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Appropriate form to cite the abstract of this publication is:

Example:
Chávez Sáenz V. *et al* (2018). Brain Activity Quantification for Pain Index Estimation Using a BCI: A System Proposal in: Lozano Muñiz S (Ed.). Biotechnology Summit IFBRESCHNSASTS AC, México. pp. 25.

Biotechnology summit Year 2018, No 1, this is a biannual publication edited by International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C. (IFBRESCHNSASTS AC). listed in the Yearbook of International Organizations with reference number: J8276, at Union of International Associations. Address: La Troje Lot 25 M3, Hacienda Real, Tuxtepec, Oaxaca, ZC 68375, MÉXICO www.bio.edu.mx info@bio.edu.mx Phone +52 1 287 1288 936. Brand: International Biotechnology Foundation IMPI 1503555. Executive editor: Susana Lozano Muñiz, president@bio.edu.mx. ISSN: 2618-0464 was obtained in ISSN International Centre. Responsible for the last update of this number, Dr. Susana Lozano Muñiz, La Troje Lot 25 M3, Hacienda Real, Tuxtepec, Oaxaca, ZC 68375. Date of last modification, September 11, 2019. Vol 1, Year 1, Issue 1.


Biotechnology Summit 2018 Summit 2018
Vol 1 Year 1 Issue 1, ISSN: 2618-0464, http://www.bio.edu.mx/ v.bio.edu.mx/

http://www.bio.edu.mx/biotechnology-summit-2018/

November 2018

ISSN: 2618-0464

Editor Susana Lozano Muñiz

International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society, A.C.
Non-Profit Organization

International Biotechnology Foundation

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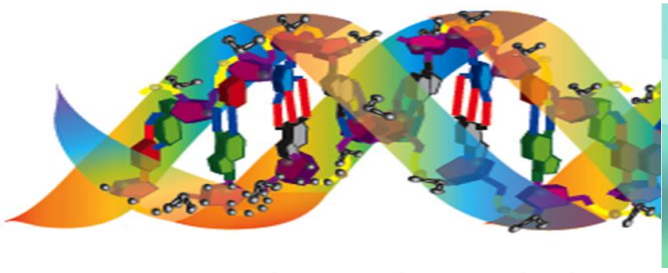


Welcome to the Biotechnology Summit 2018

Dear Professors, scientific leaders, businesspersons & entrepreneurs, public employees, government employees & young students:

The main theme of Biotechnology Summit 2018 (BS18) are the challenges of health, food, agricultural, bio-business, entrepreneurship, marketing and multidisciplinary area. We do believe all of us can make contributions in some areas of Biotechnology.
On behalf of BS18 Organizing Committee, it is a great pleasure for us to welcome you to attend the Biotechnology Summit 2018 (BS18). November 19 to 23, 2018. Hosted by the Universidad del Papaloapan (UNPA), Campus Tuxtepec of Oaxaca; co-sponsored / co-organized by several Research Centers in Biotechnology of Mexico and/or various organizations. Among those are Universidad del Mar, Sistema de Universidades del Estado de Oaxaca, International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C., Mexican Society of Biotechnology and Bioengineering, Nacional and Oaxaca delegations, National Council for Science and Technology Press.

No.	Color Area	Biotech Activities
I	Red	Human Health, Medical, Diagnostics and Tissue Engineering;
II	Yellow	Nutritional Biotechnology: Food, Nutrition Science and Nutraceuticals.
III	Blue	Marine (aquatic) Biotechnology: Aquaculture, Fish Health and Nutrition, Aquatic Animals Reproduction, Cloning and Genetic Modifications, Fisheries Pests and Disease Control
IV	Green	Agricultural and Livestock Biotechnology: biotechnologies for production, processing and storage, biofertilizers and agrobiochemicals, agricultural pests, disease control, ecology and rational wildlife management, preservation of biodiversity, plant, pet, and farm-animal, tissue culture, and health, nutrition, reproduction, cloning, and genetic modification, plant micropropagation, bioremediation & environmental biotechnology, sustainable and renewable energy generation, bioremediation and environmental biotechnology, bio-fuel production, biotechnology for competitive production using new material and new energy sources.
V	Brown	Desert biotechnology: space and geomicrobiology
VI	Black	Bioterrorism: human and animal pathogen control, biowarfare, biocrimes.

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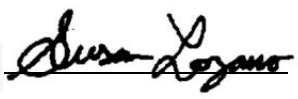
VII	Purple	Patents, IPR: strategy for intellectual property protection, patents, publications, inventions
VIII	White	Industrial biotechnology (gene-based)
IX	Gold	Bioinformatics: nanobiotechnology, microelectronic and microelectromechanical systems (MEMS), micro systems technology (MST), nano electro mechanical systems (NEMS) micromachines
X	Grey	Classical biotechnology (fermentation): industrial biotechnology: classical fermentation & bioprocess/bioengineering technology; engineering and technological equipment for bioproduction; output of science-intensive bioproducts.
XI	Transparent	Bioethics, biotechnology, and society: tools for assessment of the support to the scientific sector, including its biotechnological potential and human resources.
XII	Iris	Multidisciplinary area: biochemistry, molecular biology & biotechnology, applications based on OMIC´s
XIII	Indigo	Integrating science, education and manufacturing, early childhood stimulation in the culture of health, nutrition, sport, art, science, biotechnology & society as the use of Information and Telecommunication Technologies TIC´S
XIV	Platinum	Synthetic biology
XV	Silver	Biobusiness, Bioentrepreneurship & Marketing; Development Economics, strategy for innovative development of the national economy, improvement of the system of the S&T and innovation activities management.

Sincerely yours,



Susana Lozano-Muñiz.
President, BS14 Organizing Committee,
President, International Foundation for Biotechnology Research & Early Stimulation in the Culture of Health, Nutrition, Sport, Art, Science, Technology & Society A.C., and
President, Mexican Society for Biotechnology & Bioengineering Oaxaca Delegation.

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Ministry of Agriculture, Livestock, Rural Development, Fisheries and Food (SAGARPA)

Welcome

First of all, I appreciate the invitation to this event on behalf of my boss Eng. Jesús Caña Morales, since it is considered essential the inter-institutional coordination that can be given to schools.

I also congratulate the young people from other areas that participate in this forum, since the fact that they are from different careers and it does not mean that they limit their abilities and knowledge; on the contrary, they reinforce the skills that they can obtain in this institution.

I say this from my own experience. I consider myself still young and proud of having obtained the position in which I am: Head of the Rural Development Support Center (CADER 01) TUXTEPEC of the Rural Development Secretariat (SADER). It was through competition where they applied a series of exams and check my knowledge to deserve that position, besides that it was a challenge since I am the first woman in this charge. All my predecessors are male, great professionals, breaking with the stigmas and paradigms in an office led by them, conducting to mutual adaptation.

I must say that I did not study at a Mexican university. I had the opportunity and obtained a scholarship, and studied at EARTH University in Costa Rica, where the scheme is very similar to UNPA. If it were this University or school where you studied, you make yourself to apply your interest, decision and conviction to overcome all difficulties. It is not easy I know because to enter this university from another country I also had to compete among more than 3000 candidates and only entered 100 from 25 different countries. They will think that it is by economic level and the high school college in which I studied. However, it is not like that, I am the eldest of a humble family that worked and studied, and the college where I studied was the Forest Technological High school Center N ° 3 where I graduated through Community Development, nothing to do with Agronomy.

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This university is private and expensive, although I did not pay anything since I managed to obtain a full scholarship. After my graduation, I worked in the private initiative in the verification of works, activity for a civil engineer. Later in business administration. As you can see activities other than my profession but in which the challenges did not stop me since the university and professors like those that I see here, people with passion and dedication provided me the bases for any activity, and the main thing there, is the security that gives an adventurous spirit and insatiable thirst for knowledge. It is true it takes time, but if something can be sure is that if you want to be great agents of change, it can be done. I see it gladly because I had the opportunity to talk with the speakers here, very young girls doing masters and doctorate, with the desire to continue learning. They come from far away, Jalisco and Guadalajara, in a brief talk they tell me that they are studying and that by participating in these events they learn more since they have the challenge of being able to transmit their knowledge to new people.

As you can see, it is a matter of interest and effort. And you have the opportunity to practice, spoil and correct, even if nurses can go home and practice, in your communities, make the change, take advantage of your teachers like Dr. Cirilo Nolasco Hipilito, 10 days after arriving from Japan, here they are treasures available, which I see with the enthusiasm to teach. And that we young people have that possibility to grow in the environment where we are, and that women can go much further if we just try and take the challenge.

I can only congratulate the efforts of the organizers and participants for these activities to continue, that we hope to have alternatives in our programs, but in direct ways with you students, be spokespersons of what is offered in our Secretaries and Departments. Thank you much every one, and our hopes for an excellent event.

ENG. Teresa de Jesus Orozco Gonzalez

Head of the Rural Development Support Center (CADER 01) TUXTEPEC of the Rural Development Secretariat (SADER)

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Extended abstract

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Speaker Abstract

Title of Speech**: Enhancing the production of Lactic Acid by Repeated Batch Fermentation and a Thermophilic Lactic acid Bacteria**

*Corresponding: **Cirilo Nolasco Hipolito**
*Affiliation Institute of Biotechnology. Universidad del Papaloapan. Circuito Central #200, Col. Parque Industrial, CP. 68301. Tuxtepec, Oaxaca. Mex.7
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*Abstract:

Rapid lactic acid fermentation (LAF) based on repeated batch fermentation allows the reuse of Lactic Acid Bacteria (LAB) with high lactic acid (LA) production is reported. In this process LAF time was decreased significantly. Pre-cultural conditions established to obtain the best response of the system. LA concentration was 100 g/L in the first fermentation cycle and 110 g/L from the second to fifth cycle during less than 20h of fermentation each. Cell concentration was around 3.5 g/L and the volumetric productivity was 4.5 g/Lh. In conclusion, repeated bath fermentation was an effective method to perform a fast LAF using a thermophilic LAB without risk of contamination.

* Biography:

Originally from Tuxtepec, Oaxaca. Graduated from the University of Kyushu. MSc. Instituto Tecnologico de Veracruz, IBQ Graduated from the Technological Institute of Tuxtepec. Has been:
Director of R & D of Microbial Technology for the company Necfer Corporation, in Kurume, Japan and for the company of Chemical Engineering JGC Corporation of Japan.
Associate Professor at the University of Malaysia in Sarawak, and ¨ Professor of IPN Mexico.
International consultant for the United Nations Organization for Industrial Development.

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Business consultant in Japan, Malaysia, Singapore, Indonesia and Thailand.
He has published more than 50 international articles.
He is currently the Professor at UNPA.
Title of Speech: **Agrotransformation as a metabolic research and improvement tool for plants**

*Corresponding Author Full Name: **Edgar García López**
*Affiliation: **Universidad del Papaloapan Cathedra CONACYT**
*Area: **Plant Biotechnology**
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*email: **egarcia@unpa.edu.mx**
orcid.org: **0000 0002 1169 797X**
ResearchGate: **Edgar García**

*Abstract:

*Agrobacterium sp. i*s a group of bacteria that inhabits the soil and is pathogenic to plants. Through a complex mechanism, using a group of proteins called Vir proteins coded in a plasmid that inserts a fragment of it the T-DNA, into the plant's genome. This T-DNA is then expressed as part of the host genome and produce opines which are used by *Agrobacterium* as substrates, and induces a typical phenotype in the infected cells depending of the species causing the disease. In the case of *Agrobacterium rhizogenes* it produces roots from infected sites with high branching and hairiness, and accelerated growth.

These roots, named *hairy roots,* are widely used in plant biotechnology as model *in vitro* cultures to study different aspects of plant species, such as metabolism, its regulation, foreign gene expression and the production of secondary metabolites with biological activity. In our work group, we have used the agrotransformation technique to enhance steviol glycosides in *Stevia rebaudiana* and to elucidate its biosynthetic regulation.

Biography:

Edgar García López (1983) Born in Mexico City. Studied biotechnological engineering in UPIBI-IPN and Masters and Doctoral degrees in Biotechnology and Bioengineering in Cinvestav-IPN. Author of 8 articles since 2014 in the area of plant biotechnology.

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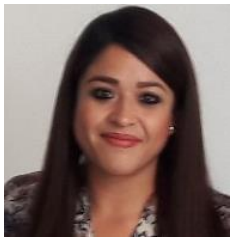
Title of Speech: **Brain Activity Quantification for Pain Index Estimation Using a BCI: A System Proposal**



Author: **Chávez Sáenz Velia**
Co- Authors: **Torres-Argüelles Vianey, Ochoa Carlos Alberto, Martínez Gómez Erwin**
***Affiliation:** Universidad Autónoma de Ciudad Juárez, México
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ResearchGate: https://www.researchgate.net/profile/Velia_Chavez

*Abstract:

Pain has been recognized by the International Association for the Study of Pain as the fifth most important vital sign after breathing, pulse, body temperature and blood pressure and has been considered as a severe and disabling condition that negatively affects the quality of life of approximately a fifth of the adult population in the Western world. Nowadays, the methods for pain assessment are subjective and easily affected by external influences. Considering the actual need, this article presents a proposal to implement a system based on the quantification of brain activity to estimate a pain index through signal analysis with Fractal Geometry as the main tool. This proposal arises from the current need to develop a tool able to provide quantitative information of pain. Currently there are several pain studies using electroencephalographic signals as a biomarker, however they are performed with specialized equipment. The novel aspect of this research is the proposal to use a brain computer interface (BCI) in an application to study and measure the intensity of pain.

* Biography:

Mrs Velia Chávez Sáenz is an engineer in mechatronics with a master degree in manufacturing with a major in automation. Currently is a student of the doctoral program in technology at the Universidad Autónoma de Ciudad Juárez. She´s been focused on the research of electroencephalographic signals as a part of her formation on applied engineering field.

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Title of Speech: # LC–MS/MS proteomic analysis of growth arrested *Bacillus subtilis* cells

***Corresponding Author Full Name: López-Torres Adolfo[1]**
Co- Authors Full Name: Castro-Cerritos Karla[1] and Pedraza Reyes Mario[2]

***Affiliation:** [1]Institute of Applied Chemistry, University of Papaloapan, Tuxtepec, Oaxaca, Mexico.
AREA COLOR: Red (Epigenetics)
[2]Division of Natural and Exact Sciences, Department of Biology, University of Guanajuato, Guanajuato, Mexico
***e mail:** adolfo_lopez_torres@hotmail.com

Abstract

Biological phenomena are frequently studied in cells during active replication in ideal laboratory conditions, however in nature the microorganisms remain under long periods of inactivity by scarcity of nutrients and are constantly confronted to stressing conditions. It has been suggested that during these conditions arise the mechanisms that promotes genetic variability, antibiotic resistance, pathogenesis, cancer and evolution. Starved *B. subtilis* cells experience a mutation frequency 100 times higher respect to replicating cells; furthermore, several strains show an even more elevated mutagenic rate. In order to further advance our knowledge on the metabolic conditions underlying this hypermutagenic phenotype, a high-throughput LC–MS/MS proteomic analysis was performed in growth-arrested cells deficient in a transcriptional regulator NrdR. Using these approach 749 proteins were identified, and 137 of them were differentially expressed in the NrdR-deficient strain. The proteomic analysis revealed an altered content in proteins associated with the stringent response, nucleotide metabolism, DNA repair, and cell signaling in amino acid-starved cells of the Δ*nrdR* strain; overall, our results exhibit a complex proteomic pattern reminiscent of a disturbed metabolism during starvation. Then LC-MS/MS is a valuable tool to evaluate global proteomic changes in specific biological conditions.

Biography:

Adolfo López Torres is an academic of University of Papaloapan with expertise in chromatographic methods (HPLC-DAD, FLD, MS; GC-FID, ECD, MS) with applications in metabolomics, proteomics, lipidomics and epigenetics. His work has been published in several international journals accumulating more than 180 citations in the last 7 years.

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Title of Speech: **Artificial Switchable Catalysts**

*Corresponding Author Full Name**: José Antonio Morales Serna**
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*Email: **joseantonio.moralesserna@gmail.com**
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*ResearcherID: **B-8100-2017**
*Scopus Auth***or ID: 8675025600**
*Twitter**: @JAMoralesSerna1**

*Abstract:

Inspired by biocatalytic systems, artificial switchable catalysts have recently received increased attention because of their ability to perform specific and complex tasks because of external stimuli such as light irradiation, pH variation, introduction of metal ions, or modification of the reaction conditions. In this context, my current research consists on the design, synthesis and evaluation of novel catalysts that can switch between ON/OFF states. The catalytic and switchable properties of these systems can be evaluated under homogeneous and heterogeneous conditions:

i) A rotaxane-based switchable asymmetric organo-catalyst has been synthesized in which the change of the position of the macrocycle reveals or conceals an acyclic, yet still highly effective, chiral organo-catalytic group. This allows control over both the rate and stereochemical outcome of a catalyzed asymmetric Michael addition (homogeneous catalysis).
ii) The use of montmorillonite, modified with a super-acid, in the presence of hydroquinone as a radical scavenger and under a nitrogen atmosphere, induced the formation of tetrasubstituted furans as the major product from benzoins. In the absence of a radical scavenger, the only products obtained were 1,2-diketones (heterogeneous catalysis).

Biography:

José Antonio Morales Serna obtained his MSc degree from the Universidad Nacional Autónoma de México (UNAM) under the supervision of Dr Jorge Cárdenas, investigating the synthesis of cyclodepsipeptides. He carried out his PhD degree (2009) in carbohydrate nanotechnology at the Universitat Rorvira i Virgili (URV), Tarragona Spain, under the supervision of Prof Sergio Castillón. In 2012 he joined Prof. David A. Leigh's group as a Newton Post-Doctoral Fellow at the University of Manchester (UoM), UK. Since 2018, he is a Lecturer at Universidad del Papaloapan, Oaxaca, Mexico.

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*Title of Speech: **I+D from the laboratory to the company**
*Corresponding Author: **Gabriel Guillen Solis**
Co- Authors: **Rosaura Aparicio Fabre**
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*Area: **Silver Biotechnology, Molecular Biology**
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AREA COLOR Silver

***Youtube Video: **En busca de la medicina de precisión**
<https://www.youtube.com/watch?time_continue=5&v=j8ueITnKK4s>
<https://www.forbes.com.mx/media-videos/en-busca-de-la-medicina-de-precision/>

*Abstract:

Innovative leader of the company Grupo QUAE S de R L, which in its initial stage was financed with partners' funds. However, these resources were insufficient for the company to achieve a profitable scale since it is a project of high technology. The additional investment required by the spinoffs has a high transaction cost because the innovator is generally focused on the positive aspects and does not take into account most potential problems. On the contrary, investors generally focus in the true high risk and demand a higher profitability that justifies it, resulting in a smaller investment than optimal. Therefore, for an innovation ecosystem to be truly successful it is required that all its elements interact appropriately, as has been the case with the successful experience of the GENO+ platform from the Laboratory of Genomic Specialties.

* Biography:

Academic Technician of the Institute of Biotechnology with more than 20 years of experience in research. Chemical Bacteriology and Parasitology by profession, graduated from the National School of Biological Sciences of the IPN, with a Master in Microbiology and a Doctorate in Biochemical Sciences from the Institute of Biotechnology. Member of the National System of Researchers with a distinction in Level I since 2013. Innovator generating ideas that bring value to his companies, QUAE and GENO +, through his direction in Innovation and Development Management. Platform developer of GENO +, which is designed to improve its patient's quality of life through nutrigenomics, pharmacogenomics and physical activity.

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Workshop Speaker Abstract:

*****Workshop Title of Speech: **Metagenomics and statistical analysis**
*Corresponding Author: **Luis Uribe Espejo Galicia**
Co- Authors: **José Francisco Pulido Barajas**
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*Abstract:

To make a correct diagnosis it is necessary to understand the background of the techniques available in order to be able and carry out a correct protocol for the diagnosis. Once you have the protocol for the diagnosis you need to determine the type of distribution of the samples to determine the types of statistical tests for the validation of the protocol and determine if it meets the minimum criteria necessary to be able to use the protocol as a technique for the diagnosis of diseases. For this reason we want to give a workshop that deals with a program of molecular biology techniques for the detection of DNA. To make an identification in the workshop you will see how the design of oligonucleotides depends on the PCR modality (anity, end point and qPCR, Multiplex and for sequencing), determination of the best technique for diagnosis, determine the distribution of your sample (binomial, normal, Fisher and others) to perform statistical tests such as specificity, predictive value, sensitivity, robustness among others. There will also be a correlation between disease from SNP and an introduction to graph correlations between mutations (GWAS and CIRCUS PLOT).

* Biography:

Luis Uribe Espejo Galicia (1993-to date) is a former student of the Universidad del Papaloapan, who is studying a master's degree in molecular medicine at UAEM, skills: data analysis, development of molecular diagnostic techniques, massive sequencing & metagenomics. He is currently the Professor at UNPA.

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Workshop Speaker Abstract:

***Title of Speech:** "Biotechnological panorama in the industry

*__Corresponding Author:__ Mauricio Díaz Sánchez

*Affiliation: Grupo T4 OLIGO

AREA COLOR Platinum Synthetic biology

***Affiliation: Grupo T4 OLIGO**

***Contact number: 7771078941**

***Email: secuenciacion@t4oligo.com**

***Abstract:** M.C. Mauricio Díaz Sánchez from Company Grupo T4 OLIGO, gave a lecture at the Papaloapan University auditorium as part of the Metagenomics and statistical analysis preconference Workshop, "Biotechnological panorama in the industry, in the framework of the Biotechnology Summit 2018. During his presentation, the manager Operations Manager and Founding Partner of Grupo T4 OLIGO specialist in synthesis of molecular biology spoke about the only company producing artificial DNA in Mexico and Latin America owners with more than 80% of the current market in our country. Who referred to the importance of working as a team, risking capital, to offer an excellent service to increase the customers' portfolio; as a positioning strategy with the purpose of specifying the perspective and future projection of the development of new services for the client as generating tools that allow them to analyze, modify and understand the genomic sequence. «Because we cannot be able to own our biological information and be able to access it in a matter of seconds, and compete with the leading foreign supplier»

The company based in the Agrobiotek park in Irapuato, Guanajuato, works with scientists CINVESTAV with those who developed LUCi, a portable laboratory that uses an open platform which allows the producer and analyst to analyze samples of deoxyribonucleic acid (DNA) to detect infections, bacteria or diseases in 60 minutes. T4 Oligo is a pioneer company in Mexico and the Bajío region, which is a molecular genetic bio business, with ISO 9001 standards and others which guarantee quality even better than abroad and re-send an oligo if this fails, among its guarantees. The purpose of this conference was to invite students to apply for scientific, technological (thesis) and social service stays of no less than 4 months; as a support to young people and talents search strategy since it is a company in constant expansion.

* Biography:

Mauricio Díaz Sánchez (1993-to date) Originally from Ozumba, Estado de México. Graduated from the Universidad Autónoma del Estado de Morelos. MSc. Instituto de Biotecnología UNAM. Has been: Director of R & D of T4 oligo, Chief Operator Manager of T4 oligo

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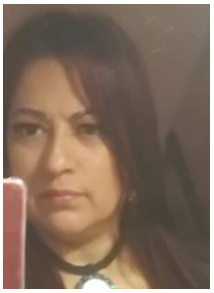
*Title of Speech: **USDA ARS SRRC Borlaug Fellowship**



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***Abstract:**

The Borlaug International Agricultural Science and Technology Fellowship Program (Borlaug Fellowship Program or Borlaug) was established in March 2004 to honor the Nobel Laureate Dr. Norman E. Borlaug. The program promotes agricultural productivity, food security, trade and economic growth by providing training and collaborative research opportunities to early and mid-career scientists, researchers, or policymakers from developing and middle-income countries. Borlaug is implemented by the United States Department of Agriculture's Foreign Agricultural Service, Office of Capacity Building and Development, Trade and Scientific Exchanges Division (USDA/FAS/OCBD/TSED). Since the program's inception, USDA has sponsored more than 850 Borlaug Fellows from 69 countries. Borlaug Fellows spend 8-12 weeks in the United States and work individually with U.S. scientists in their field. The Fellows learn new research techniques, gain exposure to the latest scientific developments in various fields of agriculture, access fully-equipped laboratories and libraries, and learn about unique public-private partnerships that help fund agricultural research and science. The program provides Fellows with opportunities to establish long-term contacts with U.S. scientists and to apply newly gained knowledge from U.S. institutions to their country's research and development programs. Mentors from the United States coordinate the Fellows' training, and they visit the Fellows' countries upon completion to continue the collaboration. Training venues include U.S. land-grant universities, USDA or other U.S. government agency research facilities, not-for-profit institutions, and international agricultural research centers. Link to the FY19 recruitment cycle…. USDA Fluid Review (closes 12.31.18) -- https://www.fas.usda.gov/programs/borlaug-fellowship-program

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IMPI LOGO AND BRAND AS INTERNATIONAL BIOTECHNOLOGY FOUNDATION) <http://www.bio.edu.mx>, the publication in 2011 of the International Biotechnology Color Journal ISSN 2226 0404, in collaboration with Dr. Sotres UNAM and Dr. Zuniga and actually the editorial board is:

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Area I

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Human Health & Diseases, Biomedicine, and Human Tissue Engineering.

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Brain Activity Quantification for Pain Index Estimation Using a BCI: A System Proposal

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Abstract

This article presents a proposal to implement a system based on the quantification of brain activity to estimate a pain index through signal analysis with Fractal Geometry as the main tool. This proposal arises from the current need to develop a tool able to provide quantitative information of pain. Currently there are several pain studies using electroencephalographic signals as a biomarker, however they are performed with specialized equipment. The novel aspect of this research is the proposal to use a brain computer interface (BCI) in an application to study and measure the intensity of pain.

Key words: ●Brain activity ● Pain ● BCI ● Fractal Analysis.

Introduction

Pain has been recognized by the International Association for the Study of Pain as the fifth most important vital sign after breathing, pulse, body temperature and blood pressure (Lynch, 2001). Although there are different types of pain, chronic pain is a severe and disabling condition that negatively impacts the quality of life of approximately a fifth of the adult population in the Western world (Ploner, Sorg, & Gross, 2017). Currently, chronic pain is associated with personal and social costs. Since individuals with chronic pain often have a reduced quality of life and unmet therapeutic needs (Davis et al., 2017), generating a negative impact on the daily productivity of people given that within the consequences of chronic pain is the significant intensity of pain, depression and anxiety (Bromley Milton et al., 2013). Currently, chronic pain impacts the quality of life of approximately a fifth of the adult population in the Western World (Ploner et al., 2017).

Nowadays, the methods for pain assessment are subjective and affected by external influences. Considering this situation, brain activity has been considered as a biomarker because studies related to chronic pain have reported this problem is considerably related to the functioning and structural reorganization in the nervous system (Tatu et al., 2018). It is the result of the activation of multiple areas of the brain, which is known as the pain matrix (Garcia-Larrea & Bastuji, 2017; Mano & Seymour, 2015).

In order to study pain from what could be considered its central processing unit (the brain), studies have been conducted to measure brain activity through electrical

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potentials, which provide estimates of the synaptic action to large scale, closely related to behavior and cognition (Xu & Zhong, 2018). Recent experimental data presented evidence that the functioning and behavior of the brain may be different in individuals with chronic pain. Compared to individuals who do not suffer from any type of pain (An, Wang, Cope, & Williams, 2017; Davis & Seminowicz, 2017; Morton & Jones, 2016) whose measures of electrical activity in response to painful physical stimuli using EEG, show that attention significantly affects electrocortical responses to painful stimuli (Blöchl, Franz, Miltner, & Weiss, 2015).

So far, most cases that handle a pain analysis approach, using electroencephalography, have used specialized laboratory equipment (An et al., 2017; Furman et al., 2018; Hadjileontiadis, 2015; Kumar, Kumar, Trikha, & Anand, 2015) which implies that the study is expensive, inaccessible and requires specialized knowledge for the management of the equipment.

