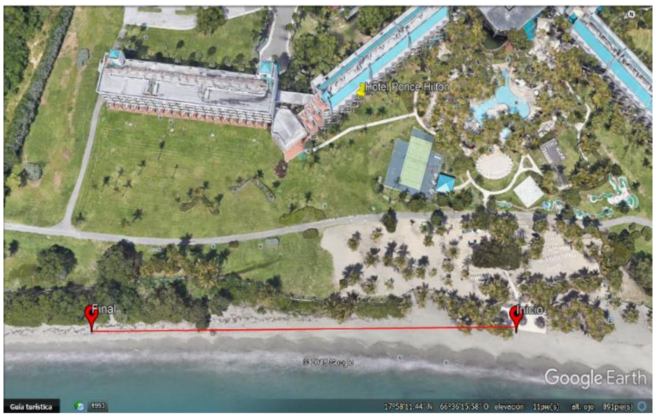
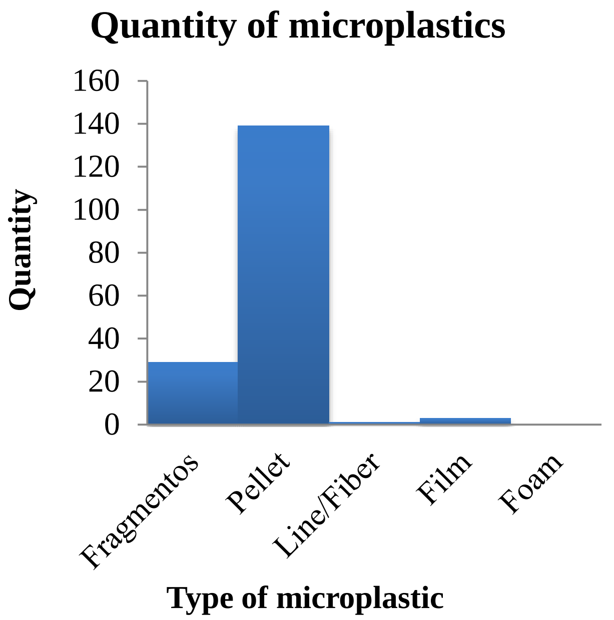


Figure 1 Image taken from Google Earth 2018

Results



Graph 1 Total description of microplastics for five months

To discuss the research question: What kind of microplastics are found on the Guancha beach in Ponce Puerto Rico?

Based on the descriptive and inferential results of the research, the presence of different microplastics is demonstrated in the coast of Guancha de Ponce, Puerto Rico. The most abundant type of plastic micro found in this research was the pellets, which predominated in the quadrants during the five months of sampling. This type of micro plastic, pellet, is found in hygiene products such as exfoliators or granular toothpaste that have a composition based on synthetic developments (Sabaté, 2016).

Another abundant category of microplastics found in the investigation were the fragments; those of greater length were found in the month of November 2017 and February 2018. Secondary microplastics are fragments generated by the decomposition of larger pieces. The fragmentation of plastics in the sea occurs through photodegradation, physical impacts and other processes, and results in the generation of a greater number of smaller particles (Tanaka & Takada, 2016).

As for the category of fibers and filaments, they were found in February in smaller quantities. According to Masura (2015), the fibers that come off synthetic clothing and rope are micro-plastics that also end up in bodies of water. The production of plastics has increased exponentially since the beginning of the 1950s, reaching 322 million tons in 2015 (Lusher, A.L., Hollman, P.C.H., Mendoza-Hill, J.J., 2017). This figure does not include synthetic fibers, which accounted for an additional 61 million tons in 2015 (Lusher, A.L., Hollman, P.C.H; Mendoza-Hill, J.J., 2017). To discuss the research question: What is the difference in the amount of microplastics at the Guancha beach in Ponce Puerto Rico and the sampling quadrants?

A nonparametric test was carried out for three or more independent samples. As the results obtained from the Kolmogorov-Smirnov test confirmed the assumption of non-normality of the data, the Kruskal Wallis test was carried out. The researchers found that the data were less than 0.05, which indicated that they do not follow a normal distribution (See Table 1).

The Kruskal Wallis test was carried out to test whether there is no significant difference in the amount of micro plastics in the Guancha beach of Ponce Puerto Rico and the sampling quadrants. The results of the test were not significant, $\chi2$ (N = 20) = .1.883, 4.762 p = .792. This p-value, when compared to a significance of 0.05 (p> 0.05), indicates that the null hypothesis is not rejected. Therefore, there is no significant difference in the amount of micro plastics in the Guancha beach of Ponce Puerto Rico and the sampling quadrants (see Table 2).

In Table 3 it is observed that the average ranges between the quadrants are similar. According to the sampling and analysis of these, it was demonstrated that there was not a significant quadrant with more microplastics than another one in the coast of La Guancha in Ponce. That is, microplastics were found along the coast during the months of November 2017 to March 2018.

Normality test

Normality test of Shapiro-Wilk			
	Statistical	gl	Sig.
Micro plastics	.550	20	.000
Mass	.678	20	.000

Table 1 Normality Test

Statistical test^{a,b}		
	Microplastics	Mass
Chi squared	1.883	4.762
gl	3	3
Sig Asymp.	.597	.190
a. Kruskal Wallis test		
b. Group variable: Quadrants		

Table 2 Kruskal-Wallis test

Ranks			
	Quadrants	N	Average range
Microplastics	1	5	7.80
	2	5	11.70
	3	5	12.50
	4	5	10.00
	Total	20	
Mass	1	5	6.00
	2	5	10.10
	3	5	12.30
	4	5	13.60
	Total	20	

Table 3 Range descriptive test

Conclusion

Based on the descriptive results (Graph 1) and inferential results of the research (Table 2), the presence of different microplastics in the coast of Guancha de Ponce, Puerto Rico is demonstrated. The most abundant type of microplastic found in this investigation were the pellets, then the fragments, fibers and filaments. Based on the statistical test (Table 2) it is concluded that during the months that the microplastics were analyzed on the Guancha de Ponce coast, there is no significant difference in the amount of microplastics found in the sampling quadrants. That is, microplastics were described in all the quadrants during the months of the study.

Acknowledgement

We thank the Pontificia Universidad Católica de Puerto Rico and the Puerto Rico Program Louis Stokes Alliance for Minority Participation (PR-LSAMP) for their support in the research. Also, Dr. Zaira Mateo, Dr. Alma Santiago, Dr. Carlos Lugo, Prof. Piel Banchs and Profa. Carmen Reyes for her support and confidence in the development of the research.

References

Departamento Científico de Greenpeace. (2016). *Plásticos en el pescado y el marisco.* . Obtenido de http://www.greenpeace.org/espana/Global/espana/2016/report/plasticos/Plasticos_en_el_pescado_y_el_mariscoLR.pdf

Galloway, T. S., & Lewis, C. N. (2016, March 1). *Marine microplastics spell big problems for future generations.* Retrieved from PNAS: http://www.pnas.org/content/113/9/2331.full.pdf

GESAMP (2015). "*Sources, fate and effects of microplastics in the marine environment: a global assessment*" (Kershaw, P. J., ed.). (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 90, 96 p.

Greenpeace Organization. (5 de septiembre de 2016). *Greenpeace identifica cuáles son las empresas que más microesferas de plástico utilizan en sus productos cosméticos.* Obtenido de Greenpeace: http://www.greenpeace.org/espana/es/news/2016/Septiembre/Greenpeace-identifica-cuales-son-las-empresas-que-mas-microesferas-de-plastico-utilizan-en-sus-productos-cosmeticos/#

Lusher, A.L.; Hollman, P.C.H.; Mendoza-Hill, J.J. 2017. *Microplastics in fisheries and aquaculture: status of knowledge on their occurrence and implications for aquatic organisms and food safety*. FAO Fisheries and Aquaculture Technical Paper. No. 615. Rome, Italy.

Masura, J. (2015). Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. NOAA Technical Memorandum, NOS-OR&R-48.

McDermid, K. J., & McMullen, T. L. (2004). Quantitative analysis of small-plastic debris on beaches in the Hawaiian archipelago. Science Direct, 790-794.

Ogunola OS, Palanisami T (2016) Microplastics in the Marine Environment: Current Status, Assessment Methodologies, Impacts and Solutions. J Pollut Eff Cont 4:161. doi:10.4172/2375-4397.1000161

PhMj. (2014, September 23). The Beaches of Puerto Rico. Retrieved from Geocaching: https://www.geocaching.com/geocache/GC5DGRC_the-beaches-of-puerto-rico?guid=f54ae531-23e0-48ca-b030-ef8642d5d7a1

Reisser, J., Shaw, J., Hallegraeff, G., Proietti, M., Barnes, D. K. A., Thums, M., … Pattiaratchi, C. (2014). Millimeter-Sized Marine Plastics: A New Pelagic Habitat for Microorganisms and Invertebrates. PLoS ONE, 9(6), e100289. http://doi.org/10.1371/journal.pone.0100289

Sabaté, J. (20 de septiembre de 2016). Qué son las microesferas de plástico y cómo empeoran nuestra vida. Obtenido de eldiario.es: http://www.eldiario.es/consumoclaro/cuidarse/microesferas-plastico-pasta-dientes-exfoliantes-contaminacion-mar_0_558194828.html

Sheavly, S. B. (2010). National Marine Debris Monitoring Program. U.S. Environmental Protection Agency.

Tanaka, K., & Takada, H. (2016, September 30). Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Retrieved from Nature: https://www.nature.com/articles/srep34351?WT.feed_name=subjects_physical-sciences#methods

Yu, X., Peng, J., Wang, J., Wang, K., & Bao, S. (2016, April). Occurrence of microplastics in the beach sand of the Chinese inner sea: the Bohai Sea. Retrieved from Research Gate : https://www.researchgate.net/publication/301781432_Occurrence_of_microplastics_in_the_beach_sand_of_the_Chinese_inner_sea_the_Bohai_Sea

5 Gyres . (s.f.). Citizen Science Protocols. 5 Gyres Institute, 4. Obtenido de 5 Gyres Institute.

The versatile biological and chemical reactivity of quinoline derivatives, a source of innovation for the chemist

La versátil reactividad química y biológica de los derivados de quinolina, fuentes de innovación para el químico

GARZA-ORTIZ, Ariadna†*, RAMIREZ-DE LA CRUZ, Flor de María, LÓPEZ-GUTIÉRREZ, Tomás J. y MEX-ÁLVAREZ, Rafael M

Universidad Autónoma de Campeche, Facultad de Ciencias Químico-Biológicas, Instituto Nacional de Investigaciones Nucleares

ID 1° Author: *Ariadna Garza-Ortiz* / **ORC ID:** 0000-0003-2585-1285, **Researcher ID:** T-6565-2017, **CONACYT-ID**: 37870

ID 1° Coauthor: *Flor de María Ramírez de la Cruz* / **ORC ID:** 0000-0002-6794-0334, **CONACYT-ID**: 6480

ID 2° Coauthor: *Tomás J. López Gutiérrez* / **ORC ID:** 0000-0002-3554-1347, **Researched ID**: H-9457-2018 y **CONACYT-ID**: 444757

ID 3° Coauthor*: Rafael M. Mex-Álvarez* / **ORC ID:** 0000-0003-1154-056, **Researcher ID**: H-4911-2018

Received: February 09, 2018; Accepted: May 30, 2018

Abstract

Innovation is a particularly important word for the chemical industry as it is daily exercised when looking for drugs with improved biological effect and reduced side effects. It is also fundamental in the development of new materials, supplements, colorants among other chemically important products. The quinoline moiety is a versatile system used for several purposes as it has been stated in specialized literature. Chemically speaking quinoline, 1-aza-naphthalene or benzo[b]pyridine is a well-known heterocyclic compound present in several organisms with various potential applications in the pharmaceutical, chemical and material science areas. Maybe one of its most remarkable application of such a type of compounds is the used of related quinoline compounds in the treatment of malaria. This work is minted to summarizing the general synthetical procedures as well as the innovative applications of quinoline related systems with final thoughts about the potential uses in the chemical industry in general and interest in the pharmaceutical industry.

Quinoline, Malaria, Innovation

Resumen

Innovación es un término muy importante para la industria química al ser un ejercicio diario en la búsqueda de nuevos fármacos con actividad biológica mejorada y efectos secundaros mínimos, así como en el desarrollo de nuevos materiales, suplementos, colorantes entre otros productos químicamente importantes. La quinolina es un sistema orgánico muy versátil empleado con diferentes propósitos como queda de manifiesto en la revisión de la literatura especializada. La quinolina, 1-aza-naftaleno o benzo[b]piridina, es un compuesto heterocíclico bien conocido, presente en diversos organismos y con aplicaciones en las áreas farmacéuticas, química y en el desarrollo de materiales. Tal vez una de las aplicaciones más reconocidas de este tipo de compuestos es su uso en el tratamiento de la malaria. El objetivo de este trabajo consiste en resumir procedimientos de síntesis más reconocidos, así como las aplicaciones innovadoras de sistemas derivados de quinolina en diferentes áreas, con ideas finales sobre los potenciales usos de tales derivados en la industria química en general, pero con principal interés en la industria farmacéutica.

Quinolina, Malaria, Innovación

Citation: GARZA-ORTIZ, Ariadna, RAMIREZ-DE LA CRUZ, Flor de María, LÓPEZ-GUTIÉRREZ, Tomás J. y MEX-ÁLVAREZ, Rafael M. The versatile biological and chemical reactivity of quinoline derivatives, a source of innovation for the chemist. ECORFAN Journal-Bolivia. 2018. 5-8: 33-42.

---

* Correspondence to Author (email: arigarza@uacam.mx)

† Researcher contributing as first author.

