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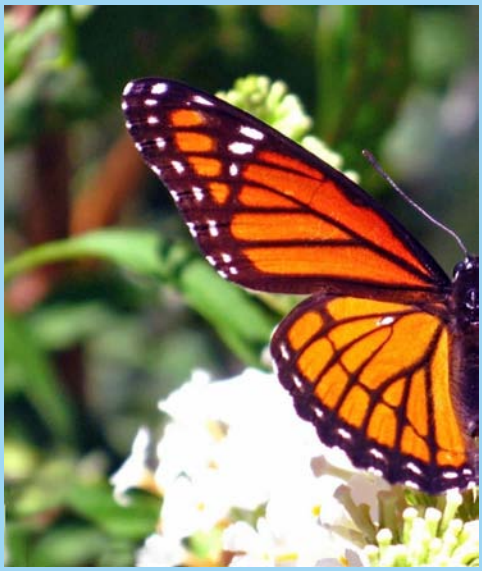

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PORTADA/ COVER

Nuestra portada combina imágenes de los temas abordados en los artículos de este ejemplar.

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Illustration of the diversity of topics covered in this issue.

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UNIVERSIDAD INTERAMERICANA DE PUERTO RICO
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CONTENIDO
CONTENTS

FARMACOLOGÍA-BIOQUÍMICA/ PHARMACOLOGY-BIOCHEMISTRY

MENSAJE DEL RECTOR
DR. RAFAEL RAMÍREZ RIVERA

Felicitamos al claustro y a sus estudiantes por continuar desarrollando proyectos de investigación de envergadura y vanguardia. Estos esfuerzos pueden verse reflejados en este quinto volumen de *Inter Scientific* que a pesar de los acontecimientos que han afectado nuestro país durante el pasado año, se ha podido gestar. En esta ocasión se presenta trabajo de mayor complejidad y un alto grado de proyectos en el campo de la farmacología. Esta publicación, definitivamente, deja en manifiesto el proceso investigativo inherente a las ciencias. Extendemos nuestra felicitación al Decanato de Asuntos Académicos por su gestión incansable en promover la investigación y el apoyo incondicional a esta publicación.

MESSAGE FROM THE CHANCELLOR
DR. RAFAEL RAMÍREZ RIVERA

Congratulations to the faculty and students for continuing to develop research projects of scope, span and vanguard. The efforts can be reflected in the publication of the fifth volume of *Inter Scientific* which besides the events that have affected our island during this past year, it has been possible. In this occasion the work presented reflects a higher degree of complexity and a high number of projects in the field of pharmacology. Definitely, this publication shows the investigative process inherent to science. We extend our congratulations and thanks to the Dean of Academic Affairs for their tireless work in promoting research and the unconditional support for this publication.

MENSAJE DE LA
DECANA DE ASUNTOS ACADÉMICOS
DRA. ANNETTE VEGA

La presentación del quinto volumen de *Inter Scientific* continua enmarcando la meta de fomentar la investigación en facultad y estudiantes. Lograr esta publicación representa el esfuerzo y dedicación de un grupo de colaboradores particularmente durante un año que ha estado lleno de retos para nuestro país. Deseamos agradecer a todos los colaboradores, facultad, estudiantes y personal administrativo que trabajaron para que este ejemplar de la revista científica del Recinto de Arecibo fuera una realidad.

Exhortamos a la comunidad universitaria a continuar apoyando esta publicación que presenta los nuevos conocimientos generados por nuestra facultad y estudiantes al igual que los generados por compañeros de otras instituciones que comparten los mismos en este medio.

MESSAGE FROM THE DEAN OF ACADEMIC AFFAIRS
DR. ANNETTE VEGA

The presentation of the fifth volume of *Inter Scientific* continues envisioning the goal of promoting scientific research among the faculty and students. Achieving this publication represents the effort and dedication of a group of collaborators particularly durign this year that has been full of challenges for our country. We wish to acknowledge the work of all the faculty, students and staff that helped make possible the publication of this issue of the Arecibo Campus' scientific journal.

We encourage our community to continue supporting this project that presents the new knowledge generated by our faculty and students as well as knowledge generated by our colleagues from other institutions that share those throught this means.

DESDE EL ESCRITORIO DE LA EDITORA
DRA. LIZBETH ROMERO-PÉREZ

La generación de nuevos conocimientos es una de las tareas claves de las Instituciones de Educación Superior. En el campo de las ciencias naturales esto es un hecho vital y fundamental. En este, nuestro quinto volumen de *Inter Scientific*, compartimos tres estudios completos realizados por estudiantes a nivel graduado y sub-graduado. Dos de estas investigaciones fueron realizadas en la Universidad de Puerto Rico, Recinto de Mayaguez y la otra en nuestro Recinto de Arecibo de la Universidad Interamericana de Puerto Rico.

La primera investigación nos presenta la mariposa monarca presente en el bosque seco de Guánica, y la preferencia de dieta en su estado de larva. Este estudio titulado *Host plant preference of Danaus plexippus larvae in the subtropical dry forest in Guánica, Puerto Rico*, tiene como objetivo conocer los hábitos alimenticios de esta especie para poder desarrollar métodos para su conservación. El segundo estudio *Predatory behavior and prey preference of Leucauge sp, spiders in Mayaguez, Puerto Rico* evalua este género de arañas y su comportamiento. Ambos proyectos de investigación fueron dirigidos por el Dr. Alberto Puente. El tercer proyecto incorpora la metagenómica. Esta tecnología de vanguardia, permite evaluar los organismos presentes en una muestra particular, en este caso una muestra de suelo. El estudio titulado *Construction and screening of soil metagenomic libraries: identification of hydrolytic metabolic activity* pretende establecer bibliotecas metagenómicas aislando ADN del suelo y utilizarlas para identificar actividad enzimática nueva que podría ser aplicable a la industria, agricultura, medicina, entre otros. Este proyecto fue dirigido por la profesora María Pagán. En la sección *La Investigación en el Campus*, encontrará resúmenes de otras investigaciones que se han estado realizando en los campos de farmacología y bioquímica.

FROM THE EDITOR'S DESK
DR. LIZBETH ROMERO-PÉREZ

The generation of new knowledge is one of the key tasks of the Higher Educacion Institutions. In the field of natural sciences this is a vital and fundamental fact. In this, our fifth volume of *Inter Scientific*, we shared with you three complete research studies done by graduate and undergraduate studets. Two of these projects were developed at the University of Puerto Rico, Mayaguez Campus, and the other in the Arecibo Campus of the Inter American University of Puerto Rico.