Currently the development of brain computer interface technology has allowed to generate a new focus in this type of studies, allowing researchers to access information about brain activity at an accessible cost, thus having a new aspect of research (Dehzangi, 2018; McCrimmon et al., 2017; Purcell, Fraser, & Vuckovic, 2017). Although there are several studies of pain considering the evaluation of brain activity and many others using computer brain interface technology, the information is scattered.

The objective of this article is to present a system proposal based on the Brain Activity Quantification for Pain index estimation acquiring the signals through a scientific context BCI. The signals will be acquired in subjects with pain and without pain. These acquired signals will be subjected to a comparative study and later those obtained in the state of pain, will be transformed to time series to perform a fractal analysis that allows obtaining a quantitative data of the pain.

Materials and Methods

Signals acquisition device

It is proposed to perform signal acquisition using the Emotiv EPOC+ device (Figure 1a). This is a neuroelectrical detection system that captures and amplifies brain waves generated by different mental "actions". This device is able to obtain the signals of the 14 channels: AF3, F7, F3, FC5, T7, P7, O1, O2, P8, T8, FC6, F4, F8, AF4, based on the international system 10-20, which is an approved method to describe the location of electrodes in the scalp, for the EEG record, as shown in Figure 1b. This device also handles a filter for frequencies of 0.2 to 45 Hz, which can take up to 128 samples per second in each channel (Emotiv, 2014) .

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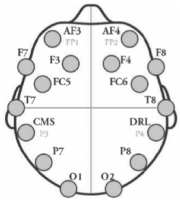
(a)

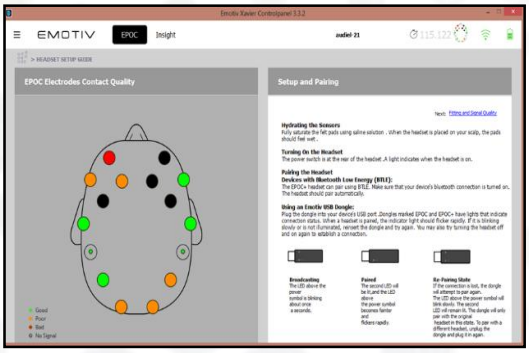


(b)

Figure 1. (a) Emotiv EPOC + (Emotiv, 2014), (b) Positioning system 10-20 (Lay & Pizarro, 2015).

Software

Among the tools that Emotiv provides with the EPOC+ headband, is the Emotiv Xavier software development kit (SDK). This is composed by Emotiv Xavier control panel, version 3.3.2 and Emotiv Xavier test bench, version 3.1.20. The control panel allows the user to see the communication status between the headset and the computer, as well as the connection status of each of the 14 signal acquisition channels (Figure 2, left image). The test bench is the platform on which the acquired signals can be visualized in real time and where the person in charge of obtaining the signals can establish the acquisition parameters (Figure 2, right image).



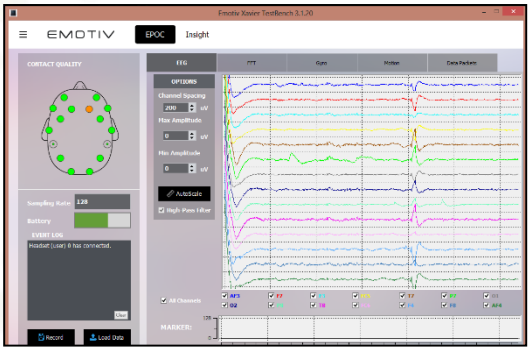


Figure 2. Emotiv Xavier control panel and *Test Bench* (Chávez, Torres, Herrera, & Hernández, 2016)

The software that Emotiv provides will be used to know the connection status of the device and to perform a general analysis of the behavior of the signals, however since it is not possible to perform the manipulation of the signals, it is proposed to establish a connection with additional software. Based on a literature review, there is evidence that the EPOC+ has established a connection with development software such as Matlab (Prince et al., 2016; Szalowski & Picovici, 2015). Based on this, it is proposed to establish the connection of the device with Matlab to perform a transformation of the signals to time series and then to be able to continue with the analysis.

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Signal analysis

The proposed method for the signal analysis based on acquiring the EEG signals of subjects in two states, with pain and without pain. The acquired signals then transformed into time series to apply fractal analysis. Fractals, were initially developed by (Mandelbrot, 1983) and represents a mathematical set that process a high degree of geometric complexity and can model numerous phenomena, providing an adequate mathematical framework to study the irregular and complex forms of these phenomena. Fractal dimension is a statistical quantity that indicates how much a fractal fills the space when it is observed at finer scales, being an effective index to measure the characteristic of complex objects and surfaces, such as littorals, mountains, clouds and textures (Long & Peng, 2013). Used as a measurement to estimate and quantify the complexity of the shape, structure or texture of objects. Its central feature is that can be used to accurately characterize the irregularity of a physical structure that may not be treated in general by Euclidean geometry. Due to the above, fractal tools have found wide applications in the analysis of EEG signals (Kobayashi & Nakagawa, 2016; Ruiz-Padial & Ibáñez-Molina, 2018).

The tools of the Fractal Geometry, useful for the analysis of complex systems from time series and signals, as the Re-scaled Range Dimension, the Power Spectrum Dimension, the Variogram and the Wavelet, with which the roughness of the signal is measured in terms of the Hurst exponent. Consists on an unbiased measure of the roughness of the images. Time series and signals, relating H to the fractal dimension (D) according to a simple rule based on Hardy (1916) conjecture: $H = 2-D$, where D is the fractal dimension, 2 is the dimension of the Euclidean common space within which the fractal is embedded; and H is the exponent of Hurst (Tapiero, 1996). H considered as an efficient tool to express the roughness of complex systems in quantitative, dimensionless and normalized terms that facilitates the detection of critical points in the non-linear behavior of these systems. The Figure 3 presents the methodology to implement and obtain the proposed results.

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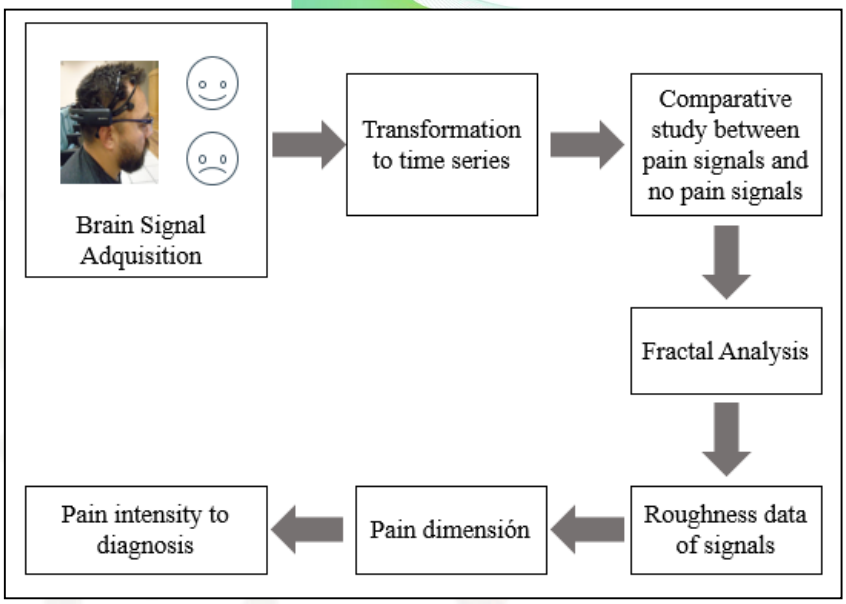

Figure 3. Methodology of the proposed system

Fındıngs/Results

The expected results of the proposed system are at first a comparative study between the acquired signals in subjects with pain and without pain. Subsequently the roughness of the signal is calculated in terms of the Hurst exponent. This quantitative data will allow to dimension the intensity of the pain and then generate a diagnosis.

Being more specific, the expected results in the system implementation process are:

- Test plan for acquisition of EEG signals in subjects with pain.
- Comparison of time series generated from brain activity in a group of subjects contemplating two states (with pain and without pain).
- Fractal analysis of EEG signals to acquire the roughness of the signal based on their behavior.
- Quantitative data of pain intensity.

Discussion

Based on a literary review previously conducted, we found evidence that valuable studies have been conducted that present joint methods to develop a quantitative pain index (An et al., 2017; Kumar et al., 2015; Schmidt et al., 2012). These studies carried out with specialized laboratory equipment implies that the study is expensive, inaccessible and requires specialized skill for handling the equipment. Development of brain computer interface technology generates a new approach in this type of studies, allowing researchers to access information about brain activity at an affordable cost.

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The literature review also shows that BCIs are giving satisfactory results in applications such as rehabilitation and treatment of people with various clinical conditions, control of devices such as didactic robots (Holewa & Nawrocka, 2014; Yordanov, Tsenov, & Mladenov, 2017). Detection of drowsiness for safety when driving vehicles (Ben Dkhil, Neji, Wali, & Alimi, 2015) and reliable information from a BCI to analyze different wave types that can be obtained, such as P300 and SSEVP (Szalowski, 2016). Based on this, it is considered that a BCI is a viable option to develop the proposed system.

Conclusion

The proposed system is based on an exhaustive review of literature from which it is demonstrated that the pain is a social and economic problem that has a negative impact on people's daily productivity and quality of life. It is associated with personal and social costs; since individuals with chronic pain often have a reduced quality of life and unmet therapeutic needs (Davis et al., 2017). Based on this, there is an urgent need to develop an objective, quantitative and reliable indicator of pain monitoring that offers the possibility of making accurate diagnoses in a short time.

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(Lumbar) Vertebrae Displacer Design for Sensing System

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Summary

The vertebral body bares great efforts before breaking, given the enormous capacity of the spongy bone to absorb energy. Vertebra resistance diminishes with age, especially after 40 years old. A mass loss of 25% represents a resistance decrease of 50%. The average resistance to fracture due to the vertebral bodies ranges between 600 and 800kg. The vertebral body fractures before the intervertebral disk does.

The instability of the spine is defined when the spinal canal is incapable of maintaining normal relations between the vertebrae. The instability shows up when there is the possibility of displacement of the relative vertebral positions before scarring, or if the fragments are capable of moving with an eventual neurological injury, when there is disruption of two out of the three columns, there is instability. (Displacement of the vertebra of 1mm).

In this work, the design of a spine vertebrae machine (displacer) is presented, in which a 3D computer design is used to make the displacer model, considering the displacement criteria that have to be complied with when the vertebra is moved with the displacer, meaning that the vertebra has to move 1mm or less so that the sensing system captures this movement and registers it. As a result, the 3D finished displacer is obtained, already manufactured for future tests with the respective specimens and the sensing system.

Key words

●Displacer ●3D design ●SolidWorks® ●Vertebra

Introduction

The movements of the spine are determined by passive elements (joint process, disc, ligament, bone structures) and active elements (muscles). Therefore, the malfunction of any of these elements brings forth a modification in the movement of the spine [Beristain-Lima, 2010].

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A cinematic study of the spine can take place from two different points of view:

-From segmental mobility. Exclusively one spinal segment.
-From the global mobility of the spine.

The sum of the limited motions between the adjacent vertebra allows an important degree of mobility to the spine overall, as seen in Figure 1 [Vázquez-Machorro,2019] [Beltrán-Fernández, 2015]. The different motions of the spine are Flexion, Extension, Bending, and Rotation.

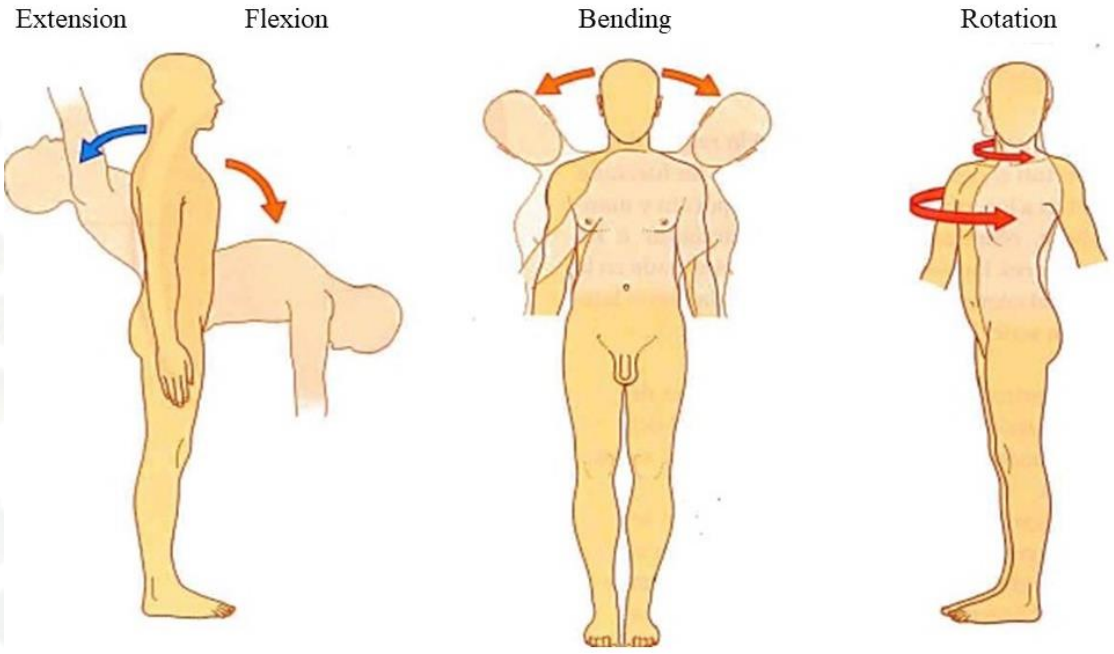

Figure 1. Motions of the spine.

On Table 1, the degree of spinal articulation for each area of the body according to the motion is appreciated [Vázquez-Machorro,2019] [Villaseñor-Chávez, 2015].

Table 1.- Summary of the movements of the spine

Motion	Cervical	Dorsal	Lumbar
Flexion	40º	20º	60º
Extension	75º	25º	35º
Lateral Bending	(30º - 45º)	20º	20º
Rotation	(45º - 65º)	35º	5º

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The objective is to design a machine (displacer) where a vertebra or a vertebra segment can be mounted, to sense their displacement, given the fact that a sensing system cannot be implanted in a human being and tests need to be made to validate it, hence the necessity to develop said machine.

Materials and Methods

The steps followed to 3D modeling and manufacturing of the displacer were the following:

1.- A sketch was made, according to the requirements the displacer needs to comply with, for example:

- The displacement of the vertebra has to be 1mm or less.
- The displacer should not be of large size because small specimens will be being worked on (less than 7cm).

2.-The modeling was done on SolidWorks®, in Figure 2, part of the modeled displacer can be seen [Gómez-González, 2009].

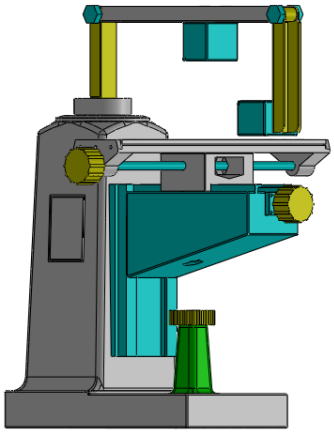

Figure 2. Displacer in 3D.

3.- Once the 3D model was finished, it was proceeded to print the model in a 3D printer, the pieces were placed for the printing and the metal cutting. In figure 3, the arrangement of the pieces of the displacer can be observed.

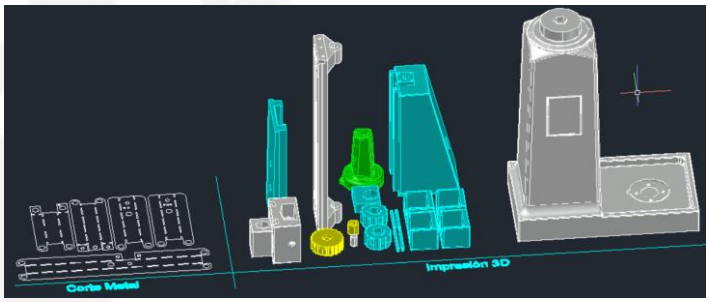

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Figure 3. Arrangement of pieces.

4.- After that, the pieces were cleaned to eliminate any residue the bending of the metal pieces was done and then the pieces were mounted to verify the proper function of the displacer before giving it the final touch.

5.- Finally, once the correct function of each and every single one of the displacer components was verified, it was proceeded to give it the final touch, that is to say, the sanding and painting of each piece was done.

Results

In Figure 4, the vertebrae displacer with its respective finishes can be seen, as well as its mechanisms to be able to displace the vertebrae in the 3 requested axes for their sensing.

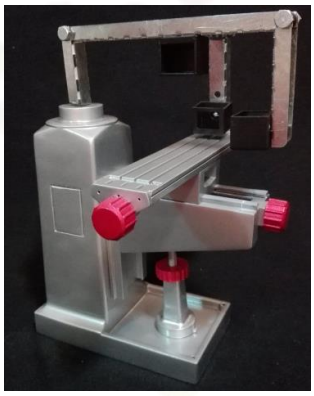

Figure 4. Vertebrae displacer with the final touches.

In Figure 5 and Table 2, the identification of the parts that compose the displacer can be seen. In Figure 6, the simulation of a vertebra already mounted on the displacer can be appreciated.

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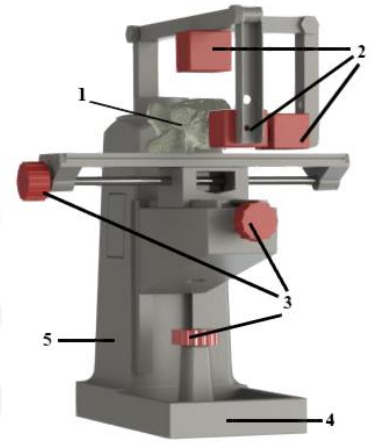

Figure 5. Identification of the displacer components.

Table 2.- Displacer components

Number	Component
1	Specimen
2	Sensors (in x, y, z)
3	Displacing knobs (x, y, z)
4	Base
5	Support

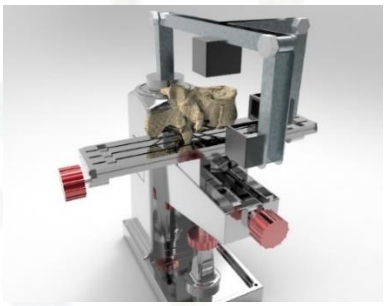

Figure 6. Mounting of lumbar vertebra on the displacer.

Discussions

In accordance to the previous results and other existing designs, the most similar thing is the exoskeleton for human mobility. They focused on helping the human body to walk or carry very heavy materials. Our machine is designed to be capable of testing the displacing of the vertebrae; this is to verify the sensing system functioning, and in a future, be able to implant the system in humans, for a better diagnosis and patient follow up.

Conclusions

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The design and the manufacturing of the vertebrae displacer complies with all the necessary requisites to be able to hold the sensing tests in the future; which is primarily, to move the vertebra or vertebrae in the three axes (x, y, z), with a displacement of 1mm or less. This displacer can be used on any section of the spine; besides the fact that it is a small machine, it can be disassembled quite easily and moved to another place without a problem; not taking a lot of space is another advantage.

Acknowledgments

The authors of this work are thankful to the Instituto Politécnico Nacional, Sección de Estudios de Posgrado e Investigación de la Escuela Superior de Ingeniería Mecánica y Eléctrica Unidad Zacatenco y al Consejo Nacional de Ciencia y Tecnología (CONACYT) for the given support.

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3D Biomodeling for the Acquisition of anthropometric parameters for the spine

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Abstract

The spine, also known as rachis, is a bony structure in the form of a pillar supporting the trunk. It has a structure consisting of 33 vertebrae of which only 24 are true, distributed as follows: from top to bottom, it has seven cervical vertebrae; the first two vertebrae of this section are atlas and axis. Follow 12 thoracic vertebrae that are supporting ribs and finally, 5 lumbar vertebrae.

Currently the hospital units depend on various methods of 3D reconstruction as a form of technique to help in the decision-making in cases of diagnosis of certain diseases and scheduled surgeries, these reconstructions are known as bio modeling. Computational bio modeling is a useful tool for processing or developing morphological analysis of human body parts. Thanks to this, the bio modeling has helped science a lot in recent years.

In this paper, we develop a methodology of a bio modeling for 3D reconstruction of the human spine obtained from CT scans. By developing this methodology, we can parameterize and visualize the backbone almost exactly as its original morphology with the purpose of obtaining specific measures and perform the development (fabrication) and analysis of it in the future.

Keywords Biomodeling ● Methodology ● Scans ●Vertebra

Introduction

The human being is a vertebrate, which represents the end of a long evolution to colonize the land. Their locomotor apparatus, whose main support is the spine (rachis). The rachis, which is the main axis of the body, has consisted of short bones stacked with each other and movable in relation to each other [Fuerte-Hernández, 2014].

This osteoarticular set serves both as axis to the frame of the body and protect the spinal cord. With it is intended to route the information to the brain that is protected by the skull

on top of the rachis that transmits orders to all body muscles. Humans share the same structure with the gorilla, their closest relative; as they are able to maintain the standing position, but not continuously since both structures, although very similar, are not the same Figure 1 [Villaseñor-Chávez, 2015].

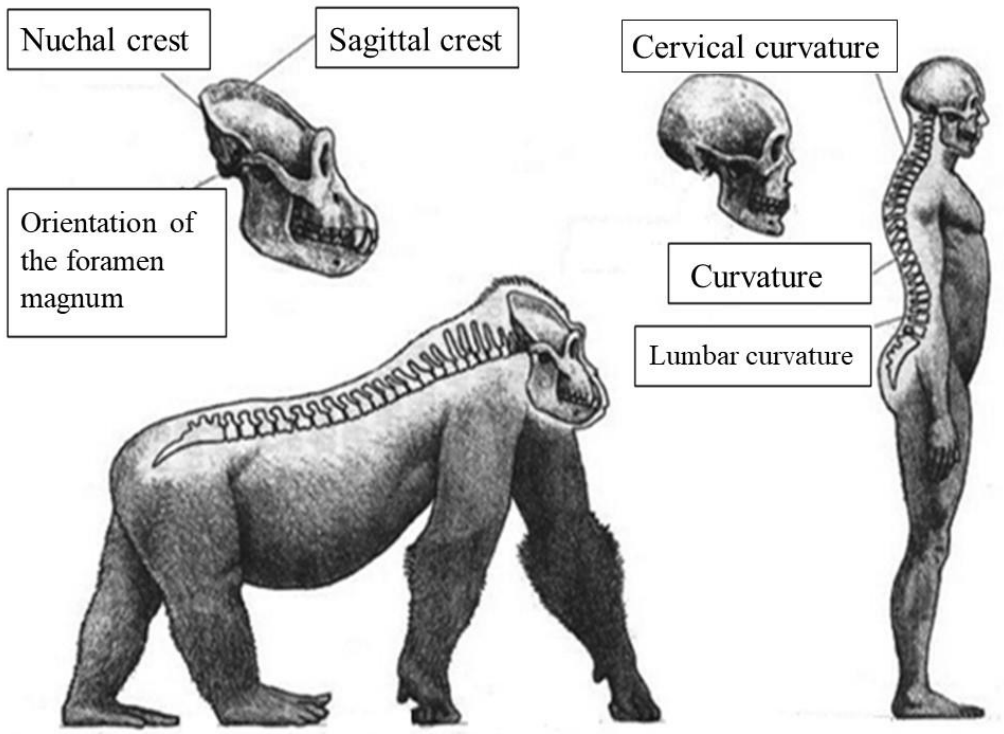

Figure 1. Difference spine.

The movements of the spine are determined by different elements: passive (articular processes, disk, ligaments, and bone structures) and active (muscles). Therefore, the dysfunction of one of these elements results in a change of motion of the spine. This structure ensures three key features for functionality, which are:

1. Providing rigidity to withstand axial loads.
2, Protecting the central nervous system (spinal cord and nerve roots).
3. Providing adequate mobility and flexibility.

Computing bio modeling is a term used to refer to the ability to reproduce the morphologies of the human body or some other morphological bodies [Vázquez-Machorro, 2019]. The modeling is used to describe the process of using radiant energy to capture and process biological data in order to reconstruct a 3D model of the specimen and make a physical model Figure 2 [Gamarra-Rosado, 2014] [Adrián-Romero, 2007].

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The objective of this paper is to find the dimensions of the spine or subject.

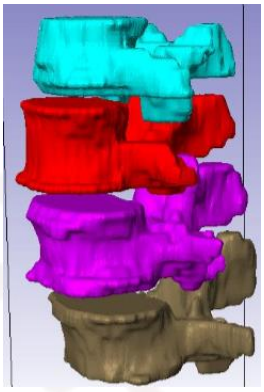

Figure 2. Bio modeling of L2-L5 vertebrae.

Materials and methods

The three steps followed for the bio modeling 3D reconstruction of the spine were preprocessing, processing and post-processing:

1. The preprocessing is the acquisition of medical data, in this case, it would be the medical images of the patient or test subject. In this process, CT scans were used, since such images provide real-time capture, obtain images of various tissues and are almost as exact as reality. To start the generation of the model, we used scans with a DICOM CT extension.

2. Processing, in this step, the import of DICOM files to a specialized program that is able to display images in a wide range of processing so you can start generating the bio modeling 3D reconstruction of the structure, figure 3.

3. Thereafter, the work area is delimited and tissue type is selected by the upper and lower threshold value, which allows differentiating the different types of biological tissue, Figure 4.

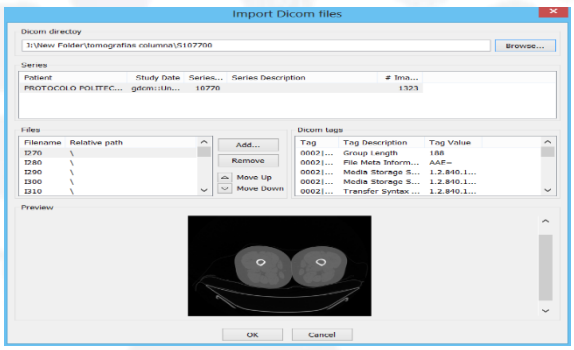

Figure 3. Import DICOM files.

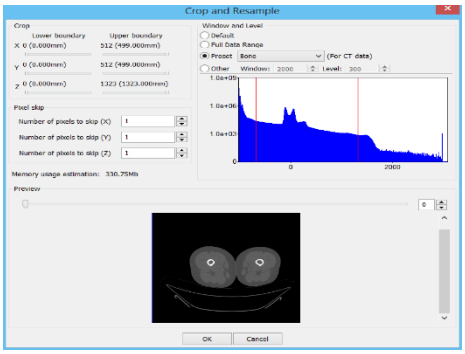

Figure 4. Selection of the type of tissue.

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4. Then, the area of interest begins the outline, Figure 5. On figure 6 shows the filled vertebra, step 4 and 5 must be performed in all the tomographic sheets containing the spine.

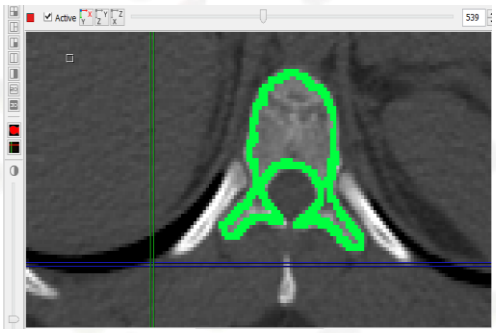

Figure. 5. View of the contoured vertebra.

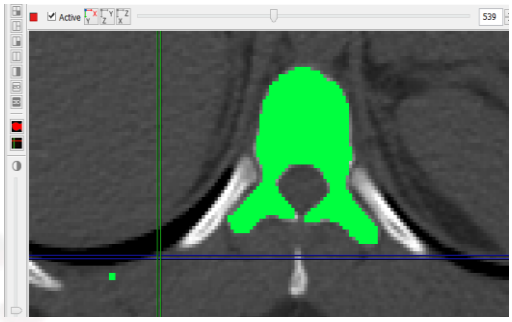

Figure. 6. View contoured vertebra

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Results

Once the 3D reconstruction of the spine is obtained, and the file moved to an STL format. As this extension allows importing it into a computer design program. Once the file is sent to a design platform, data found in this study are obtained. Table 1 shows the found data and underlines the most important ones because they are the vertebrae with larger and smaller dimension.