1. Introduction

Chemistry is considered in general terms as the central science. Thanks to the development of chemical concepts, society has benefited from the creation of textiles, plastic materials, drugs for the treatment of common and emerging diseases, specialized foods, dyes, flavorings, among others.

Heterocyclic chemistry as a branch of chemistry is an area of intense development and application. Of the commercially successful drugs internationally an important percentage contains heterocyclic functions. Heterocyclic chemistry is the science that is responsible for the study of the physicochemical properties and reactivity of heterocyclic compounds, which are described in general terms as cyclic organic compounds with at least one of the components of the cycle being a different carbon element. The non-carbon atoms present in the cycle are termed heteroatoms, nitrogen, oxygen and sulfur heteroatoms being more common. Of the most recognized and studied heterocycles, six-membered heterocycles with aromatic nitrogen, sulfur and oxygen occupy an important place.

Quinoline is a heterocyclic system resulting from the fusion of a benzene ring and a pyridine ring, both aromatic and six-member cycles (Figure 1). The quinoline molecule contains a C9H7N molecular formula and a molar mass of 129.15 g / mol. It was isolated for the first time in 1834 by Runge from coal tar and in 1842 it was obtained from the alkaline pyrolysis of cinchonina, an alkaloid derived from the cinchona tree. It has a partition coefficient of 2.04 a pKb = 4.85 and a pKa = 9.5. It is considered a weak base that can form salts with acids and shows a reactivity similar to that of pyridine and benzene. It presents electrophilic substitution reactions, attributable to its aromatic nature, as well as nucleophilic substitution reactions, mainly driven by nitrogen with its free electron pair. It is a molecule frequently present in pharmaceutical compounds and associated with several pharmacological effects[1,2].

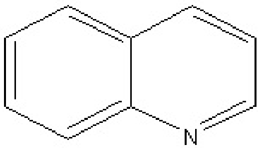


Figure 1 Structural formula of quinoline

Quinoline derivatives have been described in the specialized literature and available in the clinic as antibacterials [3,1], antifungal [4,5,6,7], anti-inflammatory [8,9,10], antimalarials [11,2], antileishmania [12,13], anticancer [14,15,16,17], antiviral [18,19,20], among others.

In addition to the obvious applications in therapeutics, systems derived from quinolines have been used in the preparation of nanomaterials and mesostructures with notable electronic and photonic applications [21,22,23,24]. The potential of the quinolines derivatives is very promising in areas such as the synthesis of agrochemicals and pesticides, preparation of dyes for tissues and foods, chemical sensors, among many more [25],[26].

This work is intended to describe the most important synthesis procedures and in general the reactivity and biological application of quinoline and quinoline derivatives, which in recent times are subject to study due to the wide variety of biological and synthetic applications. According to our knowledge, there is no work that summarizes these relevant aspects of the chemical and biological reactivity of quinoline and its derivatives. Due to the wide application, that this family of compounds can have in chemical and pharmacological processes, this material is fundamental for the processes of innovation in the subject, of the following years. Section 2 will review the main processes of synthesis of quinoline and quinoline derivatives, with special interest in those processes friendly to the environment.

Section 3 of this work will address some of the properties and reactivity characteristics of this family of heterocyclic compounds. Section 4 will describe the main biological applications of quinoline-derived compounds and the most recognized biological activity structure relationships. Section 5 summarizes the latest trends in patents generated for compounds with quinolinic structure and their potential applications.

Finally, the most important research areas on the biological properties of quinoline and its derivatives will be discussed with the intention of presenting those that imply clear sources of innovation (Section 6).

2. Synthesis of quinolines and quinolinic derivatives

The synthesis of new molecules is an activity without limits, which probably scientists, we will not finish exploring. In the past, the synthesis of new molecules was basically due to the study of the reactivity of these, that is, new molecules were synthesized in order to study the reactivity and to test the theories and hypothesis that, on chemical knowledge, that were being formulated in the way. For example, the development of many aromatic compounds was the result of the verification of the aromaticity theory. In the past synthesis was very dependent on the intuition of the chemist, since there were no concrete rules, known and studied.

Currently the synthesis of new molecules obeys a more rational design. There are very specific objectives and characteristics of the biological or chemical reactivity that a new molecule must have and that makes the synthetic steps to be analyzed very carefully. The synthesis of a molecule, which commonly refers to a family of molecules, will have specific reactive, spatial, connective, and biological characteristics. A combination of factors that make the task of synthesis quite complicated, but that facilitate the possibility of adapting the molecule or family of molecules to useful functions for humanity, such as the development of new drugs, more powerful and with fewer side effects.

Fortunately, in this work of designing new molecules, the chemist is no longer alone, he has at hand the development of computer systems and statistical packages that analyze experimental and theoretical databases to establish, for example, qualitative biological activity structure relationships and quantitative, and that investigate in detail the structural needs and physicochemical properties of the new molecules that will be used for the treatment of some disease. The same reasoning can be established for the development of catalysts, materials, paints and coatings, etc. In addition, the development of innumerable techniques and experimental procedures for the characterization of proteins, active sites, catalytic sites, to name a few, generates knowledge about the functional and structural characteristics of proteins and any chemical compound with which the new molecule can interact efficiently.

The theoretical calculation methodologies that are based on molecular mechanics, quantum mechanics and semi-empirical, allow calculations to be made for the estimation of the optimal geometry and the energy contents of new molecules and bonding sites, which also contribute to facilitate the design needs rational of new molecules. Finally it is important to mention that the better knowledge of the molecular bases of the pharmacological action, of the catalytic processes, among others, have made possible a more delicate analysis of the design of new molecules.

Although there is a vast number of synthetic strategies for obtaining quinolines [27] and its derivatives, the search for new synthesis processes continues to represent an attractive area of research. The first successful methods of quinolin synthesis comprise reactions of heterocyclic ring closure from ortho-substituted anilines and aniline derivatives without ortho-substitution. Skraup, Austrian chemist, in 1881 obtained quinoline from anilines and glycerin in a medium of sulfuric acid or phosphoric acid and an oxidizing agent (nitrobenzene, arsenic acid, lead dioxide or ferric salts and the quinoline is believed to be formed via the formation of acrolein (Figure 2) The main limitation of this synthesis is that quinlines substituted in the pyridine ring can not be obtained by this method.

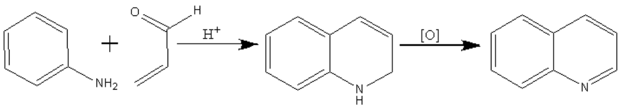


Figure 2 Chemical equation of synthesis of quinoline proposed by Skraup

In 1887 the Conrad-Limpach method was described, which is frequently used in the preparation of quinolones, precursors of several compounds of pharmaceutical importance. In this process, anilines, beta-ketoesters, which at high temperatures produce the ethanol elimination and the formation of the nitrogen heterocycle are used.

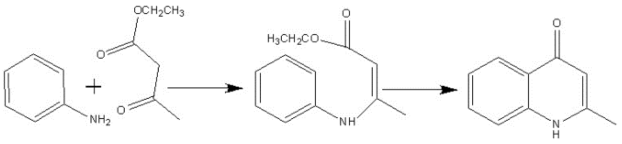


Figure 3 Chemical synthesis equation of 4-quinolone, quinoline precursor, Conrad-Limpach method

Another synthesis process widely used in the production of quinolines is the Friedländer reaction (1883), which imparts greater versatility in the synthesis of substituted quinolines in both rings. It is a synthesis procedure still in force, although with modifications. It involves the reaction of an ortho-substituted aniline and a carbonyl compound that contains methyls or methylenes in the alpha position. A condensation of amino group and an aldol condensation are suggested for the formation of the heterocycle (Figure 4). Its main limitation lies in the ease of synthesis of precursors.

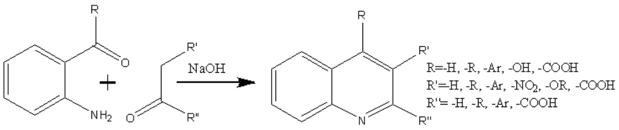


Figure 4 Chemical equation of synthesis of quinoline proposed by Friedländer

The synthesis of 4-carboxyquinoline acids of Pfitzinger is obtained from ketones and isatinic acid27,28 (obtained In situ, from isatin), or modification from enaminones29 in aqueous medium (Figure 5).

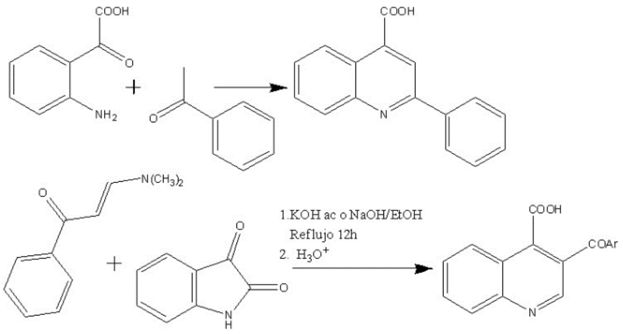


Figure 5 Pfitzinger synthetic method of quinolinic derivatives

Zhou[30] reported the synthesis of polysubstituted quinolines from 2-amino aromatic ketones and alkynes with good yields (Figure 6).

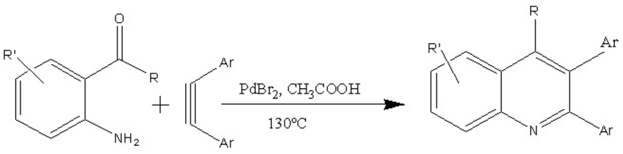


Figure 6 Synthetic method quinolinic derivatives according to Zhou

Efforts to develop cleaner methodologies resulted in synthesis protocols such as the one proposed by Zhao[30], in which quinoline derivatives are obtained in aqueous medium and facilitated by a silver catalyst, in which a high economy of atoms and few residues are observed (Figure 7).

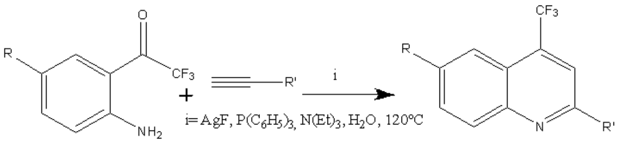


Figure 7 Synthetic method quinolinic derivatives according to Zhou

Other less popular methodologies, but yielding quinolin derivatives, aimed at obtaining compounds with pharmacological activity have been described in the literature [31].

One of the most innovative proposals in recent times, consists of the synthesis of derivatives of 1,4,6,8-tetrahydroquinolines [32,33], One of the most innovative proposals in microwave-assisted, at room temperature and above all avoiding the use of extreme reaction conditions (high temperatures, aggressive reagents) and the generation of large amounts of by-products and waste. The synthetic methodology is described in Figure 8 recent times, consists of the synthesis of derivatives of 1,4,6,8-tetrahydroquinolines.

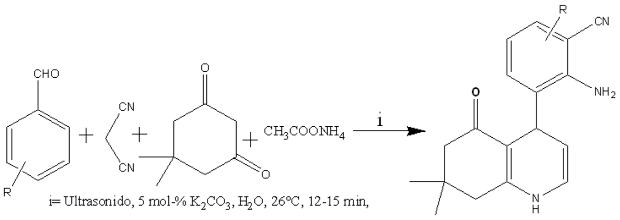


Figure 8 Synthetic method used to obtain tetrahydroquinolines using ultrasound radiation

Other quinoline derivatives with important applications are the 4-hydroxyquinoline-2-ones and the 2,4-quinolindiones, of which significant synthesis efforts have been developed and their physicochemical and biological properties described in the literature[34]. Using the Web of Science database, using the theme corresponding to the synthesis of quinolines and derivatives, there are about 3609 citations between 1980 and 2018. In 1980, 31 documents were published reporting novel synthesis processes of quinoline derivatives and 2017, said databases accounted for 244 jobs.

This represents an increase of almost 800% and trends indicate that this trend will continue.

3. Chemical reactivity of quinoline and quinolinic derivatives

As already mentioned in the introduction, quinoline derivatives are of interest in different areas of science due to their potential applications. For example, quinoline derivatives have a wide range of pharmacological activities and among them we can mention antimicrobial, antiparasitic, antifungal, cardiovascular, antiviral, antimalarial, antidiabetic, among others. This diversity of pharmacological applications directly depends on the physicochemical properties of such heterocyclic systems.

And not only that, quinoline derivatives have been used for the fluorescent recognition of metal ions, in optical, electrochemical devices, in the preparation of pigments, nanomaterials and alloys.

Quinoline is a very stable light yellow liquid. A slightly higher density than water (1.093 g / mL, 25 ° C), a melting point of -17 to -13 ° C, a boiling point of 237 ° C and a vapor pressure of 0.07 mmHg at 20 ° C. It is very little soluble in water and very soluble in benzene, dichloromethane, to mention a few.

Quinoline is a molecule that exhibits fluorescence of the S1 state (n, π *) and phosphorescence of the T1 state (π, π *) in the vapor phase. In addition, quinoline is an organic molecule that emits a T1 (π, π *) - S0 fluorescence in vapor phase[35].

.

The chemical properties of quinoline come mainly from the pyridine portion of the heterocycle. It is also undeniable that in part their reactivity is explained in terms of the aromaticity of the molecule. Quinoline is a flat molecule, with carbons in sp2, cyclic hybridization and having 10 aromatic electrons, as predicted by Huckel's formula. The nitrogen in the quinoline has a pair of electrons that does not participate in the aromaticity, for which, like pyridine, the quinoline presents basic properties, with a pKa = 4.9. From this fact, the natural tendency of the quinoline to react by attacking electrophiles with the pair of free electrons of the heteroatom, including the protons.