The first research project presents the population of the monarch butterfly at the dry forest in Guánica and its preferential diet in the larvae state. This study titled *Host plant preference of Danaus plexippus larvae in the Subtropical Dry Forest in Guánica, Puerto Rico,* has as an objective to know the feeding habits of this specie in order to develop conservation and management methods. The second study, *Predatory behavior and prey preference of Leucauge sp, spiders in Mayaguez, Puerto Rico,* evaluates this gender of spiders and their behavior. Both research projects were directed by Dr. Alberto Puente. The third project incorporates metagenomic. This vanguard technology allows the evaluation of organisms present in a particular sample, in this case a soil sample. The study titled *Construction and screening of soil metagenomic libraries: identification of hydrolytic metabolic activity,* pretends to establish metagenomic libraries by isolating DNA from soil and use them for the detection of novel enzymatic activity that could have an impact in the industry, agricultura, medicine, among others. This project was directed by profesor María Pagán. In the section *Research on Campus* you will find abstracts for other research projects being worked in the fields of pharmacology and biochemistry.

Host plant preference of *Danaus plexippus* larvae in the subtropical dry forest in Guánica, Puerto Rico

Preferencia de plantas hospederas por parte de las larvas de *Danaus plexippus* en el bosque seco de Guánica, Puerto Rico

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ABSTRACT

The monarch butterfly *Danaus plexippus* is present in the subtropical dry forest in Guánica, Puerto Rico. Understanding its feeding behavior is important in order to develop management strategies for that population. The diet of the monarch butterfly changes on each life stage. During larval stage it feeds on plants of the families Asclepiadaceae and Apocynaceae.To assess its plant preference they were exposed to host and non-host plants of the previously mentioned families. It was observed that larvae arrived more and spent more time in host plants over non-host plants. Also, that they seem to prefer *Calotropis procera* over *Asclepias curassavica*, but no significant differences were observed with ANOVA p-value >0.05. More individuals and plant composition should be examined.

RESUMEN

La mariposa monarca, *Danaus plexippus*, está presente en el bosque seco de Guánica, Puerto Rico. Comprender el comportamiento alimenticio es importante para poder desarrollar estrategias de manejo de dicha población. La dieta de la mariposa monarca cambia durante cada etapa de vida. Durante la etapa de larva se alimenta de plantas de las familias Asclepiadaceae y Apocynaceae. Para estudiar la preferencia de las larvas por las plantas, estas fueron expuestas a plantas hospederas y no hospederas de las familias antes mencionadas. Se observó que las larvas llegaron con mayor frecuencia y pasaron más tiempo en las plantas hospederas que en las plantas no hospederas. También que parecían preferir *Calotropis procera* sobre *Asclepias curassavica*, pero no se observaron diferencias significativas con ANOVA p-valor >0.05. Se deben examinar más individuos y la composición de la planta.

KEYWORDS *Asclepias curassavica*, *Calotropis procera*, feeding behavior, butterfly larvae

PALABRAS CLAVE *Asclepias curassavica*, *Calotropis procera*, forrajeo, larva de mariposa

INTRODUCTION

Danaus plexippus commonly known as monarch butterfly undergoes one of the most extensive animal migrations, traveling from North America to Mexico over the winter, although not all individuals migrate in the winter. Recent studies associate the migration behavior with circadian clocks using magnetoreception of two cryptochromes that are light dependent (Reppert, Gegear & Merlin, 2010). Monarchs possess an orange aposematic coloration that warns their predator of their toxicity. Also, it serves as Müllerian mimicry to other species of butterflies, like viceroy butterfly (*Limenitis archippus*) (Zhan et al., 2014). This toxicity is provided to them through the plants they feed on.

The monarch butterfly undergoes different life stages and possess different host plants in each of them. The larvae feeds of different plant families. It has been found in North America that the larvae feeds on around 27 species of *Asclepias* known as milkweeds because of the milky latex they contain (Zalucki, Brower & Alonso, 2001). These plants have cardiac glycosides; compounds that makes them distasteful for predators (Erickson, 1973). A study showed that female monarchs prefer ovipositing on *Asclepias humistrata*, in North America (Zalucki, Brower & Malcolm, 1990). Glycoside concentration in the plant affects negatively the physiology of the larvae, indicating the significance of the host plants. Oviposition is not cued by the concentration of glycosides, but in a plant with low cardiac glycoside adults are more toxic (emetic) providing them with better protection and therefore survival (Dixon, Erickson, Kellet & Rothschild, 1978).

Another factor affecting the feeding behavior of monarch butterflies is resource competition. They have been forced to use

other host plants outside the family Asclepiadaceae. In Barbados for example, the milkweed bugs (*Oncopeltus spp.)* share the host plant *Asclepias curassavica* with *D. plexippus*, since the plant has been eliminated the monarch butterfly is forced to survive on other milkweed, *Calotropis procera* (Blakley & Dingle, 1978).

In Puerto Rico, monarch butterflies can be found in *Asclepias* sp. and in *C. procera*. Puerto Rican monarch butterfly differs in morphology and in migration habits from North American populations. In Puerto Rico, monarchs have smallest and roundest forewings, they reproduce year around and are non-migratory. (Altizer & Davis, 2010) Puerto Rican butterflies have also been found to be more susceptible to parasites such as *O. elektroscirrha* which is present in milkweeds. (Harvell, Altizer, Cattadori, Harrington & Weil, 2009) To our knowledge no studies have been conducted to analyze the feeding behavior of monarch larvae in Puerto Rico. To contribute to the conservation of this species is important to understand which plant they prefer during the larval stage. This information can give insight as what measures to follow to establish protection plans. Here we are comparing the preference of *D. plexippus* larvae between two host plants and a non-host plant. It is expected that the larvae will favor the host plants, based on previous studies in which host plants are preferred.

MATERIALS AND METHODS

Site Description

The study site is located inside the subtropical dry forest in Guánica, a town at the South of Puerto Rico with coordinates 17o57'12.426o N and 66o50'o49.075 W. *D. plexippus* larvae were collected to the south of the dry forest. *C. procera* can be found along the shore of Tamarindo Beach harboring a population of *D. plexippus*.

Soils here are derived from limestone rock, alkaline and shallow, (Roberts, 1942) and have suffered from logging, farmland runoffs, grazing, and other anthropogenic activities (Colón-Molina & Lugo, 2006). It is among the driest subtropical forests with an annual precipitation of 860 mm having low species richness and biomass (Murphy & Lugo, 1986).

