Scientific Caribbean Foundation (SCF)

Virtual Winter 2022
SCF Research Symposium

Biological Sciences – Chemical Sciences

Biomathematics

Saturday, December 10, 2022

Puerto Rico-Dominican Republic-Chile

SCIENTIFIC CARIBBEAN FOUNDATION (SCF)

ARE PROUD TO HOST THE

VIRTUAL WINTER 2022 SCF RESEARCH SYMPOSIUM

SHOWCASING UNDERGRADUATE AND HIGH SCHOOL STUDENTS' MENTORED RESEAR**CH**

Leadership at

SCIENTIFIC CARIBBEAN FOUNDATION, INC.

Juan F. Arratia, Ph. D.
President and Founder
Research Professor and Mentor

PUERTO RICO-DOMINICAN REPUBLIC-CHILE

DECEMBER 10, 2022

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SCIENTIFIC CARIBBEAN FOUNDATION

MISSION

Scientific Caribbean Foundation (SCF) was founded by Dr. Juan F. Arratia, a 2006 US Presidential Award for Excellence in Science, Mathematics and Engineering Mentoring recipient, with the idea to continue the success of the Model Institutions for Excellence (MIE), a grant awarded by the National Science Foundation (NSF) to transform Universidad Metropolitana (UMET) into a nationally recognized undergraduate research institution, and a model in science, technology, engineering and mathematics (STEM). Mentoring of undergraduates and pre-college students by research mentors was the cornerstone of the MIE Project. Dr. Arratia was the Principal Investigator of the MIE grant at UMET. We believe that creative research is one of the best ways to prepare students to become persistent and successful in college, graduate school and professional careers. Today, the Student Research Development Center (SRDC), which is part of the SCF, is the entity that will continues the MIE strategy by impacting pre-college and university students from institutions in Puerto Rico, across the nation and abroad.

EXECUTIVE SUMMARY

The MIE ended in 2009 at UMET. The outcome of the program was over 280 UMET STEM-C majors completed their BS degrees and 175 were transferred to graduate school, with 65 achieving doctoral status (PhD, MD, VVM, Pharm D). To increase the number of BS degrees transferred to graduate school, we will continue with the strategy of an early research program and partnership with key research institutions in Puerto Rico, the US mainland and abroad. Research mentoring will be the principal component of the knowledge transfer and creative thinking activities at SCF. Project based learning, collaborative learning strategies, presentations at scientific conferences, scientific writing and co-authorship, technology literacy, and preparation for graduate school are activities that are transforming the philosophy of competitive institutions.

GOALS

The main goal of the Virtual Winter 2022 SCF Research Symposium is to encourage pre-college and undergraduate researchers to work with research mentors, develop students' written and oral communication skills, provide a forum in the Caribbean for students to foster interest in undergraduate education, particularly in STEM-C fields, and set national research standards for pre-college research presentations.

SCIENTIFIC CARIBBEAN FOUNDATION, INC.

VIRTUAL WINTER 2022 SCF RESEARCH SYMPOSIUM

CONFERENCE AT A GLANCE

SATURDAY, DECEMBER 10, 2022		Virtual
10:30–10:35 a.m.	**Opening Ceremony**	**Virtual**
	Dr. Juan F. Arratia, Research Professor and Mentor	**Virtual**
10:35–11:10 a. m.	**Poster Session** **Biological and Chemical Sciences-**	**Virtual**
11:10-11:20 a. m.	**Award Ceremony and Closing Remarks**	**Virtual**
11:20 m.	**Symposium Adjourns**	

MESSAGE FROM THE FOUNDER

Dr. Juan F. Arratia – President of the Scientific Caribbean Foundation, Inc.

December 10, 2022

Dear Students:

The Virtual Winter 2022 SCF Research Symposium is the culmination of the activities and dissemination process of the Virtual Fall 2022 Saturday Research Academy. For a period of four months, since August 2022, students from private and public schools from Puerto Rico, Dominican Republic and Chile worked long hours using Internet with the guidance of faculty mentors in research projects in science, technology, engineering, mathematics, and computer science (STEM-C) fields.

One of the objectives of the Virtual Winter 2022 SCF Research Symposium is to offer young, motivated student researchers the opportunity to gain experience and to practice their English communication skills in a formal professional scientific meeting. A second objective is to give students from Dominican Republic, Puerto Rico, and Haiti a forum for the presentation of the outcomes and findings of their research projects to research mentors, family members, and the educational community at large.

Scientific Caribbean Foundation, Inc. is proud of the results obtained by the students and their research mentors in the Virtual Fall 2022 Saturday Research Academy Program. I hope your experience inspires you and your peers to select STEM-C as your field of study soon.

My sincere appreciation goes to SCF staff, research mentors and pre-college and undergraduate research students for their effort and commitment to implement the Virtual Winter 2022 SCF Research Symposium.

Sincerely yours,

Juan F. Arratia, Ph. D.
Founder and President
Research Professor and Mentor
Scientific Caribbean Foundation, Inc.

Research Mentors' Bio Sketches

Juan F. Arratia, PhD
Research Professor and Mentor
Scientific Caribbean Foundation, Inc.