It also shows reactivity similar to pyridine against nucleophilic substitution reactions such as displacement, hydrogen substitution, among others. Because it is an aromatic compound, it is expected to present typical reactions of electrophilic aromatic substitution (SEAR). Unlike pyridine, where the aromatic electrophilic substitution is difficult, in quinoline and its derivatives is much easier since there are two aromatic cycles and of them the carbocycle is more reactive by not having the influence of nitrogen in the heterocycle. That is why by means of SEAR substituents are introduced in the carbocyclic aromatic ring, mainly in the C5 and C8, favored both by the electron distribution and by the stability of the intermediates formed (Figure 9).

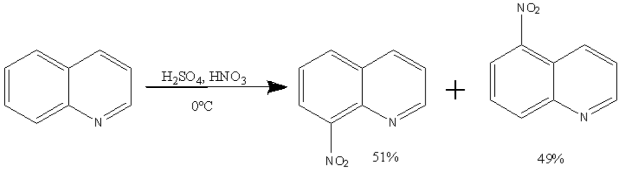


Figure 9 Example of electrophilic substitution reaction in quinoline

Unlike the SE_{ar} favored in the carbocycle, it is possible to incorporate substituents in the heterocyclic ring by addition reactions after the binding of an electrophile to the nitrogen in the C2 of the derivative (Figure 10). The most activated positions for nucleophilic attack are C2 and C4.

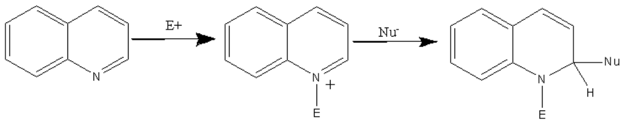


Figure 10 Quinoline reactivity and justification of addition reactions on pyridinic ring

The behavior of the quinoline against the reduction (hydrogenation) depends on the nature of the reducing agent. In the presence of molecular hydrogen and a nickel or platinum catalyst, the pyridine heterocycle is first hydrogenated, forming tetrahydroquinolines. A prolonged hydrogenation results in the hydrogenation of the carbocycle as well[27]. The reduction is more selective in the presence of lithium aluminum hydride. In the same way in the case of oxidation, this depends on the oxidizing agents. It is important to mention that, since the oxidation depends on the availability of electrons, due to the deficient character of pi electrons of the pyridine ring in the quinoline, the carbocyclic ring will be the main oxidation site.

In the presence of potassium permanganate the breakdown of the benzene ring is generated with the formation of carboxylated derivatives of pyridine[27] (Figure 11).

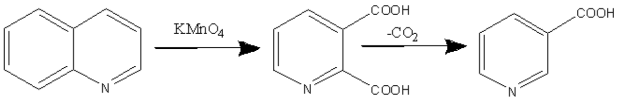


Figure 11 Chemical equation of quinoline oxidation

In addition to the heterocyclic reactivity characteristic of quinoline, it is a very good binder that can react with transition metals, lanthanides and actinides to form coordination compounds[36,37,38,39,40,41].

In the case of metals, when you have coordination compounds, it has been discovered that many times the biological effect of the binder (the organic portion) is increased by the presence of the metal[42,43,44] and in that sense it is that compounds of coordination with different metals have been prepared, among which ruthenium, gold, cobalt, nickel, zinc, copper and some metals of the family of the lanthanides and actinides described in the specialized literature stand out.

4. Biological applications of quinolines and quinolinic derivatives

The quinolinic derivatives were administered for the treatment of diseases certainly since ancient times, even before the identification of said compounds. The use of remedies obtained from leaves, fruits, barks, herbs, flowers, animals and insects, have been described in ancient documents of Indian, Egyptian and Chinese culture, to name a few. Perhaps one of the naturally occurring compounds that has received the most attention is quinine. Quinine is an alkaloid, organic nitrogenous compound that is synthesized by plants, considered a secondary metabolite, whose presence is not essential for survival of the plant, but have defined functions for the protection of the same against insects and other pests that often threaten the proliferation and permanence of it. The cinchona tree, containing high concentrations of quinine in its bark, and infusions of the bark were associated with the improvement of symptoms of bacterial infections, but especially in the treatment of malaria.

It was during the Second World War that an epidemic of malaria was unleashed and, consequently, the demand for medicine stimulated several research projects aimed at the synthesis in the laboratory of the active principles extracted from plants. From these efforts arose chloroquine and various substances chemically related to it. The treatment of malaria with chloroquine continues being one of the most used alternatives in our times (Figure 12).

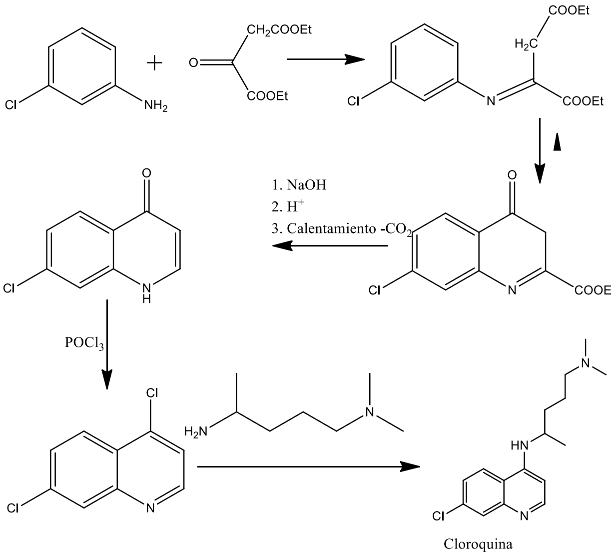


Figure 12 Synthetic route for obtaining chloroquine

Although one of the first applications of quinoline-derived compounds was as antiparasitic, for the specific treatment of malaria, quinoline derivatives have proven to be extremely versatile with respect to their application in the field of pharmacology. Important antibacterial agents are known[3,1], antifungal [4,5,6,7], anti-inflammatory [8,9,10], antimalarials [11,2], antileishmania [12,13], anticancer [14,15,16,17], antiviral [18,19,20], among others (Figure 13).

One of the areas of health where innovation is being given an important boost with respect to quinolinic compounds is in the treatment of tuberculosis. Tuberculosis is a lung infection caused by the bacterium Mycobacterium tuberculosis. TB is considered one of the most dangerous, contagious and fatal infections and represents a public health problem. Given the new threats that this disease brings, the development of new drugs is imperative, and quinoline offers an important alternative. It is believed that some quinoline-derived compounds are polar enough to traverse cell membranes and efficiently intro- duce into the bacterium.

Once inside the bacteria, it is believed that quinoline derivatives bind to DNA gyrases and thereby inhibit their proliferation by altering their DNA production[38,45].

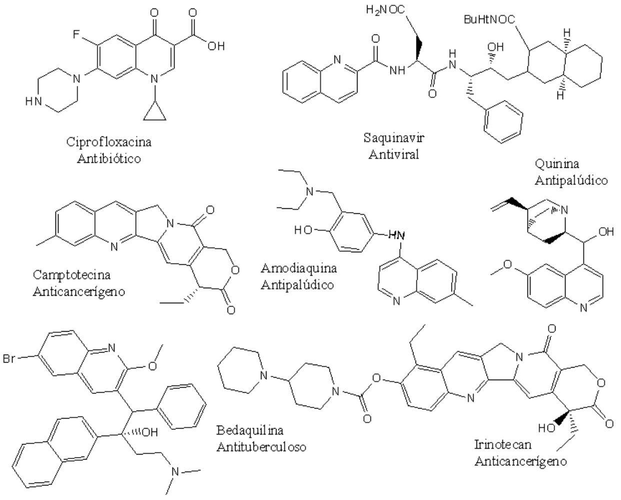


Figure 13 Compounds derived from quinoline with pharmaceutical application

5. Patents related to the synthesis of quinolinic derivatives

Starting from the wide variety of applications that the quinoline-derived compounds have, it is not surprising that there are 57727 patents available in the patent office of the United States of America, which comprise quinoline-derived compounds. There are patents related to the construction of optical and electronic devices, various organometallic and coordination compounds with outstanding optical properties, patents for molecules to be used in the treatment of conditions such as hepatitis C, prodrugs for the treatment of autoimmune diseases, antidiabetic compounds, antimalarials, potentials treatments in erectile dysfunction, anticancer, dyes, resins, adhesives.

In the same database in 2017, 19 patents were given related to the invention of products with quinoline derivatives present. In 2016, 26 patents were found, while in 2015, 35 patents.
In the particular case of patents of quinoline-derived compounds with anticancer properties, different targets of anticancer action are mentioned. Patents of quinoline derivatives have been described as inhibitors of c-Met kinase, which participates in processes of wound recovery and embryonic development, whose hyperactivity causes cancer[46].

There are also patents of anticancer compounds derived from quinolines with PI3K / AKT / mTOR inhibitory capacity, a fundamental protein for the regulation mechanisms of different cellular functions and which is very active in cancerigenic processes. Other patented quinoline derivatives are considered inhibitors of EGFR / VEGFR receptors, surface receptors to which growth factors (proteins and steroid hormones) bind, thus allowing the transmission of signals between cells. These communication mechanisms are fundamental for the development of certain types of cancer.

6. Concluding Observations

Quinoline and its derivatives are very important for their pharmacological and industrial applications, and they are also a fundamental part of many living systems. This importance is evident in the increasing number of investigations into the physicochemical and reactive properties of quinoline derivatives, with the aim of innovating with pharmaceutical products, optical devices, electronics, nanomaterials, among others.

The information contained in this document shows conclusively that quinolinic compounds continue to represent an important synthetic challenge, especially due to the wide variety of applications that this type of structure can have in different areas of science. It also demonstrates the growing interest of the industry in these systems, as the data of the number of patents indicates. In such a way that we anticipate an important development of the knowledge in synthesis and applications of these compounds, with special interest in the development of relations structure activity, that facilitate the development of better materials in the different fields of application, from finer adjustments of structures, for a biological effect or particular physicochemical property and for the benefit of humanity.

Acknowledgement

AGO is grateful for the support received via the NPTC-SEP call for the development of this work. FDMRC is grateful for the support received for the project Research on the effect of the composition and structural nature of materials in the physicochemical properties of lanthanides and actinides in solid state and solution (Stage 3), within Line 1, Study of physicochemical properties in the UV-Visible-near infrared and / or magnetic complexes of lanthanides and actinides formed with tetraquininated quinoline binders at CONACYT.

References

Aboelnaga, A. & El-Sayed, T. H. Click synthesis of new 7-chloroquinoline derivatives by using ultrasound irradiation and evaluation of their biological activity. *Green Chem. Lett. Rev.* **11,** 254–263 (2018).

Aggarwal, K. & Khurana, J. M. Synthesis and application of an indenoquinoline dione conjugate as a dual fluorescent and colorimetric pH sensor. *J. Lumin.* **187,** 457–465 (2017).

Banerjee, B. Recent Developments on Ultrasound-Assisted Synthesis of Bioactive N-Heterocycles at Ambient Temperature. *Aust. J. Chem.* **70,** 872–888 (2017).

Bharadwaj, S. S. *et al.* Design, synthesis and pharmacological studies of some new quinoline Schiff bases and 2,5-(disubstituted- 1,3,4)-oxadiazoles. *New J. Chem.* **41,** 8568–8585 (2017).

Carlos, C.-C., Naytzé, O.-P., Ariadna, G.-O. & Irma, R.-O. Crystal structure of bis-{μ-(E)-2-[(2-oxido-phenyl-imino)-meth-yl]quinolin-8-olato-κ4 O,N,N′,O′}bis-[di-butyl-tin(IV)]. *Acta Crystallogr. Sect. E* **73,** 4–7 (2017).

Carta, A. *et al.* Quinoline tricyclic derivatives. Design, synthesis and evaluation of the antiviral activity of three new classes of RNA-dependent RNA polymerase inhibitors. *Bioorg. Med. Chem.* **19,** 7070–7084 (2011).

Casado-Sanchez, A. *et al.* Effect of electronic and steric properties of 8-substituted quinolines in gold (III) complexes: Synthesis, electrochemistry, stability, interactions and antiproliferative studies. *J. Inorg. Biochem.* **174,** 111–118 (2017).

Chen C (Tianjin Longbogen Pharmaceutical Co., L. . Preparation of quinoline derivatives for treating solid tumor and leukemia. (2014).

Chen, M., Chen, H., Ma, J. W., Liu, X. Y. & Zhang, S. Y. Synthesis and anticancer activity of novel quinoline-docetaxel analogues. *Bioorg. Med. Chem. Lett.* **24,** 2867–2870 (2014).

Chen, Y. L. *et al.* Synthesis and antiproliferative evaluation of certain 4-anilino-8-methoxy-2-phenylquinoline and 4-anilino-8-hydroxy-2-phenylquinoline derivatives. *Bioorg. Med. Chem.* **14,** 3098–3105 (2006).

da Silva, E. N. *et al.* Synthesis, Characterization, Cytotoxic Activity, and Interactions with CT-DNA and BSA of Cationic Ruthenium(II) Complexes Containing Dppm and Quinoline Carboxylates. *Bioinorg. Chem. Appl.* (2017). doi:10.1155/2017/2562780

de la Guardia, C. *et al.* Antiviral Activity of Novel Quinoline Derivatives against Dengue Virus Serotype 2. *Molecules* **23,** (2018).

Dogan, F., Bilici, A., Yildirim, M. & Kaya, I. 6-Hydroxyquinoline Oligomers Emit White Light. *Sci. Adv. Mater.* **6,** 1957–1964 (2014).

Elghamry, I. & Al-Faiyz, Y. A simple one-pot synthesis of quinoline-4-carboxylic acids by the Pfitzinger reaction of isatin with enaminones in water. *Tetrahedron Lett.* **57,** 110–112 (2016).

Fang, Y. M. *et al.* Synthesis, Antifungal Activity, and SAR Study of Some New 6-Perfluoropropanyl Quinoline Derivatives. *J. Heterocycl. Chem.* **55,** 240–245 (2018).