Sampling Technique

Larvae were collected in two separate days from *C. gigantean* present in Tamarindo Beach. On Friday, December 8, 2017, a total of 22 larvae were collected, while on Monday, December 11, 2017, 18 larvae were collected for a total of 40 individuals. These were divided as Trial #1 and Trial #2, respectively, but were exposed to the same treatments.

After collection of individuals, they were taken to the Animal House at the University of Puerto Rico at Mayagüez. Treatments were performed inside round plastic pools previously cleaned with ethyl alcohol and left to dry for 10 minutes. Animals were handled using vinyl powdered gloves. All individuals were marked with different colors and patterns for identification. For each trial, larvae were put in the center of the pools and left to acclimatize for 5 minutes. Two different plant species were introduced after this period on opposite sides of the pool to observe which plant was preferred by the larvae. Treatments were divided in (1) with two host plants and (2) with one host plant and one non-host plant. The second treatment was performed two times with different host plants and same non-host plants, *A. curassavica* and *C. procera* are the host plants of the larvae and *Allamanda cathartica* was the non-host plant.

Larvae were allowed 30 minutes to choose between the two plants and observations were taken in terms of how many individuals reached which plant and how much time they spent in a certain plant. Statistical analysis implemented consists of ANOVA and Bonferroni test to assess significant differences in plant choices.

RESULTS

In Trial #1, 19 monarch butterfly larvae arrived to *C. procera*, 14 to *A. curassavica* and 5 to *Allamanda cathartica* (Figure 1). However, during Trial # 2 both host plants received a similar number of larvae with *Calotropis procera* obtaining 22 and *A. curassavica* 22 (Figure 1). For *A. cathartica* the number increased from 5 to 18. ANOVA analysis ($p>0.05$) indicated that there is no significant difference between the treatments.

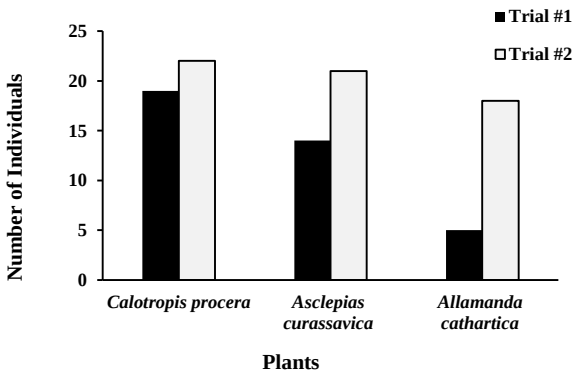

Figure 1. *D. plexippus* plant preference. Number of individuals (larvae) that arrived to each plant on the two trials performed.

Larvae spent more time in *C. procera* with 313.13 minutes accumulated between the two trials, followed by *A. curassavica* with 271.5 minutes and *A. cathartica* with 185.7 minutes. The ANOVA ($p>0.05$) indicates that the differences in time are not significant. The average time per individual was 14.23 minutes

in *C. procera*, 13.58 minutes in *A. curassavica*, and 9.77 minutes in *A. cathartica* (Figure 2). When the corresponding ANOVA is performed for this average time the differences are not significant either ($p>0.05$).

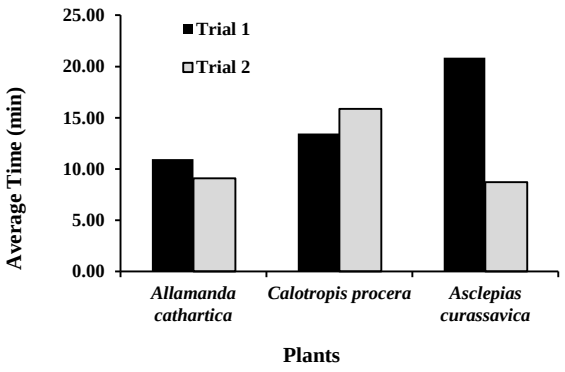

Figure 2. Average time spent by *D. plexippus* larvae on each plant.

Bonferroni test was employed to compare the different treatments, but no significant differences were observed.

DISCUSSION

Larvae arrived and spent more time in host plants, as was expected. The fact that there is no significant difference between the two host plants is indicative that these are suitable plants for the monarch butterfly larvae. Yeargan and Allard (2005) compared two hosts, *Asclepias syriaca* and *Cynanchum laeve*, for *D. plexippus* and found that both plants are ideal for the development (host) of the monarch. Our findings suggest that it might be happening the same between *A. cathartica* and *C. procera*.

Several other causes can influence the election or rejection of a larvae towards a plant. Some larvae may have not been attracted to the plants due to the way the leaves are placed and the pool cleaning procedure. According to Gomez-Domínguez (2012), some butterflies need physical and chemical stimuli. The ethyl alcohol might have blocked the chemical factors necessary for some larvae to detect their food. Moreover, individuals that looked sick or made chrysalis were removed from the observations, which reduces the sample size affecting negatively the results. Individuals that looked big enough to make the chrysalis, did not follow the same behavior as smaller ones. They were not interested in the plants as much as in looking for a place to settle and make the chrysalis.

The preference for *C. procera* might be due to the concentration of cardiac glycosides in the plant. Evolution, also has an important role for the election of *C. procera* as a main food source, since milkweeds are susceptible to parasites all year round in Tropical areas (Harvell et al., 2009) along with resource competition with other insects (Blakley and Dingle, 1978).

Even though no significant differences were observed in the election of the *D. plexippus* for any treatment, more individuals arrived and spent more time in *C. procera*. Further controlled research needs to be conducted with a higher number of individuals. Additionally, some similar methods to that of Mattila and Otis (2003) could be followed, which allowed the larvae to be for a longer period and the oviposition was observed in the host plants. According to Mattila and Otis (2003), few studies have been conducted outside the genus *Asclepias* as hosts. In addition, the concentration of glycosides in the host plants and non-host plants also needs to be assess because it is an important aspect of the butterfly plant election for oviposition.