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Table 1.- anthropometric dimensions of the vertebrae.

VERTEBRAE	BODY WIDTH	WIDTH apófisis	LONG VERTEBRA
L 5	47.68 mm	8456 mm	68.43 mm
L 4	44.12 mm	78.58 mm	72 mm
L 3	44.20 mm	82.68 mm	78.9 mm
L2	42.32 mm	73.71 mm	70.39 mm
L 1	41.25 mm	64.04 mm	63.42 mm
T 12	36.9 mm	37.59 mm	61.1 mm
T 11	34.7 mm	45.67 mm	60.20 mm
T 10	32.86 mm	52.16 mm	57.88 mm
T 9	30.82 mm	51.46 mm	57.24 mm
T 8	28.71 mm	51.21 mm	54.09 mm
T 7	27.73 mm	52 mm	55.51 mm
T 6	25.97 mm	53.13 mm	52.3 mm
T 5	27.01 mm	50.8 mm	57.27 mm
T 4	25.9 mm	49.66 mm	58.39 mm
T 3	26.8 mm	51.53 mm	59.60 mm
T 2	27.3 mm	55.3 mm	59.33 mm
T 1	26.88 mm	60.02 mm	56.30 mm
C 7	22.4 mm	52.11 mm	52.67 mm
C 6	20.41 mm	53.48 mm	44.23 mm
C 5	17.68 mm	51.11 mm	40.13 mm
C 4	15.56 mm	45.56 mm	40.47 mm
C 3	16.63 mm	46.67 mm	38.70 mm
C 2	14.01 mm	48.90 mm	43.23 mm
C 1	13.99 mm	47.05 mm	40.05 mm

Discussions

According to past results, the obtained data has been compared with the dimensions found in the literature and show us a similarity to those obtained in the development of this research, since the bio modeling gives us an accuracy of the morphology of the vertebrae.

Conclusions

The acquisition of the data was acceptable because. Although they were not equal to literature data because each human being has a different morphology. It shows that using the 3D bio modeling is rather reliable because it uses CT scans that show an image almost exact to that of the patient. Moreover, these image data may be used for their future analysis as for their construction, although we found some drawbacks, such as computational resources when using the 3D bio modeling because of the high consumption of computing resources.

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Acknowledgments

The authors of this work are thankful to the Instituto Politécnico Nacional, to the Sección de estudios de Estudios de Posgrado e Investigación de la Escuela Superior de Ingeniería Mecánica y Eléctrica Unidad Zacatenco y al Consejo Nacional de Ciencia y Tecnología (CONACYT) for the support given.

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Design of an SNP Panel Related to Cancer

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Palabras claves, DNA and SNP.

Key words: SNP Single nucleotide polymorphisms

Abstract

Cancers are a group of diseases caused by the growth and spread of cells (Catherine-Sánchez, 2013). Causing 16% of deaths worldwide (OMS, 2017) Cancer is associated with mutated genome. Genomic changes are multifactorial (AMS,2014;Aguilar Cordero *et al.* 2012;Siemiatycki *et al*, 2004) but most of the factors converge in the formation of free radicals (Cox et at 2018;Kajatt et at, 2014) causing chemical modification in the bases and consequently mutations (Sanz-Montero, et al 2004). The detection of changes in DNA sequences has helped to establish the molecular basis of cancer and its possible etiologies. Currently there are 7,628 SNPs related to 31 types of cancer reported up to December 31, 2017, distributed heterogeneously in chromosomes with the exception of the Y chromosome. Of the 7628 SNPs, their bioinformatic analysis was performed to determine the detectable SNPs by designing a sequencing technique by MinION to detect the most possible SNPs through exome sequencing.

Introduction

Cancers are a group of diseases caused by the uncontrolled growth and spread of cells by alteration in their cell cycle, mainly due to changes in their genome[1]. The genomic changes are multifactorial (ACS, 2014; Aguilar Cordero et al, 2012; Siemiatycki, J. *et al.* 2004), but most factors converge in the formation of free radicals (Portillo, 2014), which can cause genetic damage, for example causing chemical modification of reactive groups in the nucleotide bases (Sanz-Montero et al, 2004). Due to the new lifestyles, which entail low consumption of vegetables, high intake of fats and carbohydrates, the incidence of cancer has increased by more than 50% in the last three decades. By 2015, cancer is the cause of 16% of deaths worldwide (OMS, 2017).

Cancer is the fifth cause of death worldwide, in 2012 it caused 8,201,575 deaths, representing 16% of all deaths due to illness worldwide (OMS, 2017). Cancer development may be due to many factors, but mainly to the combination of exogenous factors that are related to the lifestyle and endogenous factors that predispose the person to the disease due to their physiological, mental and genetic state (Lagunas-Rangel *et al*, 2016). The most important endogenous factor is the genetic factor, because it cannot be

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eliminated, it contributes to develop the disease and is constant throughout the patient's life, which allows it to be used as a biomarker for diagnosis. The detection of Biomarkers for the diagnosis of cancer has been carried out for more than 40 years (Santillán-Garzón, 2015). The first analyzes were carried out using the PCR technique (Cox et at, 2018; Kajatt et at, 2014) and with this diagnostic procedure it could detect between 1 to 6 biomarkers per study. The form of analysis was insufficient to diagnose correctly (Esser et al, 2017), due to the large number of genetic variations that have been associated with neoplasms (Lay-Sona et al, 2015). Currently third-generation sequencing technologies (NGS) are being used for detection of several biomarkers associated with cancer (Morris et al, 2014). Currently there are sequencing equipment that allows detection of thousands of SNPs; but due to its high cost, and its bioinformatic requirement, panels are designed to only detect the desired SNPs, considerably reducing costs. Being the reason for this work to present the design of an SNP panel related to all cancers detectable in blood present in the databases until December 31, 2017.

Material and methods

Bibliographic search of genes

A database was made of the genes that have been reported that are detectable in peripheral blood. To determine all the genes that have been reported in blood, the GEO database of the NCBI was used (https://www.ncbi.nlm.nih.gov/geo/).

Obtaining a gene database related to cancer

With the genes that are expressed in blood, a phyton language script was used to determine the number of genes that are expressed in blood and have been associated with some pathology of neoplasia. In order to obtain the information, working with specialized databases used in Cancer: intogen (https://www.intogen.org/search), Cosmic (https://cancer.sanger.ac.uk/cosmic), NIH (https://www.cancer.gov) and MalaCards (http: / /www.malacards.org/).

Polymorphisms related to cancer

From the genes related to tumors, we searched for SNPs with a momio ratio (OR) greater than one, using four pages specialized in mutations and gene variations, SNPedia (https://www.snpedia.com/), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), MalaCards (http://www.malacards.org/) and GWAS Catalog (https://www.ebi.ac.uk/gwas/).

Chromosomal position of each of the mutations.

Each of the mutations that were selected as possible biomarkers, chromosomal localization carried out by means of the PhenoGram program, modifying the script in RUBY language. It was given the condition that in the genes that have more than 20 mutations in its sequence, it was only positioned a mutation for all mutations found at a distance of no more than 2,500 bases in both directions of the gene.

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Circus plot

With the data collected from the SNPs, a Circus plot analysis was performed, in order to make a correlation of all the data obtained from the SNPs obtained with the chromosomes, for its design the circus plot library of R program was used, the intensity of each line qualitatively represents the number of mutations for cancer type with said disease.

Interactions of genes with cancer

A database of tumors with the obtained genes then were correlated with at least 3 types of selected tumors, with this data a map of interaction between the genes, grouping them by the type of cancer that has been reported, to determine the impact that such gene has to develop several types of cancer. To do this, used the Cytoscape version 3.0 program with the cy3sbml version 0.2.7 app.

Results

Bibliographic search of genes

We found a total of 15744 genes expressed in peripheral blood, covering 26.11% of all genes present in the human genome (GRCH38.p11), with chromosomes 1, 6 and 19 (Figure 1) standing out, expresses the greatest number of genes in blood, including among them three 26.39% of the genes detectable in blood.

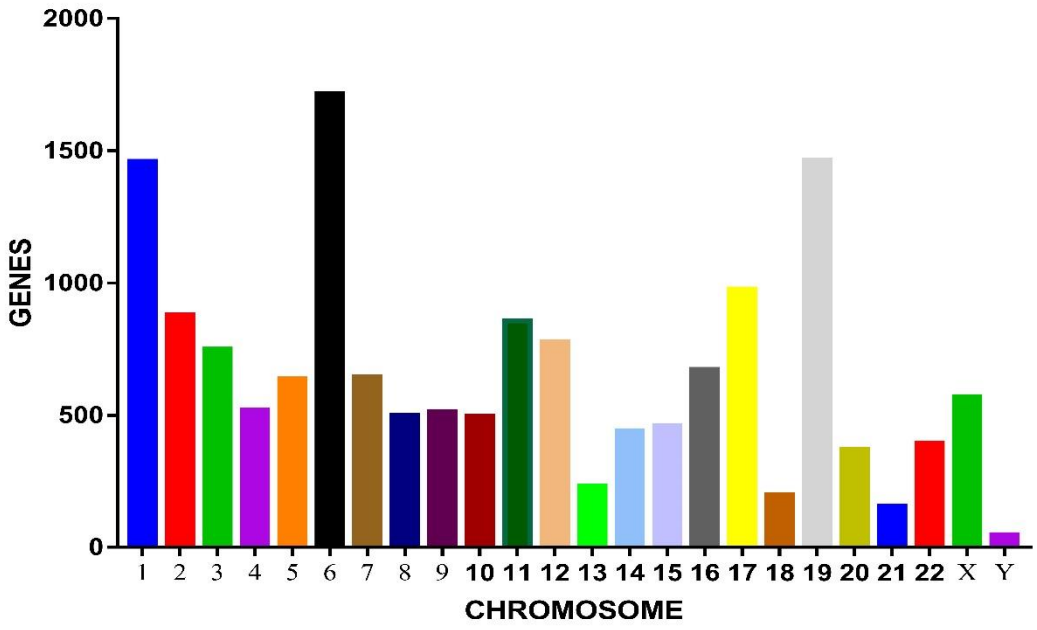


Figure 1 Gene expression in blood

Database of genes related to cancer

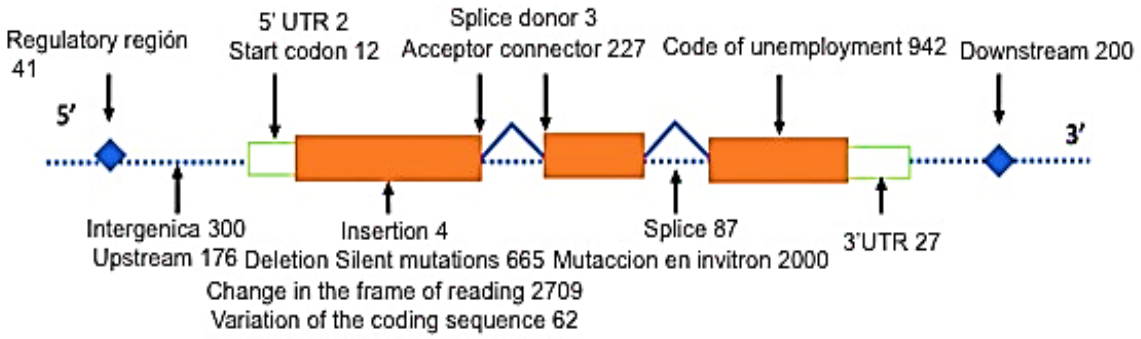
Of the 15744 genes that are expressed in blood, 9.32% (1468 genes) have a relationship with some neoplasm, 54 genes being related to more than three types of neoplasms

(Figure 4) that can be detected in blood using the database reported until December 31, 2017.

Polymorphisms related to cancer and its analysis

In 1468 cancer-related detectable genes in blood, and 7,628 SNP found in 31 types of cancers (Table 1). Figure 3 shows the position in the genome of each SNP, possibly due to the costs of sequencing the complete genome, most of the studies were obtained using RNA-seq. It was determined that the great majority of mutations validated for clinical use, are in transcribed regions (Figure 2). Possibly due to the costs of sequencing complete genomes, there is 726 SNP that has the ability to regulate the expression of transcripts (209 downstream, 176 upstream, 41 regulatory regions and 300 intergenic). Three hundred and seven teen SNPs related to splice alternatives (3-splice donor, 227-splice acceptor and 87 mutations) in introns that allows an alternative splice formed with the introduction of the intron to the mature transcript. There are two thousand mutations in introns related to transcription factors 4439 SNPs in the mature transcript (41 in the UTR regions and 4398 in the exons).

Figure 2 Position of mutations in the genes



Circus Plot

The Circus plot analysis allowed the correlation of 31 types of cancer reported in the databases. The Y chromosome is not included because in the database generated, obtained no sequence of the chromosome, it complied with the requirements established to perform the analysis, the information that allows us to have this database, allows us to visualize more clearly the data obtained in data mining (Figure 5).

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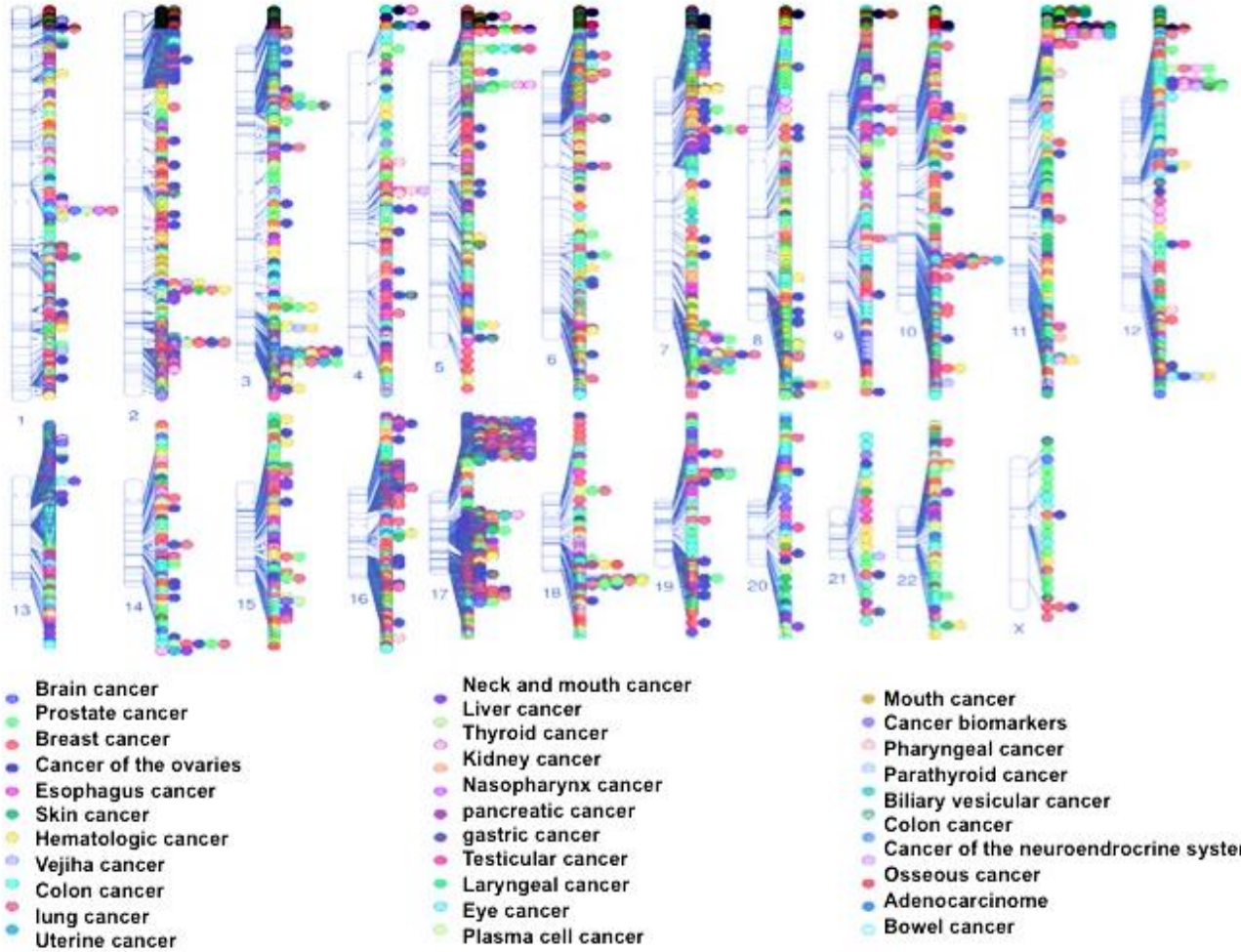

Figure 3 Chromosomal localization of the SNPs related to 31 cancer in the human genome

Discussions

More than 25% of the genes expressed in blood and it is possible to detect with molecular biology techniques from a blood biopsy. Chromosomes 1, 6, and 19 have the highest number of genes expressed in blood, possibly due to linked genes. An example is the short arm of chromosome 6, it contains all the genes belonging to the major histocompatibility complex (Catherine-Sánchez 2013, Siemiatycki, J. *et al.* 2004), which are expressed constantly by the cells of the immune system such as the lymphocytes that it is circulating in blood (Catherine-Sánchez 2013, Cox, D. R., Kartsonaki, C. & Keogh, R. H., 2018). Of the 15744 genes expressed in blood, 9.32% (1468 genes) have at least one related to some neoplasm. Moreover, a maximum of 7,628 SNPs with clinical relevance in detected cancer. The SNP characterized in 31 types of cancer. These cancers are the most common in human population such as breast and skin cancer. Cancers with the highest mortality rate characterized, such as lung cancer and gliomas due to its great impact on society. From the 7,628 SNPs that have clinical significance 4,398 SNPs

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detected by the mRNA, allow the screening panel to detect SNP cancer related. Currently validated the technique to determine if you can detect the 4398 SNP expressed by mRNA.

Conclusion

There are 15,744 genes reported as of December 31, 2017, expressed 1468 genes related to a neoplasm, there are 7,628 SNPs related to at least one neoplasm, up to a maximum of 4,389 SNPs detected in the genes expressed in blood.

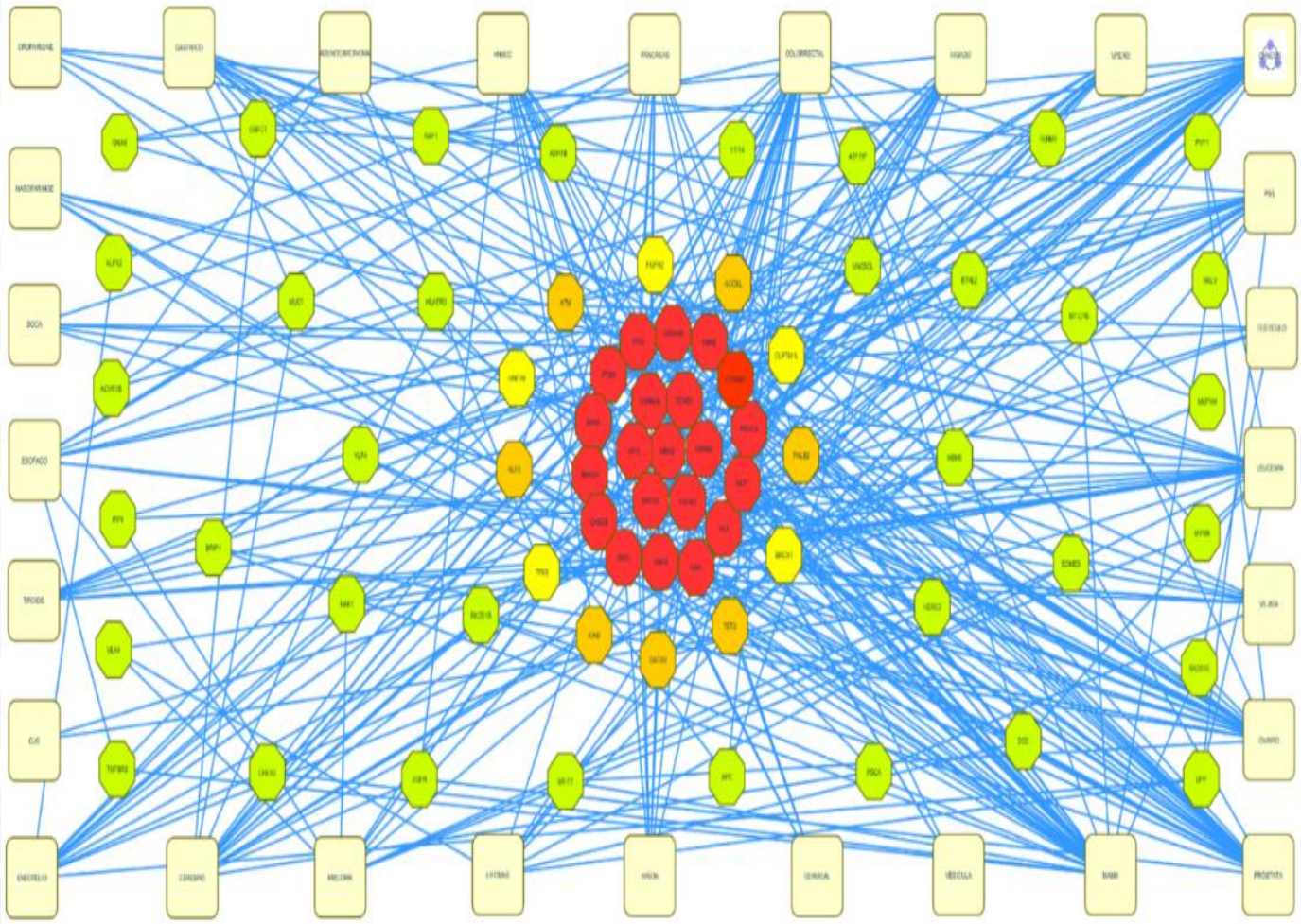

Figure 5 Map of interactions of cancers with genes

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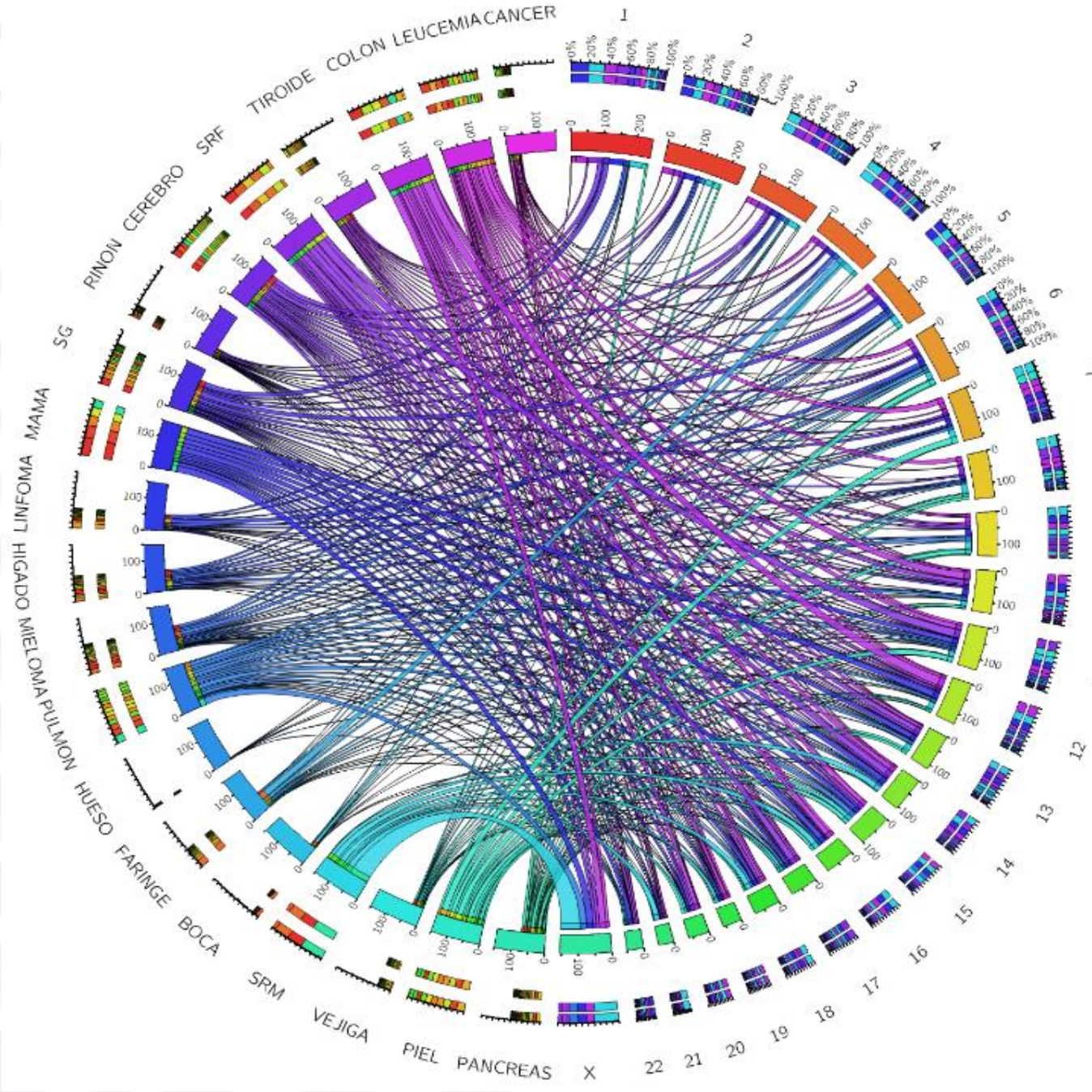

Figure 6 Circus plot for the interaction of cancers with chromosomes.

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Table 1.- SNP related to cancer

CANCER	Subtype	GENES	SNP	Articles
Adenocarcinome	2	9	9	10
Cancer biomarker	1	29	62	4
Cervical cancer	1	6	21	4
Colorectal cancer	2	178	1543	35
Mouth cancer	1	15	18	2
Brain cancer	3	78	144	18
Cervical cancer	1	9	29	5
Esophagus cancer	4	69	101	9
Liver cancer	1	21	243	7
Bone cancer	2	9	10	2
Larynx cancer	1	6	6	1
Breast cancer	1	156	5568	50
Eye cancer	1	3	102	1
Oropharyngeal cancer	1	11	11	1
Ovarian cancer	4	131	4346	9
Pancreatic cancer	1	65	91	9
Parathyroid cancer	1	1	12	1
Skin cancer	5	125	183	11
Prostate cancer	1	235	498	31
Lung cancer	2	100	2167	24
Kidney cancer	3	20	100	6
Testicular cancer	1	22	45	9
Thyroid cancer	1	24	80	5
Bladder cancer	1	33	57	11
Gallbladder cancer	1	3	15	2
Gastric cancer	2	25	86	6
Nasopharynx cancer	1	16	32	4
Endometrial cancer	1	27	66	7
HNSCC	1	65	290	2
Leukemia	4	129	234	20
Lymphoma	3	70	100	11
Multiple myeloma	1	66	121	6

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Protein expression optimization and *in vitro* production of biocomparable bovine interferon-gamma in *Escherichia coli*

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Abstract

Bovine interferon-gamma (BoIFN-γ) is a key regulatory cytokine of the cellular mediated immune (CMI) response; IFN-γ overproduced in bovines infected with the intracellular pathogen *Mycobacterium bovis*. Disease diagnostics is performed through the commercial *in vitro* Bovigam™ test based on direct measure of BoIFN-γ in blood of infected animals, however, this test is expensive and requires it importation, not been considered as a routine test yet for routine diagnosis of bovine tuberculosis. Some attempts made to produce BoIFN-γ in *E. coli*, with the disadvantage that is often present in inclusion bodies, thus tedious purification and renaturation treatments is required. This work aims to obtain a complete and soluble BoIFN-γ molecule from *E. coli* by the used of an optimized gene codon strategy, attempting to produce a bovine protein in a bacterial expression system. BoIFN-γ was purified from *E. coli* BL21 strain from gene His and expressed by tag metal affinity chromatography. Recombinant BoIFN-γ detected in cell lysates as soluble protein and the protein immunologically detected with specific monoclonal antibodies. Bovine immune protein satisfactorily produced in *E. coli* for downstream applications with diagnostic potential.