Garza-Ortiz, A., Maheswari, P. U., Siegler, M., Spek, A. L. & Reedijk, J. Ruthenium(III) chloride complex with a tridentate bis(arylimino)pyridine ligand: Synthesis, spectra, X-ray structure, 9-ethylguanine binding pattern, and in vitro cytotoxicity. *Inorg. Chem.* **47,** (2008).

Garza-Ortiz, A., Uma Maheswari, P., Siegler, M., Spek, A. L. & Reedijk, J. A new family of Ru(ii) complexes with a tridentate pyridine Schiff-base ligand and bidentate co-ligands: Synthesis, characterization, structure and in vitro cytotoxicity studies. *New J. Chem.* **37,** (2013).

Ghorab, M. M., Ragab, F. A. & Hamed, M. M. Design, synthesis and anticancer evaluation of novel tetrahydroquinoline derivatives containing sulfonamide moiety. *Eur. J. Med. Chem.* **44,** 4211–4217 (2009).

Gopal, M., Shenoy, S. & Doddamani, L. S. Antitumor activity of 4-amino and 8-methyl-4-(3diethylamino propylamino)pyrimido 4 ’, 5 ’: 4,5 thieno (2,3-b) quinolines. *J. Photochem. Photobiol. B-Biology* **72,** 69–78 (2003).

Guo, M. *et al.* Synthesis and biological evaluation of rhodanine derivatives bearing a quinoline moiety as potent antimicrobial agents. *Bioorg. Med. Chem. Lett.* **23,** 4358–4361 (2013).

Hussain, H., Al-Harrasi, A., Al-Rawahi, A., Green, I. R. & Gibbons, S. Fruitful Decade for Antileishmanial Compounds from 2002 to Late 2011. *Chem. Rev.* **114,** 10369–10428 (2014).

Jenekhe, S. A., Lu, L. D. & Alam, M. M. New conjugated polymers with donor-acceptor architectures: Synthesis and photophysics of carbazole-quinoline and phenothiazine-quinoline copolymers and oligomers exhibiting large intramolecular charge transfer. *Macromolecules* **34,** 7315–7324 (2001).

Katritsky, A. R. & Pozharzky, A. F. *Handbook of Heterocyclic Chemistry*. (Elsevier Scienc Ltd., 2000).

Kaya, I., Er, G. & Temizkan, K. Synthesis, characterization and fluorescence properties of azomethine polymer containing quinoline unit. *Polym. Bull.* **75,** 1809–1822 (2018).

Kumar, V. *et al.* Synthesis of quinoline based heterocyclic compounds for blue lighting application. *Opt. Mater. (Amst).* **50,** 275–281 (2015).

Madkour, H. M. F., El-Hashash, M. A. M., Salem, M. S. & Mahmoud, A. O. A. Synthesis, antileishmanial and cytotoxicity activities of fused and nonfused tetrahydroquinoline derivatives. *Res. Chem. Intermed.* **44,** 3349–3364 (2018).

Mandewale, M. C., Thorat, B., Patil, U., Kale, B. & Yamgar, R. Developments in quinoline synthesis: a review. *Heterocycl. Lett.* **5,** 475–488 (2015).

Mandewale, M. C., Thorat, B., Shelke, D. & Yamgar, R. Synthesis and Biological Evaluation of New Hydrazone Derivatives of Quinoline and Their Cu(II) and Zn(II) Complexes against Mycobacterium tuberculosis. *Bioinorg. Chem. Appl.* (2015). doi:10.1155/2015/153015

Manikandan, A., Ravichandran, S., Sathiyanarayanan, K. I. & Sivakumar, A. Efficacy of phenyl quinoline phenol derivatives as COX-2 inhibitors; an approach to emergent the small molecules as the anti-inflammatory and analgesic therapeutics. *Inflammopharmacology* **25,** 621–631 (2017).

Martinez-Bulit, P. *et al.* 2,6-Bis(2,6-diethylphenyliminomethyl)pyridine coordination compounds with cobalt(II), nickel(II), copper(II), and zinc(II): Synthesis, spectroscopic characterization, X-ray study and in vitro cytotoxicity. *J. Inorg. Biochem.* **142,** (2015).

Meghdadi, S. *et al.* Benign synthesis of quinolinecarboxamide ligands, H(2)bqbenzo and H(2)bqb and their Pd(II) complexes: X-ray crystal structure, electrochemical and antibacterial studies. *J. Coord. Chem.* **70,** 2409–2424 (2017).

Mohssen, H. F., Ali, N. M. & Ali, H. A. Synthesis of new quinoline derivatives from methylene Meldrum’s acid and screening the biological properties. *Res. J. Pharm. Biol. Chem. Sci.* **8,** 476–498 (2017).

Mukherjee, S. & Pal, M. Medicinal Chemistry of Quinolines As Emerging Anti-inflammatory Agents: An Overview. *Curr. Med. Chem.* **20,** 4386–4410 (2013).

Musiol, R. Quinoline-based HIV Integrase Inhibitors. *Curr. Pharm. Des.* **19,** 1835–1849 (2013).

Phan, L. T. *et al.* Synthesis and antibacterial activity of a novel class of 4 ’-substituted 16-membered ring macrolides derived from tylosin. *J. Med. Chem.* **47,** 2965–2968 (2004).

Pinz, M. P. *et al.* 7-Chloro-4-phenylsulfonyl quinoline, a new antinociceptive and anti-inflammatory molecule: Structural improvement of a quinoline derivate with pharmacological activity. *Regul. Toxicol. Pharmacol.* **90,** 72–77 (2017).

Proisl, K., Kafka, S. & Kosmrlj, J. Chemistry and Applications of 4-Hydroxyquinolin-2-one and Quinoline-2,4-dione-based Compounds. *Curr. Org. Chem.* **21,** 1949–1975 (2017).

Prajapati, S. M., Patel, K. D., Vekariya, R. H., Panchal, S. N. & Patel, H. D. Recent advances in the synthesis of quinolines: a review. *Rsc Adv.* **4,** 24463–24476 (2014).

Reddy, S. M., Rao, D. S., Sudhamani, H., Kumari, P. G. & Raju, C. N. New phosphoramidate derivatives of 5-nitroquinolin-8-ol: synthesis, spectral characterization, and evaluation of biological activity. *Phosphorus Sulfur Silicon Relat. Elem.* **190,** 2005–2012 (2015).

Subhedar, D. D. *et al.* Novel tetrazoloquinoline-rhodanine conjugates: Highly efficient synthesis and biological evaluation. *Bioorg. Med. Chem. Lett.* **26,** 2278–2283 (2016).

Siddekha, A., Azzam, S. H. S. & Pasha, M. A. Ultrasound-Assisted, One-Pot, Four-Component Synthesis of 1,4,6,8-Tetrahydroquinolines in Aqueous Medium. *Synth. Commun.* **44,** 424–432 (2014).

Umamatheswari, S. & Sankar, C. Synthesis, identification and in vitro biological evaluation of some novel quinoline incorporated 1,3-thiazinan-4-one derivatives. *Bioorg. Med. Chem. Lett.* **27,** 695–699 (2017).

Vandekerckhove, S., Tran, H. G., Desmet, T. & D’Hooghe, M. Evaluation of (4-aminobutyloxy)quinolines as a novel class of antifungal agents. *Bioorg. Med. Chem. Lett.* **23,** 4641–4643 (2013).

Xu, M. *et al.* Insecticidal quinoline and isoquinoline isoxazolines. *Bioorg. Med. Chem. Lett.* **24,** 4026–4030 (2014).

Zhang, H. R. *et al.* Synthesis, characterization and biological evaluation of a cobalt(II) complex with 5-chloro-8-hydroxyquinoline as anticancer agent. *Appl. Organomet. Chem.* **30,** 740–747 (2016).

Zhou, W. & Lei, J. Palladium-catalyzed synthesis of polysubstituted quinolines from 2-amino aromatic ketones and alkynes. *Chem. Commun.* **50,** 5583–5585 (2014).

Article
ECORFAN-Bolivia Journal
June 2018 Vol.5 No.8 43-47

Mitigation of greenhouse gases in ruminants through acetic acid as a food additive

Mitigación de los gases de efecto invernadero en los rumiantes a través del ácido acético como aditivo alimentario

CRUZ-HERNÁNDEZ, Ángel Raúl[1]*†, MALFAVÓN-MEJÍA, Janett[1], LUCIO-DOMÍNGUEZ Rodolfo[1], BEDOLLA-CEDEÑO, José Luis Carlos[1] y GONZALES-RONQUILLO, Manuel[2]

[1] Universidad Michoacana de San Nicolás de Hidalgo, Facultad de Medicina Veterinaria y Zootecnia, Av. Acueducto y Tzintzuntzan sn. Col Matamoros, 58130 Morelia Michoacán México
[2]Universidad Autonoma del Estado de México. El Cerrillo Piedras Blancas, Toluca, Estado de México. C.P. 50090.

ID 1° Author: *Ángel Raúl, Cruz-Hernández* / **ORC ID:** 0000-0001-9809-1323

Received: February 09, 2018; Accepted: May 30, 2018

Abstract

The objective was to evaluate the result of the addition of acetic acid in sheep feed to mitigate the production of greenhouse gases. The research was carried out in the Faculty of Veterinary Medicine and Zootechnics in the Posta unit where the greenhouse gas production was evaluated with the experimental method carried out with two adult sheep being the donors of the rumen liquid In this method of assessment of pounds-force per square inch (PSI) in vitro using 125 ml glass bottle in an incubator at a temperature of 39 degrees Celsius with stubble and bran inoculated with acetic acid in different inclusion degrees 10, 15 and 20 for 96 hours in incubation taking measurements every 3 hours to observe the constant variation. It was compared with standard samples where only food was found without the addition of acetic acid. Each incubated sample was evaluated as a whole; each measure was evaluated in PSI taken with which a comparative analysis of these measurements and proportions was made, respectively. It was determined that the samples of stubble and bran with a degree of inclusion at 20% showed a marked difference in the reduction of greenhouse gases while the other inclusion degrees 15% and 10% reduced that production compared to the control group. It is concluded that acetic acid contributes to the mitigation of greenhouse gases with a level of 10% inclusion in the diet of sheep based on stubble and bran.

Gases Greenhouse Effect, Acetic Acid, Ruminants

Resumen

El objetivo fue evaluar el resultado de la adición ácido acético en la alimentación de ovinos para mitigar la producción de gases de efecto invernadero. La investigación se realizó en la Facultad de Medicina Veterinaria y Zootecnia en la unidad Posta donde se evaluó la producción de gases de efecto invernadero con el método experimental realizado con dos ovinos adultos siendo los donadores del líquido ruminal En este método de valoración de libras-fuerza por pulgada cuadrada (PSI) de manera in vitro utilizando frasco de 125 ml de vidrio en una incubadora a una temperatura de 39 grados centígrados con rastrojo y salvado inoculados con ácido acético en distintos grados de inclusión 10,15 y 20 durante 96 horas en incubación tomando medidas cada 3 horas para observar la variación constante. Se comparó con las muestras estándar donde se encontraba solo alimento sin adición de ácido acético. Se evaluó en conjunto cada muestra incubada, se valoró cada medida en PSI tomadas con lo que se realizó un análisis comparativo de estas medidas y proporciones respectivamente. Se determinó que las muestras de rastrojo y salvado con un grado de inclusión al 20 % mostró marcada diferencia en la reducción de gases de efecto invernadero mientras que los otros grados de inclusión 15% y 10% redujeron dicha producción comparada con el grupo testigo. Se concluye que el ácido acético contribuye a la mitigación de gases de efecto invernadero con un nivel de inclusión al 20% en la dieta de ovinos basada en rastrojo y salvado.

Gases Efecto Invernadero, Ácido Acético, Rumiantes

Citation: CRUZ-HERNÁNDEZ, Ángel Raúl, MALFAVÓN-MEJÍA, Janett, LUCIO-DOMÍNGUEZ Rodolfo, BEDOLLA-CEDEÑO, José Luis Carlos y GONZALES-RONQUILLO, Manuel. Mitigation of greenhouse gases in ruminants through acetic acid as a food additive. ECORFAN Journal-Bolivia. 2018. 5-8: 43-47.

---

* Correspondence to Author (email: dr.angelraulcruz@gmail.com)
† Researcher contributing as first author.

Introduction

There are gases from the Earth's atmosphere that give rise to the so-called greenhouse effect, its importance lies in the increase in the temperature of the planet, keeping it at a range of values fit for life. The most important gases are: water vapor, carbon dioxide (CO2), methane (CH4), nitrous oxide (N2O), chlorofluorocarbons (CFC) and ozone (O3) (Morgan, 2003). Climate change is a phenomenon of great relevance, due to its implications for the economy of any country; particularly, in Mexican livestock, the negative impact is evident (Clark & Scholten, 2013).

Livestock will accelerate climate change, mainly through the emission of greenhouse gases (GHG), such as: methane (CH4) and carbon dioxide (CO2); produced in the anaerobic fermentation of carbohydrates (cellulose, starch, sucrose) of their diet, in addition to nitrous oxide (N2O) (Carmona, Bolivar, & Giraldo, 2005).

The microbiota constituted by bacteria, archaea, fungi and protozoa develops in an anoxic environment producing one of the largest quantities of methane (Espinoza Velasco, Ramírez-Mella, & Sánchez Villarreal, 2018). In the rumen, the structural carbohydrates (cellulose) of food are anaerobically fermented by the action of millions of bacteria and protozoa, resulting in: 65% CO2, CH427% and other gases, heat and short chain fatty acids.