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Predatory behavior and prey preference of *Leucauge* sp. spiders in Mayaguez, Puerto Rico

Comportamiento de depredación y preferencia de presa de arañas del género *Leucauge* en Mayagüez, Puerto Rico

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ABSTRACT

Silk-using spiders, such as the species from the superfamily Araneoidea, have distinct behaviors when potential prey fall into their webs. We studied several spiders of the genus *Leucauge* on the campus of the University of Puerto Rico at Mayaguez to observe this behavior with detail. We captured and presented prey of different sizes directly on the webs of *Leucauge* sp. and observed their reactions and consequent behavior. A list of behaviors was compiled from all observations. We found that the spiders attacked and consumed most prey smaller than themselves, and discarded or ignored prey that was too large or too small, compared to the spider under study. Except for one prey item, all prey that were ultimately consumed by the spiders were attacked within one minute of being placed on the web, suggesting that they recognize potential prey rapidly after it falls on their webs. Ants that were placed on the web were all attacked within one minute or ignored completely, depending on whether they were actively moving in the web or not. This suggests that ants are considered a threat by the spiders rather than a food source. We conclude that *Leucauge* sp. are generalist predators with a preference for small, "defenseless" prey such as flies and bugs; however, we believe that more sample size and prey diversity is needed to narrow down preferences.

RESUMEN

Las arañas que usan tela, tales como las especies de la superfamilia Araneoidea, poseen comportamientos distintivos cuando su presa cae en sus telas. Estudiamos a las arañas del género *Leucauge* en los alrededores de la Universidad de Puerto Rico, Recinto Universitario de Mayagüez para observar este comportamiento detalladamente. Se capturaron y presentaron presas de diferentes tipos y tamaños en las telas de *Leucauge* sp. y se observó sus reacciones y comportamiento consiguiente. Una lista de comportamientos fue compilada de todas las observaciones. Se encontró que las arañas atacaban y consumían a la mayoría de las presas de menor tamaño que la araña, y descartaban o ignoraban a la presa que era muy grande o muy pequeña, comparado con la araña bajo estudio. Con la excepción de una presa, todas las presas que fueron eventualmente consumidas por las arañas fueron atacadas dentro de un minuto de haber sido puestas en la tela, sugiriendo que ellas reconocen a una presa potencial rápidamente cuando caen en sus telas. Las hormigas que fueron puestas en la tela fueron atacadas dentro de un minuto o ignoradas completamente. Esto sugiere que las hormigas son consideradas un peligro por las arañas en vez de una fuente de alimento. Concluimos que *Leucauge* sp. son depredadores generalistas que prefieren presas pequeñas e "indefensas" tal como moscas y hemípteros; sin embargo, creemos que se requiere un mayor tamaño de muestra y diversidad de presa para acortar estas preferencias.

KEYWORDS Tetragnathidae, Diet, Responses, Orb weaver spider

PALABRAS CLAVE Tetragnathidae, Dieta, Respuestas, Araña tejedora

INTRODUCTION

Many spider species employ ambush tactics to catch prey, the most common being the web-building method. Spiders of the genus *Leucauge* (Family: Tetragnathidae) are members of the web-building superfamily Araneoidea and are relatively common in the tropics, encompassing a total of 173 known species.

Leucauge sp. can mostly be found in and around low bushes and tall grass, usually in "colonies" of several individuals and their respective webs. These spiders have been known to employ several strategies that aid in prey foraging: some species attach themselves with a thread of silk to the central hub of their webs to move with ease around the web (Yoshida, 2000). Also, their abdomens are adorned with a variety of colors that may well aid them in attracting prey to their webs (White, Dalrymple,

Herberstein, & Kemp, 2017). Their sexual behavior has also been studied (Aisenberg & Barrantes, 2011; Eberhard & Huber, 1998). However, not many studies have focused on their behavioral habits and prey preference, if any.

The purpose of this experiment was to determine what kind of prey these spiders prefer and the respective behavior in relation to the predation of the prey. After looking at the size of the spiders and the location and apparent fragility of the webs they build, we hypothesized that these spiders prefer prey that are smaller than themselves. For this purpose, we captured prey in the surrounding areas of different sizes and types to place in the spiders' webs to gather the relevant data.

MATERIALS AND METHODS

Study Areas

Leucauge spiders were found around the campus of the University of Puerto Rico in Mayaguez (UPR-RUM) between the months of August and December. The climate was relatively humid, as rain is common in Mayaguez. A total of six locations were established and visited periodically: a palm tree near a road (Area A); a fallen tree stump near Area A (Area B); a fallen tree in the forest near the Biology Department's building (Area C); around plants in the forest floor (Area D); the grass outside the Entomology Lab (Area E); and outside the *Luis de Celis* building (Area F). Observations were done during the day or afternoon.

Main Experiment

For this study, prey items were placed on the webs of *Leucauge* sp. to study their behavior and prey preference. Prey items included insects from the orders Blattodea, Hymenoptera, Lepidoptera (larva and adults), Diptera, Dermaptera, Hemiptera and Orthoptera, as well as spiders from an indeterminate taxon. Blattodea included a single urban cockroach; Hymenoptera included ants (Family: Formicidae); Lepidoptera included urban, case-building moth larva, an unidentified moth adult and a single plume moth (Family: Pterophoridae); Diptera included a single mosquito (Family: Culicidae), a single moth fly (Family: Psychodidae) and several unidentified flies; Dermaptera included a single unidentified earwig; Hemiptera included three leafhoppers (Family: Cicadellidae) and a single unidentified bug; and Orthoptera included a single cricket nymph (Family: Gryllidae).

Prey items were collected in the field via sweeping with bug-catching nets or direct hand capture. All captured prey, excluding Lepidoptera larvae and the Blattodea specimen, were found near the areas of study. Once captured, prey was placed in small plastic containers. The size of the prey items was measured with a ruler, in centimeters, from the top of the head to the last segment of the abdomen. Before spider feeding sessions, the containers were placed in refrigerators for 1-10 minutes depending on how far the area was from the refrigerator used. This was done to subdue prey for easier handling. Once at the site of study, the size of the spider under study was measured with a ruler, in centimeters, from the back legs to the front legs. Prey were either dropped onto the spider web or placed with tweezers; prey was placed as close to the spider's web's central hub area as possible. The "central hub" refers to the center of the web, where web strings converge and where the spider normally rests waiting for prey. Once prey landed on the web, the spider's behavior was observed for approximately 15 minutes. Prey that happened to fall on the web during observatory sessions were also taken into account. Since we returned to the same areas to acquire observational data without discriminating between the individual spiders, it is safe to assume that most of the spiders were used on more than one study session for each area, but on different days. This experiment was inspired in part by Yoshida's (1989) work on *Gasteracantha* spiders.

Figure 1. Study areas A to C. Area A- between leaves of a palm tree, near a road. Area B- fallen tree trunk, close to Area A. Area C- fallen tree in forest near the Biology Department.