Dr. Juan F. Arratia was born in Pomaire, Chile. He graduated from Universidad Técnica del Estado with a BS in Electrical Engineering in 1973. He was awarded a MSc in Engineering from Louisiana Tech University, Ruston, Louisiana, in 1979 and a Ph.D. in Electrical Engineering from Washington University, St. Louis, Missouri in 1985. He has taught and conducted research at universities in Chile (Universidad Técnica del Estado and Universidad Austral de Chile), Puerto Rico (Universidad Interamericana de Puerto Rico and the University of Puerto Rico-Mayaguez), and in the US mainland at Washington University, St. Louis, and Louisiana Tech University, Ruston, Louisiana. He has lectured and given conferences on advanced automation, robotics, vision systems, artificial intelligence, total quality management and science and engineering education in Chile, Bolivia, Ecuador, Guatemala, Panama, Mexico, Brazil, Nicaragua, Perú, Canada, Spain, the Netherlands, Turkey, Japan, Philippines, Singapore, Australia, China, Puerto Rico and in the US mainland. He was the Advanced Manufacturing Manager for Medtronic, Inc., a leading pacemaker company, and is a consultant in advanced automation for pharmaceutical and medical devices companies in Puerto Rico. From 1998 to 2008, he was the Director and Principal Investigator of the Model Institutions for Excellence (MIE) Project, a National Science Foundation sponsored program based at Universidad Metropolitana in San Juan, Puerto Rico. From 2008 to 2018, he was the Executive Director of the Ana G. Méndez University System (AGMUS) Student Research Development Center, designed to disseminate MIE best practices at Universidad del Turabo and Universidad del Este. For twenty year he was part of AGMUS and during his tenure he wrote proposal to NSF and was awarded more than 85 million USD for MIE, CCCE, AGMUS Institute of Mathematics, MRI-AMISR, MRI-Puerto Rico Laser, Administration of Arecibo Observatory, among others. Since 2018 to present he is the President of Scientific Caribbean Foundation in San Juan Puerto Rico. In November 2007, he was awarded the Presidential Award for Excellence in Science, Mathematics and Engineering Mentoring at a ceremony in the White House in Washington DC.

Fabiola D. Pagán Torres is currently a rising senior at the University of Puerto Rico at the "Bayamón" Campus pursuing a bachelor's degree in Biology. She has passed through enrichment opportunities in different areas of science. During her senior year of high school, she started to get involved in research. Everything started by attending to the Pre-College Saturday Academy of the Ana G. Méndez System sponsored by the National Science Foundation. Here she was able to complete two pre-college level scientific investigations. After that, she had the opportunity to attend an internship at the University of Vermont, where she worked with Dr. Vigouroux. Over time, the opportunity of mentoring was given in the Saturday Research Academy. She applied the skills learned from her past mentors to the new generation of researchers. From that time through now, she has mentored over 30 students on what research is. Later on, another opportunity arrives to participate in the SNURF Program of the University Of Vermont under the guidance of Dr. Ballif. Here the research was focused on being able to study the protein called TLT1 more thoroughly. During this process, she acquired skills that are essential in molecular biology. From learning how to culture cells, do immunoprecipitation, SDS-Gels, and western blots. She also worked three years as a NASA Teacher Assistant in STARBASE, P.R., a STEM focus program of the Department of Defense. Her long-time goal in science is to acquire an M.D /Ph.D.

Britney Hopgood is a chemistry professor at Universidad Ana G. Mendez Recinto de Carolina. She is a Ph.D. student at the University of Maryland Baltimore County. She has worked on a multitude of research projects, from the fields of chemistry to the fields of applied physics and engineering. She has worked at NASA LaRC and with the Puerto Rico Science Trust. She has also traveled to present at many conferences and participated in leadership workshops and workshops in which she learned about different technologies in the field of photonics. She has been invited to participate in many outreach initiatives to make the STEM field more accessible to all. Given her passion for teaching, she founded a company to offer to tutor young people of all grades called Britney Hopgood Tutoring.

Research Presenters' Bio Sketches

Sofía De León is a junior at Academia María Reina in San Juan, Puerto Rico. She was born in San Juan, Puerto Rico and is currently sixteen years old. She is very interested in biology and the human body; however, she is quite fascinated with the nanotechnology known as CRISPR. Her passions include reading books, spending time with family, traveling, and running. She is currently enrolled in Dr. Arratia's program Fall 2022 Saturday Research Academy and her research poster consists of how disabling a protein using CRISPR can be used to treat Cancer. Her future plan is to become a doctor and keep investigating/researching topics that fascinate her.