A cow of 500 kg of weight can produce between 400-450 liters of methane per day (Berra, 2002). Research to mitigate ruminal methane gas focuses on feeding, selection of low emission animals, intensification of animal production, secondary compounds of plants, chemical inhibitors, nitrate, immunization, defaunación, reductive acetogenesis, bacteriophages, predictive models and inventories of emissions by region (Ungerfeld, Escobar Bahamondes, & Muñoz, 2018).

The reduction of GHG emissions is affected by the global demand for meat and milk, which will double by 2050, which is why the manipulation of diets through the addition of acetic acid is necessary (FAO, 2014).

The production of methane increases with the digestion of the fiber, due to the increase in the amount of acetic acid in relation to propionic acid, so the most digestible forages and quality fibers such as soybean husk generate more methane. While the fermentation of starch generates changes in ruminal pH, not suitable for the development of methanogenic and higher production of propionic acid, due to the stimulation of amylolitic bacteria, which leads to a decrease in the generation of methane (Alvarez Nivia, 2018).

The emission of CH4 and N2O have an impact on animal production, work is being carried out in the search and implementation of practices aimed at mitigating their emissions, with the aim of impacting the profitability of the production units (Alayón Gamboa, and others, 2018).

Hypothesis

The addition of acetic acid in the feed of sheep, reduces the emission of greenhouse gases.

Objective

The objective of the present investigation was the addition of acetic acid in sheep feed to mitigate the production of greenhouse gases.

Materials and methods

The present investigation was carried out at the facilities of the FMVZ-UMSNH, posta unit, which is located on the Morelia-Zinapécuaro highway, Km 9.5, in the municipality of Tarímbaro, Michoacán, México.

The in vitro phase was carried out with ruminal liquid of two ovines on a corn silo diet, fresh and ground oats, bran and molasses, food and water ad libitum. 1lt of ruminal fluid was extracted with gold-ruminal probe and vacuum pump, placing in amber glass bottles of 125 ml with 10% ruminal fluid and 90% buffer solution (Posada, Noguera, & Bolibar, 2006).

- For the solution of macro minerals (1L): 5.7gr of sodium phosphate, 6.2gr of potassium phosphate and 0.6gr of magnesium sulfate are weighed. 500ml of distilled water was placed in the flask and placed on a magnetic stirrer; to which the reagents were added one by one, the solution is passed to a volumetric flask and it is poured up to 1L.

- For the solution of micro minerals (25ml): 3.3gr of calcium chloride, 2.5gr of manganous chloride and 0.25gr of cobalt chloride are weighed. A 25ml beaker is placed in the magnetic stirrer, 15ml of distilled water is added, adding the minerals, the 25ml sample is poured and the solution is placed in the flask. 0.999g of total food was weighed, incubated in a water bath at 39.5ºC, a sample of acetic acid was added, incubating at 39ºC x 96 hrs and measuring at 3, 6, 9, 12, 24, 36, 48,60, 72 and 96 hrs, with pressure transducer.

After incubation and measurement, statistical and mathematical calculations were performed to observe variations in gas production.

Results

The production of gases within the flasks with ruminal liquid of stubble and bran during the incubation time at 96 hrs of observation registered a significant decrease at different degrees of inclusion of acetic acid as an additive for GHG mitigation, the best results were with the 20% additive achieving a reduction of 23.1% with respect to the control. The control diet based on stubble and on the basis of bran produced in total PSI 41.27 ± 1.9 of greenhouse gases. The diet with the same base of the control but including 20% acetic acid was the best performance in the mitigation of gases with a PSI of 31.74 ± 1.8 representing the best option. The inclusion additive at 10% generated a reduction of 8.6% also compared to the control with a production of 37.7 ± 1.6 PSI of gases.

Including 15%, a 6.6% decrease in gas production was observed compared to the control in stubble and bran with a total of 38.52 ± 1.9 PSI.

Discussion

Previous studies conducted in pigs by Berra and Finster (2002) showed that incubation with a specific diet with an inclusion degree of acetic acid reduced gas production after 72 hrs of incubation with respect to the control group. A similar effect has been achieved with the incubation of acetic acid in bran and stubble the sheep. (Bruni & Chilibroste, 2001). In his research on greenhouse reduction techniques, he determined that the use of acetic acid in the diets of animals whether they are ruminants or monogastric helps with the reduction of up to 10 or 15% of the emissions, whether in excrement, burps or gases in set with better solubility of the residues shown thus better digestibility.

In both cases the studies of Bruni (2001) and Berra and Finster (2002) were about the use of acetic acid addition to the diet of the animals, however none of them has carried out studies in sheep or ruminants with acetic acid, so that the results are satisfactory because the objective of this research was met, another factor that can change but nevertheless support is the incubation time because it varies from 72 to 96 hours. However (Marrero, and others, 2014), in his research mentions that in combination with sodium bicarbonate could have more relevant results. The production of gas was higher in the control and inclusion bottles of 10 and 15% than what was observed with the bottles with an inclusion degree of 20%.

However, the aforementioned works contemplate the reduction of gas production in other species but nevertheless given this practice contemplates and complements the investigations carried out with acetic acid but this time in sheep with positive results so the research can be validated.

Conclusions

It is concluded that acetic acid contributes to the mitigation of greenhouse gases, the inclusion of 20% of the additive to the diet of sheep based on stubble and bran represents the best option; while at an inclusion level of acetic acid of 10 and 15%, although they show reduction, they are lower.

With this additive an alternative to climate change is presented with the reduction of 23.1% of greenhouse gases produced in the rumen and to be applied in bovine meat and milk with increasing global demand is a real alternative, it is recommended to conduct more research to improve the results and find new additives.

References

Alayón Gamboa, J. A., Jiménez Ferrer, G., Piñeiro Vázquez, Á. T., Canul-Solís, J., Albores Moreno, S., Villanueva López, G., y otros. (2018). ESTRATEGIAS DE MITIGACIÓN DE GASES DE EFECTO INVERNADERO EN LA GANADERÍA. Agroproductividad, 9-15.

Alvarez Nivia, D. M. (2018). EFICIENCIA DE LA FERMENTACIÓN IN VITRO DE LOS TRACTOS. Palmira: Universidad Nacional de Colombia.

Berra, G. (2002). EMISIÓN DE GASES DE EFECTO INVERNADERO;. Cordoba: Universidad Nacional de Rio cuarto.

Bruni, M., & Chilibroste, P. (2001). Simulación de la digestión ruminal por el método de la producción de gas. Alpa, 43-51.

Carmona, J. C., Bolivar, D. M., & Giraldo, L. A. (2005). El gas metano en la producción ganadera. Revista Colimbiana de Ciencias Pecuarias, 49-63.

Castillo Montenegro, P. (2018). Propuesta de la implementación de un biodigestor tipo geomembrana PVC mediante la evaluación de un reactor piloto anaerobio para la producción de biogás a partir de estiércol vacuno, residuos de cocinas industriales y aguas residuales provenientes de sani. Guatemala: Universidad San Carlos de Guatemala.

Clark, H., & Scholten, M. (2013). Reduciendo las emisiones de gases de efecto invernadero de la ganadería. Dublin: New Zealand.

De Blas, C., García Rebollar, P., Cambra López, M., & Torres, A. G. (2008). Contribución de los rumiantes a las emisiones de gases con efecto invernadero. Madrid: Universidad Politecnica de Madrid.

Espinoza Velasco, B., Ramírez-Mella, M., & Sánchez Villarreal, A. (2018). ELUCIDANDO LA RELACIÓN ENTRE LA MICROBIOTA RUMINAL Y LA EMISIÓN DE GASES DE EFECTO INVERNADERO MEDIANTE LA APLICACIÓN DE LA GENÓMICA. AGROPRODUCTIVIDAD, 3-8.

FAO. (11 de Abril de 2014). Aumentan las emisiones de gases de efecto invernadero de la agricultura. Recuperado el 15 de Diciembre de 2017, de Organización de las Naciones Unidas para la Agricultura y la Alimentación: http://www.fao.org/news/story/es/item/218907/icode/

Gonzales, E., Castillo, F., del Socorro Correa, S., & Retto, C. (2018). SISTEMA DE APROVECHAMIENTO DE RESIDUOS ORGÁNICOS DE GANADO VACUNO Y SU APLICACIÓN EN LA AGROPECUARIA CAMPOS DEL CHIRA E.I.R.L. Lima, Perú: Universidad de Piura.

Instituto Nacional de Ecología. (01 de Noviembre de 2016). Inventario Nacional de Emisiones y Compuestos de Gases de Efecto Invernadero. Recuperado el 25 de Noviembre de 2017, de Inventario Nacional de Emisiones y Compuestos de Gases de Efecto Invernadero: http://www.cnog.org.mx/archivos/expo/INECC%20Inventario%20Nacional%20de%20Emisiones%20y%20Compuestos%20de%20Gases%20de%20Efecto%20Invernadero%20Ileana%20Villalobos%20Estrada.pdf

Marrero, Y., Ruiz, O., Corrales, A., Jay, O., Galindo, J., Castillo , Y., y otros. (2014). Revista Cubana de Ciencia Agrícola. Revista Cubana de Ciencia Agrícola, 119-123.

Martínez, D. A., Vargas Vayona, J. E., Morales, E., & Melgarejo, L. M. (2014). ESTANDARIZACIÓN DE LA TÉCNICA DE PRODUCCIÓN DE GAS IN VITRO CON HECES CAPRINAS EN LA DEGRADACIÓN DE MATERIA SECA EN FORRAJES TROPICALES. Actas Iberoamericanas de Conservación Animal, 240-242.

Morgan, J. A. (2003). Organización de Estados Iberoamericanos. Obtenido de Organización de Estados Iberoamericanos: http://www.oei.es/historico/divulgacioncientifica/noticias_049.htm

OEI. (2015). Organización de Estados Iberoamericanos para la educación, la ciencia y la cultura. Obtenido de Organización de Estados Iberoamericanos para la educación, la ciencia y la cultura: http://www.oei.es/historico/divulgacioncientifica/noticias_049.htm#

Posada, S. L., Noguera, R., & Bolibar, D. (octubre-diciembre de 2006). Relación entre presión y volumen para la implementación de la técnica in vitro de producción de gases. Revista Colombiana de Ciencias Pecuarias, 19(4), 407-414.

Ungerfeld, E. M., Escobar Bahamondes, P., & Muñoz, C. (2018). PREDICCIÓN Y MITIGACIÓN DE LAS EMISIONES DE METANO DE LOS RUMIANTES. Agroproductividad, 34-39.

Article ECORFAN-Bolivia Journal
June 2018 Vol.5 No.8 48-58

Prevalence of dental caries in children of 6 to 15 years of age with disorder due to deficit of attention and hyperactivity of the Center of Multiple Attention # 3 and the USAER # 55 of the city of San Francisco de Campeche

Prevalencia de caries dental en infantes de 6 a 15 años de edad con trastorno de déficit de atención e hiperactividad en el Centro de Atención Múltiple # 3 y el Usaer # 55 de la Ciudad de San Francisco de Campeche

SANSORES-AMBROSIO, Fátima†*, ROSADO-VILA, Graciella, SÁNCHEZ-ALVAREZ, Lucía, y LLANES-CHIQUINI, Charlotte

Universidad Autónoma de Campeche, Facultad de Odontología, y Facultad de Contaduría, San Francisco de Campeche, México

ID 1° Author: *Fátima, Sansores-Ambrosio* / **ORC ID:** 0000-0001-5403-4802

ID 1° Coauthor: *Graciella, Rosado-Vila* / **ORC ID:** 0000-0002-8688

ID 2° Coauthor: *Lucía, Sánchez-Alvarez* / **ORC ID:** 0000-0002-4606-0354

ID 3° Coauthor*: Charlotte, Llanes-Chiquini* / **ORC ID:** 0000-0001-8389-5943

Received: February 09, 2018; Accepted: June 25, 2018

Abstract

Children are the most vulnerable population for dental caries, an endemic disease that needs prompt attention due to the repercussions on the general health of the subject; This condition that begins with the enamel affection. This study explored this condition in children with ADHD, which is attention deficit hyperactivity disorder (ADHD). Here is born the following question: What is the prevalence of dental caries in children from 6 to 15 years of age with attention deficit hyperactivity disorder (ADHD) who come to the center of attention (CAM) multiple # 3 and USAER # 55 ADHD of the city of San Francisco de Campeche 2015?. The results of this research would be a baseline and a valid reference in the implementation of improving and promoting the activities of prevention, early diagnosis, treatment, rehabilitation of oral health of children with ADHD. In the course of this research, we worked with a total population of 135 children with attention deficit hyperactivity disorder. In this population the average age was 9.4 years, SD = 2.6 a range of 6 to 15 years the prevalence of caries was 72.3%.

Dental Caries, Tooth, Prevalence

Resumen

Los niños son la población más vulnerable para caries dental, enfermedad endémica que necesita pronta atención debido a las repercusiones en la salud general del sujeto; en esta investigación se exploró este padecimiento en niños con TDAH que es el trastorno por déficit de atención e hiperactividad adquirida. El objetivo general de este estudio plantea el siguiente cuestionamiento: ¿Cuál es la prevalencía de la caries dental en niños de 6 a 15 años de edad con el trastorno por déficit de atención e hiperactividad (TDAH) que acuden al centro de atención (C.A.M) múltiple # 3 y USAER # 55 TDAH de la ciudad de San Francisco de Campeche 2015? Los resultados de esta investigación serian una línea base y un referente válido en la implementación de mejorar e impulsar las actividades de prevención, diagnóstico precoz, tratamiento, rehabilitación de la salud bucal de los niños con TDAH. Metodología: En el transcurso de esta investigación se trabajó con una población total de 135 niños que presentan trastorno por déficit de atención e hiperactividad. Resultados: En esta población la edad media fue de 9.4 años, DE =2.6 un rango de 6 a 15 años La prevalencia de caries fue de 72.3%.