Figure 2. Study areas D to F. Area D- around plants on forest floor. Area E- grass outside the Entomology lab. Area F- outside the *Celis* building.

RESULTS

Here we detail the observed behavior of *Leucauge* sp. during the study. A list of behaviors and their definitions can be seen in Table 1. Upon prey landing on the web, we observed *Leucauge* sp. fleeing from prey three times, confronting prey five times, inspecting prey once and immediately attacking prey 10 times and 19 instances of no reaction towards presented prey. There were 10 instances of spiders pulling on the web in the direction of prey; two were ultimately followed by attack and feeding, five by discarding, and three were simply ignored. We observed two instances of spiders manipulating prey with the shorter pair of back legs while feeding. We saw three instances of spiders wrapping prey and relocating it to the hub area of the web before feeding.

A detailed registry of physical interactions with prey (or lack thereof) can be seen in Table 2. We observed one instance of a spider chasing another spider off its web. Instances of smaller spiders, most likely kleptoparasites, were seen around some of the *Leucauge* sp.'s webs; we observed one instance of the spider under study swatting the smaller spider off its web.

Table 1. Types of behavior, towards prey, observed for *Leucauge* sp. during the study.

Behavior	Definition
Flee	Flees from presented prey
Confronts	Faces presented prey, staying in same location
Inspection	Moves towars prey but does not make contact
Web pull	Pulls on web strings in the direction of the prey
Attack	Bites prey
Feeding	Feeds on prey
Attack and retreat	Bites and retreats from prey
Wrap	Wraps prey with web
Manipulation	Manipulates prey with mouthparts or legs
Relocating	Moves prey to another side of the web
Discard	Discards prey from web

Table 2. Types and quantity of prey that were presented to *Leucauge* sp. spiders and their behavior.

Prey	Behavior				Total prey
	Attacked and consumed	Attacked and retreat	Ignored	Discarded	
Blattode (cockroach)			1		1
Hymenoptera (ant)		6	10		16
Lepidoptera (moth larvae)			1	2	3
Lepidoptera (adult moth)	1			1	2
Diptera (fly)	6		1	3	10
Dermaptera (earwig)				1	1
Hemiptera (bug)	1		1	2	4
Orthoptera (cricket)				1	1
Araneae (spider)	2				2
Total	**10**	**6**	**14**	**10**	**40**

Table 3 shows the mean size difference between spider and prey by type. Table 4 shows comparison between *Leucauge* sp. behavior and size difference between the spider and the prey. Table 5 shows the comparison between behaviors observed and time that passed by before observing that behavior. Figure 3 shows a comparison between spider-prey size differences for prey that were eventually consumed. Finally, Figure 4 shows a comparison between spider-prey size difference for prey that were discarded or ignored.

Table 3. Size difference (mean) between the *Leucauge* spiders and the prey.

Prey Type	Size difference (cm)
Blattode (cockroach)	2.00
Hymenoptera (ant)	0.40
Lepidoptera (moth larvae)	0.03
Lepidoptera (adult moth)	0.05
Diptera (fly)	0.90
Dermaptera (earwig)	-0.20
Hemiptera (bug)	0.23
Orthoptera (cricket)	1.00
Araneae (spider)	0.75

Table 4. Relationship between behavior and size difference between *Leucauge* spiders and prey. Attacked and consumed (A), attacked and retreat (AR), ignored (I), discarded (D).

Prey Type	< 0.0	0.0-0.4	0.5-0.9	1.0-1.4	1.5-2.0
Blattode (cockroach)					I
Hymenoptera (ant)		AR, I			
Lepidoptera (moth larvae)	D	I, D			
Lepidoptera (adult moth)		A,D			
Diptera (fly)		A	A,D	A	I, D
Dermaptera (earwig)	D				
Hemiptera (bug)		A, I, D			
Orthoptera (cricket)				D	
Araneae (spider)			A		

DISCUSSION

All prey that were attacked by the spiders were of smaller size than the spider itself (Table 4), the majority of which had a size difference between 0.0-0.9 (Figure 3). Past a certain size difference ($x < 0.1$, $x > 1.3$cm) spiders would always ignore or discard prey. Apart from one unidentified dipteran, all prey items that were ultimately consumed by the spiders were attacked within one minute of placement in web (Table 5); four of these prey items (three dipterans and one spider) were attacked immediately after placement. The spiders in this study seemed to distinguish an item as potential prey almost immediately after it fell on their webs, likely based on the weight of the item. Both

spiders presented to *Leucauge* sp. individuals were attacked and consumed (Table 2). Diets based mostly on araneophagy have been observed in species of the family Mimetidae (Jackson & Whitehouse, 1986), Pholcidae (Jackson & Brassington, 1987), Salticidae (Jackson & Wilcox, 1993), Archaeidae (Rix & Harvey, 2011) and Scytodidae (Escalante, Aisenberg, & Costa, 2015). Aside from sexual cannibalism (Elgar, 1991; Robinson & Robinson, 1980), however, araneophagy is not common in other spiders. Furthermore, we believe that the presented spiders were opportunistically perceived as prey due to their inactivity after being placed in cold temperatures and their small size compared to the spiders under study.

Table 5. Relationship between behavior and time (min) before behavior was observed towards prey. Attacked and consumed (A), attacked and retreat (AR), ignored (I), discarded (D).

Prey Type	< 1	1-5	5-10	10-15	>15
Blattode (cockroach)					
Hymenoptera (ant)	AR				
Lepidoptera (moth larvae)			D	D	
Lepidoptera (adult moth)	A	D			
Diptera (fly)	A		A,D	D	D
Dermaptera (earwig)		D			
Hemiptera (bug)	A	D			
Orthoptera (cricket)					D
Araneae (spider)	A				

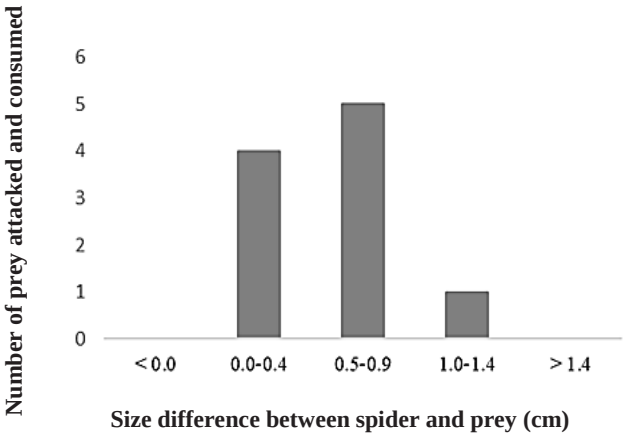

Figure 3. Total of prey attacked and consumed by *Leucauge* spiders during the study compared to size difference between spiders and prey presented.