Naydelin Zapata Arias was born in Santo Domingo, Dominican Republic. She is currently 20 years old and is studying for a degree in microbiology at the Autonomous University of Santo Domingo (UASD). She is extremely interested in everything that is the field of molecular biology, genomics, and microbial genetics. Among her passions are reading, music, going to church, participating in coastal cleaning, and helping in microbiology laboratories, especially in the preparation of bacterial culture media. In the future, she hopes to continue participating in research based on molecular biology and microbiology that can contribute to the development of treatments for bacterial resistance and the strengthening of the immune system in huma

Camila Oñate Cheuquepán *is a student who recently graduated from the Liceo las Araucarias, Curacautín, Chile. She was born in Temuco, Chile. She is currently seventeen years old. She has been very interested in microbiology and molecular biology from a few years. Her passions include venturing out to learn new languages, playing the piano, and reading. She has been part of the PROENTA-UFRO program at the Universidad de la Frontera for seven years, where she has also participated in different math Olympics regional, obtaining important distinctions. He recently completed a research internship in the scientific and technological nucleus at "BIOREN", also at the Universidad de la Frontera, where together with the Citizen Diplomacy Action Fund and Scientific Caribbean Foundation, Inc. She carried out the study, analysis and characterization of the composition of rainwater in Curacautín, Araucanía Region, Chile).*

Nailha Acacia is a microbiology student. She was born and raised in Haiti and is currently completing her 5th semester at Universidad Autònoma de Santo Domingo in the Dominican Republic. She's interested in microbial physiology, ecology, and environmental microbiology. She's still exploring possible applications for bioinformatics, genetic and molecular technics in her field. She is passionate about dance, drawing and learning new things. Her goals are to keep up with current investigations, to apply the obtained knowledge to the development of her country in the future. Her research for this SRA session is related to Azotobacter vinelandii, this is a nitrogen fixing bacterium that can be used as a biofertilizer.

SCHEDULE OF EVENTS

SATURDAY, DECEMBER 10, 2022 VIRTUAL

10:35 – 11.10 a.m. POSTER SESSION

Chairperson: Dr. Angel Arcelay

10:35 – 10:41 a.m.	**Sofía C. de León Gómez,** Academia María Reina, San Juan, Puerto Rico Disabling p21 Through Genome Editing CRISPR-Cas9 to Fight Cancer.
10:42 – 10:48 a.m.	**Naydelin Zapata Arias,** Universidad Autònoma de Santo Domingo, Santo Domingo, Dominican Republic CRISPR-Cas9: A Solution for Antibiotic Resistant Bacteria
10:49 – 10:55 a.m.	**Camila *Oñate* Cheuquepán,** Liceo las Araucarias, Curacautín, Chile Physicochemical Characterization of Rainwater, Curacautín, Chile
10:56 – 11:02 a.m.	**Elizabeth Nailha Acacia,** Universidad Autònoma de Santo Domingo, Santo Domingo, Dominican Republic Azotobacter Vinelandii: A nitrogen-Fixing Bacterium

Posters of Research Presenters

Sofía de León

Disabling p21 Through Genome Editing CRISPR-Cas9 to Fight Cancer

Sofía C. De León Gómez (sofiadl2024@gmail.com) Academia María Reina, 1879 Av. Glasgow, Urb. San Juan, 00921, P.R.
Fabiola D. Pagan Torres (fpagan.sra@gmail.com) Scientific Caribbean Foundation, 12 Camino Francisco Rivera, San Juan, 00926, P.R.

Abstract

The new biotechnology breakthrough called CRISPR Cas9 is a tool that can modify the genetic information of a cell in a cheaper, faster and more effective way. It has a variety of uses, including curing genetic diseases like cancer, an illness that can be caused by the presence of the protein p21, also known as cyclin-dependent inhibitor 1 [9,11]. The cyclin-dependent inhibitor-1 can have a dual purpose; for instance, it works as a tumor suppressor, yet it can also play an oncogenic role [8]. If scientists regulate the protein using CRISPR Cas9, then cancer patients may be able to fight this disease more efficiently [8]. Our goal is to identify a better alternative to treat cancer using this innovative tool of CRISPR Cas9. Therefore, this nanotechnology could be used to fight off cancer and hopefully help patients to have an easier recovery [8]. This research consists of scientific articles with a time limit of 2007-2022; we used fifteen (15) keywords and twelve (12) publications.

Keywords: CRISPR/Cas9; genome editing; DNA sequence; p21; cyclin dependent inhibitor; nanotechnology; manipulation; gene therapy; protein; reconstruction;

Introduction

- CRISPR "Clustered Regularly Interspaced Short Palindromic Repeats" Cas9 can alter genes by accurately cutting DNA, which is subsequently repaired naturally by the body [11]. The system's two main components are the Cas9 and a guide RNA [5].
- Cancer is a condition that affects millions of people [7]. This disease is caused when some of the body's cells grow out of control and spread to other bodily regions [12]. One of the most common treatments of cancer are chemotherapy and radiation; however, these treatments have various downsides.
- Cyclin-dependent inhibitor 1, also known as p21 is a protein that can lead to growth arrest in the cell cycle through the cyclin kinases pathway; however, it can also function in an oncogenic matter and promote cancer [8].
- The behavior and purpose of the p21 protein changes depending on its location. For instance, in a nuclear location, it has a pro-apoptotic function, but in a cytoplasmic location, it promotes anti-apoptotic and oncogenic functions [8].

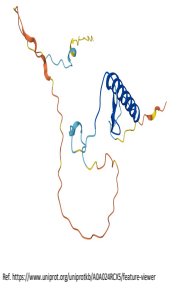
Hypothesis

Gene knockout and regulation of the p21 protein's using CRISPR Cas9 can develop a new treatment for cancer.