Key words: recombinant interferon-gamma ● gene codon usage optimization ● heterologous expression ● soluble protein synthesis.

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Introduction:

Interferons are part of a complex network of regulatory cytokines that participate in multitude of cellular activities ranging from control of cellular function and replication, to the defense of the host in response to infection (Samudzi *et al*. 1993).

Currently, based on their biological activity and physicochemical tests, classified interferon into three types: type I, II, and III (Xu *et al*. 2014).

Some cellular immune stimulation IFN-γ is produced by CD4 + T cells, CD8 + cells and Natural Killer (NK) cells response by regulating biological functions such as the

inflammatory response, activating macrophages increasing its anti-mycobacterial and anti-viral capacity (Xu *et al*. 2014).

The open reading frame of the gene sequence coding for BoIFN-γ is composed of 166 aminoacids with a molecular mass of 19,393 kDa (Cerretti *et al*. 1986). Production of BoIFN-γ attempted in mammalian expression systems (Xu *et al*. 2014); however, cell culture protein expression systems are quite expensive and tedious. Diagnosis of some infectious diseases that affects livestock sector carried out through specific tests that targets cellular immune responses. Like BoIFN-γ overproduction considered to be a diagnostic molecule hallmark of *Mycobacterium bovis* infection. It is and animal disease detected *in vitro* with the specific kit Bovigam™ that targets this molecule, however, its scarce use is limited to very few countries due its high cost of importation and management, making necessary to find an alternative method for detection of BoIFN-γ in clinical samples at a lower cost.

An optimized strategy to produce BoIFN-γ to obtain specific antibodies would allow the design of an alternative diagnostic method against livestock diseases including bovine tuberculosis in favor of animal health.

Materials and methods

BoIFN-γ optimized gene. Optimized gene sequence for *E. coli* codon usage for *in vitro* gene expression and translation of BoIFN-γ, was obtained in plasmid pUC57 from GenScript Co. (Piscataway, New Jersey, USA) based in protein sequence from NCBI Protein Bank Database (NCBI reference sequence: NP_776511.1).

Subcloning BoIFN-γ optimized gene in plasmid pET28a. Plasmid pUC57 and pET28a were digested with enzymes *Nco*I and *Xho*I, and mix reaction observed by agarose gel electrophoresis, then fragments of 443 pb and 5369 pb corresponding to BoIFN-γ gene and pET28a, respectively, purified from gel with GenElute extraction kit. Ligation reaction was performed in a 5:1 ratio (gene:plasmid) at 16°C for 18 h and electrophoresed into *E. coli* TOP10, transformants obtained on selective LB agar with kanamycin. Clones recovered and analyzed for plasmid content by isolation and endonuclease analysis as above.

Synthesis of BoIFN-γ in *E. coli* BL21. Plasmid pET28a with BoIFN-γ gene was first electrophoresed to *E. coli* TOP10, then plasmid was isolated again and transformed to chemo competent *E. coli* BL21 cells. Clones randomly selected on kanamycin agar and recombinant plasmid content analyzed as described before. Plasmid expression and protein synthesis was performed in *E. coli* BL21 cell culture at OD_{600nm} of 0.7 with 250 ml of LB broth on 1 Lt flask during 4 hr after addition of 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG). Cells pelleted by centrifugation and supernatant medium discarded, and then, cells re-suspended in PBS and 0.1% Triton X-100 and lysed by ultra-sonication. The protein supernatant obtained by centrifugation at 4°C and used to confirm the presence of BoIFN-γ

by SDS-PAGE and Coomassie staining and Western blot on nitrocellulose sheets with both, commercial mouse anti-His6x and rat monoclonal anti-BoIFN-γ antibodies. BoIFN-γ identity confirmed with *in vitro* test kit Bovigam^{TM}, a specific ELISA test for BoIFN-γ in clinical samples.

Recombinant BoIFN-γ purification. BoIFN-γ in supernatant had His-tag metal affinity purified with 5 ml Bio-Scale mini Profinity IMAC cartridges (Bio-Rad), bound proteins were eluted with 250 mM imidazole, and eluted fractions were dialyzed by membrane tubing against 25 mM HEPES (pH=7.5) 10% glycerol, 0.15 M NaCl, and then, subjected to SDS-PAGE. Protein bands were visualized by Coomassie staining and Western-blot as described above.

Results

Recombinant plasmid pET28a with BoIFN-γ gene. For gene subcloning of BoIFN-γ protein (Figure 1A) into plasmid pET28a, complete open reading frame of optimized gene (Figure 1B) was obtained from pUC57 by endonuclease restriction with *Nco*I and *Xho*I, and then, incorporated by enzyme ligation into enzyme excised plasmid pET28a (Figure 2A), and electroporated to *E. coli* TOP10 strain for plasmid multiplication.

A.

MGQGQFFREIENLKEYFNASSPDVAKGGPLFSEILKNWKDESDKKIIQSQIVSFYFKLFE
NLKDNQVIQRSMDIIKQDMFQKFLNGSSEKLEDFKKLIQIPVDDLQIQRKAINELIKVMN
DLSPKSNLRKRKRSQNLFRGRRAST

B.

ATGGGTCAGGGCCAATTCTTTCGTGAGATTGAAAACCTGAAGGAGTACTTCAACGCGAGCAGCCCGGATGTGGCG
AAAGGTGGCCCGCTGTTTAGCGAGATCCTGAAGAACTGGAAAGACGAAAGCGATAAGAAAATCATTCAGAGCCAA
ATTGTGAGCTTCTATTTTAAGCTGTTCGAAAACCTGAAAGATAACCAGGTTATCCAACGTAGCATGGACATCATC
AAGCAGGATATGTTCCAAAAGTTCCTGAACGGTAGCAGCGAGAAGCTGGAAGACTTTAAGAAACTGATCCAGATT
CCGGTGGACGATCTGCAGATCCAGCGTAAGGCGATCAACGAGCTGATTAAAGTTATGAACGATCTGAGCCCGAAA
AGCAACCTGCGTAAGCGTAAACGTAGCCAAAACCTGTTCCGTGGCCGTCGTGCGAGCACC

Figure 1. Complete IFN-γ protein from *Bos taurus* (A), and optimized gene (B) for efficient expression in *E. coli*.

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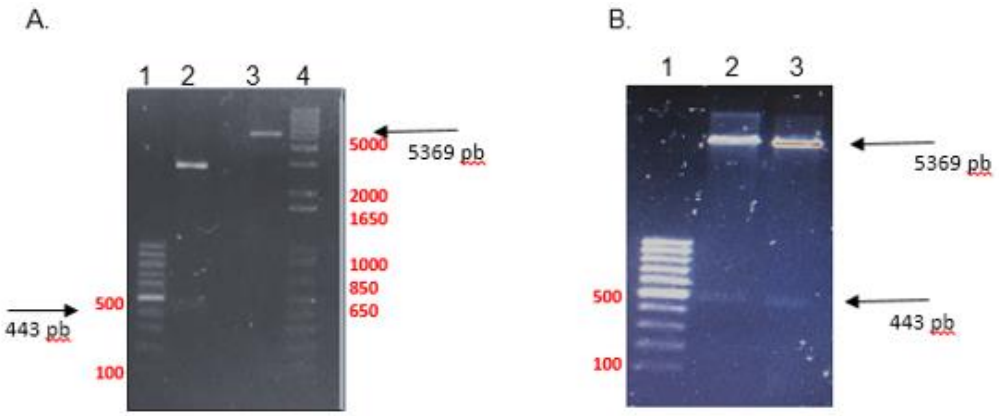

Figure 2. (A) Digested pUC57: IFN-γ (line 2), and pET28a (line 3) with *Xho*I and *Nco*I for subcloning. (B) PET28a: IFN-γ plasmid from *E. coli* BL21 strain after enzyme digestion.

Afterwards, recombinant plasmid pET28a:IFN-γ was obtained and introduced to *E. coli* BL21 strain, then analyzed as described from one selected clone (Figure 2B) for gene insert confirmation.

Bovine IFN-γ synthesis and purification. Recombinant BoIFN-γ was metal-affinity purified from lysate supernatant from *E. coli* BL21 cultures. After protein cell induction with IPTG, lysate culture was analyzed by Coomassie staining and Western blot (Figure 3A and B) with mouse anti-His tag monoclonal antibody, showing a 19 kDa band observation of BoIFN-γ in both, cellular and soluble lysate fractions, indicating a high and optimal protein synthesis.

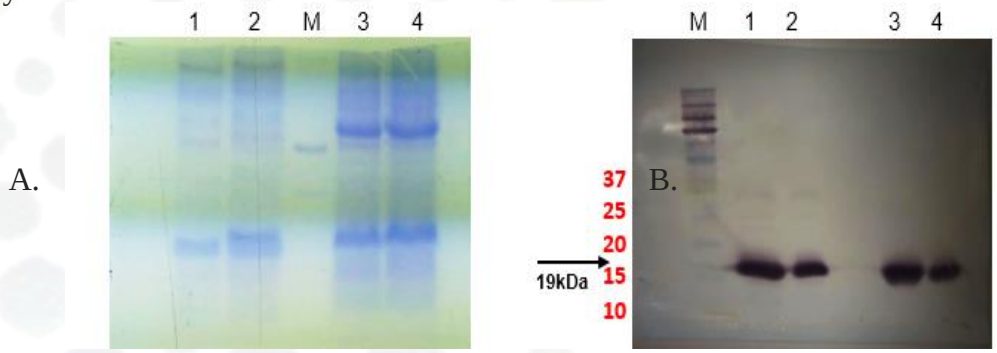

Figure 3. SDS-PAGE and Western blot of a cell lysate after protein synthesis induction in *E. coli*. Supernatant (lines 1 and 2) and pellet (lines 3 and 4) were observed by Coomassie staining (A), and by mouse anti-His tag monoclonal antibody (B). Molecular size standards are shown (M).

Then, BoIFN-γ from supernatant purified by metal affinity His-tag chromatography, and cleared bovine protein assessed by Coomassie staining after SDS-PAGE in final elution

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fractions (Figure 4), showing a good affinity of BoIFN-γ of 19 kDa in metal matrix and without need of special treatment for protein recovery.

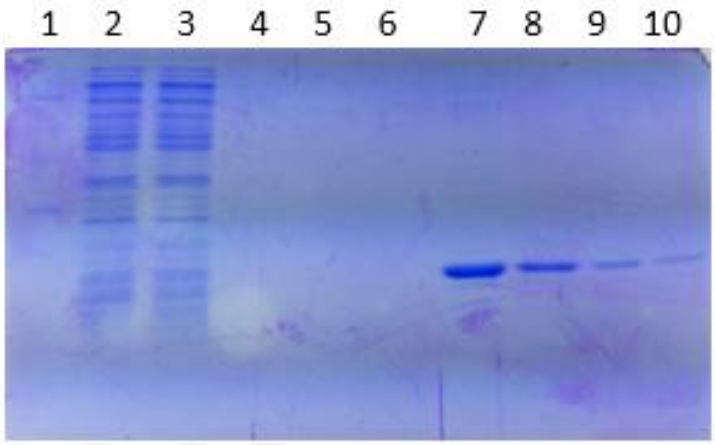

Figure 4. SDS-PAGE of supernatant from *E. coli* after metal-affinity chromathography and protein purification. Lines: 1, molecular weight standards; 2 unbound body; 3-6, buffer washed fractions; 7-10, eluted fractions with bound protein.

Immunodetection of recombinant BoIFN-γ. Identity of purified recombinant protein was assessed immunologically with monoclonal anti-BoIFN-γ antibody (R&D Systems) by Western blot (Figure 5A) with detection of a 19 kDa specific protein and, by sandwich ELISA kit Bovigam™ for *in vitro* detection of BoIFN-γ, with confirmation of interferon-gamma molecule in purified bacterial cell lysate (Figure 5B).

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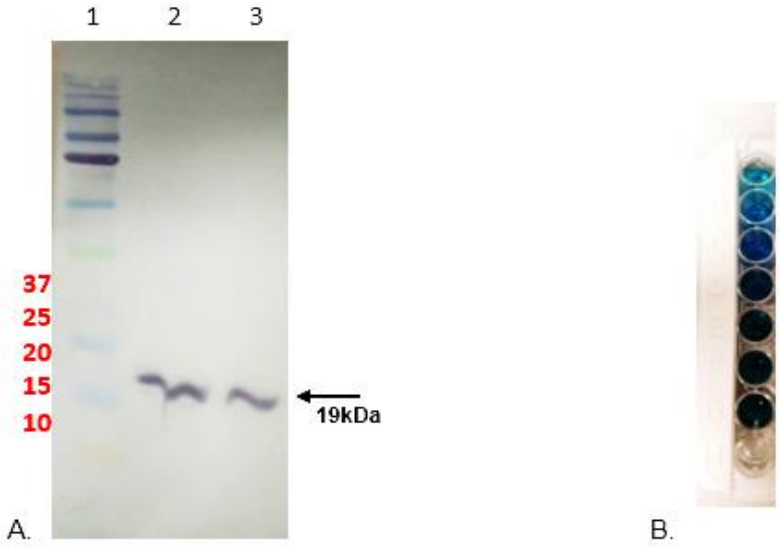

Figure 5. Specific immunodetection of BoIFN-γ by Western blot with a monoclonal antibody of recombinant (line 2) and a commercial (line 3) BoIFN-γ (A). Increasing dilutions (wells B-G) of recombinant molecule were measured with ELISA Bovigam™ kit, positive (well A) and negative (well H) controls.

Discussion

IFN-γ is a cytokine with extended interest and demand for immunotherapy and diagnosis which biological synthesis has advanced greatly since first cloning of the associated gene. Because some biosynthetic methods have considerable technical and economical disadvantages over bacterial expression systems, in this work we attempted to produce BoIFN-γ in *E. coli* to produce protein synthesis without losing yield and protein identity by taking into account the knowledge of *E. coli* codon usage and translation system. Thus, we produced a structurally comparable BoIFN-γ molecule that could be an alternative way to produce this molecule for antigenic purposes. However, considering that posttranslational modifications are needed for IFN-γ activity, bacterially synthetized molecule are in disadvantage, as has been discussed by Jalali *et al*. (2010) who have further improved biosynthesis of the molecule using eukaryotic cells. Even so, *E. coli* biosynthesis would favor high over production of animal proteins for secondary application such as production of biologicals with diagnostic potential in the biopharmaceutical industry (García *et al*. 2013).

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Conclusion

Production of BoIFN-γ in *E. coli*, besides gene codon optimization, with great success yielding a molecule with immunological identity matching that of the native one. The present work shows that biosynthesis of BoIFN-γ in bacterial expression systems can be performed by codon usage optimization of gene sequence avoiding aggregate formations of recombinant protein allowing good performing of downstream applications such as production of monoclonal and polyclonal antibodies with diagnostic potential.

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Nutritional Biotechnology: Food, Nutrition science and Nutraceuticals.

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Nutrimental characterization of a sweet food prepared with *Agave* spp. in the Mixteca Oaxaqueña

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Abstract

Oaxaca is characterized for having a great biodiversity used for the elaboration of food. The agaves (*Agave* spp.), traditionally known as "magueys" have been used by the Mixtecan communities of Oaxaca as a basic raw material to make traditional sweet food, to which complete coyul or clover plants (*Oxalis lasiandra*) is added. Considering the phenological characteristics that indicate that the plant reached its physiological maturity, the farmers select the agaves to ensure a sweet product. The ratio of the ingredients is 2:1 maguey/coyul; the cooking time is 5 to 7 hours. During the cooking process, the carbohydrates of the *Agave* hydrolyzed by the acidity of the *Oxalis* plants, breaking the fructooligosaccharides until the formation of glucose and fructose. The process characterized from three species of *Agave*: *A. nussaviorum, A. potatorum*, and *A. seemanniana*, finding that the sweet food prepared with *A. nussaviorum* reports the best values of carbohydrate content with 7.43 g / 100 g and 1.1 g of glucose / 100 g. In general, traditional sweet food can be considered as a prebiotic.

Key words: Maguey ● Coyul ● Food ● Culture.

Introduction

Fructans are the reserve molecules of the Agaves: they are fructose polymers, linked by β-type glycosidic bonds 2-1, which have a degree of polymerization of more than 10 units (Olvera *et al* 2007). In different communities of the Mixteca Oaxaqueña sweet food with Maguey (*Agave* spp.) and Coyul (*Oxalis lasiandra*) is prepared and consumed. The hydrolysis of inulin or fructooligosaccharides depends on factors such as temperature, pH and cooking time; so it is important to control these parameters, since, at high temperatures, the reducing sugars degraded by the Maillard reaction or a caramelization (Cedeño, 1995; Téllez, 1998). As mentioned, the traditional sweet is produced by the acidic and thermal hydrolysis of fructans during cooking; Acid hydrolysis is attributed to plants of the genus *Oxalis*, which have a weak acid that confers hydrolyzing properties on the reserve polysaccharide of *Agave*. This process aimed to determine the physical and chemical properties of traditional sweet food, comparing also the differences that exist in its

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preparation when using different species of *Agave*, which determines its properties and quality, as well as its impact as a prebiotic.

Materials and metods

The study comprised two stages: 1) field work for the recognition and systematization of the complete process of traditional food preparation, in six communities: San Juan Tamazola and Santa María Apazco belonging to the Nochixtlán district; Santa María Cuquila, Santo Tomas Ocotepec, Santiago Nuyoo, and San Esteban Atatlahuca of the Tlaxiaco district. 2) the physical-chemical analysis of the product, determining, a) the reducing sugars based on the official standard NMX-F-312-1978, b) Brix degrees, according to NMX-F-274-1984, c) the pH, d) fructose content by the method of Hessler (1959), based on the reaction of p-anisidine in phosphoric acid, e) the determination of glucose by the enzymatic method, making a modification to the colorimetric test (Glucose-LQ GOP-POP Liquid Spinreact®). Finally, the bromatological analysis in order to recognize the nutritional value of the sweet food was carried out according to Trinder (1969) and the regulations of the American Organization of Analytical Chemistry, (Horwitz 1975).

Results and discussion In the elaboration of the traditional sweet food pieces of three species of *Agave* are used: *A. nussaviorum A. potatorum*, and *A. seemanniana*; as well as stems and leaves of *Oxalis lasiandra* (Figure 1); and water in sufficient quantities.

Figure 1. Ingredients: *Oxalis lasiandra* (A) and *Agave potatorum* (B).

The proportion of *Oxalis lasiandra-Agave* spp. is used to make the sweet food in the communities is approximately 2:1; that is, for each Kg of Coyul used, two Kg of Maguey were needed. The cooking temperature of the sweet food oscillated between 85 and 95 °C and the time that this process lasted was from 5 to 7 hours.

Regarding the pH of the water used for the cooking of the traditional food, the values were very close to the neutral (between 6.49 and 7.72) in almost all the communities studied,

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except in Santa María Apazco, where the pH value was alkaline (9.17). With respect to the *Agave* used as a raw material, the pH values were acidic in all the samples. For its part, the pH of the resulting sweet food presented acid values between 3.15 and 3.86, which gives it a bittersweet taste characteristic of the food (Figure 2).

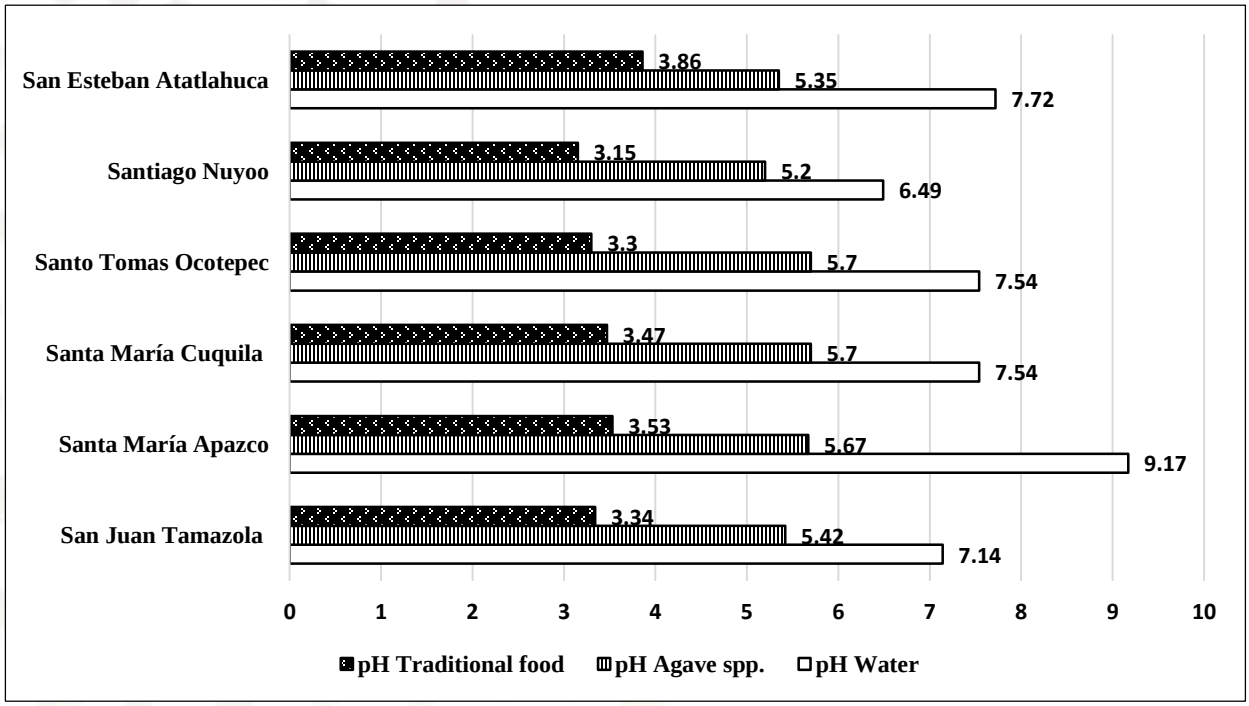

Figure 2. pH values of the finished sweet food and the ingredients.

During the cooking of the sweet food, samples were taken every hour, with the purpose of recognizing the kinetics of maguey hydrolysis, which had its beginning under pH conditions of 4.04, continuing with a hydrolytic process of pH below 4 and concluding with a final product with a pH value of 3.96 (figure 3A). On the other hand, the behavior of the Brix degrees during the cooking of the traditional food, start with values of 7.5, which gradually increased until reaching values of a little more than twice the initial ones; The above can be explained if we consider that the acid pH of *Oxalis* directly influences the fructo-oligosaccharides breakdown.

The preliminary bromatological analysis practiced on the samples of the traditional sweet indicates that the protein values of *A. potatorum* are better than the content in any of the industrialized sweets. Regarding the values of energy content, sweet food has a very important value, since it helps consumers lead a healthier nutritional life (Table 1).

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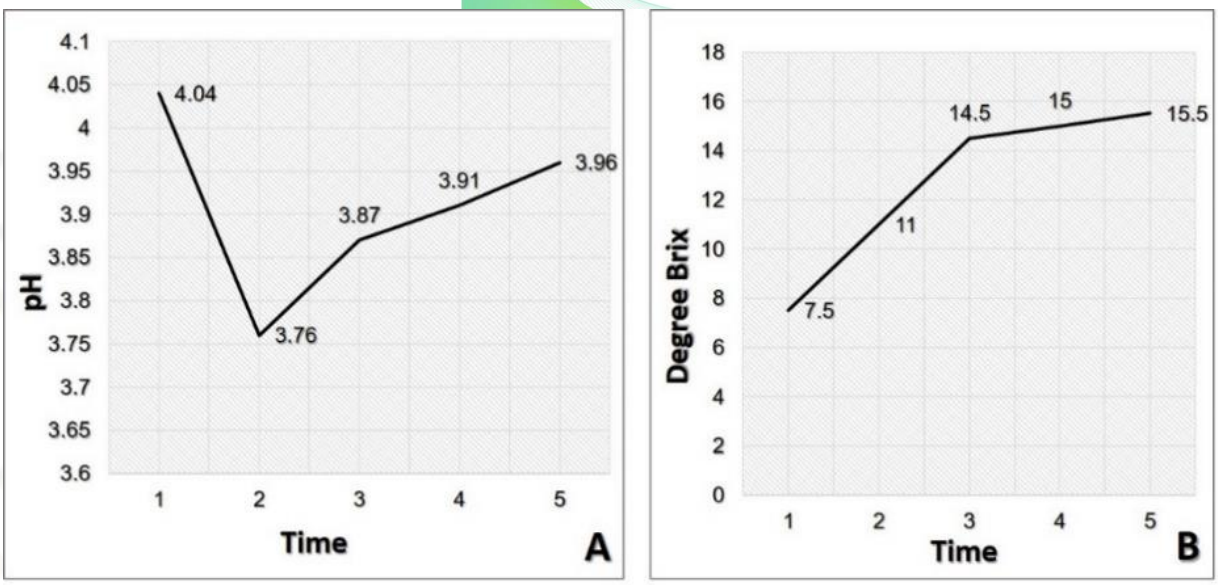

Figure 3. Kinetics of hydrolysis (A) and the behavior of Grades Brix (B).

Table 1. Bromatological results of traditional sweet food

Parameter	**g/100 g sample *(A. potatorum)***
Protein	0.35
Ethereal extract	0.19
Raw fiber	1.72
Ashes	0.31
Humidity	91.00
Carbohydrates	6.43
Energetic content	28.83 kcal/100 g= 120. 68 KJ/100 g

Table 2 shows the difference between the contents of available reducing sugars (ARD) and total reducing sugars (ART) in the finished sweet food, which can be assumed that the fructooligosaccharides are not completely hydrolyzed; that is to say that in the sweet there are still whole fructans. With regard to glucose and fructose content, the best values are present in *Agave nussaviorum*.

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Table 2. Physicochemical parameters and content of available reducing sugars (ARD) and total reducing sugars (ART) of the traditional sweet food.

Parameter	Cooking (h)	pH	Brix degree	ARD (%)	ART (%)	Glucose (g)	Fructose (g)
A. potatorum	3.30	3.6	11	6.5	7.9	1.32	6.05
A. seemanniana	2.20	3.5	7	2.7	3.4	0.02	2.03
A. nussaviorum	3.0	3.3	15	9.2	10.8	1.05	8.91

As a result of the semi-structured surveys applied in the villages where the study was conducted, the Mixteco names were identified with those that identify the *Agave*, the *Oxalis*, the traditional processed sweet, as well as the name of the place where the materials were harvested (Table 3).

Table 3. Mixteco names of the raw materials, of the place of *Agave* obtaining, as well as of the processed sweet food.

Villages	*Agave*	*Oxalis*	Processed sweet food	Place where the *Agave* was obtained
San Juan Tamazola	***Yavi pasmo***	***Ntúje***	***Yavi ntu ˈdu*** (Sweet *Agave*)	***ntu ˈve ntiyi*** (The cross)
Santa Maria Cuquila	***Yavi tikunchi***	***Ntu ˈja***	***Yavi kaa ˈo*** (*Agave* to eat)	***xi ˈi yuku*** (Next to the hill)
Santa María Apasco	***Yavi***	***Tinduza***	***Yavi saanto ˈo*** (Edible *Agave*)	***Sa ˈa kava nu ˈu ntiku*** (Hill of burning)
Santiago Nuyoo	***Yavi a yao***	***Nuxia***	***Yavi ya ˈo*** (Agave to eat)	***Siki Yuku*** (Above the hill)
Santo Tomas Ocotepec	***Yavi tikunchi***	***Ntu ˈja***	***Yavi vixi*** (Sweet *Agave*)	***Yute ranchu*** (Ranch river)
San Estaban Atatlahuca	***Yavu***	***ntu ˈja***	***Yavu vixi*** (Sweet *Agave*)	***Yuku ntichi*** (Hill inclined)

Conclusions

During the characterization of the traditional sweet in six communities of the Mixteca region of Oaxaca, it found that three different species of *Agave* are used: *A. nussaviorum*, *A. potatorum*, and *A. seemanniana*. The phenotypic characteristics referred to the state of maturity of the *Agave*, are of great importance since they assure the elaboration of a traditional sweet of an excellent quality. The proportion of the ingredients *Agave* spp. and *Oxalis lasiandra* is 2:1. The cooking temperature is in the range of 80 to 95 ° C, with a cooking time of 5 to 7 hours. The bromatological analysis of this traditional Mixteco sweet

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food made it possible to recognize its nutritional composition and this should serve to emphasize not only its cultural value, but above all its nutritional value. The determinations of the sugars applied to the sweet food indicate that the fructose content is 7.43 g / 100 g of sample and 1.10 g of glucose / 100 g of sample, which indicates that the food is very rich in fructose, which is the basic structural unit constituting the fructooligosaccharides of the *Agave* used. In the case of the Mixtec names of the ingredients and the process, it was found that in each town the evolution of this living language is accentuated.