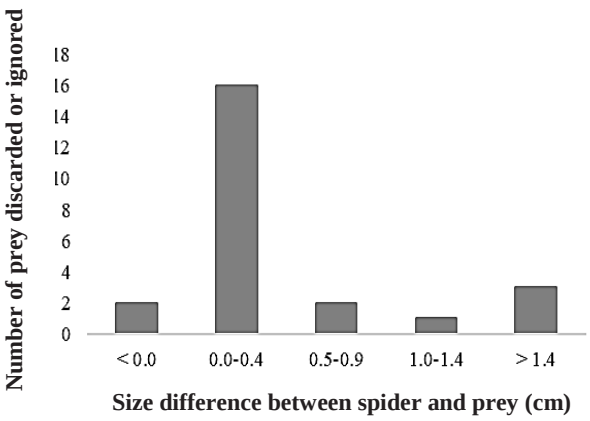

Figure 4. Total of prey discarded or ignored by *Leucauge* spiders during the study compared to size difference between spiders and prey presented.

Prey items that were ignored or discarded from the web may not have been considered prey by the spider; the former, a precaution to avoid damage from a dangerous animal, as most prey items ignored where of near equal size to the spider (Figure 4); the latter, an attempt to "clean up" the web. This discarding behavior was also observed by Henaut, Ibarra-Nuñez, & Williams (2001). We observed one instance of a spider discarding a prey item of the same type and of similar size to one presented earlier, which the spider had promptly attacked. This raises the question of why it discarded the prey item instead of "saving" it for later consumption. Food catching behavior is uncommon in invertebrates but has been observed in orb weaving spiders such as *Nephila eludes* (Champion de Crespigny, Herberstein, & Elgar, 2001). For now, it seems that the *Leucauge* sp. studied do not partake in this behavior. All active (moving) ants placed on the web were attacked within one minute and promptly ignored, while inactive (non-moving) ants were ignored completely. Henaut et al., (2001) had similar results, were only a small percentage of ants were attacked by the *Leucauge* species they studied when compared to the other prey presented. It is likely that the ants were recognized as dangerous and not worth risking serious injury from their bites or stings.

Spiders make use of their webs not only to capture prey, but to assess prey size, location and activity (Landolfa & Barth, 1996; Suter, 1978; Zschokke, Henaut, Benjamin, & García-Ballinas, 2006). The *Leucauge* spiders studied were often seen pulling on the web strings in the direction of presented prey (Table 1), perhaps to further assess information on prey before interacting with it. In total, there were 10 instances of spiders pulling on the web in the direction of prey. No correlation was found between the spider-prey interaction after the web pulling and the prey type, although a small majority (five in total) were discarded after web pulling. Though not part of the original experiment

design, we observed and documented one instance of a *Leucauge* spider chasing another *Leucauge* spider off its web. As well, instances of smaller spiders, possibly kleptoparasites, were seen around some of the *Leucauge* sp.'s webs; on one occasion, the spider under study swatted the smaller spider off its web. The *Leucauge* spiders seem to actively fend off intruding spiders if they are detected. These interactions with other spiders were observed on a single individual, which was also the only one seen to have more than one *Leucauge* spider in the same web.

From the data we were able to collect, we conclude that *Leucauge* sp. are generalist predators with a preference for "defenseless" prey, such as dipterans and some hemipterans, that are neither too big nor too small when compared to the spider. However, sample size in this study was small compared to works done on other web-building spiders, with most presenting hundreds or even thousands of prey items to said spiders (Nentwig, 1983; Yoshida, 1989). The study did not have much diversity in terms of prey selected for the study, with most prey items being from the class Diptera or the family Formicidae. Hemipterans presented did not include the taxons that produce chemical weapons such as the pentatomids, which Yoshida (1989) tested on his spiders. Finally, only two specimens were presented that were bigger than the spider itself; as such, we cannot confirm with certainty whether *Leucauge* sp. show preference for prey smaller than themselves or not. Future projects concerning the genus *Leucauge* could focus on obtaining more evidence of their predatory behavior, as well as identifying the spiders by species to link said species to their respective behavioral traits. Studies with spiders reared in a laboratory to control their diet, as well as studies at night to contrast time interval preferences, could be done as well.

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Construction and screening of soil metagenomic libraries: identification of hydrolytic metabolic activity

Construcción de bibliotecas metagenómicas de suelo e identificación de actividad metabólica hidrolítica

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ABSTRACT

Soil is a complex system that houses many microorganisms that are capable of hydrolyzing organic compounds. The metagenomic study of a soil sample allows the identification of the microbial diversity and their function. The objectives of this research were to construct metagenomic library from a topsoil samples collected in Quebradillas, Puerto Rico, and to identify or detect if hydrolytic activity (lipase, cellulase and amylase) is present in this library and in a previous one created. To construct the metagenomic library the selected size of environmental DNA (eDNA) was ~40 Kb. The eDNA was ligated with a fosmid cloning vector (pCC1FOS) and inserted in a bacteriophage Lambda to further transform *Escherichia coli* cells (EPI300-T1^{R}) and select in media with chloramphenicol. Fosmid vectors of positive clones were isolated and digested using BamHI. Trybutyrin agar, Starch agar and Carboxymethyl Cellulose Agar (CMC) were used to screen enzymatic activity of clones from both libraries. Results show that 55% of the tested clones from the Quebradillas library exhibit amylase activity, and 15% were positive for cellulose. Of clones evaluated from the previous library 45% were positive for amylase. Positive clones from both libraries should be further analyze, by sequencing or by means of other enzymatic tests to characterize enzymes, and to discover novel metabolic activities.