Caries Dental, Dientes, Prevalencia

Citation: SANSORES-AMBROSIO, Fátima, ROSADO-VILA, Graciella, SÁNCHEZ-ALVAREZ, Lucía, y LLANES-CHIQUINI, Charlotte. Prevalence of dental caries in children of 6 to 15 years of age with disorder due to deficit of attention and hyperactivity of the Center of Multiple Attention # 3 and the USAER # 55 of the city of San Francisco de Campeche. ECORFAN Journal-Bolivia. 2018. 5-8: 48-58.

---

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

1 Introduction

Children are the most vulnerable population for dental caries, an endemic disease that needs prompt attention due to the repercussions on the general health of the subject; This condition begins with the enamel affection, continues to the dentine and immediately damages the vasculo-nervous package that nourishes the tooth and can cause severe damage to the stomatognathic system and consequently throughout the body's economy.

Given this pathophysiological development, permanent vigilance of the infant population is necessary to minimize the damage. This study explored this condition in children with ADHD, which is attention deficit hyperactivity disorder (ADHD). Systemic alteration characterized by being a neurological disorder manifested by hyperactivity, impulsivity, inattention or lack of concentration, suitable for learning and behavior for the child's degree of development, present emotional and behavioral difficulties.

The scientific reports show that being the behavioral alterations that predominate in these children, the attitude of the parents should be according to the condition, a fact that is not observed frequently and the dental care of this population demands from the professional a management specific behavior that allows good results.

This study is the first performed in this child population with the aforementioned condition, observing a very high prevalence of caries, especially in boys. There was a greater affection of the permanent dentition in those between six and ten years of age in both boys and girls; the fact of having a high number of decayed first molars stands out.

In developing countries such as ours, the situation of high prevalence of caries in children without ADHD and responding to the therapeutic problem entails very high costs. The oral attention generated by caries can start very early in any child and therefore in children with this syndrome and in both cases, but especially in the second specialized attention is required due to the difficulty of behavioral management in the odontologic consultation.

The dental morbidity and mortality that caries can generate in children has been demonstrated. Authors from all continents have addressed the study of this disease considering variables of various kinds: environmental, clinical, sociodemographic, etc.

For this reason, the actions undertaken in the field of health research have as a priority task the search for determinants in pathological processes. In the case of children with ADHD the situation worsens as they present other variables that can intervene in the process of caries such as behavioral and oral environment; they permanently ingest highly sweetened syrups that make the oral environment more inhospitable; Adding carelessness in hygiene measures and diet.

The results discharged here will serve as a baseline for the exploration of variables mentioned here.

1.1 Problem Statement

The diagnosis of early childhood caries should be a priority in all health systems; oral care in children is very required, a reality that emanates from the high prevalence and severity that this disease has in children, a population vulnerable to this condition.

The need that derives from the high prevalence of this disease is little covered in the treatment and rehabilitation, both in quality and quantity. This situation is aggravated due to the fact that in most of the children, behavioral management in dental care is one of the greatest obstacles. Given this premise, it is relevant to have information that addresses the situation of dental caries in children with Attention Deficit Hyperactivity Disorder (ADHD); the information obtained will serve as an argument to stimulate parents of the importance of early detection of dental caries in these children. This research work addresses attention deficit hyperactivity disorder (ADHD) that can affect the behavior, learning and development of the infant; these children have brief attention intervals and are very active or hyperactive, characteristics that will impact the oral rehabilitation of these patients.

In developed countries there are reports of the prevalence of dental caries in children with systemic alterations of various kinds. Dental caries, and oral disorders of another nature are unique problems in the dental care of patients with ADHD. The lack of early attention, together with the chronic nature of oral diseases, complicates other behavioral or developmental problems, the panorama that presents itself with more edges in developing countries such as Mexico. Campeche is an entity in which no information has been reported in order to make decisions regarding preventive measures, early diagnosis and treatment of these patients that constitute a challenge in care due to the nature of their systemic disease.

From here arise the following questions:

What is the prevalence of dental caries in children from 6 to 15 years of age with attention deficit hyperactivity disorder (ADHD) who come to the multiple care center (CAM) # 3 and USAER # 55 ADHD in the city of San Francisco de Campeche 2015?

What are the risk indicators for dental caries in patients who attended clinic 1 of the Faculty of Dentistry of the Autonomous University of Campeche

1.2 Justification

It is known that the balanced development of the stomatognathic system is established from early ages and since there are multiple alterations that can affect it, it is necessary to recognize the oral characteristics; of infants that guarantee adequate oral physiology in adulthood; since oral diseases are considered a public health problem due to their high degree of frequency, such as cavities; to date there are no data that demonstrate the oral health status of children with Attention Deficit Hyperactivity Disorder (ADHD), and if there is any difference with school children who do not have it.

The competent authorities have reported that many children have Attention Deficit Hyperactivity Disorder, this disorder is basically characterized by inattention or lack of concentration, hyperactivity, impulsivity.

Which occurs between 5 and 17% of children in schools in Mexico and other countries; given these characteristics are children who need permanent medication and behavioral decisions of parents according to the condition, factors that have an influence on oral health. The present investigation is justified from two perspectives: the first being a study that will give its contribution to oral health to a selected population and the second because of the importance in the social approach. In fact throughout this investigation will be studied the caries that is a product of the cariogenic diet, drugs and difficulty for a correct oral hygiene.

It is intended to evaluate a group of individuals, which socially has a considerable impact, not only on the correct development and well-being of the affected person, but also on the relatives; as a result of the responsibility and personalized care required by children with ADHD. For this reason this study is relevant, since it presents a series of dental peculiarities that require a specific management, as is the case of: application of psychological techniques, physical restraint and pharmacological therapy.
The results of this research would be a baseline and a valid reference in the implementation of improving and promoting the activities of prevention, early diagnosis, treatment, rehabilitation of oral health of children with ADHD.

2 Theoretical framework

Dental caries has been affecting humanity since prehistory, it is a disease with a multifactorial etiology, influenced by various cultural, social and technological factors of modern society, with large variations in prevalence and incidence, difficult to explain, characterized by a large clinical polymorphism.

The World Health Organization (WHO) defines dental caries as a localized pathological process, of external origin, that begins after the eruption, determined by a softening of the hard tissue of the tooth and evolves towards the formation of a cavity. Dental caries is a multifactorial and infectious chronic dynamic process or disease that occurs in the tooth structure producing a progressive deterioration.
It starts in the periphery of the enamel or root cement, advancing centripetally towards the dentine until the pulp affection, reaching the total destruction of the tooth.

The etiology of caries has been attributed to several factors that include aspects from the social, cultural, economic, political, ethnic, psychological and biological points of view. To understand the etiology more clearly, we can consider that there is a susceptible host (child-teeth); a causative agent such as microorganisms present in the host; and an environment with the necessary conditions.

The anatomical and morphological characteristics of the temporal dentition give rise to any caries process initiated in a temporary molar having a rapid evolution producing the early destruction of the enamel and the dentin, and the affection of the pulpal tissue. The repercussions in the child, have great relevance because it alters the functions of chewing, phonation, aesthetics and preservation of the stomatognathic system and interfere with distance causing pathology in heart, kidneys, joints and other organs.

At present, its appearance is associated with socio-cultural, economic, environmental and behavioral factors. It affects between 60% and 90% of the school population according to WHO. The approach of the presence of dental caries in children with attention deficit is important since they tend to the loss of a greater number of teeth and decay than normal children. Probably due to the fact that attention deficit and hyperactivity make it very difficult for them to have a discipline regarding the food and consumption of junk food, as well as proper oral hygiene.

On the other hand, it may also be due to the possible side effects of the drugs, including xerostomia. ADHD stands for Attention Deficit Hyperactivity Disorder. It is a neurobiological disorder originating in childhood that involves a pattern of attention deficit, hyperactivity and / or impulsivity, and that is often associated with other comorbid disorders.

Food and habits have an influence on growth and development.

In the case of ADHD patients, their eating styles are not correct and often dysfunctional: lack of appetite, food phobias, alterations in mealtimes, nocturnal feeding, rejection of certain types of food, preference only for attractive foods, etc.

These dysfunctions are caused in part by drugs for the treatment of ADHD, which translate into malnutrition patterns, growth problems, physical and mental performance, sleep disturbances, fatigue and oral health. Children with attention deficit hyperactivity disorder tend to have a higher number of tooth loss and decay than normal children.

Probably due to poor oral hygiene and high consumption of sugary foods. To correct this situation it is essential to establish good oral hygiene habits, regular visits to the pediatric dentist to establish efficient preventive measures and designed in a personalized way, control of the diet.

The help of trained professionals to attend them is essential, since they are often classified as "children problems" or "spoiled". It is necessary to inform the pediatric dentist of the diagnosis, medications and specific problems so that together you can find the best way to help the child.

As time has gone by, the percentages of people suffering from this condition have increased, while the age at which they can be diagnosed has been decreasing. Although a few years ago the prevalence of ADHD was estimated at 4% -6%, the latest epidemiological studies give figures that are around 20% and even the most prudent ones place the prevalence above 10%.

The symptoms of attention deficit / hyperactivity disorder (ADHD) have a great impact on the development of the individual and interfere in their social, emotional and cognitive functioning, and cause an important morbidity and dysfunction not only in the child, but also in the group of classmates and in your family.

Attention Deficit Hyperactivity Disorder (ADHD) has 3 main symptoms: Lack of attention

- Hiperactivity- Impulsivity.

Hyperactivity is probably one of the easiest symptoms to recognize, for its evidence and for being the best known by the general public. The person suffering from hyperactivity is characterized because:

- It moves at times when it is not appropriate
- It is hard to remain still when necessary
- Speech in excess
- Noises constantly, even in quiet activities
- Has difficulty relaxing
- Change activity without finishing any
- There is a lack of consistency

The symptoms of lack of attention are probably the most difficult to perceive at childhood ages. The person suffering from attention deficit is characterized because:

- Has difficulty maintaining attention for a long time
- Does not pay attention to details
- Has difficulty completing tasks
- He has difficulty listening, following orders and instructions
- He is disorganized in his tasks and activities
- Usually lose or forget objects
- Easily distracted
- It does not conclude what starts
- Avoid activities that require a sustained level of attention

Change conversation frequently He has difficulty following the rules or details of the games. Impulsivity is probably the least frequent symptom of the three core symptoms of ADHD. The person suffering from impulsivity is characterized because:

- He is impatient
- He has problems waiting for his turn
- Do not think before acting
- Constantly interrupting others
- He has arrogant answers: spontaneous and dominant
- Tends to "touch" everything

In order to be able to consider a possible diagnosis of ADHD, the following criteria must be met marked by the DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, fifth edition).

- He manifests these behaviors or some of them disproportionately compared with other children of his age and with respect to his degree of development.
- He is present from an early age (before the age of 12).
- Affects in at least two different environments of the child's life: school, social and / or family.
- Significantly impairs their quality of life.
- It is not caused by a medical, toxic or other psychiatric problem.

Three types of ADHD have been established according to the level of symptoms experienced by the individual. Description of the three types of ADHD:

1. Predominantly inattentive. It is difficult for the individual to organize or finish a task, pay attention to details, or follow instructions or a conversation. The affected person is easily distracted or forget the details of the daily routines.

2. Predominantly hyperactive-impulsive. The person is restless and talks a lot. It is difficult to remain calm for a long time (to eat or to do a job).

3. Combined. The affected person experiences the symptoms of the previous types equally.

The cause of ADHD (Attention Deficit Hyperactivity Disorder) is still uncertain today. It is a heterogeneous and complex neurobiological disorder, which can not be explained by a single cause, but by a series of genetic conditions together with other environmental factors and psychosocial factors; although it is not considered critical.

Hence the importance of having a qualified healthcare professional to perform the diagnosis that has extensive experience in the diagnosis of Attention Deficit Hyperactivity Disorder. The diagnosis of this disorder must be based on a thorough assessment to exclude other possible causes of the child's difficulties, including information about his family, his teachers and an evaluation by health professionals in different areas: child psychiatrists, child psychologists, pediatricians, neuropsychiatrists, neuropsychologists, etc.

The evaluation of this complex disorder must be done from a multiprofessional perspective that addresses both the psychological, educational and medical evaluation.

There is no single test that alone allows an exclusive and reliable diagnosis. To establish the final diagnostic judgment it is necessary to gather information from the different sources that surround and intervene in the child and make systematic observations of their behaviors and the retrospective assessment of their behavior from the earliest ages.

The ADHD evaluation must include:

1. A complete medical examination to evaluate the general health of the child and discard problems of visual, auditory, anemic or lack of vital components for their health.
2. A professional psychological evaluation to have a clear idea of the emotional condition of the child, including tests of intellectual capacity and cognitive development.
3. A family assessment for which behavioral scales are used.
4. A school evaluation that includes the child's academic and behavioral history in the classroom.
5. The encephalogram (EEG) would only be indicated in the presence of focal signs or when there is a clinical suspicion of epilepsy or degenerative disorders.
6. The diagnosis will be based on compliance with DSM-V criteria, which are the diagnostic criteria established by the American Academy of Psychiatry (1994) or the ICD-10 (1992) criteria recognized and established by the WHO.

The DSM (Diagnostic and Statistical Manual of Mental Disorders) is the diagnostic and statistical manual of mental disorders. It is the system of classification of mental disorders most used worldwide, providing descriptions, symptoms and other useful criteria for the diagnosis of mental disorders.

It is reviewed periodically, according to the investigations, studies and discoveries made. The CIE is the International Classification of Diseases (ICD: International Statistical Classification of Diseases and Related Health Problems) conducted by the World Health Organization. The treatment of ADHD should be multimodal and individualized, taking into account the patient and his / her family.