Methodology

Figure 1. Methods use during the process of the research. This research was elaborated based on scientific articles with a time limit of 2007-2022, a combination of fifteen (15) keyword and twelve (12) publications. *Created with BioRender.com*

Results



Ref. https://www.uniprot.org/uniprotkb/A0A024RCK5/feature-viewer

Functions of p21:
Regulates cell division and growth by constraining the cell cycle through the cyclin kinases pathway. At times, it can inhibit apoptosis; however, it can also prevent it.

Location: Nucleus
In the nucleus, p21 constrains the activity of cyclin dependent kinases Cdk1 and Cdk2. Also, it can restrain the transition from G1 phase into S phase or from G2 phase into mitosis after DNA damage, so it can be repaired.

Location: Cytoplasm
In the cytoplasm, the protein behaves as an anti-apoptotic. If there is little damage done to the DNA then, the amount of p21 is increased and cell cycle arrest is induced and the protein functions as anti-apoptotic. Moreover, if there is substantial DNA damage then, the expression of p21 is decreased, allowing the cell to undergo apoptosis. Also, it binds to and suppresses caspase 3, and kinases ASK1 and JNK, apoptotic kinases.

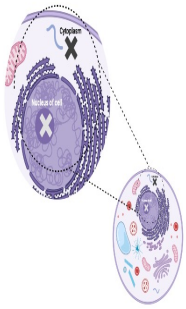


Figure 3. Function and location of p21. The cyclin-dependent protein kinase (CDKs) holoenzyme complex contains a kinase catalytic subunit associated with a regulatory cyclin partner. In the nucleus, the expression of the gene is specifically in the nuclear body which is extra-nucleolar nuclear domains and the nucleoplasm which is the part of the nuclear content other than the chromosomes or the nucleolus. https://www.ebi.ac.uk/QuickGO/term/GO:0005654 *Created with BioRender.com*

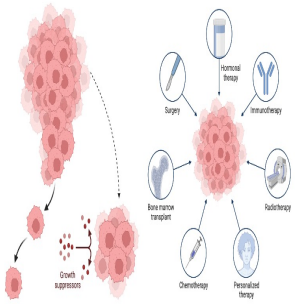


Figure 4. Cancer replication and therapies available. In normal cell division, the multiplication of cells is controlled and if a cell's DNA is damaged, then apoptosis, the cell's death, will be initiated; however, cancer cells do not have this form of programmed death and the malignant cells grow out of control and spread to other bodily regions. Regarding the therapies available, this proposal will be classified as personalized therapy. *Created with BioRender.com*

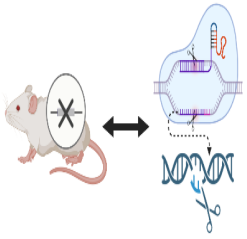


Figure 5.. Gene knockout and CRISPR-CAS9 as possible mechanisms to develop new treatments. CRISPR-CAS can cut CDKN1A, the gene responsible for encoding p21, and replace it with one that is instructed with lowering the levels of p21 [2].
Decreasing the p21 expression in cancer patients will increase the chances of apoptosis taking place in damaged cells, reducing the chances of cancer.[8] *Created with BioRender.com*

Conclusion

Therefore, the location of the p21 is crucial as the cyclin-dependent inhibitor 1 can act in an oncogenic way in the cytoplasm of the cell. That being the case, if the gene that is responsible for the creation of p21 is manipulated then, the amount of p21 can be controlled and this method can be used to control the spread of malignant cells.

Future works

- ✓ Keep researching CRISPR Cas9 and how it can help fight cancer to keep finding more evidence.
- ✓ Following a strict protocol, investigators could buy cells from a provider or use rats to test on to ensure that the methods are safe and hopefully be able to take it to clinical trials.
- ✓ Intend to explore if CRISPR Cas9 can be used to help people with disabilities, for instance, vision impairment, mental health conditions, deafness, and much more. If so, how effective is it and what are the safety risks.

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Acknowledgments

First, I would like to acknowledge and give special thanks to Fabiola D. Pagan Torres, my supervisor, for providing me with helpful guidance and advice during the research process. Her counseling assisted me in the title of my project, the writing, and so much more. Moreover, I would like to express my gratitude towards Dr. Juan Arratia for allowing me to be part of the Research Program, for providing me with a great mentor, and for giving me the resources necessary to complete my research. Finally, I would also like to thank my parents for letting me join this program that has allowed me to expand my knowledge.

Naydelin Zapata Arias

CRISPR-Cas9: A Solution For Antibiotic Resistant Bacteria

Naydelin Zapata Arias (allisonzapataarias@gmail.com), Universidad Autònoma de Santo Domingo, Alma Máter, Santo Domingo, República Dominicana.
Fabiola D. Pagán Torres (fpagan.sra@gmail.com), Scientific Caribbean Foundation, 12 Camino Francisco Rivera, San Juan, 00926.