RESUMEN

El suelo es un sistema complejo que alberga muchos microorganismos capaces de hidrolizar compuestos orgánicos. El estudio metagenómico de una muestra de suelo permite identificar la diversidad de microorganismos y su función. Este estudio tenía como objetivos construir una biblioteca genómica de la capa superficial de una muestra de suelo colectada en Quebradillas, Puerto Rico e identificar actividad hidrolítica (lipasa, cellulasa y amilasa) en esa biblioteca y en una generada en un estudio anterior. Para construir la biblioteca genómica el tamaño del ADN ambiental (eDNA) seleccionado fue ~40 kb. El eDNA fue ligado utilizando un vector de clonación fósmido (pCC1FOS) e insertado en el bacteriófago lambda, seguido por transfomación de células de *E.coli* (EPI300-T1R) cultivadas en Luria Bertani con cloranfenicol. Vectores de clones positivos fueron aislados y digeridos con BamHI para análisis molecular. Para detectar actividad hidrolítica se utilizaron agar tributírico, agar de almidón y agar carboximetílico de celulosa. De los clones analizados de la biblioteca generada en este estudio 55% fueron positivos para actividad de amilasa y 15% para celulosa. En cuanto a los clones de la librería del estudio anterior 45% fueron positivos para amilasa. Se deben estudiar más a fondo los clones positivos de ambas bibliotecas, sea por secuenciación o con otras pruebas enzimáticas para caracterizar las enzimas y evaluar otras actividades metabólicas adjudicadas a los microorganismos en el suelo.

KEYWORDS Soil, DNA, Enzymes, Metagenomics, pCC1FOS, *Escherichia coli*

PALABRAS CLAVE Suelo, ADN, Enzimas, Metagenómica, pCC1FOS, *Escherichia coli*

INTRODUCTION

Organisms in soil have the capacity to transform or degrade natural components necessary for life or transform compounds that can be harmful. In this way, this ecosystem is considered a main reservoir of microbial diversity and genes for the degradation or transformation of organic compounds on the planet (Suárez-Silva, 2010). The microbial diversity and genomic materials present in the soil have been studied in greater detail thanks to metagenomics (Hernández-León, Velázquez-Sepúlveda, Orozco-Mosqueda & Santoyo, 2010). Metagenomics, which can also be known as environmental and community

genomics is the genomic analysis of microbial communities by extraction and cloning of DNA (Handelsman, 2004, Suárez-Silva, 2010; Hernández-León et al., 2010; Cortés-López, Montor-Antonio, Olvera-Carranza, Pena-Castro, & Del Moral, 2014). By retrieving the metagenome from microorganisms, it is proposed that metabolites in them can be accessed by directly cloning the DNA into vectors (Gillespie et al., 2002). The industrial applications of metagenomics include identification of novel biocatalysts, discovery of new antibiotics, personalized medicine, and bioremediation (Olson & Morrow, 2012; Bashir, Pradeep Singh, & Kumar Konwar, 2014; Pieper & Reineke, 2000). In addition, metagenomics has emerged as a strategic approach to explore experimental methods such as DNA hybridization, gene expression, proteomics, metabolomics, and enzymatic screening (Popovic et al., 2017).

Screening of functional metagenomes offers a method focused on the discovery of genes of interest (Allen, An, Handelsman, & Moe, 2015). This type of analysis helps identify clones with potential applications in agriculture, medicine and the industry (Suárez-Silva, 2010). The enzyme screening depends on directly assaying proteins expressed from environmental DNA in a surrogate host (clones) over a differential agar for enzymatic activity against a specific chemical substrate and obtaining the activity through changes in color or conformation of halos surrounding the colonies (Suarez-Silva, 2010; Popovic et al., 2017). Within these identifications are those that code for amylases, lipases, esterases, among other enzymes (Suarez-Silva, 2010). This method has also greatly expanded the number of enzymes, including over 130 new nitrilases and many cellulases, carboxylesterases, and laccases (Popovic et al., 2017). A wealth of information has been uncovered by metagenomics, such as microbial diversity, uncharacterized metabolism, and increased complexity of biogeochemical pathways, and it promises to provide new enzymes and molecules with diverse applications (Bashir et al., 2014).

This research is focused on the construction of a metagenomic library from a sample of topsoil collected in a wooded area in Úcar, Quebradillas, Puerto Rico and to identify hydrolytic activity (lipase, cellulase and amylase) present in the environmental DNA (eDNA) samples of this library and a previous one.

MATERIALS AND METHODS

Sample Collection

Two soil samples were collected from topsoil of a 1) wooded area in the "Úcar" in Quebradillas, P.R. (18°27'32" E, 66°55'11" S) (Figure 1), and 2) the river bed from the "Rio Camuy" in Camuy, P.R. (18°28'48" E, 66°50'29" S) (Figure 2). Temperature and pH as well as the description of wind, weather, and soil were recorded. The samples were transported to the laboratory and DNA extraction was performed. A portion of these environmental samples was stored at 4°C and -80°C to determine which temperature was best to conserve the quality of the eDNA from the soil sample.

Figure 1. Area of soil sample collection at "Úcar", Quebradillas.

Figure 2. Area of soil sample collection at the river bed of "Rio Camuy", Camuy.

DNA extraction

Lysis of cells in the soil samples, was perform using a vortex. The high molecular weight DNA from the soil was extracted using the E.Z.N.A. soil DNA Kit (Omega, GA, USA, Cat# D5625-01) according to manufacturer's protocol (Omega, 2016). After several incubations and centrifugations, the pellet containing the eDNA was eluted with 100 µL of elution buffer and stored at -20°C.

Spectrophotometric analysis and agarose gel electrophoresis

DNA concentrations were determined with a spectrophotometer at 280 nm. DNA quality was determined by the ratio A_{260} to A_{280} (DNA-Proteins) and A_{260} to A_{230} (DNA-Humic acid). Soil may contain contaminants such as humic acid that must be eliminated because they can inhibit the activity of DNA polymerases or nucleases. Integrity of the DNA was determined by agarose gel electrophoresis.

Library Generation

Confirmation of the eDNA size (~40Kb) was done using agarose gel (1%) electrophoresis and fosmid control DNA (Epicentre Biotechnologies, WI, USA) as a marker. Reparation of insert ends was performed by the end-repair enzyme mix, 10X Buffer, dNTPs and ATP (Epicentre). The library was constructed according to the protocol used in a previous study (Huertas et al., 2017) protocol. The library was divided in master pools, kept in 50 mL centrifuge tubes, containing Luria Bertani broth with chloramphenicol (12.5 µg/mL) and 20% glycerol and stored at -80 ^{o}C.

The fosmid DNA was isolated and purified using a Miniprep kit (Qiagen, Cat#27106). Fosmid DNA was digested with BamHI to determine the presence of eDNA from soil. Digested samples were analyzed on 1% agarose gel electrophoresis.