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Area IV

GREEN

Agricultural and Livestock Biotechnology: biotechnologies for production, processing, and storage, biofertilizers and agrobiochemicals, agricultural pests and disease control, ecology and rational wildlife management, preservation of biodiversity, plant, pet, and farm-animal, tissue culture, and health, nutrition, reproduction, cloning, and genetic modification, plant micropropagation, bioremediation & environmental biotechnology, sustainable and renewable energy generation; bioremediation & environmental biotechnology; bio-fuel production; biotechnology for competitive production using new material and new energy sources.

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Diagnosis and molecular analysis from *Drosophila simulans* and *Zaprionus indianus* (Diptera: Drosophilidae), insects of agricultural importance

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Abstract

Insect pest affects to different crops, within the insects we find order Diptera, and especially the family Drosophilidae. *Drosophila simulans* and *Zaprionus indianus* are one of the main causes of crop losses. This species can be collected from variety of rotting fruits or in the process of fermentation, due to its great morphological similarity with other species in larvae stage and in some times in adult stage as *D. melanogaster*. The present work has the purpose of describing an accurate diagnostic method for the identification of *D. simulans* and *Z. indianus*, which includes the morphological study of the insects and molecular analysis of a coding region of the COI gene. With this methodology it is expected the inclusion of diagnostic techniques that allow the use of more accurate insect id tools.

Key words: Flies ● Fruit ● Phylogenetic analysis ● COI gene

Introduction

Pests are one of the main causes of crop losses. The problem is exacerbated in developing countries, like most Latin American countries, including Mexico (Ruíz-Corral *et al*., 2013). Pests that can affect a crop are fungi, viruses, bacteria, and insects. Within the insects, we find order Diptera, and especially the family Drosophilidae. This family contains the genera *Drosophila* Fallén, 1823 and *Zaprionus* Coquillett, 1902 (Figures 1a-c), among others (Markow & O´Grady, 2006). Its life cycle comprises four development stages: egg, larva, pupa and adult *(*McAlpine *et al*., 1981).

Z. indianus Gupta is an invasive species. This species is native from Africa and moved to the American continent in the 90´s with first record in Brazil. On the North American continent, detected for the first time in Mexico in 2002 and in EUA in 2005. Today, this species is present in America, Europa, Asia and Africa. *Z. indianus* is considered a secondary pest that

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can live on a wide range of fruit species (about 80 species). However, it has been able to cause economic damage as a primary pest to fig crops (*Ficus carica*) in Brazil. On the other hand, *D. simulans* is reported in several localities of Asia, Africa, America, Europe and Oceania, therefore has wide distribution. This species can be collected from variety of rotting fruits or in the process of fermentation. *D. simulans* can be confused with others species of the genus *Drosophila* (Markow & O´Grandy, 2006) due to its great morphological similarity with other species in larvae stage and in some times in adult stage as *D. melanogaster*. In this respect, detailed analysis of collections *D. simulans* was described as *D. melanogaster* (Capy & Gibert, 2004).

The present work has the purpose of describing an accurate diagnostic method for the identification of *D. simulans* and *Z. indianus*, which includes the morphological study of the insects and molecular analysis of a coding region of the COI gene. With this methodology is expected, the inclusion of diagnostic techniques that allow the use of more accurate tools, especially when pests are in a larval state since their identification is often confused with traditional techniques

Materials and methods

Morphological identification

The larva must preserve its morphological characteristics, for this it is necessary to place the larvae in a beakers with boiling water for 1 min and then place it in a bottle, Kimble clear glass straight-sided jar with ethyl alcohol 70 % to preservation (Triplehorn & Johnson, 2005). Subsequently placed in a porcelain capsule of 41 mm in diameter for the revision of the morphological structures with the stereo microscope Model Discovery V12 Carl Zeiss: perianal pad, anterior spiracle, cephalopharyngeal skeleton form, cornua dorsal, present tooth (Stehr, 1991). However, you can only identify to genus or in many cases to family with taxonomic keys.

Molecular identification

DNA extraction

The collected insects were stored in 70 % ethyl alcohol until the extraction of the nucleic acids. The extraction of DNA began by placing the insects independently in a tube and adding liquid nitrogen, was macerated with the help of a pistil to obtain a fine powder. Subsequently, 600 µL of 2 % CTAB extraction buffer was added with 1 M NaCl, pH 9.5; 4 µL of enzyme proteinase K. Subsequently, it was homogenized by inversion. Then, the samples were incubated at 65 ° C for 25 min to lyse the cells. Finally, extraction was carried out using the protocol with hexadecylmethylammonium bromide (CTAB) reported by Doyle and Doyle (Doyle & Doyle, 1991)

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Amplification by PCR and sequence analysis

PCR was performed using primers C1-J-1718 (5'-GGAGGATTTGGAAATTGATTAGTTCC-3') and C1-N-2191 (5'-CCCGGTAAAATTAAAATATAAACTTC-3'), which amplify a 525 bp fragment of the COI gene (Simons, Frati, Beckenbach, & Crespi, 1994) (Barcenas, Unruh, & Neven, 2005). The reaction mixture consisted of 97.5 µL of water (Invitrogen), 15 µL of Buffer ($MgCl_2$) (Invitrogen), 7.5 µL of $MgCl_2$ (Invitrogen), 6 µL of the primer C1-J-1718 (10 µM), 6 µL of the primer C1-N-2191 (10 µM), 3 µL of TaqPol (Invitrogen) and 3 µL of the DNA template, in a final volume of 25 µL. Thermocycling under the following conditions: 1 cycle of 95 ˚C for 2 min, 1 cycle at 95 ˚C for 15 s; 40 cycles, 50 ˚C for 30 s, 72 ˚C for 45 s and 72 ˚C for 7 min during 1 cycle final extension. The amplified products were visualized on a 1.5 % agarose gel.

The PCR products were sequenced in the Laboratorio de Biología Molecular del Centro Nacional de Referencia Fitosanitaria, using the Applied Biosystems model 3130; the sequences obtained were assembled and edited to obtain 486 bp sizes and compared in the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). In addition, they were grouped together to do a local alignment using the MEGA program v7.0.26 and the MUSCLE algorithm. A matrix with sequences of *D. simulans* and *Z. indianus* reported in the NCBI database was constructed to perform a phylogenetic analysis. The sequence corresponding to *Haematobia irritans* was used as an external group (KU599978.1). The Maximum Likelihood (ML) method based on the Tamura-Nei model was used, with 1000 bootstrap repeats. The initial trees for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of distances by estimated pairs using the Maximum Composite Likelihood (MCL) approach and selecting the topology with a higher log likelihood value with the MEGA program. v7.0.26 (Kumar, Stecher, & Tamura, 2016).

Results

All samples analyzed amplified the expected product of 525 bp (Figure 1e). The sequences obtained from samples L1, L2, L3, L4, L5, L6, L7 and M7 had 100% identity with the sequence identified as *Z. indianus* haplotype 7 cytochrome oxidase subunit I gene (KC994629.1). samples L8 with 99 % identity to the one reported with the access number (KJ463786.1) and L10 100 % identity to that reported with the access number (KC994631.1) corresponding to *Z. indianus*.

On the other hand, samples LB1, LB2, LB3 and LB4 had 99% identity with the sequence identified as *D. simulans* strain 14021-0251.167 cytochrome C oxidase subunit I (COI) gene (KJ767247.1).

In the tree with the highest probability of registration (1105.30) it is observed that the sequences of the samples L1-L10 were grouped within the node that has the sequences of *Z. indianus* (KC994629.1, EF632359.1, EF632363.1 and KC994631.1) and samples LB1-LB4 were grouped within the node having the *D. simulans* sequences (KJ767247.1 and KJ671606.1) (Figure 1e).

Discussion

The sequences analyzed by BLAST demonstrate that by using the pair of oligonucleotides C1-J-1718/C1-N-2191, a fragment of the COI gene is obtained, which identifies by means of phylogeny the identification of *D. simulans* and *Z. indianus* with more than 99% identity with respect to sequences reported in the databases for these insects. Markow & O´Grandy (2006) mentioned that without microscope, this is impossible, the females are indistinguishable even with a microscope, so that simply pooling flies which look alike could produce mixed-species cultures, because they form a group of sister species.

Conclusions

By means of the morphological and molecular analyzes of *D. simulans* and *Z. indianus* presented in the methodology described in this work, it is possible to unequivocally identify the genus and species of members of the Drosophilidae family. The diagnosis by molecular techniques allows identifying at the species level the larvae include into the Drosophilidae family, without the need to obtain or to require the adult state of the species.

Acknowledgments

We thank Abrego-Álvarez O. G. for photographs, Hérnandez-Sosa L. for their generosity in facilitating the specimens for this study and Centro Nacional de Referencia Fitosanitaria by the facilities and equipment.

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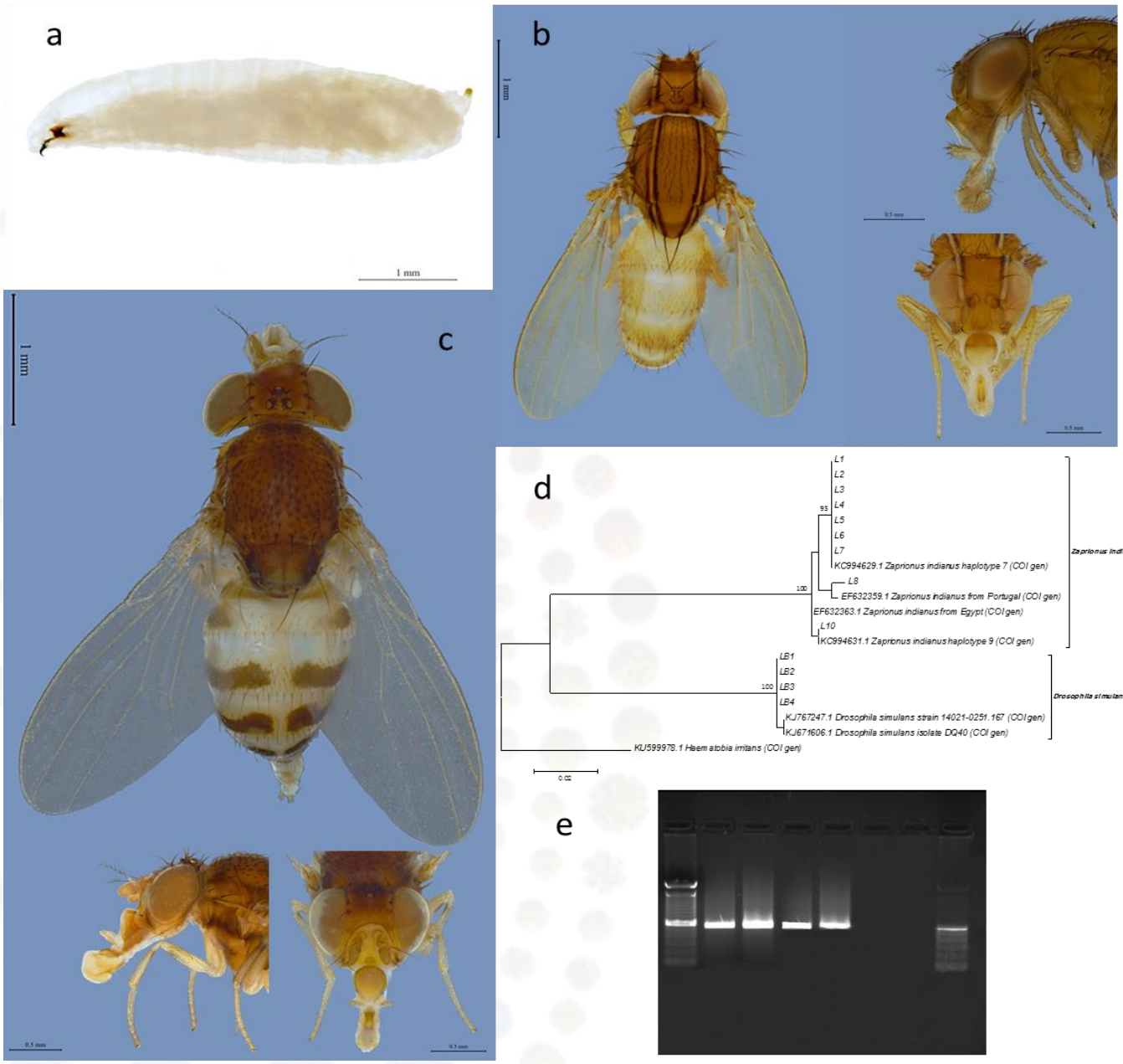

Figure 1. *D. simulans* and *Z. indianus* (Diptera: Drosophilidae). a) Larvae of *Z. indianus*; b) *Z. indianus* adult, dorsal, lateral and front view; c) *D. simulans* adult, dorsal, lateral and front view; d) The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model; e) Agarose gel of *D. simulans* larvae.

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Aseptic establishment and shoot induction from nodal explants of avocado cv. San Miguel

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Abstract

The Avocado 'Hass' is the cultivar most widely produced, however it is poorly adapted to low-lands with hot climates like Northwest Mexico, where other avocado genotypes with interesting traits are well-adapted. In the present work, we aim to establish disinfection and organogenesis protocols from nodal explants of avocado, for the conservation of germplasm adapted to Northwest Mexico. Avocado (cv. San Miguel) nodal explants were selected in orchards of Guasave, Sinaloa, and subjected to a disinfection train consisting of immersion of explants in different solutions of detergent, fungicide, bactericide, ethanol, chlorine, and antioxidants, followed by its culture under controlled conditions in two basal media (MS or Yasuda) supplemented with two microbicides (PTC[3]® or ceftriaxone). The disinfection protocol was effective to keep contamination rates below 20% and oxidation at 0% after two weeks of culture, and Yasuda medium + ceftriaxone showed the highest shoot induction rate after four weeks of culture. This protocol will be useful to establish an *in vitro* germplasm bank with avocado accessions from Northwest Mexico.

Keywords: contamination ● micropropagation ● oxidation ● *Persea americana.*

Introduction

In Northwest Mexico there are a great diversity of avocado (*Persea americana*) genotypes with outstanding characteristics over commercial varieties, for example, a higher content of α-tocopherol and β-sitosterol (Peraza-Magallanes, 2015), greater resistance to enzymatic browning (Higuera-Rubio, 2016), and greater antioxidant capacity (Valdéz-Agramón, 2017). However, these genotypes are not in any germplasm bank and thus are susceptible to genetic erosion. Germplasm banks can be created through traditional propagation (*in situ* or *ex situ*), or through *in vitro* culture techniques that allows the massive clonal propagation of plants in a short period, under controlled conditions and small spaces, in addition to guaranteeing sanity and genetic stability (Kumar and Loh, 2012). The present research seeks the *in vitro*

establishment of avocado germplasm adapted to Northwest Mexico for its conservation and biotechnological exploitation.

Materials and methods

Disinfection protocol

Tissue samples of avocado (cv. San Miguel) adult trees were collected in orchards (Figures 1a and 1b) located in Guasave, Sinaloa, then transported in coolers to the laboratory, and subjected to a disinfection process adapted from Vidales-Fernández (2002), and Ibarra-López *et al*. (2016), with modifications. Stems with multiple axillary buds were selected and washed with running tap water (Figure 1c), then cut into segments of 2 - 3 cm and washed with distilled water for 30 min, followed by three washes (10 min each) in a soapy solution. Subsequently, the explants immersed for 24 h in a fungicide-bactericide (Figure 1d) solution with: 1.5 mL/L Azimut® (azoxystrobin + tebuconazole) + 2 g/L Agry-Gent® (gentamicin + oxytetracycline) + 30 g/L sucrose. The explants were rinsed three times with sterile distilled water to remove the agrochemicals, and taken to a laminar flow hood for its surface sterilization with 70% ethanol for 30 s, followed by immersion in NaClO (3.5%) + tween-20 (0.2%) for 30 min. Then, the explants rinsed three times with sterile distilled water and immersed in a solution of ascorbic acid and citric acid (150 mg/L each) for 30 min (Figure 1e).

Aseptic establishment and shoot induction

The disinfected explants were sectioned into segments of 1 cm in length, and were cultured (Figure 1f) in MS (Murashige and Skoog, 1965) or Yasuda (Yasuda *et al*., 1985) media supplemented with 1.5 mL/L PTC^{3}® (Plant Tissue Culture Contamination Control, Phytotechlab) or 300 mg/L ceftriaxone (Cef). All four media contained 8 g/L agar, 1 mL/L Gamborg vitamins, 1.5 mL/L Azimut®, 150 mg/L ascorbic acid, 150 mg/L citric acid, 2.0 mg/L benzylaminopurine, 0.1 mg/L indolacetic acid and 0.5 mg/L giberellic acid, and the pH adjusted to 5.7. Explants were cultured during 4 weeks at 26 ºC and 16:8 photoperiod, with the following response variables recorded weekly: bacterial contamination, fungal contamination, oxidation and shoot induction. Ten replicates per treatment were used for data analysis, each replicate consisted in three vessels with one nodal explant each. Since data did not fit a normal distribution, the results were analyzed with the Kruskal-Wallis test to identify significant differences ($P < 0.05$) between treatments, using the Statgraphics Plus 5.1 software.

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Results

Standardization of disinfection protocol

In preliminary assays, it was observed that 1.5 h of treatment with fungicide-bactericide solution as recommended by Ibarra-López *et al*. (2016), was insufficient to reduce the high microbial charge of avocado nodal explants collected in Guasave, Sinaloa, leading to contamination rates up to 100% in the first trials (data not shown). Therefore, different times were evaluated (1.5 to 48 h) and found that 24 h of exposure to fungicide-bactericide solution was effective to reduce the contamination to levels below 50% without causing excessive damage to the nodal explants. Another problem was the oxidation of explants during *in vitro* establishment. Different antioxidants added to the medium were evaluated (1 g/L activated charcoal, 10 mg/L L-cysteine, 150 mg/L ascorbic acid and 150 mg/L citric acid), and it was found that ascorbic acid and citric acid were effective to reduce the oxidation of nodal explants below 50% (data not shown). Therefore, both ascorbic and citric acids were added to the culture media and used in a solution to immerse the explants after its surface sterilization in the laminar flow hood.

Aseptic establishment of nodal explants and shoot induction

Once standardized the disinfection protocol, the effects of basal media (MS or Yasuda) and microbicides (PTC^3 or Cef) on contamination, oxidation and shoot induction were evaluated. The bacterial and fungal contamination of explants was noticeable after two weeks of culture and increased progressively during incubation. After four weeks of culture, the bacterial contamination (Figure 1g) ranged from 26.5% (MS+ Cef) to 57.7% (Yasuda + PTC^3), meanwhile the fungal contamination (Figure 1h) ranged from 13.2% (MS + Cef) to 40.0% (MS + PTC^3) (Table 1). Although MS + Cef seems to be the most efficient treatment to control contamination problems, no significant differences ($P > 0.05$) observed between treatments in the four times evaluated (Table 1). Still, most of treatments achieved contamination rates below 50% that is a positive result when working with avocado adult trees as explant donors. Remarkably, the use of antioxidants (ascorbic and citric acids) as pre-treatment and added to culture media was effective to control the oxidation problems of avocado nodal explants during the four weeks of *in vitro* culture, obtaining a 0% of oxidation rate in all treatments (Table 1). Finally, the doses of plant growth regulators in culture media (see methods) were effective to induce shoots from nodal explants after 1 week of culture in some treatments. After four weeks of culture, significant differences ($P < 0.05$) on shoot induction (Figure 1i) were observed between treatments, being Yasuda + Cef the treatment with the highest shoot induction rate (83.4%), meanwhile the lowest value was observed with MS + PTC^3 (9.9%) (Table 1).

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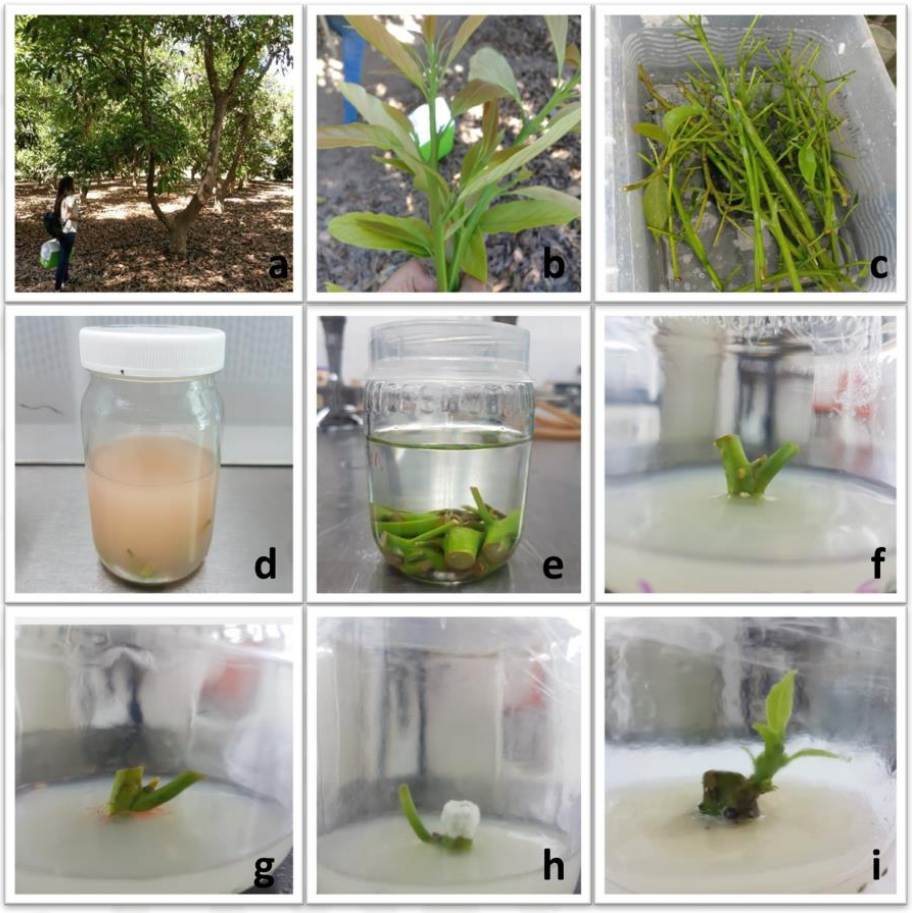

Figure 1. *In vitro* establishment and shoot induction from avocado (cv. San Miguel) nodal explants. a) and b) explant sampling from adult trees, c) washing and selection of explants, d) disinfection of explants with a fungicide-bactericide solution, e) treatment of explants with an antioxidant solution, f) *in vitro* establishment of nodal explant, g) bacterial contamination, h) fungal contamination, i) shoot induction after 4 weeks of culture.

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Discussion

Microbial contamination and oxidation of explants are the major problems during *in vitro* establishment of avocado genotypes. Dalsaso and Guevara (1988) suggested that the high percentage of bacterial contamination in avocado explants is due to endogenous pathogens possibly located in the vascular tissues of the stem, which proliferates after a few days in culture media. The disinfection protocol (Ibarra-López *et al*., 2016) with modifications (fungicide-bactericide solution for 24 h, NaOCl 3.5% for 30 min) in combination with the fungicide and bactericide added to culture media allowed us to keep the microbial contamination below 20% after two weeks. Below 50% after three weeks of culture, thus we suggest to make subcultures to fresh media every two weeks in order to keep the contamination at low rates. Explants of woody plants like avocado are very susceptible to phenolic oxidation, the phenolic compounds are exudate and accumulated in the medium, forming a black area around the explant that can interfere with the nutrient absorption and plant growth (Calderón-Estrada, 2000). In order to prevent oxidation of avocado explants, we use a combination of ascorbic acid y citric acid as pre-treatment solution and added to culture media, achieving a 0% oxidation rate during the complete experiment. Finally, we observed higher rates of shoot induction in Yasuda medium, which is a modification of MS medium with 25% of macroelements. These results are in agreement with Vidales-Fernández (2002) and Ibarra-López *et al*. (2016) who reported that a reduction in the concentration of MS macroelements is beneficial for shoot induction in avocado, although reduces the shoot length.

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Table 1. Effect of culture media on contamination, oxidation and shoot induction in avocado.

Treatment	Bacterial contamination (%)	Fungal contamination (%)	Oxidation (%)	Shoot induction (%)
1st week				
MS + Cef	0 ± 0 a	3.3 ± 10.4 a	0 ± 0 a	47.6 ± 23.5 a
MS + PTC^3	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
Yasuda + Cef	0 ± 0 a	0 ± 0 a	0 ± 0 a	60 ± 26.4 a
Yasuda + PTC^3	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 a
2nd week				
MS + Cef	9.9 ± 15.9 a	6.6 ± 13.9 a	0 ± 0 a	59.9 ± 37.9 ab
MS + PTC^3	0 ± 0 a	0 ± 0 a	0 ± 0 a	0 ± 0 c
Yasuda + Cef	3.3 ± 10.4 a	13.4 ± 28.2 a	0 ± 0 a	80.1 ± 23.3 a
Yasuda + PTC^3	9.9 ± 15.9 a	9.9 ± 15.9 a	0 ± 0 a	33.3 ± 42.3 bc
3rd week				
MS + Cef	16.6 ± 23.5 a	9.9 ± 15.9 a	0 ± 0 a	59.9 ± 37.9 a
MS + PTC^3	13.3 ± 32.1 a	30 ± 42.9 a	0 ± 0 a	0 ± 0 b
Yasuda + Cef	20 ± 28.1 a	20 ± 35.8 a	0 ± 0 a	80.1 ± 23.3 a
Yasuda + PTC^3	43.3 ± 31.7 a	26.6 ± 34.4 a	0 ± 0 a	61.6 ± 29.6 a
4th week				
MS + Cef	26.6 ± 26.3 a	13.2 ± 17 a	0 ± 0 a	66.7 ± 35.2 ab
MS + PTC^3	29.9 ± 33.1 a	40 ± 41 a	0 ± 0 a	9.9 ± 15.9 b
Yasuda + Cef	36.7 ± 36.7 a	20 ± 35.8 a	0 ± 0 a	83.4± 23.5 a
Yasuda + PTC^3	56.7 ± 22.7 a	29.9 ± 33.1 a	0 ± 0 a	66.6 ± 31.5 ab

Data were recorded weekly during one month of culture at 26 ºC and 16:8 photoperiod. Results are expressed as means ± standard deviation. Different letters within a column in each week indicate significant differences ($P < 0.05$, Kruskall-Wallis test). See methods for full media composition.

Conclusions

A protocol for *in vitro* establishment and shoot induction from nodal explants of avocado cv. San Miguel was successfully developed. The Yasuda medium supplemented with ceftriaxone is recommended to obtain a high rate of shoot induction and a low rate of microbial contamination. This protocol will be very useful to preserve and propagate other non-commercial avocado genotypes adapted to Northwest Mexico, that could be used to develop

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new avocado cultivars with high nutritional value and adaptation to high temperature and low altitude conditions.

Acknowledgements

This work was supported by SIP-IPN (20170317, 20180443).