Abstract

Studies have already developed bacteriophages modified by CRISPR-Cas whose mission is to eliminate resistant bacteria or reverse this resistance process. Cas3 and Cas9 have been identified in the development of these experiments as potential biotechnology tools (Elisa Garcia, 2019). Our goal is to find a solution to microbial resistance; with minimally invasive treatments. The microbial resistance of microorganisms, generally the most pathogenic, represents infections, including nosocomial ones. Using modified bacteriophages by CRISPR-Cas9 endonuclease may function as a modified vehicle to attack them. Therefore, obtaining a weak bacterial wall; thus, making the organisms more susceptible to therapeutic targets. We would gain an improvement in the immunological health of the individual and a possible effective treatment that would eliminate the use of broad-spectrum antibiotics. The content of this research was composed of seven (7) differents scientific articles using specific keywords during the search. With the findings, we will continue working toward identifying new antimicrobial mechanisms focusing on their effectiveness and how the evolving resistance proceeded.

Keywords: microbial resistance; CRISPR-Cas9; antibiotics; pathogens; mutation; bacteriophage; engineered;

Introduction

- Bacteriophages (phages) are viruses that specifically infect bacterial species, resulting in lysis and self- propagation. They can be used as a novel tool to combat persistent human health problems, like multidrug resistant bacteria.
- CRISPR-Cas9 may be a solution, thanks to the DNA decoding technology it presents, thus giving us the opportunity to alter the microbial structure of the most pathogenic microorganisms, making them more sensitive to antibiotics or their inactivity.

Hypothesis

Engenireed CRISPR-CAS9 batheriophages could be a solution to microbial resistance since is a minimally invasive treatment.

Methodology

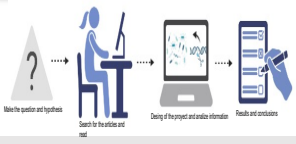

Figure 1. Methods use during the process of the research. Created with BioRender.com

Results

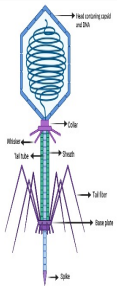

Figure 2. Bacteriophage T4. T4 phages are part of complex viruses, consisting with more than 40 different proteins form the mature virion. Its genome is encapsulated in a protein shell with a 172-kpb, linear, double stranded (ds) DNA. Leiman, P. G., Kanamaru, S., Mesyanzhinov, V. V., Arisaka, F., & Rossmann, M. G. (2003). Structure and morphogenesis of bacteriophage T4. *Cellular and Molecular Life Sciences CMLS*, 60(11), 2356-2370. Created with BioRender.com. https://images.app.goo.gl/tu2AKKCFQbXR76HAA

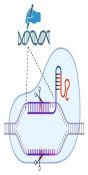

Figure 3.CRISPR-CAS9. CRISPR/Cas9 is a gene-editing technology that involves two essential components: a guide RNA to match a desired target gene and Cas9 (CRISPR-associated protein 9), an endonuclease that causes a double-stranded DNA break, allowing modifications in the genome (Redman M et al., 2016). Created with BioRender.com

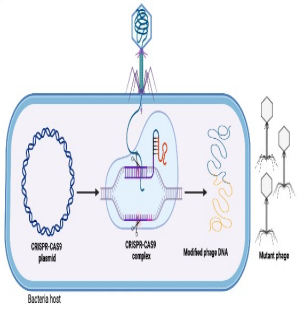

Figure 4. Development of Mutant Bacteriophage by CRISPR-CAS9. The engineered CRISPR-CAS9 plasmid will have the mutations needed to induce the phage genome by recombination. The CRISPR-CAS9 complex will bind to the target site of the bacteriophage DNA, creating a break during the infection. The results will be mutant's phages of interest. Chen Y, Batra H, Dong J, Chen C, Rao VB and Tao P (2019) *Genetic Engineering of Bacteriophages Against Infectious Diseases. Front. Microbiol.* 10:954. doi: 10.3389/fmicb.2019.00954. Created with BioRender.com

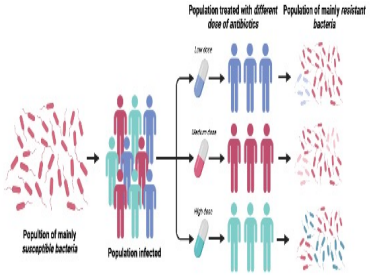

Figure 5. Antibiotic resistance. In a population of bacteria where the majority are susceptible to the antibiotic after the administration of a higher dose, the susceptible ones die and only those that present resistance to the agent survive. The result is a population of bacteria that are resistant due to the exposure of the higher dose. Created with BioRender.com

Future works

With the applications derived from CRISPR-Cas9 in antibiotic resistant bacteria, we would like to:

- ✓ Try to develop a lab protocol to proceed experiments in cultured cells and sequence the genome.
- ✓ Continue researching and experimenting with CRISPR-CAS9 to develop new antibiotic treatments.

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Conclusion

Clustered Regularly Interspaced Palindromic Repeats (CRISPR-Cas9) is a technology applied to gene editing. This allows correcting errors in the genome and activating or deactivating genes in cells and organisms quickly, cheaply and with relative ease. The use of bacteriophages modified by CRISPR-CAS9 is something innovative that requires more research and experimentation. Even so, in the studies that have been successful, demarcated that it is a novel strategy to combat the crisis that we are facing due to antibiotic resistance.