The objective of multimodal treatment is to reduce symptoms while reducing the complications derived from the disorder and the negative impact it can have on the lives of patients and their environment.13 This multimodal treatment of ADHD involves three approaches:

- Pharmacotherapy
- Cognitive-behavioral treatment
- Psychoeducational treatment (parents and teachers).

Broadbent JM and performed in 2009 col. a study 128 pairs of cases and controls in children with and without ADHD from Otago, New Zealand defining as a case the child who had 5 or more DMFT and control to the one who had less than 5 DMFT. The analysis showed that children with ADHD were almost 12 times more likely to have a higher DMFT than children who did not. (OR = 11.98, 95% CI 1.13-91.8). In a study of 447 children conducted in 2009, in deciduous dentition in the metropolitan area of Aburra, Saldarriaga-Alexandra et al. They report a prevalence of dental caries of 74.7% with an average and a 7.3 ± 9.1.

When studying the affected surfaces, it was concluded that the prevalence of at least one affected surface per tooth was 73.4%. 18Prakash Chandra, Latha Anandakrishna, and Ray Prayas in 2009 did a study using a total of 80 children, including 40 ADHD and 40 children without ADHD were included in the study.

A dental visual examination for dental caries was carried out and the state of oral hygiene of these children was determined. As a result, children with ADHD had a significantly higher score, the average value of caries compared with that of children without ADHD. Burns, Heidi A. Reported in a study in 2010, they did tests that showed that children with ADHD have significantly more enamel caries in the permanent and temporary dentition and a significantly higher prevalence of the total caries experience compared to the controls. In 2010 Bruce A. Dye et al. In a study conducted in the UAE in two groups of children: one with low socioeconomic status and the other group with high socioeconomic status, from 2 to 8 years of age found a prevalence of caries, for the first group from 45 to 53% and for the second group it was from 23 to 31%.

In addition, a group of 2 to 5 years of high socioeconomic status was examined, finding a prevalence of 13 to 21%. They observed that decay in permanent dentition declines in children of high socioeconomic status; there is an increase from 8 to 22% in poor non-Hispanic children between 6 and 8 years of age; and among Mexican-American children between 9 and 11 years old it increased from 18 to 55%. Ceyhan Altun et al in 2010 reported in 136 subjects with disabilities that the average CPOD was 1.58 + - 2.72 and that of ceod was 1.18 + - 2.11. The ceod and DMFT showed statistically significant differences by age (p = 0.05). The presence or absence of dentobacterial plaque was studied through the Sliness and Löe Index, observing that as the disability worsens, the percentage of teeth with dentobacterial plaque increases.

In the 2010 AE Sanders et al. They conducted a study in South Australia in 1058 children who presented some deficiency at birth, according to the Apgar Registry and found a caries prevalence of 40.1%. The regression analysis showed that as more disorders presented the child at birth, according to this registry, more likely to decay in the primary dentition.

In 2011 Blomqvist perform a study in Stockholm in 555 children with and without TDH, finding that the DMFT in children with ADHD and without the presence of this disorder shows differences in terms of averages: the former had 2.8 + - 4.0 and the latter: 2.2 + -3.2. Incipient and frank caries were observed in both groups without showing statistically significant differences for the cavity in both groups: OR = (1.26, 95% CI 0.76-2.09) and for the incipient lesions OR = (1.04, 95% CI 0.59-1.82). 38% of the children with ADHD were free of caries and in the control group it was 48% .24In 2011 Ariela Hidas and co. conducted a study in 132 subjects, children, youth and young adults with and without ADHD, dividing it into 3 groups: group I did not receive medication, group 2 received medication at least one month before the study and group 3, healthy subjects.

There were no statistically significant differences in the averages of CPOD / ceod (p = 0.08) between the groups; A higher average of decayed teeth is found in group 2 compared to 1 and to control, without statistically significant differences (p = 0.06).

The authors denote a higher plaque index in children without medication than with medication with statistically significant differences (p = 0.05). Ghada SM Al-Bluwi report that the prevalence of caries for children of 5 years was 72.9% and for those of 6 years 80%, the average of ceod = 4 for those of 5 years and ceod = 4.5 for those of 6 years. In 2011, the prevalence of caries in children aged 5 and 6 years was 31.1%.

In 2011 Zaror Sànchez et al. studied 301 Chilean children from 2 to 8 years of age who had a prevalence of ITC (early childhood caries) per tooth of 70% and that of ITC by area of 52%. Average of ceod = 3. 28% of the children of 2 years presented incipient lesions.

The multivariate mathematical model for two-year-old children presents the following variables with statistical significance: breastfeeding (OR 2.9, 95% CI 1.4-6.0, p = 0.00); no bottle at night (OR = 2.6, ic 95% 1.2-5.7 p = 0.01). 27In 2012 Ramírez Puerta, Blanca Susana et al. In Colombia they reported a caries prevalence in permanent teeth of 60.4%, the average of classic and modified CPOD was 1.6 ± 1.7 and 1.7 ± 1.8 respectively. The significant caries index was 3.7 ± 1.2.

In Brazil in 2012, Paula Flavia Méndez Thaurino et al. carried out a study in 185 children from 6 to 12 years where a prevalence of caries was found in children of 6 years of 87.5% with an average of ceod = 5.2 and for permanent dentition was 74.4% with a DMFT = 2.8.

Lida García Vega et al. in 2012 they studied a sample of 108 schoolchildren of 6 and 11 years of age, to determine the prevalence of caries that was 89.8% the DMFT index was 1.7 and the CEOD 3.1% studied the frequency of brushing that was between one and two times day, the consumption of cariogenic foods was between moderate and high in 85% of children.

In 2013 Suzuki Padilla Bertha et al. in 2013 in San Luis Potosí, Mexico, a study was conducted on 595 children between 6 and 10 years of age to analyze the risk of caries and assess the frequency of caries in 12 months. At the beginning they observed an average of DMFT of 0.8, with an increase with age, children of 6 years showed an increase of 0.09 and those of ten years the increase was 0.9 with a statistically significant difference of p <0.05.

Low-risk children decreased their risk with increasing age and high-risk children with increasing age increased their risk, showing statistically significant differences p <0.05. Halcrow S.E. And col. in 2013 in a study carried out on children under 15 in Thailand and Cambodia. The prevalence of caries in the most affected tooth in the first dentition of 5.5% and in the permanent dentition was 9.9%.

A study done in 2013 in Carabobo, Cuba, Reyes et al. found in children of 12 years, a caries prevalence of 68%, highlighting that the first molars and especially the lower ones were very affected.

In 2013 Nasco Hidal et al carried out a study in the Plaza de la Revolución Municipality, Cuba, about the prevalence of incipient lesions to dental caries and its relationship with caries risk factors in children from 6 to 11 years of age.

A prevalence of incipient lesions was found in 22.5% of the children. Of these 74.6% had poor oral hygiene and of them 52.7% incipient lesions, children with CPOD or ceod from 0 to 2 also had incipient lesions.

In Peru in 2013 Cárdenas Flores et al. They reported in a caries prevalence study in a sample of 231 children from 5 to 3 years of age, that this was 65.8%, found a statistically significant association of (OR = 3.01), between dental caries and inadequate oral hygiene. F. Vargas-Ferreira et al. In Mexico in 2014 they report that in structural enamel defects of hypoplasia and the prevalence of dental caries in children is 32.4% (OR = 2.79, 95% CI: 1.05, 6.51). 36Nelly Molina Frechero, Denisse Durán Merino, Enrique Castañeda Castaneira and Adriana Juárez López. They made a study where the study population consisted of children of both genders; 69.5% presented caries, and the DMFT was 3.52 ± 3.7 (c = 3.37 ± 3.5, p = 0, or = 0.11 ± 0.51). 98.2% of the children with caries presented poor oral hygiene with a DMFT of 4.91 (confidence interval [IC95%: 3.99-5.84), which in children with good hygiene was 0.17 (95% CI: -0.18-0.51).

3 Methodology

At the beginning of this work the authorization of the director of special education was requested to be able to carry out the study in the multiple attention center (C.A.M) # 3 and the USAER # 55 ADHD in the city of San Francisco de Campeche.

The duration of this study was from July to October 2015. Children who met the inclusion criteria were selected. An informed consent letter was given to their parents (Appendix 1) where it was explained that this study will not harm their child physically or psychologically.
He was given the objective of the study and the impact it would have on his oral health. A folio was assigned to each child in a file attached to the odontogram, with a sequential number. The data of the sociodemographic variables were taken from the school documentation. The clinical examination was carried out by a standardized examiner in the use of the ceod and CPOD indices (Kappa = .90); was performed in the school, placing the patient in the chair in a position of 90 °, the operator in front, with natural light using plane mirror # 4, scanner # 23.

We proceeded to perform a revision of the oral cavity of each child starting in the upper right quadrant and ending in the lower quadrant on the same side. The researcher put on gloves and face masks which were discarded between each child, the annotations were made in the odontogram with a bicolor. At the end of the registration, the capture and analysis of data was carried out with the SPS 20 program. Summary and central tendency measures were reported.

4 Results

In the course of this research, we worked with a total population of 135 children with attention deficit hyperactivity disorder. In this population the average age was 9.4 years, SD = 2.6 a range of 6 to 15 years. 77.8% (105) were children and 22.8% (30) are girls.

The prevalence of caries was 72.3% (73), with 26.7% (28) of caries-free subjects in both dentitions. The average DMFT was 1.3 SD = 1.8 and a range of 0 to 8. The average CEOD was 1.9, DE = 2.1 and range from 0 to 8. (Graph 1).
The average DMFT in children was 47.8% with caries and 52.2% free of caries.

The permanent dentition of these children presented the following: 13.3% (18) of the subjects had a decayed tooth, 15.6% (21) had 2 decayed teeth, 3% (4) 3 teeth, 5.2% (7) 4 teeth, .7% (1) 5, 1.5% (2) 6 decayed teeth, .7% (1) 7. 60% of the children had permanent dentition without decay. 3% (4) had permanent teeth lost. The obturations were observed in the following way: 10.4% (14) had 1 obturated tooth, 7.4% (10) presented 2, 7% (1) 3, 1.5% (2) had 4 obturated teeth. When analyzing the average DMFT and sex we observed that children were more affected 56.6% (17) and of the total of girls 45.1% (47) was. The CPOD was analyzed by age groups, obtaining as a result that in both of them 50.0% (32) presented some decayed, lost or clogged tooth. (Graph 2).

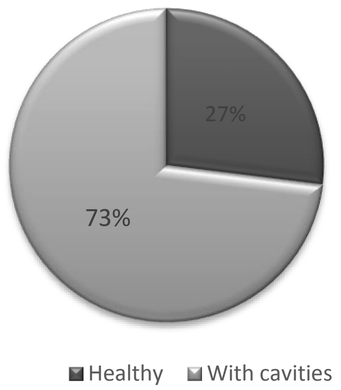
These children in the first dentition presented: 1 decayed tooth 11.8% (12), 12.7% (13) 2 decayed teeth, 8.8% (9) 3, 9.8% (10) 4 teeth, 2% (2) 5 and 6 teeth with cavities respectively and 1% (1) 8 teeth. 52% of these children had no tooth decay in the first dentition. The 10.8% (11) of the children studied had a lost or indicated tooth for extraction, 2.9% (3) had 2 teeth lost or indicated for extraction. The average ceod was 57% with caries and 43% healthy. (Graph 3).

When analyzing the average sex and sex we observed that children were more affected 65.5% (38) and of the total of girls 34.4% (20) was. Derived from these anomalies we see that 60% (81) of the first permanent molars is affected.

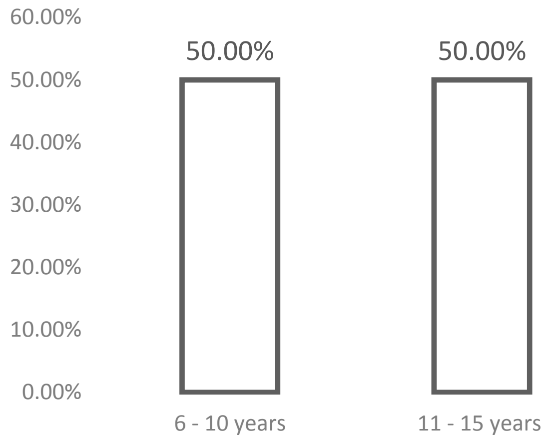
Given the importance of the first permanent molars, the analysis of their health status was made: The first lower right molar in 68.5% (89) of the children was found healthy, 18.5% (24) was decayed, 9.2% (12) sealed and 3.8% (5) was lost. In the study there were 5 children who did not have this tooth. The first lower left molar in 72.3% (94) of the children was healthy, 18.5% (24) were decayed and 9.2% (12) were filled.

The first upper left molar was observed in 89.7% (104) healthy, 8.6% (10) with decay, and 1.7% (2) with obturation. The first right upper molar in 87.6% (106) healthy, 10.7% (13) decayed and 1.7% (2) obturado.

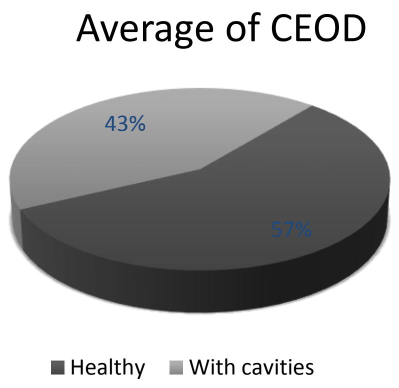
The affection of the first permanent molar with age groups showed that the group of children who presented between 6 and 10 years of age 74% (60) had it, while in the group of 11 to 15 years 26% (21) he presented it. Of the total of children with some first permanent molar affected, 79% (64) were children and 21% (17) were girls.