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In vitro tissue culture of papache (*Randia echinocarpa*) and evaluation of its antioxidant activity

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Abstract

Randia echinocarpa is an endemic plant from Northwest Mexico, and recent studies have shown that its fruit have antioxidant, antimutagenic and antidiabetic activities. However, its wild populations have decreased by deforestation and are at risk. Therefore, we aim to establish a system for *in vitro* tissue culture of *R. echinocarpa* and to evaluate its antioxidant activity. Foliar and internodal explants from eight weeks-old *in vitro* plantlets were cultured in six media with different concentrations of BAP and IAA, and the antioxidant activity of extracts from calli, leaves and internodes was assayed by ABTS and DPPH methods. All treatments achieved a 100% of explants with callus, however the treatment with 1 mg/L IAA + 1 mg/L BAP was selected since obtained callus with higher biomass and friable texture. The antioxidant activity of calli from internodal explants was higher than that of calli from foliar explants in both assays (ABTS and DPPH). Interestingly, the antioxidant activity of foliar explants from 8 weeks-old *in vitro* plantlets was significantly higher than those of internodal explants and calli tissues. The present work will be useful for propagation and conservation of *R. echinocarpa* and to optimize the accumulation of its bioactive compounds.

Keywords: antioxidant ●callus ● tissue culture ● *Randia echinocarpa*.

Introduction

Randia echinocarpa is commonly known as 'papache' in Sinaloa, and has been employed in traditional Mexican remedies for cancer, malaria and diabetes; as well as for kidney, lung, circulatory and gastrointestinal diseases (Bye *et al*., 1991; Cortés, 2000). Santos-Cervantes *et al*. (2007) reported for the first time the antioxidant and antimutagenic activities of the papache fruit. Subsequently, Cano-Campos *et al*. (2011) obtained methanolic extracts from papache fruit with strong antimutagenic activity, which was associated with the presence of linoleic acid, palmitic acid and β-sitosterol. Finally, Cuevas-Juárez *et al*. (2014) isolated soluble melanins from 3 endemic Mexican plants: *Vitex mollis*, *R. echinocarpa* and *Crescentia alata*, which showed remarkably high antioxidant and antidiabetic activities *in vitro*. Unofficially, it is known that wild populations of papache have been reduced

considerably by deforestation and overexploitation of its fruit. However, some alternatives for the conservation of this species at risk, such as traditional propagation (*in situ* or *ex situ*), and *in vitro* culture allows the clonal micropropagation of a large number of seedlings in short periods and small spaces, maintaining controlled conditions and guaranteeing health and genetic stability (Kumar and Loh, 2012). Have been reports of *in vitro* culture on plants of the Rubiaceae family, such as *Randia dumetorum* (Begum *et al*., 2003), *Moringa citrifolia* (Sreeranjini and Siril, 2014), and *Gynochthodes umbellata* (Anjusha and Gangaprasad, 2017). However, up to now there are no reports in the literature on *in vitro* culture of *R. echinocarpa*. Therefore, in the present work we seek to establish a system for calli induction in *R. echinocarpa* and to evaluate its antioxidant activity.

Materials and methods

Papache fruits collected in the Ocoroni sierra region of Sinaloa. The seeds were extracted from the pulp (Figure 1a) and subjected to surface sterilization according to Cruz-Mendívil *et al*. (2011) and then germinated *in vitro* in four culture media with different concentrations of MS salts (0.5 and 1X) and sucrose (15 and 30 g/L). Seed were kept in dark during two weeks (Figure 1b), and then under 16:8 photoperiod and 26ºC. The response variables were the percentage of germination, percentage of plantlets with cotyledons and true leaves, and the number of leaves per plantlet. Subsequently, foliar and internodal explants from eight weeks-old plantlets were cultured in six callus induction media with different concentrations of indoleacetic acid (1 and 2 mg/L IAA) and benzylaminopurine (0.2, 0.6 and 1 mg/L BAP). The response variables were the percentage of explants with callus, as well as the size, texture and color of calli. Finally, the antioxidant activity of methanolic extracts from calli, leaves and internodes was determined using the ABTS and DPPH methods. Data was subjected to ANOVA and media comparison by Tukey's Honest Significant Difference test, using the Statgraphics Plus 5.1 software.

Results

Seed germination

After eight weeks of culture, the germination percentage of papache seeds ranged from 63.3 to 83.3% (Table 1), the percentage of plantlets with cotyledons ranged from 19.9 to 49.9%, and the percentage of plantlets with true leaves (Figure 1c) ranged from 13.3 to 26.6%, meanwhile, the number of leaves per plantlet ranged from 0.8 to 2.8. No significant differences were observed between treatments, thus the germination medium with 0.5X MS and 15 g/L sucrose was selected for resources efficiency purposes.

Calli induction

A 100% of explants with callus observed in all evaluated media, however, some physical differences were observed between treatments (Table 2). The explants cultured with 2 mg/L

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IAA formed green, compact and small calli (Figure 1e and 1g), while explants cultured with 1 mg/L IAA formed creamy, friable and large calli (Figure 1d and 1f). Since the medium with 1 mg/L AIA + 1 mg/L BAP obtained calli with higher biomass and friable texture, this treatment was selected for further analysis since it is optimal for cell suspension culture and genetic transformation procedures.

Antioxidant activity

Methanolic extracts obtained from calli and the initial explants (leaves and internodes) as controls. With ABTS assay, the antioxidant activity values ranged from 272.7 to 1,166.4 µmol TE/100 g db. The activity of calli from internodal explant was significantly higher ($p \leq 0.05$) to that of calli from leaf explant using ABTS, however the activities of both calli extracts were significantly lower ($p \leq 0.05$) than that of foliar explants (Table 3). On the other hand, with DPPH assay (Table 3) the values of antioxidant activity ranged from 49 to 345-µmol TE/100 g db. There were no significant differences between the two types of callus using DDPH, but again the antioxidant activity of leaf explants was significantly higher ($p \leq 0.05$) than the rest of samples (Table 3).

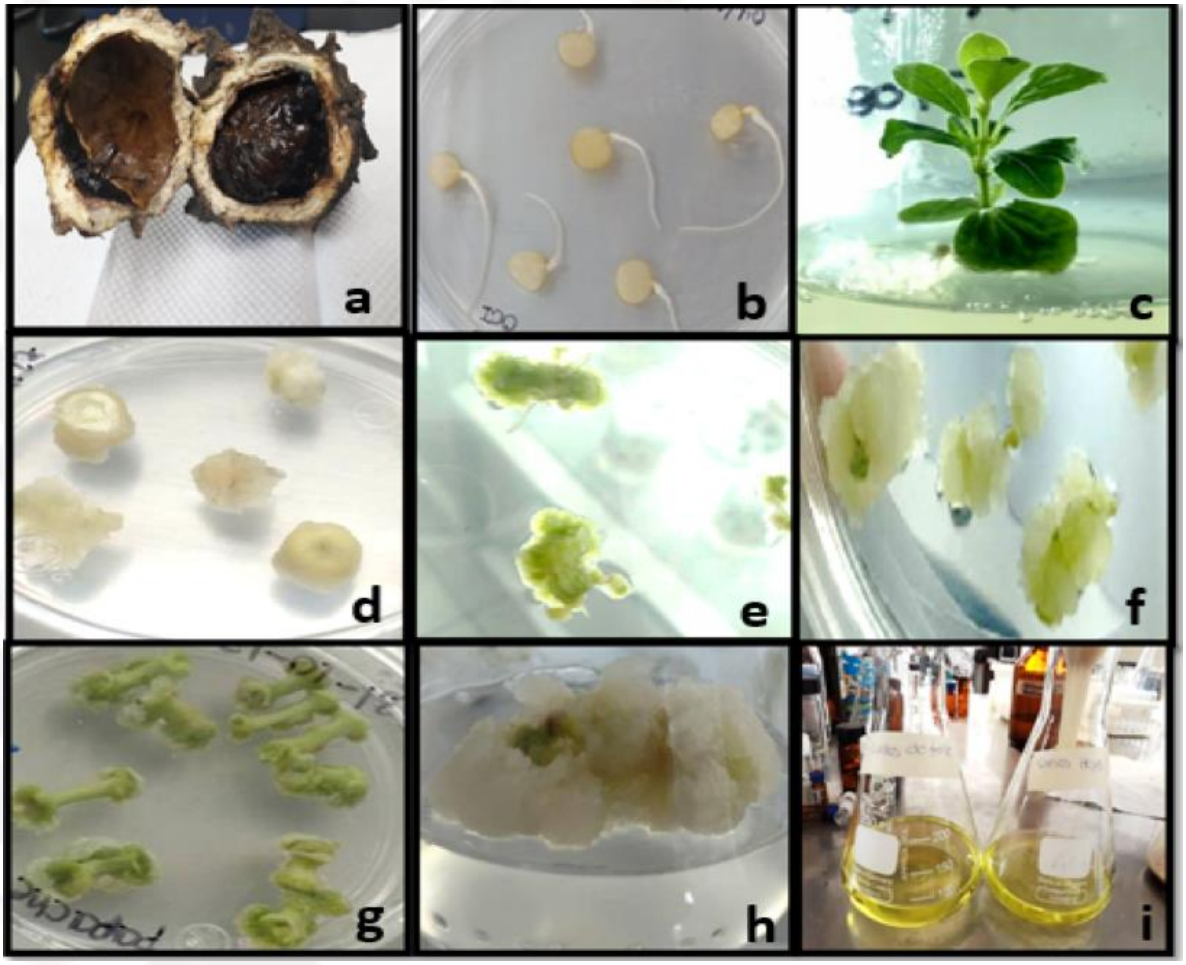

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Figure 1. Seed germination and *in vitro* callogenesis of papache. (a) Mature fruit. (b) Germinated seeds after two weeks in the dark. (c) Plantlet with true leaves after eight weeks of culture. (d, f) Creamy, friable calli from leaves and internodes after six weeks in 1 mg/L IAA + 1 mg/L BAP. (e, g) Compact green calli from leaves and internodes after six weeks in 2 mg/L IAA + 1 mg/L BAP. (h) Calli from leaves with the largest size recorded after 12 weeks in 1 mg/L IAA + 1 mg/L BAP. (i) Methanolic extracts of calli from leaves and internodes.

Table 1. Effect of MS salts and sucrose on papache *in vitro* germination

MS (X)	Sucrose (g/L)	Germination (%)	Plantlets with cotiledons (%)	Plantlets with true leaves (%)	Number of leaves per plantlet
0.5	15	73.3 ± 43.8	33.3 ± 31.4	26.6 ± 26.3	2.8 ± 2.2
0.5	30	63.3 ± 48.4	19.9 ± 28.1	13.3 ± 23.3	0.8 ± 1.3
1	15	80.0 ± 42.1	49.9 ± 36.0	26.6 ± 30.6	2.1 ± 1.5
1	30	83.3 ± 36.0	33.3 ± 35.1	16.6 ± 23.5	2.3 ± 2.3

Data recorded after 8 weeks of culture at 26ºC and 16:8 photoperiod. Results are expressed as means ± standard deviation.

Table 2. Effect of phytohormones on calli induction from papache explants

		Calli characteristics			
BAP (mg/L)	IAA (mg/L)	Texture	Color	Size	Explants with callus (%)
0.2	2	Compact	Green	*	100
0.6	2	Compact	Green	**	100
1	2	Compact	Green	**	100
0.2	1	Friable	Cream	**	100
0.6	1	Friable	Cream	**	100
1	1	Friable	Cream	***	100

Data recorded after 12 weeks of culture at 26ºC and 16:8 photoperiod. Size scale: *small, **medium, ***large.

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Table 3. Antioxidant activity of methanolic extracts from calli and explants of papache.

Extract	Antioxidant activity (µmol TE/100 g db)	
	DPPH	ABTS
Calli from leaves	49.3 ± 10.0c	343.0 ± 10.1c
Calli from internodes	56.0 ± 9.0c	533.2 ± 7.0b
Leaves	345.5 ± 17.3a	1,166.4 ± 76.0a
Internodes	86.6 ± 4.2b	272.7 ± 3.3c

Results are expressed as means ± standard deviation. Different letters within a column indicate significant differences (HSD, $p \leq 0.05$). µmol TE/100 g db = Micromoles of Trolox equivalents/100 g of sample on dry basis.

Discussion

The reduction in the concentration of MS salts had been reported as favorable for the development of different plant species. Pedroza and Caballero (2009) observed a good development and vigor, rapid growth, and high percentage of survival in propagules of *Marchantia polymorpha* by using a 0.25X MS medium; indicating that low concentrations of mineral salts favors good vegetative development. On the other hand, Uribe *et al*. (2008) indicated that the high nitrogen content in 1X MS medium could affect the survival and viability of the explants, since nitrogen-rich media may favor tissue necrosis. In this sense, diluting the culture media favors the development and vigor of the explants, obtaining healthier plantlets with more green and expanded leaves.

Regarding callogenesis, Anjusha and Gangaprasad (2017) reported callus induction and *in vitro* production of anthraquinone in *Gynochthodes umbellata* (Rubiaceae) from leaf explants, being the concentration of 1 mg/L 2,4-D the most effective to produce yellow, friable callus and with a higher anthraquinone content. Meanwhile the maximum dry and fresh weight of calli were obtained in explants cultured in 1 mg/L 2,4-D + 1 mg/L BAP, similar to (1mg/L IAA + 1mg/L BAP) treatment in the present work for higher biomass production.

The levels of antioxidant activity of papache calli and explants in this work are higher than those reported in other medicinal plants such as *Acorus calamus* (2.55 µmol TE/100 g db), *Berberis aristata* (3.59 µmol TE/100 g db), *Plantago ovata* (0.79 µmol TE/100 g db), and *Withania sominifera* (1.13 µmol TE/100 g db) (Surveswaran *et al*., 2007). For instance, the antioxidant activity by ABTS was up to 100 times higher in the calli from internodes and up to 1000 times higher in the leaf explants, compared to the above-mentioned medicinal plants, which suggests that the *in vitro* culture of papache can be a good alternative for the production of bioactive compounds.

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Conclusions

An efficient system for *in vitro* tissue culture of papache (*R. echinocarpa*) was established for the first time. The calli with higher biomass and friable texture were obtained when culturing foliar or internodal explants in 1 mg/L IAA + 1 mg/L BAP. Among the evaluated extracts, the leaf explants from *in vitro* germinated plantlets presented the highest antioxidant activity by ABTS and DPPH assays. The present work settles the basis of future research aimed at the propagation and conservation of *R. echinocarpa*, as well as the optimization of *in vitro* culture conditions to increase the accumulation of bioactive compounds.

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New reports of mycorrhizal fungi associated with *Agave nussaviorum* García- Mendoza, from Oaxaca

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Key words: Mycorrhiza ● A. Nussaviorum ● symbiosis

Abstract

Arbuscular Mycorrhizal Fungi (AMF) are symbiotic microorganisms of terrestrial plant species. They were recently regrouped in the Phylum Glomeromycota. In Oaxaca the diversity of AMF in agroecosystems, low deciduous forest, arid and semi-arid ecosystems, in symbiosis with different agaves has been studied, such as in *Agave potatorum*, A. *angustifolia*, among others. The objective of the work was to identify at a genus level the AMF associated with *Agave nussaviorum* from two different semi-arid ecosystems: from the Mixteca and Sierra Norte de Oaxaca. In addition, highlight the importance and potential of this interaction for agricultural crops in this region. Carried out in the municipality of San Juan Tamazola, Nochixtlán (N1) and San Miguel del Rio, Ixtlán (N2), Oaxaca. The spores extracted and mounted on slides for observation under the microscope. The characteristics used to determine the genus and species of the AMF were: the color and size of the spore, the ornamentation of the supporting hyphae color observed in the stereoscopic microscope, the coloration of the spore layers and sheets. The results were compared with the INVAM database. Five different genera of AMF associated with *A. nussaviorum* were identified. There were no previous report on the association of this agave with AMF since this agave was not known until 8 years ago.

Introduction

One of the most important interactions at the evolutionary level and of great agricultural importance are the arbuscular mycorrhizae, due to the fundamental role they have played for million years ago, since the association that forms with the roots of the plants has been indispensable for them. Mycorrhizae are beneficial associations that were established between the various species of fungi and the roots of most vascular plants, this symbiosis

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displays a biotrophic effect since the plant provides carbon compounds from photosynthesis to the fungus also a protected microhabitat among many other benefits. *Agave nussaviorum* is a recently described species García Mendoza (2010). There have been reports of AMF species associated with different agaves within the State of Oaxaca, such as *A. angustifolia*, and *A. potatorum*. There is no information about *Agave nussaviorum* nor to the association between it and the AMF, therefore, the objective of the work was to identify at genus level the AMF associated with *A. nussaviorum* and compare its diversity in two regions: The Mixteca Oaxaqueña and the Sierra Juárez.

Materials and methods

Completely random sampling, collecting in two municipalities, San Juan Tamazola (N1), Nochixtlan, and at San Miguel del Rio (N2), Ixtlán de Juárez Oaxaca. Used the Jenkins (1964) technique to extract the spores. The spores isolated with a stereoscope microscope and grouped according to color and size, mounted in preparations based on Schenck and Pérez (1990) with the PVLG and Melzer reagents used. The characteristics used to determine the genus and species of the AMF were: the size of the spore and its color observed in the stereoscope, as for the coloration and number of the layers was made with the help of the microscope, and the results compared with the INVAM database

Results and Discussion.

Mounts of 20 morphotypes were made, ten of each AMF sites associated with *A. nussaviorum*, some of which are shown in figure 1, of the genera found in samples N1 and N2, are: *A) Glomus B) Dentiscutata C) Gigaspora D) Rhizophagus E) Glomus and F) Diversispora* to mention a few. Of these for N1, three gendera is reported: *Gigaspora, Ambispora and Septoglomus*; and for N2 four genera are reported: *Dentiscutata, Rhizophagus, Diversispora and Racocetra*. Although the conditions of both sites sampled, are very different regarding climate and soil conditions, the two places are natural habitat of the *Agave nussaviorum* despite being considered endemic to the Mixteca Oaxaqueña by its discoverer (Dr. Abisaí García Mendoza 2010), and on the other hand they also coincided in *Glomus, Rhizophagus, Dentiscutata, Diversispora.*

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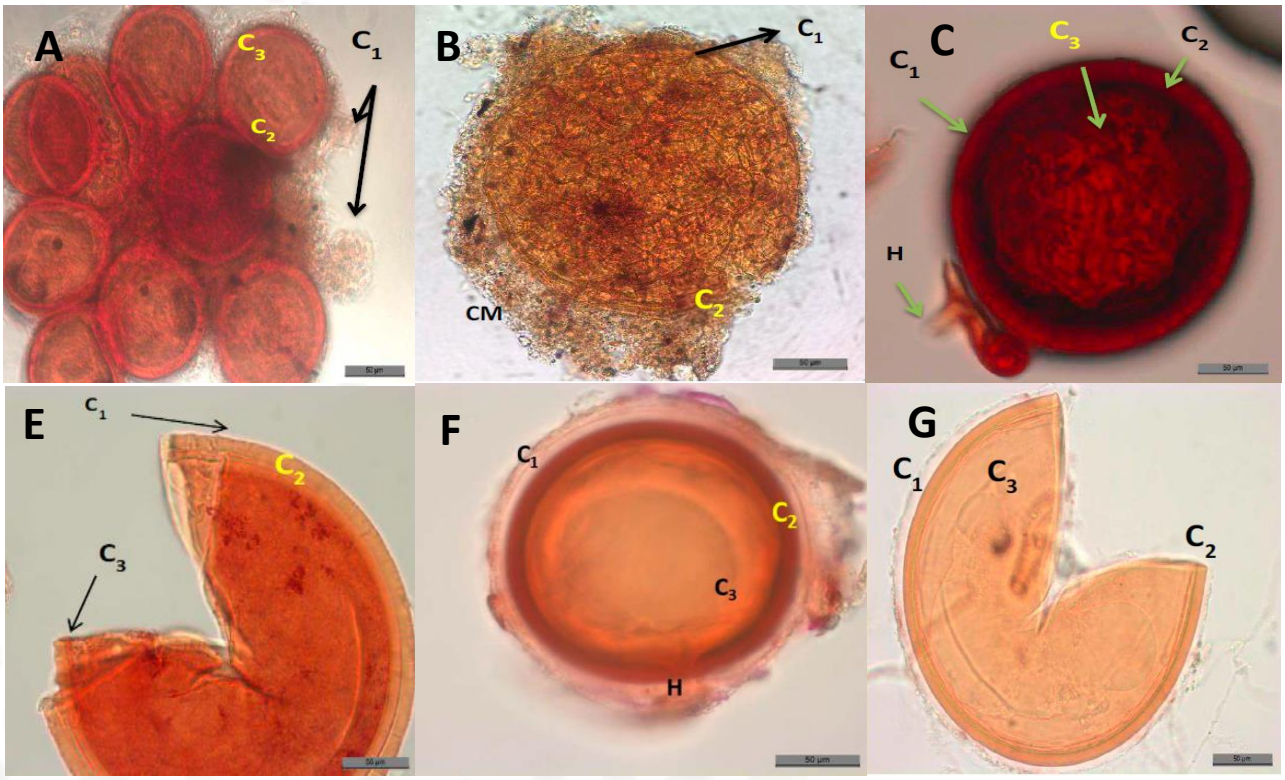

Figure 1. Genus of AMF associated with *A. nussaviorum*, from left to right: *A) Glomus, B) Dentiscutata, C) Gigaspora, D) Rhizophagus, E) Glomus and F) Diversispora*

Discussion.

There are currently no reports regarding *Agave nussaviorum* and its association with AMF that makes this research the first on this interaction. In previous reports regarding this symbiotic association between AMF and agaves of other species, it has been mentioned that there is a great variety of genera, but despite these, it is still necessary to continue investigating their importance and contributions to the plants.

Conclusion.

Seven different genera of AMF associated with *A. nussaviorum* are reported, it is noted that there is no previous report on the association of this agave with AMF since this agave was not known until 8 years ago. It is also important to highlight the importance of these fungi because of the unfavorable habitat conditions they are in, and perhaps their interaction may

help the plants to subsist. With it all these results, there is still more to discover about this agave and its association with mycorrhizal fungi

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Vicenin-2 from *Moringa oleifera* leaves extraction

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Abstract

Moringa oleifera Lam is a plant that contributes multiple and varied benefits, it can be used as a nutritional supplement, elaboration of medicines and for industrial purposes. The flavonoids extracted from this plant have been studied for their antioxidant and healing properties. In the present work, it was tested to obtain flavonoids from *M. oleifera* leaves by a conventional solid-liquid technique, using ultrasound (sonication) and microwaves assisted extraction using different solvents. The Vicenin-2 total content was determined using high-resolution thin layer chromatography (HPTLC) and high performance liquid chromatography coupled to mass (HPLC-MS) to identify the presence of vicenin-2. The best results obtained using ultrasound-assisted extraction with 100% methanol (0.9969 mg of vicenin-2). Also the presence of other compounds that eluted to different retention times from the standard. A compound proved to be an isomer of vicenin-2 according to the molecular weight, determined by the mass spectrum.

Key words: Extract ● Moringa ● Vicenin-2 ● HPTLC

Introduction

Moringa Oleifera Lam., also known as "the tree of life" is native to India and Africa. Considered one of the most useful trees in the world because each part of the Moringa tree can be used for food, medicine and industrial purposes (Moyo *et al.*, 2011). The plant contains numerous phytonutrients including flavonoids, which are secondary metabolites with beneficial health effects, such as the prevention of DNA damage on normal cells and the promotion of apoptosis of cancer cells (Rodríguez-Pérez et al., 2016). The total flavonoid content and its corresponding antioxidant activity may vary widely depending on the applied extraction conditions (Pakade et al. 2013). To identify the chemical components of the plant material it is important to follow an efficient extraction procedure that provides sufficient extraction in quantity and quality, decreasing the possibility of the extracted compounds modifying its structure during the process (Vinatoru 2001). Traditional methods, such as distillation, solvent extraction and cold compression, continue to be used, but significant improvements can be achieved with the application of ultrasound or microwave technologies. therefore, the objective of this study was to identify the presence vicenin-2 in a methanol extract of leaves of *M. oleifera* obtained by different extraction methods such as microwave assisted extraction, ultrasound assisted extraction and maceration.

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Materials and methods

The plants of *Moringa Oleifera* Lam. were obtained from laboratory of the Centro de Desarrollo de Productos Bióticos, at the municipality of "Yautepec de Zaragoza", Morelos; at N 18 ° 49 ´ 44.278, W 099 ° 05 ´ 34.296 and with an altitude of 1064 masl. The extracts made from leaves of the plant were dried in a stove at low temperature. The extraction of the flavonoids was done by maceration and with the assistance of sonication and microwaves, using various solvents such as: hexane, ethyl acetate and methanol. The extracts obtained were concentrated in a rotosteamer (Buchi, RE 111). Through analysis of flavonoids by high-resolution thin layer chromatography (HPTLC), only the methanolic extracts were used, having as stationary phase HPTLC chromatographic plates of silica gel 60 F254 (Merk) of 10 x 10 cm and mobile phase n-butanol, N-propanol-acetic and Acid-water. For HPLC-MS analysis, a system controller (CBM-20 [a]) with 2 binary pumps (LC-20AD), degasser (DGU-20A5R), autosampler (SIL-20AC), column furnace (CTO20A), an array detector of SPD-M20A UV-Vis photodiodes and a mass spectrometer (LCMS-2020) Simple quadrupole with electrospray ionization source (ESI) (LCMS-2020).

Results

The results obtained by HPTLC are shown in figure 1, where we could observe the possible presence of the compound (Vicenin-2) in an RF range from 0.55 to 0.6 (yellow Bands) in the extracts obtained by sonication, microwaves and maceration. It should be mentioned that for the case of extractions made by maceration the bands were more tenuous than with the other methods.

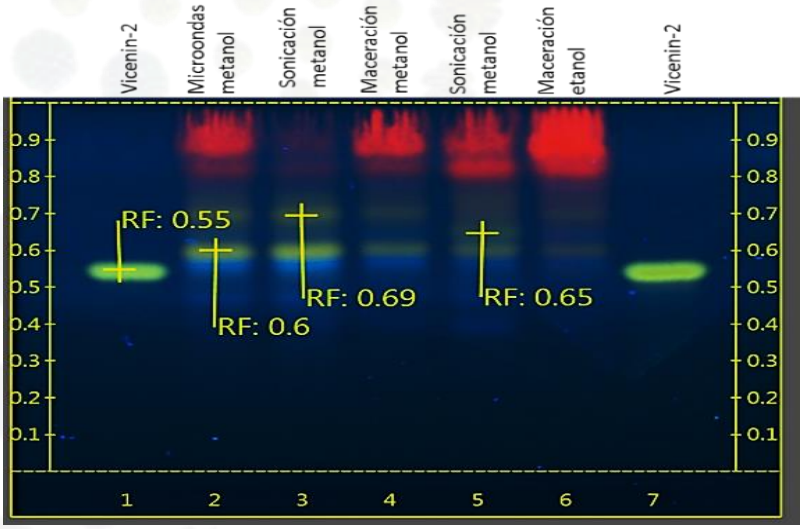

Figure 1. Chromatographic profile of methanolics extracts of *M. oleifera* leaves with different extraction methods: (1 and 7) vicenin-2, (2) microwave-assisted extraction, (3 and

5) extraction by sonication, (4) maceration extraction, (6) ethanol extraction by maceration method.

Table 1 shows the results of the quantification of vicenin-2 in which the extraction by sonication, the highest amount of vicenin-2 (0.9969 mg/ml) was obtained compared with microwave extraction (0.4399 mg/ml) and by maceration with methanol (0.2352 mg/ml).