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Ms. Fabiola Pagán-Torres
Universidad Autónoma de Santo Domingo
Elizabeth Nailha Acacia

Camila *Oñate* Cheuquepán

Physicochemical characterization of rainwater, Curacautín, Chile.

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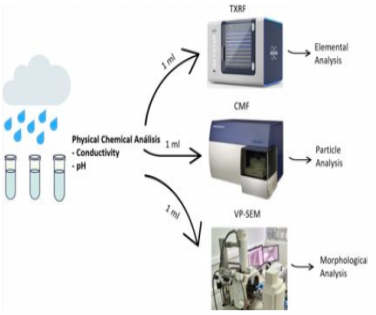
Abstract

Over the last decade and due to global warming, Chile has been immersed in a water crisis, a major problem is the waste of potentially potable water in rural areas. For this reason, a physicochemical characterization of rainwater from Curacautín, Chile. The investigation included 3 sampling stations where water was collected without contact with any surface. The characterization included the measurement of pH and conductivity, elemental quantification with Total Reflection X- ray Fluorescence (TXRF), particle analysis by Flow citometry (FCM) and morphological analysis by Electron scanning microscopy (SEM). The analyses showed that for the 3 sampling points analyzed, the parameters of conductivity and concentration of chlorine, aluminum, iron, copper, nickel and zinc are outside the ranges required for potable water according to the Chilean norm, instead of, pH, magnesium and aluminum concentration showed values within the allowed limit. The cytometry analysis was showed an increase in two of the samples when compared to the control and the micrographs (SEM), so further microbiological study would be beneficial to complement these studies.

Introduction

Water scarcity was defined as a decrease in the quality and quantity of available freshwater, in detriment to human health and/or economic activity (Carrasco P et al., 2018.). The sustainable management of rainwater could be approached resource for different uses such as agricultura and drinking water, but the requirements were different(Llopart-Mascaró et al., 2010 y Rojas-Valencia et al., 2012). The quality of rainwater was not been studied in detail and in several rural localities its physical, chemical and microbiological conditions were unknown (Arcila & Zúñiga, 2014), so the study of these is of great importance to evaluate its possible uses and it also helps us to demonstrate the contribution of pollutants emitted by natural and anthropogenic sources to the atmosphere (Morera et al., 2012 y Hernández et al., 2016). Therefore, the present study aims to evaluate the physicochemical and elemental composition of rainwater extracted on 7th August, 2022, from the IX region in the area of Curacautín to determine its potential use, mainly in the agricultural area.

Methodology



Rainwater was collected in sterile conical tubes of 50 ml. Approximately 1 ml of each tube was subjected to physical chemical analysis (pH, conductivity), elemental analysis by TXRF, particle analysis by Flow Cytometry and Morphological analysis by Scanning electron microscopy.

Results

Table N°1.Physicochemical Analysis in water

Sample code	pH	Conductivity (µS)
Sample 1	11.01	1.49
Sample 2	10.70	1.81
Sample 3	10.35	1.52

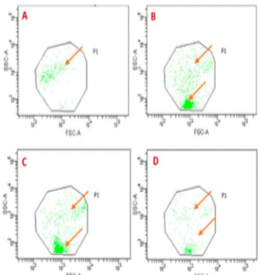


Figure 1. Particle analysis by Flow Cytometry. A) Control water. **B)** Sample 1. **C)** Sample 2. **D)** Sample 3. Flow Cytometer FACs Canto II, Becton Dickinson-USA.

Table N°2. Elemental Analysis by Total Reflection X- ray Fluorescence (TXRF)

Element	Unit	Sample 1	Sample 2	Sample 3
Magnesium (Mg)	mg/L	373,70	674,40	316,00
Aluminium (Al)	mg/L	10,32	17,73	11,10
Silicon (Si)	mg/L	1,15	2,22	1,51
Phosphorous (P)	mg/L	0,49	1,03	1,08
Sulfur (S)	mg/L	13,73	14,36	10,36
Cloro (Cl)	mg/L	177,40	175,60	183,50
Potassium (K)	mg/L	1,94	1,99	2,11
Calcium (Ca)	mg/L	0,43	0,49	0,48
Manganese (Mn)	mg/L	0,06	0,10	0,08
Iron (Fe)	mg/L	0,14	0,15	0,24
Cobalt (Co)	mg/L	ND	0,01	ND
Nickel (Ni)	mg/L	0,02	0,04	ND
Copper (Cu)	mg/L	0,05	0,08	0,04
Zinc (Zn)	mg/L	0,04	0,06	0,03

ND: No detected

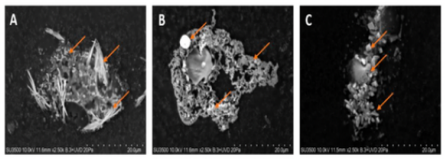


Figure 2. Morphological Analysis by Scanning Electron Microscopy. A) Control water. **B)** Sample 1. **C)** Sample 2. **D)** Sample 3. Scanning Electron Microscope SU3500, HITACH-Japan.