Graph 1 Prevalence of dental caries



Graph 2 Average CPOD by age



Graph 3 Average CEOD with caries

6 Conclusion

This population of children with ADHD presented a high prevalence of caries; I emphasize that there is a large number of healthy children, but we also find children who have 8 caries in the first and / or second dentition. I note that the number of children with cavities in the second dentition was greater. The carious component was higher in the averages of DMFT and CEOD. The condition in permanent teeth was observed equal in both age groups. Temporary tooth decay was observed more in children from 6 to 10 years of age. The lower molars were the most affected in this population. The affection of the first permanent molar was observed more in the children and those who were more affected were the smaller children.

References

Bimstein E, Wilson J, Guelmann M, Primosch R. (2008). Oral Characteristics with Attention-Deficit Hyperactivity Disorder. Spec Care Dentist 2008; 28(3):107-110.10.

Bruce A. Dye, Oscar Arevalo &Clemencia M. Vargas; (2010) United States.Trends in Pediatric Dental Caries by Proverty Status in the Unite States, 1988-1994 and 1999-2004: International Journal of Pediatrics Dentistry.

C.Zaror, P. Pineda & Ju. Orellana. (2011). Prevalence of Early Childhood and Associated Factors in two and four year-old Chilean Children.Int. J. Odontosmat.

Ceyhan Altun y col. Cartagena de Indias (2010) Prevalencia de Caries Dental y Factores Familiares en Niños Escolares de Cartagena de Indias, Colombia; Eur J Dent. Oct 2010.Apgar score and dental caries risk in the primary dentition five yeay olds. Australian Dental Journal.

Claudia Girón. (2014) Nutrición, Caries y Transtorno de Deficit de Atención con Hiperactividad (TDAH). Revista Sonrie. Junio.

Cuenca E, Baca P. Rev. Cubana Estomatol. (2009). Odontología Preventiva y Comunitària: Principios Métodos y Aplicaciones

Da Silva Freixinho A, Massao Miasato J. Da Gama Silveira R. (2015) S.L. Estudio clínico Epidemiológico de Prevalencia de Caries en Niños de 0 a 30 Meses y Determinación de los Factores de Riesgo en una Población de la Ciudad de Berisso.

Dr. Ignacio Castro viejo (2007). Urgencia en Odontologia Estomatología

Fernández J, Costa F, Bartolomé B, Beltri P, cols. (2012). Tésis Acta Odontólogica Venezolana

Fernández M, Bravo B. Madrid: Médica Ripano (2006). Prevalencia Severidad de Caries Dental en Niños Institucionalizados de 2 a 5 años.

González H, Brand S, Díaz F, Farfán M, González V, Range W, Catanho N, González R, Devera R. Madrid (1990). Prevalencia de Caries Rampante en Niños Atendidos en el Centro Odontopediátrico Carapa, Hidas, A. Fux, N. Birman, I. Matot, D.Steinberg, M. Moskovitz (2011).

Higashida B. Antímano Venezuela (2006). Odontología Preventiva. Primera Edición. México: McGraw-Hill; 2000:188- 89.8.

H. Burns, S.L. Myers, S. Beiraghi, A. Emery (2011) Trastornos por Déficit de Atención e Hiperactividad (TDAH). Asociación Española de Pediatría. Madrid.

M. Blomqvist, S. Ahadi, E. Fernell, U. Ek and G. Dahllöf (2010) Dental Caries en Adolescents with Attention Deficyit Hyperactividit y Disororder: a Popupation-Based Follow-up Study. EJl. October 119: 381-385.25.

Saldarriaga A., C. Arango, M. Cossio, A. Arenas, C. Mejía, E. Mejía, L. Murillas.- Bimstein E, Wilson J. Valle de Aburrá (2009)Prevalencia de Caries Dental en Preescolares con Dentición Decidua Area Metropolitana del Valle de Aburrá. Revista CES Odontología.2

Thomson WM-. (2005) Is Attention-Deficit Hyperactivity Disorder a Risk Factor for Dental Caries? JE-BDPM.

Valdés Miyar, Manuel tr. American Psychiatric (2013). Association. DSM-5 Barcelona The Size and Burden of Mental Disroders and Other Disorders of the Brain in Europe 2010. ENP.2011; 21:655-79. Broadbent JM, Ayers KM

Wittchen HU, Jacobi F, Rehm J, etal. (2010) Europe.The Size and Burden of Mental Disroders and Other Disorders of the Brain in Europe. ENP.2011; 21:655-79. Broadbent JM, Ayers KM.

Instructions for authors

[Title in Times New Roman and Bold No. 14 in English and Spanish]

Surname (IN UPPERCASE), Name 1st Author†*, Surname (IN UPPERCASE), Name 1st Coauthor, Surname (IN UPPERCASE), Name 2nd Coauthor and Surname (IN UPPERCASE), Name 3rd Coauthor

Institutional Affiliation of Author including Dependency (No.10 Times New Roman and Italic)

ID 1st author: (ORC ID - Researcher ID Thomson, arXiv Author ID - PubMed Author ID - Open ID) and CVU 1st author: (Scholar-PNPC or SNI-CONACYT) (No.10 Times New Roman)

ID 1st coauthor: (ORC ID - Researcher ID Thomson, arXiv Author ID - PubMed Author ID - Open ID) and CVU 1st coauthor: (Scholar or SNI) (No.10 Times New Roman)

ID 2nd coauthor: (ORC ID - Researcher ID Thomson, arXiv Author ID - PubMed Author ID - Open ID) and CVU 2nd coauthor: (Scholar or SNI) (No.10 Times New Roman)

ID 3rd coauthor: (ORC ID - Researcher ID Thomson, arXiv Author ID - PubMed Author ID - Open ID) and CVU 3rd coauthor: (Scholar or SNI) (No.10 Times New Roman)

(Report Submission Date: Month, Day, and Year); Accepted (Insert date of Acceptance: Use Only ECORFAN)

Abstract (In English, 150-200 words)

Objectives
Methodology
Contribution

Keywords (In English)

Indicate 3 keywords in Times New Roman and Bold No. 10

Abstract (In Spanish, 150-200 words)

Objectives
Methodology
Contribution

Keywords (In Spanish)

Indicate 3 keywords in Times New Roman and Bold No. 10

Citation: Surname (IN UPPERCASE), Name 1st Author†*, Surname (IN UPPERCASE), Name 1st Coauthor, Surname (IN UPPERCASE), Name 2nd Coauthor and Surname (IN UPPERCASE), Name 3rd Coauthor. Paper Title. ECORFAN Journal. Year 1-1: 1-11 [Times New Roman No.10]

---

* Correspondence to Author (example@example.org)

† Researcher contributing as first author.

Instructions for authors

Introduction

Text in Times New Roman No.12, single space.

General explanation of the subject and explain why it is important.

What is your added value with respect to other techniques?

Clearly focus each of its features

Clearly explain the problem to be solved and the central hypothesis.

Explanation of sections Article.

Development of headings and subheadings of the article with subsequent numbers

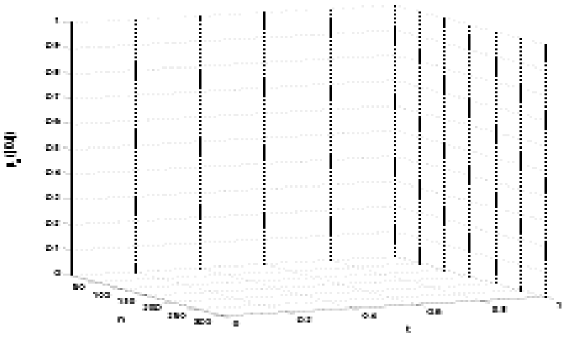
[Title No.12 in Times New Roman, single spaced and bold]

Products in development No.12 Times New Roman, single spaced.

Including graphs, figures and tables-Editable

In the article content any graphic, table and figure should be editable formats that can change size, type and number of letter, for the purposes of edition, these must be high quality, not pixelated and should be noticeable even reducing image scale.

[Indicating the title at the bottom with No.10 and Times New Roman Bold]



Graphic 1 Title
Source (in italics)

Should not be images-everything must be editable.

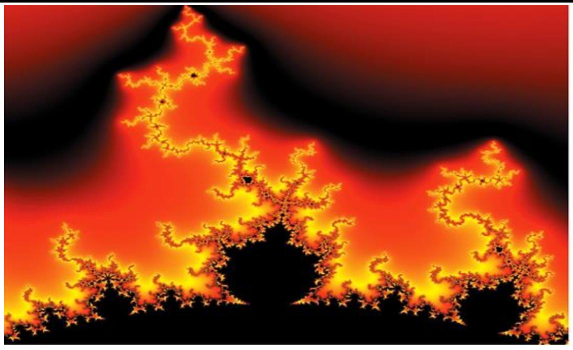


Figure 1 Title
Source (in italics)

Should not be images-everything must be editable.

Table 1 Title
Source (in italics)

Should not be images-everything must be editable.

Each article shall present separately in **3 folders**: a) Figures, b) Charts and c) Tables in .JPG format, indicating the number and sequential Bold Title.

For the use of equations, noted as follows:

$$Y_{ij} = \alpha + \sum_{h=1}^{r} \beta_h X_{hij} + u_j + e_{ij} \tag{1}$$

Must be editable and number aligned on the right side.

Methodology

Develop give the meaning of the variables in linear writing and important is the comparison of the used criteria.

Results

The results shall be by section of the article.

Annexes

Tables and adequate sources thanks to indicate if were funded by any institution, University or company.

Conclusions

Explain clearly the results and possibilities of improvement.

References

Surname (IN UPPERCASE), Name 1st Author†*, Surname (IN UPPERCASE), Name 1st Coauthor, Surname (IN UPPERCASE), Name 2nd Coauthor and Surname (IN UPPERCASE), Name 3rd Coauthor. Paper Title ECORFAN Journal. Year [Times New Roman No. 8]

Use APA system. Should not be numbered, nor with bullets, however if necessary numbering will be because reference or mention is made somewhere in the Article.

Use Roman Alphabet, all references you have used must be in the Roman Alphabet, even if you have quoted an Article, book in any of the official languages of the United Nations (English, French, German, Chinese, Russian, Portuguese, Italian, Spanish, Arabic), you must write the reference in Roman script and not in any of the official languages.

Technical Specifications

Each article must submit your dates into a Word document (.docx):

Journal title
Article title
Abstract
Keywords
Article sections, for example:

1. Introduction
2. Description of the method
3. Analysis from the regression demand curve
4. Results
5. Thanks
6. Conclusions
7. References

Author Name (s)
Email Correspondence to Author
References

Intellectual Property Requirements for editing:

-Authentic Signature in Color of Originality Format Author and Coauthors

-Authentic Signature in Color of the Acceptance Format of Author and Coauthors

ECORFAN®

Sucre-Bolivia a _____ de ____ de 20__

Originality Format

I understand and agree that the results are final dictamination so authors must sign before starting the peer review process to claim originality of the next work.

____

Article

____

Signature

____

Name

ECORFAN®

Sucre-Bolivia a _____ de ____ de 20__

Authorization form

I understand and accept that the results of evaluation are inappealable. If my article is accepted for publication, I authorize ECORFAN to reproduce it in electronic data bases, reprints, anthologies or any other media in order to reach a wider audience.

____

Article

____

Signature

____

Name

ECORFAN Journal-Bolivia

"Bdellovibrio bacteriovorus as a possible biological control agent"
ARANDA-VIVAS, Leonardo Iván, SÁNCHEZ-VARELA, Alejandro, RODRÍGUEZ-LUNA, Isabel Cristina, & ELUFISAN, Temidayo
Instituto Politécnico Nacional

"Driving training and evaluation using the Big-Rig HD1 driving simulator"
ÁGUILA-FLORES, Arturo, CORTÉS-MALDONADO, Ismael, CORTES-GARCÍA, Franco & PÉREZ-MENDOZA, Viridiana
Universidad Politécnica de Tlaxcala

"Molecular identification of Aeromonas spp. isolated from water in northeastern México"
SANCHEZ-VARELA, A., ORTEGA-BALLEZA, J. L, RODRIGUEZ- LUNA, I. C. & GUO, X.
Instituto Politécnico Nacional

"Interaction between Aedes aegypti CPB1 and viral proteins"
LÓPEZ-LÓPEZ, Edgar & BARRIENTOS-SALCEDO, Carolina
Universidad Veracruzana

"Description of microplastics on the coast of La Guancha in Ponce, Puerto Rico"
COLÓN-ORTIZ, Abner J. & FEBLES-MORENO, Karelys
Pontificia Universidad Católica de Puerto Rico

"The versatile biological and chemical reactivity of quinoline derivatives, a source of innovation for the chemist"
GARZA-ORTIZ, Ariadna, RAMIREZ-DE LA CRUZ, Flor de María, LÓPEZ-GUTIÉRREZ, Tomás J. y MEX-ÁLVAREZ, Rafael M
Universidad Autónoma de Campeche

"Mitigation of greenhouse gases in ruminants through acetic acid as a food additive"
CRUZ-HERNÁNDEZ, Ángel Raúl, MALFAVÓN-MEJÍA, Janett, LUCIO-DOMÍNGUEZ Rodolfo, BEDOLLA-CEDEÑO, José Luis Carlos y GONZALES-RONQUILLO, Manuel
Universidad Michoacana de San Nicolás de Hidalgo
Universidad Autónoma del Estado de México

"Prevalence of dental caries in children of 6 to 15 years of age with disorder due to deficit of attention and hyperactivity of the Center of Multiple Attention # 3 and the USAER # 55 of the city of San Francisco de Campeche"
SANSORES-AMBROSIO, Fátima, ROSADO-VILA, Graciella, SÁNCHEZ-ALVAREZ, Lucía, y LLANES-CHIQUINI, Charlotte
Universidad Autónoma de Campeche

2410 4191
ISSN 2410 - 4191
www.ecorfan.org