Table 1. Concentrations of vicenin-2 obtained with different methods of extraction

	Area	Quantity of Vicenin-2 (mg / mL)
Vicenin-2	0.02916	1.0000
Methanol Sonication	0.02907	**0.9969**
Methanol Microwave	0.01283	0.4399
Methanol Maceration	0.00686	0.2352

Results by HPLC-MS (Figure 2) show the retention time at 9 minutes of the standard (vicenin-2). In figure 2a, the chromatogram of the methanol extract obtained by sonication shown in which the compound of interest can be identified (red arrow). Depending on its mass (figure 2b), the molecular weight of the vicenin-2 was 593 m/z. There were also other compounds that eluted to different retention times from standard (Figure 3a), one of these compounds proved to be an isomer of vicenin-2 according to the molecular weight determined by the mass spectrum (Figure 3b).

a)

b)

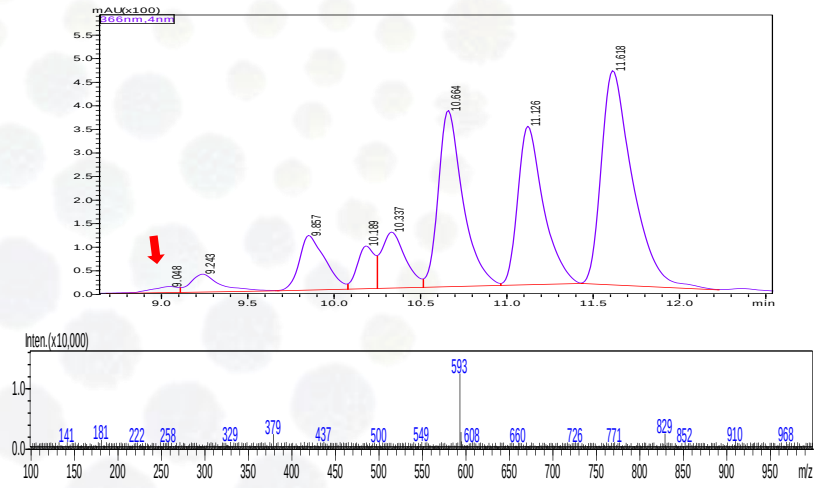

Figure 2. a) Chromatogram by HPLC of the methanol extract of leaves of *M. oleifera* by sonication vicenin-2. b) Analysis of the mass spectrum of vicenin-2.

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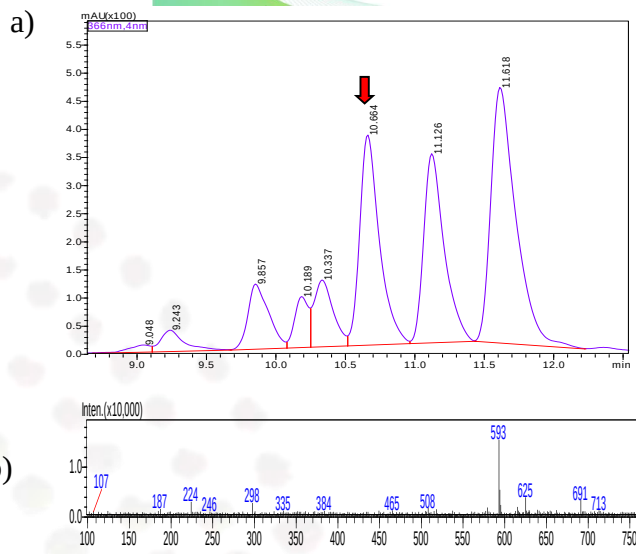

Figure 3. Chromatogram of HPLC of methanol sonication extract of leaves of M oleífera **a**) Isomer of Vicenin 2. **b**) Analysis of the mass spectrum of the Vicenin-2 Isomer

Discussion.

Muhammad *et al.* (2013), identified vicenin-2 by HPLC-MS in *M. oleifera* leaves of an aqueous fraction of a methanol extract by maceration, which had a retention time of 12 minute. Regard to the presence of other observed compounds different to the standard, Islam *et al.*, (2014), identified vicenin-2 in *Artemisia capillaris* and mention that there may be isomers of this compound.

Conclusion

In analysis by HPTLC was possible to identify vicenin-2 in the extracts tested, however, with the extraction assisted with sonication was obtained the highest amount of vicenin-2, the analysis of HPLC-MS of this extract allowed to identify vicenin-2, in addition to the presence of other compounds in which an isomer is found. The method of extraction by sonication can be an alternative for the extraction of flavonoids from vegetables, with better results than conventional methods.

Acknowledgements

The authors thank the National Polytechnic Institute and the National Council of Science and Technology for financial support and the BEIFI Program.

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Vol 1 Year 1 Issue 1, ISSN: 2618-0464, http://www.bio.edu.mx/

Establishment of *in vitro* cultures of *Crataegus monogyna*, with anxiolytic compounds production potential

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Abstract

Traditional medicine used infusions of *Crataegus monogyna* to treat anxiety. Here tested *In vitro* cultures with *C. monogyna* seeds to obtain compounds showing anxiolytic activity potential. Total phenol and flavonoid contents of extracts obtained from leaves of adult plants also were analyzed to be compared with those obtained from the *in vitro* callus cultures. *Crataegus monogyna* seeds were cultivated for 35 days in Murashige & Skoog (MS) medium supplemented with kinetin (KIN; 0-2.0 mg/L) in combination with 2,4-dichlorophenoxyacetic acid (2,4-D; 0-4.0 mg/L). Callogenesis occurred in 65% of the evaluated treatments and was directly proportional to the amount of auxin present in the media. Significantly better induction percentages (60-70%) were obtained with treatments containing 2,4-D (2.0 and 4.0 mg/L) with KIN (0.5 and 2.0 mg/L). We also carried out extractions with different solvents (water, ethanol, and water:ethanol (4:6)) from the leaves, evaluating two biomass-to-volume ratios (1:10 and 1:30) on the extraction yield. Regarding the content of phenols, the hydroalcoholic extract at the 4:6 proportion with the 1:10 biomass-to-volume ratio showed the highest content (55.7 ± 0.14 mg GAE g^{-1} DW) and extraction yield (17.7 ± 7 mg extract g^{-1}); while the ethanolic extract with the biomass-to-volume ratio of 1:10 showed the highest flavonoid content (9.5 ± 0.08 mg QE g^{-1} DW).

Key words *Crataegus monogyna* ● *in vitro* culture ● anxiolytics ● phenols

Introduction

Anxiety is an adaptive response to stress that becomes pathologic when in absence of a stimuli, the physiological responses persist uncontrollably and excessively (Gelfuso *et al*. 2014). According to the World Health Organization, anxiety is most frequent than common mental disorders and drug abuse; in Europe and USA, anxiety represents one of the main health problems in terms of medical attention, sick leave, disability and premature mortality (WHO, 2013) and in Mexico, anxiety is the most frequent psychological disorder (14.3%) (Medina *et al*. 2003). Treatment frequently includes synthetic anxiolytic drugs like diazepam; however, multiple side effects have been observed. Therefore, a high number of patients with anxiety (~43%) seek treatment outside the allopathic paradigm, being plants the most preferred (Dominguez, 2010). In traditional medicine, *Crataegus monogyna* commonly known as "hawthorn" is highly valued for its medicinal properties on which anxiolytic effects

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are among them, being flower and leaf infusions commonly used to “calm nerves” (de Sousa *et al.* 2015).

Materials and methods

Establishment of aseptic seed cultures of *C. monogyna*

C. monogyna seeds were disinfected superficially by immersion in a soapy solution (1% w/v) for 15 minutes, followed by immersion in an ethanol solution (70% v/v) for 30 seconds, and finally in a sodium hypochlorite solution (0.6, 1.2 and 1.8% (v/v) for 10 and 20 minutes, respectively).

Callus induction from seeds of *C. monogyna*

The disinfected seeds were inoculated under aseptic conditions in Murashige & Skoog medium (MS; 1962), supplemented with plant growth regulators (PGR) at different concentrations: kinetin (KIN; 0.0, 0.5, 1.0, 2.0 mg/L) and 2,4-dichlorophenoxyacetic acid (2,4-D, 0.0, 0.5, 1.0, 2.0, 4.0 mg/L), complemented with 3.0 g/L of sucrose and 2.4 g/L of phytagel as a gelling agent. The culture medium was adjusted to a pH of 5.7 ± 0.1 before autoclaving at 121°C for 15 min. Cultures were then incubated at 25 ± 2°C under a 16-h light photoperiod.

Production of extracts from leaves of *C. monogyna*

Dried and pulverized samples of adult specimens’ leaves biomass (1:10 and 1:30 w/v) were macerated in different solvents (water, ethanol, and water:ethanol (4:6)). The ground leaves were purchased at the Herbal Remedies Lab, Rosa Elena Dueñas S.A. of C.V. To obtain the aqueous extract with a 1:30 w/v ratio, infused the biomass during 30 minutes in distilled water boiled for 5 minutes. Subsequently, adjusted the volume to 30 mL and stored in an amber container. The ethanolic and ethanolic:aqueous extracts were obtained by adding the solvent and the biomass to an amber flask and exposed to a water bath at 40°C for 60 minutes under continuous agitation.

For all macerated samples (1:30 and 1:10), extraction was assisted by ultrasonic bath (Ultrasonic Cleaner, SK2210HP, China) at 40ºC and 53kHz for 40 minutes. The resulting mixture was vacuum filtered, the supernatant was recovered and dried by lyophilization (Labconco, 7670521, USA), after which its weight (DW) was determined to be stored in an amber bottle at 4°C until further use.

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Total phenol quantification

Total phenol quantification from the leaves was achieved following the Folin-Ciocalteau methodology (Sánchez-Rangel *et al.* 2013). A calibration curve was constructed with gallic acid and the results are expressed as mg GAE/g biomass, DW.

Flavonoid quantification

Flavonoid concentration from the leaves of *C. monogyna* was quantified (Pękal & Pyrzynska, 2014) methodology. A calibration curve constructed with quercetin and the results expressed as mg QE/g biomass, DW.

Results

Establishment of aseptic seed cultures of *C. monogyna*

The seed treatment with sodium hypochlorite at 1.8% for 20 minutes showed the lowest percentage of contamination (0%) and seed exposure to this treatment did not have any effect on the germination process, which began 15 days after seeding, showing a 100% germination at 60 days.

Callus induction in *C. monogyna* seed cultures

For callus induction, the 2,4-D auxin treatment showed the highest performance, which also exponentially increased with concentration (Fig. 1), performing at its best in the treatments containing 2 and 4 mg/L of 2,4-D with KIN (0.5, 1.0, and 2.0 mg/L). Auxins are involved in the cellular division and successfully evaluated in many works for callus induction.

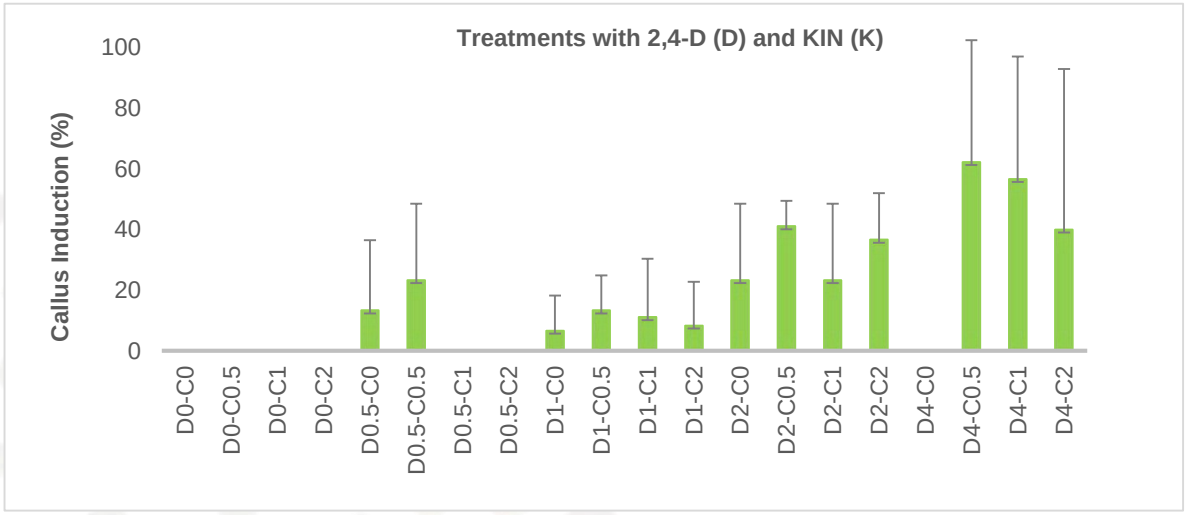

Figure 1. Percentage of callus induction in *C. monogyna* seeds at 30 days of incubation in MS medium. The data shows the average of two replicates ± SD.

Visually, the calli had a low-friability appearance (Fig 2A), the coloration was white and yellow with some red-pink pigmentation (Fig 2B). However, after carrying out three subcultures, the medium containing 2 mg/L of 2,4-D was the treatment that demonstrated an increase not only in growth but also in friability (Fig. 2C).

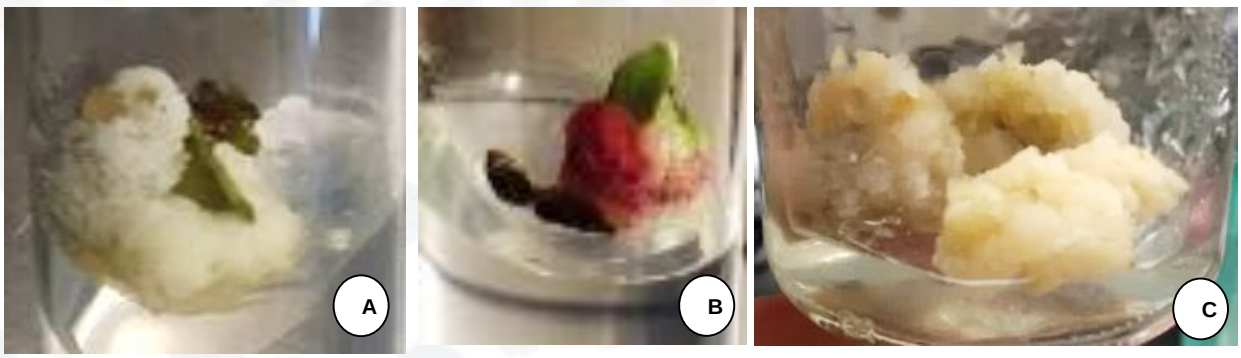

Figure 2. *C. monogyna* calli: A) callus culture in MS medium with 4mg/L of 2,4-D and 1 mg/L of KIN (D4-C1) producing compact and embryonic callus, 30 days after cultivation; B) callus culture in MS medium (D2-C2) producing red-pink pigments; and C) callus culture, after 90 days of culture (D2-C0) producing friable callus.

Phenol and flavonoid content in leaves of *C. monogyna*

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In general, the data obtained shows that the 1:10 ratio had the best extraction yield. On the other hand, the water:ethanol solvent (hydroalcoholic extract) had the highest effect on the extraction of phenols and flavonoids, followed by the ethanolic and the aqueous extracts, respectively. Regarding the total phenols content, the hydroalcoholic extract from 1:10 biomass to solvent ratio, showed the highest value. While, the highest content of flavonoids was obtained from the ethanolic extract at the 1:10 biomass to solvent ratio (Table 1).

Table 1. Extraction yield, phenol and flavonoid contents by different solvents in two biomass-to-volume ratios from the *C. monogyna* leaves.

Biomass: solvent ratio (m/v)	Type of Extract	Extraction yield (%)	Phenol Content (mg GAE/g biomass DW)	Flavonoid Content (QE/g biomass DW)
1:30	Aqueous	06.90±0.07^{d}	16.9±0.068^{d}	0.69±0.01^{d}
	Ethanolic	12.95±0.32^{b}	32.6±0.088^{c}	1.33±0.02^{c}
	Hydroalcoholic	12.72±0.87^{b}	37.8±0.23^{bc}	1.51±0.02^{c}
1:10	Aqueous	10.5±0.05^{c}	19.0±0.09^{d}	2.6±0.04^{c}
	Ethanolic	14.16±0.17^{b}	30.0±0.14^{c}	9.5±0.08^{a}
	Hydroalcoholic	17.67±0.04^{a}	55.7±0.14^{a}	6.8±0.02^{b}

The data represents the average of two replicates ± SD. Different letters represent significant differences from the Fisher's LSD test ($p \leq 0.05$).

Discussion

Several reports in plants from the genus *Crataegus* have demonstrated the formation of calli with phenolic compounds production (Bahorun *et al.* 2003; Bahri-Sahloul *et al.* 2014; Eisawy Elgengaihi *et al.* 2009). However no reports have previously shown the influence of plant growth regulators such as 2,4-D and KIN in seed cultures of *C. monogyna* as an initial explant in the induction of callus. Given that *C. monogyna* is widely exploited worldwide for its medicinal properties, it is important to carry out studies that allow the optimization of calli induction in this species.

Some studies indicate that exposing leaf explants of a species of *Crataegus* to an equal concentration of auxin and cytokinin is the best combination to induce callus formation with a high duplication rate (Eisawy *et al.* 2009; Lotfi *et al.* 2015). In contrast, our results show that in seeds of *C. monogyna*, at a higher concentration of auxin in combination with a lower concentration of cytokinin induces a higher percentage of callus production and accelerates growth. Bahorun *et al.* (1994) found similar results when using flower buds of *C. monogyna*.

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Such differences may be due to its genetic constitution and the required hormone levels that aid callus growth from different plant components. For example, high levels of auxin promote cell proliferation and the formation of embryonic calli (Gliwicka *et al.* 2013). Among synthetic auxins, 2,4-D is the most effective inducer possibly because it triggers auxin and stress responses simultaneously.

Regarding the content of total phenols and flavonoids, the differences observed in the extracts may be due to the ability of solvents to extract the compounds from the plant material with the temperature and extraction time used (Garrido *et al.* 2013). This could also explain the yielding percentage of the hydroalcoholic extract (1:10 w/v), which demonstrated that the solvent used in the extraction process plays an important role in the activity of the extracts by considering the polarity of both, the solvent and phenolic compounds (Ricco *et al.* 2010). By using a polar solvent assisted by an ultrasonic bath, the cell membrane breaks down and improved extraction occurs, which would explain the higher yielding percentage in the hydroalcoholic extracts. All extracts contained phenolic compounds in significant amounts, which is important because phenols are among the compounds on which medical properties of numerous plant species are based, including those involving anxiolytic activity. However, such activity in *C. monogyna* remains to be investigated.

Conclusions

The plant growth regulators, 2,4-D and KIN, promote callus induction in *C. monogyna* seeds. Regarding the extraction of phenols and flavonoids from *C. monogyna* leaves, the solvent and the biomass to volume ratio used have a significant effect.

Acknowledgements

The authors are thankful to CONACyT, Mexico for partially funding this project through the Innovation Stimulus Program.

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Biotechnology Summit 2018
Vol 1 Year 1 Issue 1, ISSN: 2618-0464, http://www.bio.edu.mx/

Agave angustifolia phytosterols microwave-assisted extraction

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Abstract

In Mexico, traditional medicine used *Agave angustifolia* in the treatment of inflammation and other diseases. On the other hand, the composition and bioactivity of plant extracts depends on the extraction technique used. In previous studies the extraction of secondary metabolites of *A. angustifolia* was carried out using a conventional extraction technique such as maceration, nevertheless it has disadvantages in terms of selectivity, extraction yield, long times and toxicity of solvents required. In this work, β-sitosterol β-D-glucoside was obtained from the stems and leaves of *A. angustifolia* by maceration and microwave extraction (MAE) to select the best process in terms of the amount obtained and the extraction time. β-sitosterol β-D-glucoside was identified and quantified by high performance thin layer chromatography (HPTLC). The results showed that with MAE a greater amount of β-sitosterol β-D-glucoside by reducing the extraction time (from hours to minutes) and using environmentally friendly solvents (ethanol).

Key words microwave-assisted extraction ● β-sitosterol β-D-glucoside ● high-performance thin-layer chromatographic.

Introduction

Previous works with *A. angustifolia* reported different biological activities, among those the anti-inflammatory activity of acetone extract of *A. angustifolia* Haw and *A tequilana* Weber attributed to the presence of catalasaponin-1 (Monterrosas-Brisson N *et al* 2013). Besides, measured the anti-inflammatory effect of the acetone extract of *A. angustifolia*, from two active fractions, the isolation and identification of active compounds. For the characterization of the pharmacological activity used the acute inflammatory model of ear mouse oedema induced with TPA. The mainly fraction consisted of 3-O - [(6'-O-palmitoyl) -β-D-glucopyranyl] Sitosterol, β-sitosteryl glucoside and stigmasterol (Hernández-Valle E *et al* 2014). Both works used traditional maceration extraction technique. Traditional extraction presents different drawbacks such as long extraction times, use of large quantities of toxic solvents; low yields of the active compounds of interest. The MAE, has demonstrated a superior extraction efficiency compared to conventional extraction methods, with short extraction time, as well as using environmentally friendly solvents (Mustapa AN, Marti A,

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Mato RB, Cocero MJ 2015). The aim of this work was to develop a precise, efficient and rapid extraction method for phytosterols of *Agave angustifolia* Haw through MAE.

Materials and methods

It used dried and powdered plant material from stems and leaves of 5-year-old *A. angustifolia*. The MAE extraction conditions for the phytosterol were 200 W of microwave power, ethanol as solvent at 70°C, for different extraction times (7'30", 10', 12'30" and 15'). Then compared with maceration extraction (MAC) using ethanol as solvent for 48 hours. After lyophilized all samples, used HPTLC chromatographic technique for the identification and quantification of β-sitosterol β-D-glucoside. The sample solutions were spotted with nitrogen gas in the form of bands of 6.0 mm width with the Camag microlite syringe on pre coated silica gel glass plate 60F254 (20 cm X 10 cm with 250 µm thickness); using a Camag Linomat V. The mobile phase was 35 mL of toluene: ethyl acetate: formic acid 5: 5: 0.5. Linear ascending development in 20 X 10 cm twin through a saturated glass chamber; and the length of chromatogram run was 7 cm. The optimized chamber saturation time for mobile phase was 20 min at room temperature (22°C approx.) at relative humidity of 48°C. After the scanning, HPTLC plates were dried. Scanning densitometry was performed with Camag TLC scanner IV in the reflectance absorbance mode from 200 to 600 nm operated by Vision Cats software (2.3 Camag) with the help of a deuterium and tungsten lamp. After the development HPTLC plate was dipped in 4-hydroxybenzaldehyde reagent followed by drying it in an oven at 110°C for 5 min. The β-sitosterol β-D 1mg/mL reference standard was used. For the preparation of the experimental samples, used 5 mg of extract dissolved in 1 ml of methanol. A calibration curve was made for the quantification (Xiao XH, Yuan ZQ, Li GK 2013; Jirge S, Tatke P, Gabhe, SY 2010).

Results

The presence of β-sitosterol β-D-glucoside at Rf 0.11 and a fraction of β-sitosterol at Rf 0.67 (Figure 1) confirmed in the ethanolic extracts from the stem and leaves by MAE, which showed the same Rf of the reference compounds. Besides, the concentrations of β-sitosterol β-D-glucoside obtained with different times by MAE and maceration (MAC) extractions from leaves and stems of *A. angustifolia* (Table 1).

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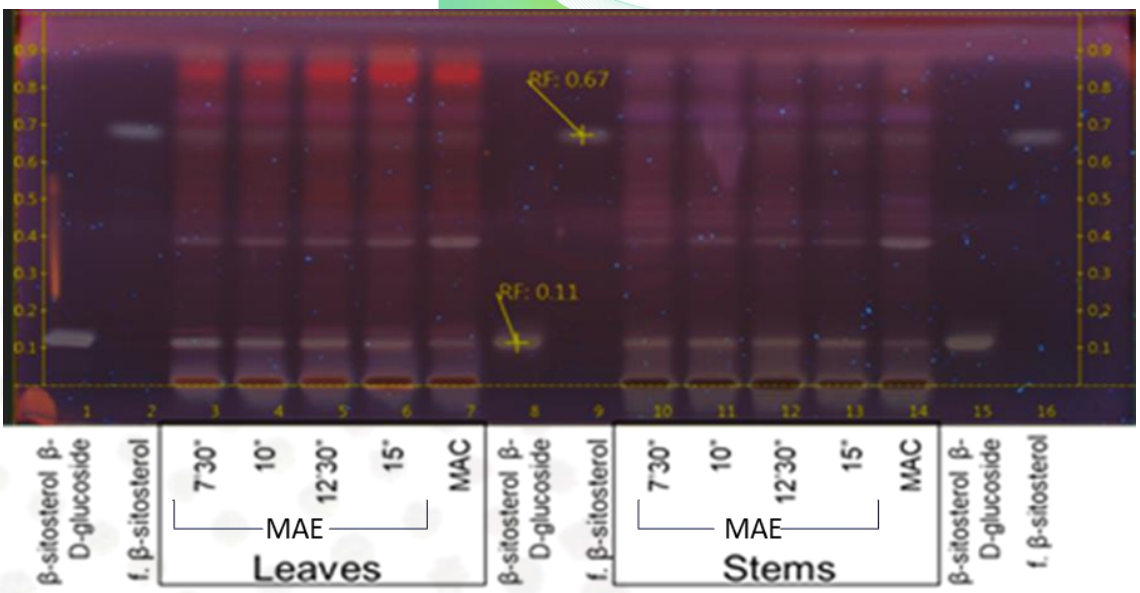

Fig. 1. HPTLC plate of β-Sitosterol β-D-glucoside and ethanolic extracts of leaves and stems of *A. angustifolia* by MAE at different times and maceration (MAC) 48 h

Table 1. Concentrations of β-sitosterol β-D-glucoside in leaves and stems extracts of *A. angustifolia* obtained by MAE at different times and maceration (MAC) 48 h. n = 3.

Extraction time	mg of ß-sitosterol ß-D- glucoside / 1 g of dried extract	
	Leaves	Stems
MAE 7'30"	121.43	178.57
MAE 10"	119.84	146.83
MAE 12'30"	110.32	121.43
MAE 15'	107.14	118.25
MAC	40.48	64.29

Discussion

Different factors must be examined to determine the effectiveness of medicinal plant extracts. Among them, the β-sitosterol β-D-glucoside by MAE, the bioactive compounds in this work identified in an ethanol extract, in addition to using environmentally friendly solvents (ethanol). Comparing our results with other works where they identifed the same bioactive compound, but using a maceration technique with a long extraction time and toxic solvents (acetone) (Hernández-Valle E *et al* 2014). On the other hand, the microwave radiation to the plant materials improved the efficiency and reduced the extraction time in comparison with

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the maceration extraction, whose hexane extract of *Sisymbrium irium* obtained 0.00210 mg / g of β-sitosterol β-D-glucoside (Al-Massarani S 2017), lower than the obtained in this work.

Conclusions

The MAE constitutes a useful tool to extract phytosterols from *A. angustifolia* with a greater amount extracted and processing time reduction (hours to minutes).

A perspective of this work is the characterization of β-sitosterol β-D-glucoside present in *A. angustifolia* extract obtained by MAE based on the UHPLC-ESI analysis.

Acknowledgement This work has been carried out with the financial support provided by CONACYT, CeProBi-IPN and the BEIFI-IPN scholarship.

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Area X

GREY

Classical Biotechnology (Fermentation): Industrial Biotechnology; Classical Fermentation & Bioprocess/Bioengineering Technology; Engineering & Technological Equipment for Bioproduction; Output of Science-Intensive Bioproducts.

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Shiitake (*Lentinula edodes* Pegler) Radial growth evaluation in different solid culture media

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Abstract

In order to produce vegetative mycelium of *Lentinula edodes* Pegler, its growth evaluated and characterized in three different solid culture media: potato dextrose agar (PDA), malt extract agar (EX) and Sabouraud agar (S). The incubation at 26 ± 2 ° C; consecutive measurements were made every 24 h with a Vernier, up to a maximum of 216 h. The Minitab 16 program showed that there is no significant difference between the strain and the culture media; the regression and adjustment analysis in Matlab R2014a calculated and determined the different polynomial parameters that best fits the experimental data, allowing a better perspective analysis. Radial growth rate (RG) showed that the ITO-03 strain (isolation from the spores) has the best growth adaptation to the three solid media analyzed.