Discussion and conclusions

The rainwater samples showed pH values higher than those permitted for drinking water according to NCh 1333 y NCh 409, which accepts values between 6.5 and 8.3. Conductivity (1.49; 1.81 and 1.52 µS) is within normal ranges (0.75 a 7.5 µS/cm2). Elemental analysis (TXRF) showed elevated values for Mg (373.7; 674.4 and 316.0) even though these values do not report toxicity by regulation. The values for Cl (177.4; 175.5 and 183.5 mg/L) are within the expected range (< 200 mg/L), furthermore, elements were identified that are regulated for their toxicity, such as Al, Fe, Cu, Ni and Zinc, all under the permitted limit, except for Al. Particulate matter analysis (FCM) showed an increase in particulate material in two defined populations compared to the control, when comparing these data with the micrographs (SEM), we were only able to observe saline deposits, consistent with the presence of particulate matter, but we cannot discard that they correspond to bacteria or fungi present in the samples because no microbiological analysis was performed.

Future Works

- Implementation of a rainwater collector prototype.
- Development of Microbiological Analysis techniques

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Elizabeth Nailha Acacia

Azotobacter vinelandii: A nitrogen-fixing bacterium

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Universidad Autónoma de Santo Domingo[1], Universidad Ana G. Mendez Recinto de Carolina[2], Scientific Caribbean Foundation[3]

ABSTRACT

The biological fixation of nitrogen is an indispensable metabolic process limited to bacteria and archaea that sustains life on earth. All living organisms require nitrogen to ensure vital functions and structures. This study uses bioinformatics methods to compare available genomes for different *Azotobacter vinelandii* strains, a well-known diazotrophic bacterium often used as a biofertilizer or bioplastic producer. In this study, the possible phylogenic relation to the nifH region of different diazotrophs is proposed. Different microbiology methods were employed to isolate and cultivate nitrogen-fixing microorganisms from the soil. Results showed that growth in a nitrogen-free environment is challenging, and that very small mutations occur in the *Azotobacter vinelandii* strains. No apparent relations have been established between the nifH sequences, respiratory system, and environments of the different diazotrophs.

INTRODUCTION

Azotobacter vinelandii is a soil bacteria that has been studied for over 100 years by various scientists. It presents a great interest in agriculture due to its metabolism as a nitrogen-fixing bacteria.[1] Discovered in 1903 in Vineland's soil and adopted as a model organism, *A. vinelandii* is known for its genetic pliability, its natural competence, for producing multiple valuable polymers (bioplastic and alginate), production of biofuel, and specifically for its nitrogen-fixing prowess; which could reduce the need of synthetic fertilizer in agriculture and industrial fermentations.[2] Even though nitrogen is a clue element in the biogeochemical cycle that all organisms should metabolize as it is part of all known amino acids and nucleic acids (DNA, RNA), the ability to fix N_2 from the atmosphere and convert it into NH_3, NO_3, and others is limited to bacteria and archaea[3].

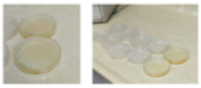
According to the Dos Santos team, this limitation can be explained by the following factors: the enzyme that catalyzes N_2 reduction called nitrogenase is oxygen-sensitive, and fixing $N2_2$ costs a lot energetically (16MgATP) for a single nitrogen molecule; thus, nitrogen is a poor catalyst, and the nitrogenase is a complex metalloenzyme that relies on a consortium of metal-Sulphur with cofactors as Fe, Mo or V for its activity. Thanks to the work of Sebatul and co-workers, since 2009, the *A. vinelandii* genome has been available and consists of a circular chromosome of 5,365,318 bps. A. vinelandii is also polyploid and can contain almost 80 copies of its genome. The most studied mechanisms are nitrogen fixation by *A. vinelandii* and its secretion of alginate polymers from forming cysts or other processes. There has been significant interest shown for its respiratory protection system beyond its high respiration rate because of being an aerobic bacteria with a nitrogenase oxygen-sensitive enzyme that must physiologically control the concentration of O2 in the cytoplasm. *A. vinelandii* has also been studied for its various biotechnological potentials, including the production of hydrogen from the nitrogenases, natural polymers of industrial interest and application of nitrogenase to alternative substrates.

Nowadays, this bacterium has yet to be fully explored via bioinformatic methods. In this project, we will study the genomic conservation of the different strains of *Azotobacter vinelandii*, isolate and cultivate diazotrophic microorganisms, and compare the growth rate of either diazotrophic bacteria or non-diazotrophs to understand how challenging this metabolism is, and finally study a possible relation between the nifH gene and the respiratory system of different diazotrophs.

METHODOLOGY

Microbiological Methods

1. Medium preparation: An accommodation of Ashby's agar was prepared for the medium. Components' measurements were calculated with a *OHAUS*™ – Scout-Pro electronic balance to get the following quantities of substances: Agar *Bacto*™ 3g; Dipotassium Phosphate(K_2HPO_4) 0,04g; Magnesium Sulphate 7 Hydration ($MgSO_47H_2O$) 0,04g; Sodium Chloride (NaCl) 0,04g; Hydrated Calcium Chloride ($CaCl_2.2H_2O$) 1g; Mannitol 4g . All the chemicals were put in a flask of 400ml. 200ml water was measured with a graduated cylinder and poured into the previous flask forming the medium solution. The solution was mixed homogeneously using *Thermo Scientific*™ hot plate with 60ºC and 300rpm. The pH was measured through a pH meter. The medium was sterilized via steam autoclave at 121ºC, 15 psi for 15 minutes. After this process, we poured the medium solution into 10 Petri dishes; two of those dishes received three drops of Phenol red as an indicator of the future acidification of the medium.



2. Sampling: On the next day, the soil samples were isolated from the UASD campus on the following site: Science Faculty Park, the Park in front of the Pedro Mir Library, the Sportive Camp, and finally, in front of the High Technology Laboratory. About 20 g of soil was taken in each location with a spoon and collocated in a different plastic cup. All the soil samples were cultivated in the previously described medium with a swab onto a Petri dish through the streak plate method and were incubated at 35ºC for six days.

3. Microbiological analysis: The biochemical identification was done through the following test TSI, Catalase, Indole, and Mannitol fermentation following the Flores- Gallegos' methods'[4] . The morphological identification was made through microscopic observation of the colonies after a Gram stain (macro-observation and single bacteria observation).

Bioinformatic Methods

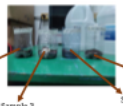
The different Azotobacter vinelandii strains genomes were obtained from NCBI All database. Alignment was done through the window version of ClustalX2.1. The genome analysis and comparison were made through GeneDoc by highlighting either the most conserved sequences or the different mutations present in the genomes. For the phylogenetic's study of the nifH gene from different diazotrophs, all the sequences were also obtained from NCBI in FASTA format and collocated in Phylogeny.Fr in A la Carte option considering a root or common ancestor. Through Figtree, we got cladograms of the previous tree. We also compare the different nifH sequences strictly through the neighbor joining.

RESULTS

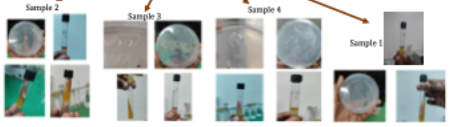
Microbiology

Microbial growth of bright cream colonies and small translucent colonies was observed in all the agar plates, meaning that the microorganisms metabolized the mannitol as the only carbon source. Also, nitrogen fixation was confirmed by the phenol red indicator by the fuchsia coloration of the plate, indicating the formation of NH3 and alkalinity of the medium.

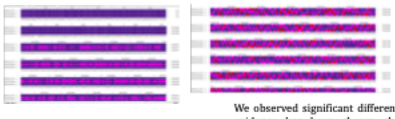
The microscopic observation after the Gram stain revealed different morphologies, like rod-shaped, spherical, and in pairs (diplobacilli).



We got the following results for the biochemical test:
- Sample 1: indole (-) , TSI (+ for all sugars)
- Sample 2: indole (+), TSI (+ for glucose, gas liberation)
- Sample 3: indole(+), TSI (+ for glucose), Catalase (+)
- Sample 4: indole (-), TSI (+ for glucose), Catalasa (+)

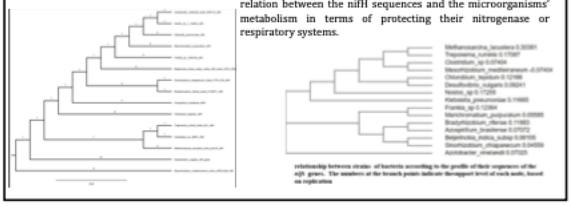


Bioinformatics



Of the 3 Azotobacter vinelandii strains, Dj and CA6 are the most conserved sequences.

We observed significant differences in each tree. No apparent evidence has been shown about a possible phylogenetic relation between the nifH sequences and the microorganisms' metabolism in terms of protecting their nitrogenase or respiratory systems.



CONCLUSION AND FUTURE WORKS

We have successfully isolated different diazotrophs from the soil. Biochemical and microscopic results suggest that those microorganisms can be different bacteria, archaea, or strains of *Azotobacter vinelandii*. More molecular tests are needed for the exact identification. In GeneDoc, we observed that DJ and CA6 count with more conservative sequences than CA. Relevant comparisons in the growth rate of diazotrophs and extremophilic diazotrophs have yet to be done because of the differences in the observation period, chosen medium for the culture, incubation temperature, and others. None of the elaborated trees present anaerobic, aerobic, or symbiotic relations through the formed clades from the nifH analysis. In future works, we propose:

- Continue to characterize microbiological samples of the *Azotobacter vinelandii* nitrogen-fixing bacteria through molecular technics and then determine growth rate and compare them to other nitrogen-fixing bacteria.
- Determine the phylogenetic tree of *Azotobacter vinelandii* and compare it to other bacteria.
- Further study the mutations of *Azotobacter vinelandii* and compare them to other nitrogen-fixing bacteria.
- Further study of possible phylogenetic relationships for the nifH gene considering environmental factors and the metabolism of the microorganisms.

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