Key words: *Lentinula edodes* ● edible fungi

Introduction

The division Basidiomicetes, includes the most evolved fungi. Most edible fungi belong to these taxa. The aerial gross part that is collected for consumption is only the fruiting bodies. In general, edible basidiomycetes are usually symbionts and/or saprophytes and grow on organic matter or soils rich in humus (Finimundy *et al* 2014). The Shiitake (*Lentinula edodes* Pegler) known for its sensory, nutritional and functional attributes, is the second most cultivated edible mushroom on the planet, preceded only by the white (*Agaricus bisporus*) (Finimundy *et al* 2014). The pileus of the fungus is very desirable while its stipe is usually separated and is not commercialized due to its high fiber content (Rivera *et al* 2017). Shiitake has been studied for its various biological activities, such as antioxidant, hypocholesterolemic, hypoglycemic, antibacterial, antiviral, cardiovascular system regulator, anticancer and immunomodulator activities attributed to its secondary metabolites; Due to all these chemical and biological characteristics not only the culture but the study of its bioactive compounds worldwide has been intensified (Hobbs, 2000).

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Materials and methods

Vegetative isolation

The strain of Shiitake (*Lentinula edodes* Pegler) coded as strain ITO-01 was acquired through a commercial company. Once the fruiting body was obtained, vegetative and spore isolation was processed. The fruiting bodies of *Lentinula edodes* were cut in a laminar flow hood; cutting sections was performed with a sterile scalpel and placed in glass Petri dishes sterilized with dry heat (200 °C for 1 h). Petri dishes were prepared with sterilized malt extract agar at 15 lbs for 15 min, adding Ampicillin at 300 mg/L. Cuttings of the fruiting bodies were approximately one cm^2 thick; 50% of them were washed in distilled water with 1% sodium hypochlorite for 5 min, while the rest were not washed. They were sown in a sterile area, and the Petri dishes were identified with the letter "A" (fruiting bodies without washing) and "B" (fruiting bodies with washing). They were incubated at 26 °C for 7 days, recording their growth. This strain was coded ITO-02 strain and stored at 4.4 ° C in sloped test tubes, containing malt extract agar (Mata *et al* 1990).

Spore isolation

The Shiitake spores obtained by placing the fruiting body (cap) of the mushroom on a circle of white filter paper in a sterile area, incubating at 26 °C for 5 days. The presence of spores was observed as a change in the color of the filter paper; Pieces of the filter paper containing the spores were cut and placed inside a test tube with 5 mL of sterile distilled water, then, serial dilutions were made up to 10^{-6}, inoculating 1 mL of each dilution with a Drigalski loop in duplicate. Later sown in Petri dishes with malt extract agar, incubated at 26 ° C for 48 h until the growth complete saturation of the Petri dish was achieved. The obtained strain was coded ITO-03 and stored at 4.4 ° C in test tubes tipped with malt extract agar.

Radial growth kinetics

To promote mycelial growth of strains ITO-01, ITO-02 and ITO-03 of *L. edodes*, sowing was carried out in triplicate with the help of a sterile borer with internal diameter of 0.8 cm. Petri dishes with malt extract agar (EX, Bioxon), potato and dextrose agar (PDA, Bioxon) and sabouraud agar (S, Bioxon), were sterilized in an autoclave at 15 lb. for 15 min. The Petri dishes were incubated at 26 °C, measuring the growth zone with a Vernier every 24 h. The results analyzed with the software Matlab R2014a for model determination and Minitab 16 for statistical analysis.

Results

For the isolation by spores and the vegetative isolation, two fruiting Shiitake bodies were collected, produced from a culture bag containing forest residues as a substrate (figure 1a).

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In the case of vegetative isolation, the best growth of the mycelium was achieved from the fruiting bodies without washing at 5 days, reaching its maximum growth at 12 days of incubation (figure 1b). For the isolation by spores, it was decided to take a spore of the 10^{-3} dilution at 36 h of incubation, achieving its maximum development in the reseeding at 10 days of incubation (figure 1c).

The growth rate of *L. edodes* showed no significant difference with respect to the strain type ($F = 1.92$, $P = 0.26$), nor with respect to the solid culture medium used ($F = 2.29$, $P = 0.217$). The experimental data, and the adjustments made by the software Matlab R2014a are shown in figure 2.

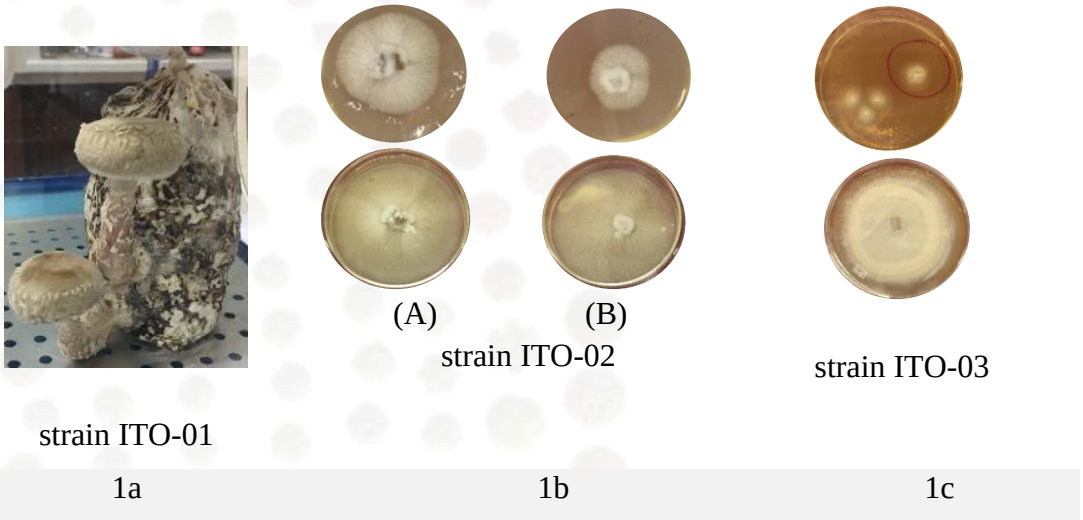

Figure 1. Isolation from the fruiting body of the strain ITO-01 (1a), vegetative "A" without wash and "B" with wash (1b), spores in the 10-3 dilution (1c).

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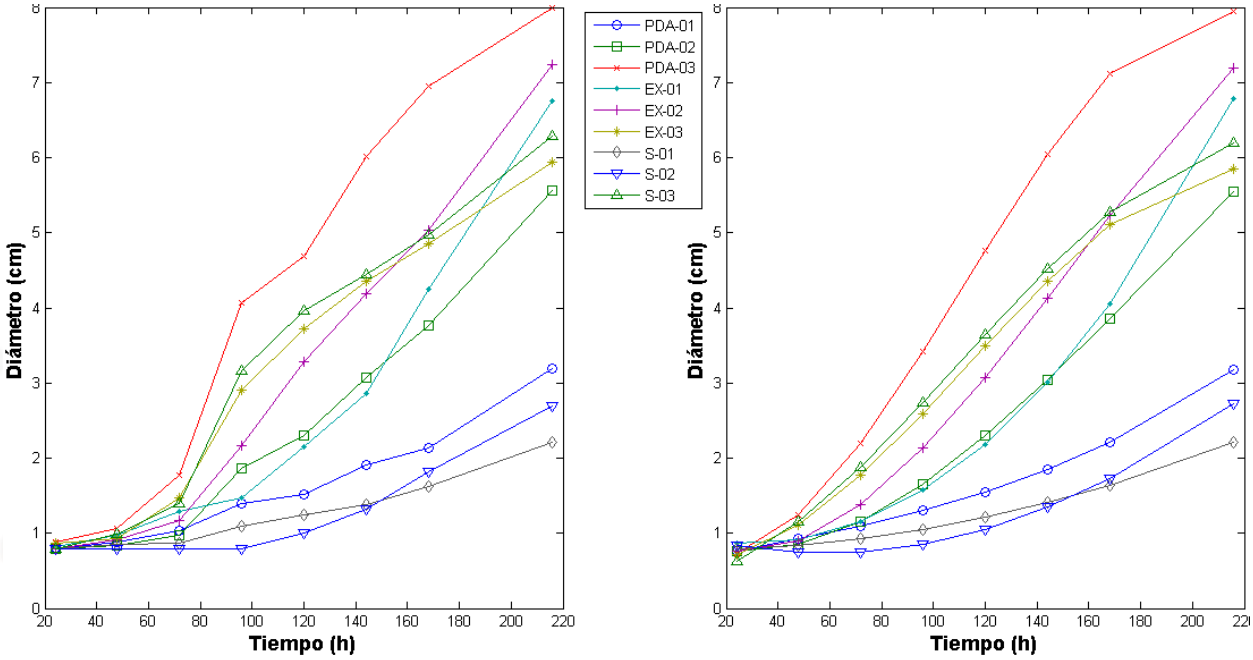

Figure 2. Radial growth kinetics with experimental data (a) *vs* adjusted radial growth kinetics based on the third order polynomial model (b).

Regarding the strain ITO-03, the three solid media presented similar growth capacity with respect to the other two strains. The regression model related the growth diameter of the strain (Y) with respect to the incubation time (x). Table 1 shows the comparative data with respect to the model and RG (radial growth rate). The measurements showed significant difference in the structure and form of growth of each strain in the three culture media analyzed.

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Table 1. Mycelial growth of Shiitake (*Lentinula edodes* Pegler)

STRAIN	CULTURE MEDIA	DIAMETER (cm)	Polynomial $f(x) = P_1x^3 + P_2x^2 + P_3x + P_4$ MODEL	R^2	RG ($cm*h^1$)
ITO-01	PDA	3.2	$f(x)_{P01} = 1.372E^{-07}x^3 - 2.943 * 10^{-06}x^2 + 0.006092x + 0.6225$	0.9936	0.011
ITO-01	EX	6.75	$f(x)_{EX01} = 9.293E^{-08}x^3 + 0.0001447x^2 - 0.00877x + 0.9962$	0.9966	0.028
ITO-01	S	2.217	$f(x)_{S01} = 9.779E^{-09}x^3 + 2.778E^{-05}x^2 + 0.0002129x + 0.7693$	0.|9945	0.007
ITO-02	PDA	5.567	$f(x)_{P02} = -4.605E^{-07}x^3 + 0.0002584x^2 - 0.01297x + 0.9273$	0.9952	0.022
ITO-02	EX	7.233	$f(x)_{EX02} = -9.64E^{-07}x^3 + 0.0004448x^2 - 0.02276x + 1.067$	0.9964	0.03
ITO-02	S	2.7	$f(x)_{S02} = -5.897E^{-08}x^3 + 0.0001001x^2 - 0.01114x + 1.052$	0.9929	0.009
ITO-03	PDA	8	$f(x)_{P03} = -1.974E^{-06}x^3 + 0.0006657x^2 - 0.0187x + 0.8245$	0.9868	0.033
ITO-03	EX	5.933	$f(x)_{EX03} = -1.161E^{-06}x^3 + 0.0003959x^2 - 0.007395x + 0.6829$	0.9857	0.024
ITO-03	S	6.283	$f(x)_{S03} = -9.339E^{-07}x^3 + 0.0003109x^2 - 0.003326x + 0.3774$	0.9758	0.025

Discussion

The most recent research focuses on the evaluation of culture media that facilitate the growth of fungi for study and research. The present work determined that culture media of potato dextrose agar (PDA) favors the mycelial growth of Shiitake (*Lentinula edodes*), followed by malt extract agar (EX) and the Sabouraud agar (S) respectively. This agrees with what was obtained by Suárez and Holguin (2011), who evaluated synthetic culture media PDA, OGY (oxytetracycline-yeast extract and glucose), S, concluding that the PDA favored mycelial growth followed by culture media OGY and the sabouraud agar respectively. On the other hand, Suárez (2012) used liquid fermentation in the production of *Lentinula edodes*; the culture medium used was PDA in agitation of 100 rpm, at 38 °C for 15 days, reporting that the biomass obtained is almost 20% on day 12 of agitation, and with a percentage of polysaccharides above 80%.

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Conclusion

The mycelium produced by the three strains, was more vigorous in Sabouraud agar. Such mycelium managed to occupy the entire Petri dish; in contrast, the mycelium grown in the other two culture media colonized only the center without developing throughout the Petri dish (Figure 2b); and it is reaffirmed with the graphs for S01, S02 and S03, which have a uniform growth trend. The previous reflected in the characteristics of the strains, such as the aerial mycelium of white color and cotton texture. The best radial growth speed of the mycelium in the three culture media was observed in the ITO-03 strain; while the ITO-02 and ITO-01 strains showed their greater growth capacity in the malt extract agar.

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Area XI

TRANSPARENT

Bioethics, Biotechnology, and Society: Tools for Assessment of the Support to the Scientific Sector, Including its Biotechnological Potential and Human Resources.

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Relevance and biotechnological potential in obtaining biofilms from agave waste in producing communities

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Abstract

Recently biotechnology has had a great impact in different areas of knowledge. However, these applications have influenced the receiving communities of this technology in the modification of paradigms of consumption, procurement and unconventional processes, having social implications. The study was in the regions of Morelos and Jalisco, since the regions in both states have the advantage of agave plant production for alcoholic beverages. This industry process generates residues that are not used. These residuals can be used for the manufacture of biofilms with different applications. For this study the use of Agave residues is proposed to obtain a sunlight protective skin membrane, with the possibility of being generated by the communities themselves, allowing the responsibility and decisions making that have individual and collective implications for the well-being of their community. With the biotechnological transformation of residuals from the agave, this waste is valued, the generation of products that favor heritage conservation for the community, as well as having a value as a consequence of the reduction of the pollution problem due to the effective use of waste.

Key words: agave ● biofilm ● waste ● communities.

Introduction

The reuse of post-consumer organic waste nowadays is a worldwide concern due to the great environmental impact and increased production of these wastes (FAO, 2010). Particularly in the case of Mexico, annually large quantities of residual lignocellulosic fibers are generated from different manufacturing activities, such as the production of mezcal and tequila, which are one of the most representative industries in Mexico (Hernández *et* al., 2000). The

production of this waste generates a source of important pollution for the communities that makes it putting at risk their health, the conservation of the landscape and their own heritage. These processes lead to the exposure of physical risks associated with climate that include among others, exposure to the sun for hours during the workday, leading to a wide variety of health hazards (McGuire *et* al., 2015). Recent research has demonstrated that residual lignocellulosic fibers can be transformed for the production of biofilms with different applications and potentialities. In addition to the premise of the use of plants that abound in the communities that in turn, generate organic waste such as *Opuntia ficus-indica* and *Aloe Barbadensis Miller*. This socio-technical-environmental work aims to generate biofilms through processes in the area to promote the conservation of the patrimony agave landscape through the recovery of residual materials.

Materials and methods

Residual samples of Agave

The biological material was obtained from the residual fiber that remains after cooking, grinding and extracting the fermentable juice from the Agave pineapple core. The residual bagasse was dried at 60 ° C, in a tray dryer until 5% moisture content of was reached. It was ground in a mechanical mill (Pulvex®) obtaining fibers between 1 and 4 cm in length. *Agave Tequilana Weber* was obtained from the Tequila, Jalisco (A) area, while the *Agave angustifolia Haw* variety was obtained from the area of Tlalquiltenango, Morelos (B), Mexico. To form the membranes, *Opuntia ficus-indica* and *Aloe Barbadensis Miller* mucilage were used, which were obtained from the same regions where the agave residuals fiber were obtained.

Obtaining the mucilage

For the extraction of the two mucilage in test, thorns were removed cut and crushed separately. In the case of the *Opuntia ficus-indica* (cladodes) mucilage (Figure 1) and the mucilage (Figure 2) of *Aloe Barbadensis Miller* leaves were milled in an industrial blender with rotating blades. Both samples were homogenized with water in a ratio of 1:5; cooking was carried out for 20 min at 85 ° C (pH 4-4.5), then filtered through a sieve and then passed through N.150 sieve to finally centrifuge at 4000 rpm / 15 min (Bekman, model Avanti J - 25), following the procedure developed by Abrajam (2008).

Obtaining biofilms

Mixtures of both types of fibers (A and B) were prepared in a solution of *Opuntia ficus-indica* and *Aloe barbadensis* Miller mucilage with constant magnetic stirring while heating up to 80

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° C. The solution also contained fructans. The suspension was emptied in Petri dishes (Figure 1), after which they were allowed to dry at 60 ° C for 48 hours in an oven (VWR Scientific Products) following the (Ibarra-Trujillo et al., 2012) method. The properties of (A) and (B) films were compared for the study.

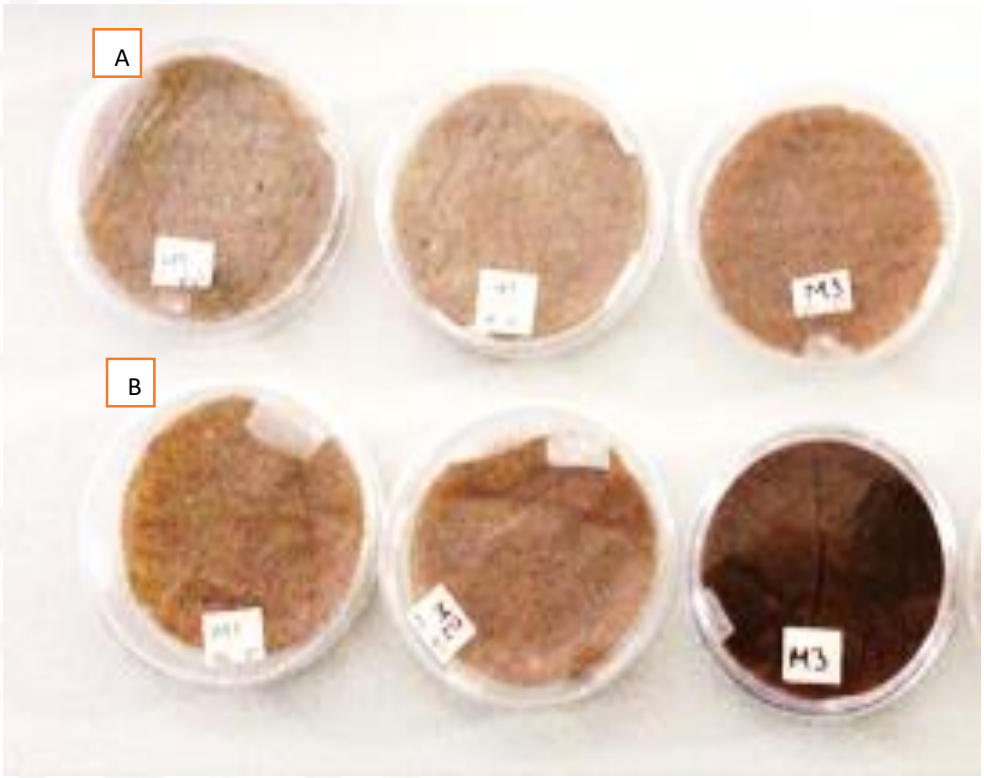

Figure 1. Biofilms obtained.

Morphological characterization of biofilms

For the morphology of the biofilm surface, transverse and longitudinal images are made by FE-SEM (Zeiss, Sigma, Germany) at 1, 3 and 5 magnifications (1z, 3z and 5z, respectively), stereoscopic and scanning electron microscopy.

Adsorption of ultraviolet radiation

The UV radiation adsorption of the biofilms was determined using a luxometer model EA30 of the brand Extech Instrument and a UV lamp model UVLMS-38 of 8 volts. The amount of light transmitted through the different biofilms was quantified. For this, the biofilms exposed

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to different wavelengths: 254, 302 and 365 nm, which cover the entire UV spectrum of light radiation. The luxmeter detector was completely covered, placing the lamp 24 cm away, inside a black box in order to eliminate the external light.

Results

The morphological surface structure in both (A) and (B) have rugoses and porosity due to the presence of agave fibers (Figure 2).

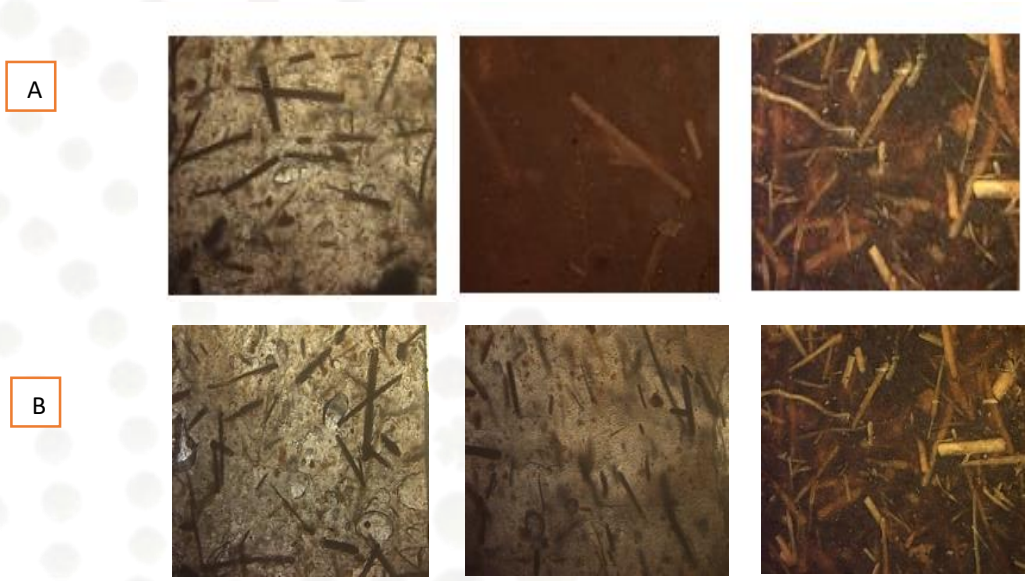

Figure 2. Stereoscopic micrographs of biofilms (A) and (B) obtained at 1z, 3z and 5z, respectively).

The conformation and adherence of the mucilage and the residual fiber to their different concentrations can be observed in different increases (Figure 3).

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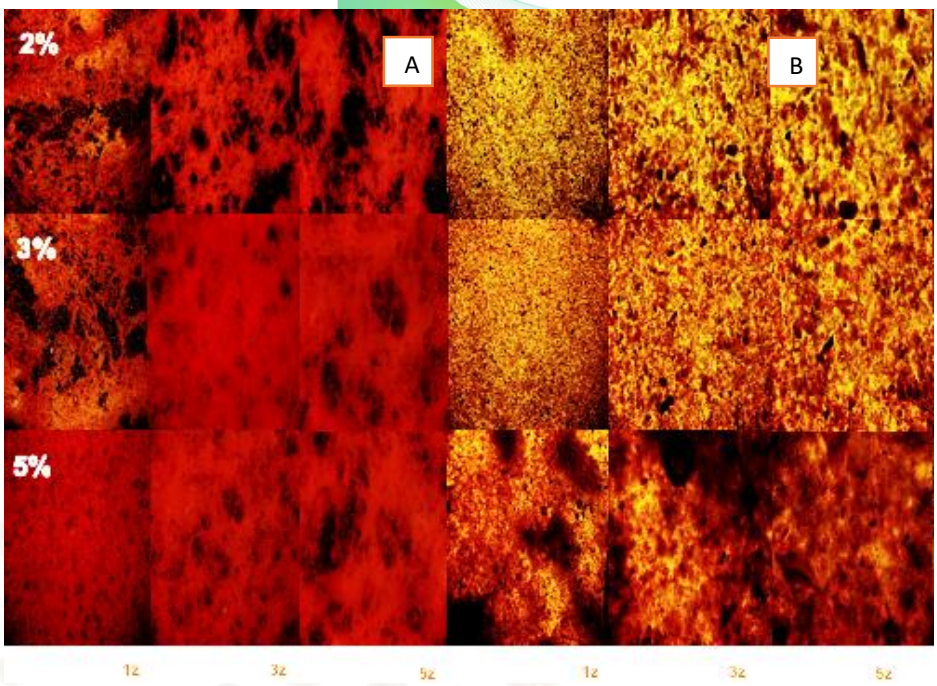

Figure 3. SEM micrographs of the biofilms of (A) *Agave tequilana* Weber and (B) *Agave angustifolia* Haw.

In Figure 4, the percentages of UV light or radiation absorbed by the different biofilms are presented, with the effect of impeding the passage of UV light in all of them.

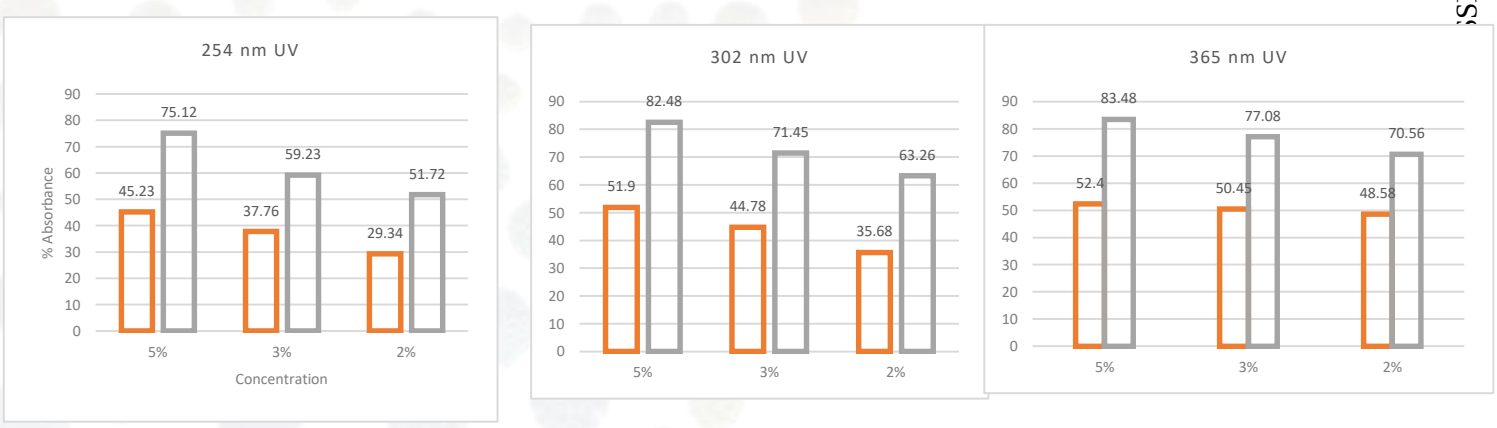

Figure 4. Absorbance according to residual agave fiber concentration.

Discussion

The porous morphological surface increases as the residual fiber concentration increases, improving its dispersion and uniform distribution along the surface, as reported by Maged *et* al., (2010), such is the case of the 2% concentration from (A) and (B) where agglomerations are observed in the distribution of residual agave fibers.

Due to the physicochemical interactions between the mucilage and the residual agave fibers, differences were observed between (A) and (B), related to the different morphology of the agave species. There is a better adhesion between the mucilage and the fiber (B). This is probably because the fiber in a polymeric matrix is more porous, and is more chemically related to the mucilage, causing adhesion and achieving a more uniform structure (Faruk, 2012).

The absorbed percentage is between 29 and 83% for the different lengths (254, 302 and 365 nm). Coinciding with that reported by Korać *et* al., 2011, who report the mucilage of cactus and aloe Vera due to their physiological and biological characteristics have a sunscreen effect. It can be observed that as the concentration of residual fiber increased, the UV light passage decreased by 20 and 30%. Similar results were described by Kapoor *et* al., 2009, in which they reported different herbs for sunscreens, an at different concentrations presented greater effectiveness at higher concentrations of the herbs.

Conclusions

The morphology of the biofilms is modified by the concentration of residual agave fibers, giving rise to a better conformation at higher concentrations. The biofilms formed absorbed between 29 and 83% of UV radiation, this behavior was directly proportional to the concentration of residual agave fibers. Recommendation for the exploitation of residues of each region was achieved, having individual and collective implications for the community, such as obtaining a protective biofilm for solar radiation for field workers of the same productive process that generates the residual.

Biotechnology Summit 2018
Vol 1 Year 1 Issue 1, ISSN: 2618-0464, http://www.bio.edu.mx/

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Biotechnology Summit 2018
Vol 1 Year 1 Issue 1, ISSN: 2618-0464, http://www.bio.edu.mx/

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Vol 1 Year 1 Issue 1, ISSN: 2618-0464, http://www.bio.edu.mx/