Bacterial strains and vectors

The library was generated using the fosmid vector pCC1FOS from the Copy Control Fosmid Library Production Kit (Epicentre Biotechnologies, WI, USA), and following the manufacturer's protocol. The bacterial host for the eDNA was the EPI300 T1 Phage T1-Resistant *E. coli* (Epicentre). *E. coli* EPI300-T1^{R} was cultured on Luria Bertani growth medium and incubated at 37 ^{o}C for 24 hours.

Culture and growth conditions

The controls for the hydrolytic activity of the enzymes amylase, lipase, and cellulase were selected using the theoretical data from Cappuccino & Sherman (2014). The bacterial strains (controls) *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Bacillus cereus* were obtained from Kiwik-Stik™, cultivated following their instructions and incubated for 24 to 48 hours at 37ºC. A Gram's staining was done for each control.

Screening of hydrolytic activity

Selected clones from the soil metagenomic libraries were culture on petri dishes with Starch agar for amylase activity, Tributyrin agar (peptone, meat extract, agar, and tributyrin) for lipase activity, and 1% Carboxymethylcellulose agar (Luria Bertani medium, agar, and CMC) for cellulase activity. To accomplish this assay, the clones and controls were organized in the same petri dish. The bacterial strain used as a positive control for amylase test was *Bacillus cereus* (ATTC® 11778); *Staphylococcus aureus* (ATTC® 25923) was used as positive control for lipase; *Bacillus subtilis* (ATTC® 11774) as a positive control for cellulase, and *E. coli* (ATTC® 25922) as a negative control for all three enzymatic tests.

Cultures were incubated at 37 ^{o}C during 2 days for lipase and amylase activity and 4 days for cellulase activity. To determine the hydrolytic activity, Starch agar was flooded with Gram's iodine to observe a yellow zone around the colony for positive results. Lipase activity was evaluated observing a clear zone around the colony. CMC agar plates were flooded with Congo red 0.1% for a period of 15 minutes at room temperature, rinsed off the colony with distilled water, and counterstained with 1M NaCl. A clear zone in the medium indicated a positive result for cellulose hydrolysis. To confirm the enzymatic activity, the clones were culture in triplicate.

RESULTS

To know at which temperature it was better to store the collected soil samples and to preserve the quality of the DNA, the samples from Úcar and Río Camuy were stored at 4 ^{o}C and at -80 ^{o}C. After a 24-hour storage period there was no evident change in the appearance of the soil compared to the freshly collected sample. Úcar sample had no change in pH, remaining at 8 (Table 1). However, the soil from Río Camuy had a decrease in pH; from 8 to 6. The "Úcar" sample had a concentration of 93.9 µg/mL and a purity ratio of 1.80, while the Rio Camuy sample had a concentration of 24.3 µg/mL and a purity ratio of 1.96 (Table 2).

Table 1. pH levels of soil samples collected from Úcar, Quebradillas and Río Camuy stored at different temperatures.

Condition	Temperature (ºC)	pH Úcar	pH Río Camuy
Fresh	22	8	8
Stored	4	8	6
Stored	-80	8	6

Table 2. Concentration and ratio 260nm/280nm of eDNA isolated from soil samples.

Sample	Concentration (µg/ml)	Ratio 260 nm/280 nm
Úcar	93.9	1.80
Río Camuy	24.3	1.96

To verify the size of the extracted eDNA a 1% agarose gel electrophoresis was performed. Copy Control DNA insert was used as a control, since it has a size of 40 Kb, which corresponds to the maximum insertion capacity of the fosmid cloning vector to be used. Results show that both samples had a fragment size

of approximately 40 Kb (Figure 3). Based on best concentration and purity the "Úcar" sample was selected to perform the soil metagenomic library.

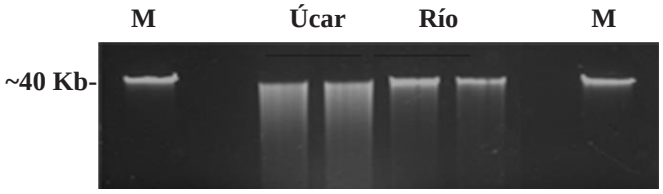

Figure 3. Agarose (1%) gel electrophoresis to verify size of eDNA from soil samples collected at Úcar, Quebradillas and "Río Camuy", Camuy. Lane M is a Copy Control Insert DNA (40 Kb).

Isolated vectors from positive clones of new and previous library were digested with BamHI. It is known that BamHI generates two fragments of the pCC1FOS fosmid: a 8,127 bp fragment and a lower molecular weight fragment of 12 bp. Additional fragments observed correspond to the eDNA inserts that fluctuate between 40,000 bp to 500 bp (Figure 4 and Figure 5).

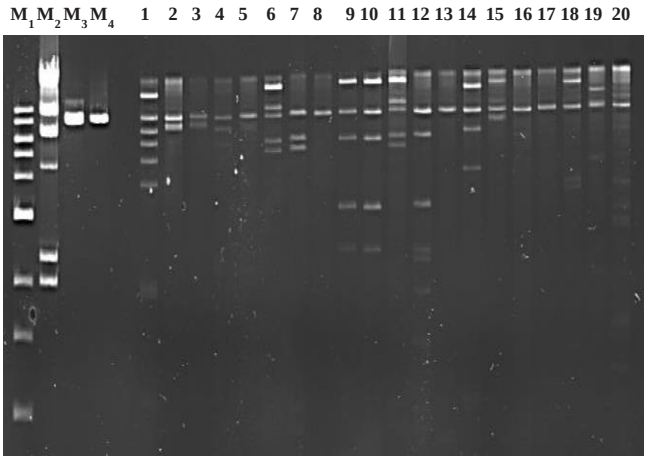

Figure 4. Agarose gel (1%) electrophoresis of the digested fosmids isolated from the 20 clones selected of the library generated in this study. Fosmids were digested with the enzyme BamHI. Marker 1Kb Ladder (lane M_1), Lambda/HindIII marker (lane M_2), pCC1FOS DNA uncut (8,139 bp) (lane M_3), pCC1FOS DNA cut (lane M_4) and recombinant fosmids digested with BamHI (lanes 1-20).

To evaluate the hydrolytic activity of clones, Trybutyrin agar, Starch agar and Carboxymethyl Cellulose Agar (CMC) were used to screen enzymatic activity of amylase, lipase and cellulose, respectively. For each of these enzymatic tests controls were used; *B. cereus* for amylase, *B. subtilis* for cellulase and *S. aureus* for lipase. *E.coli* was used in all tests as negative control.

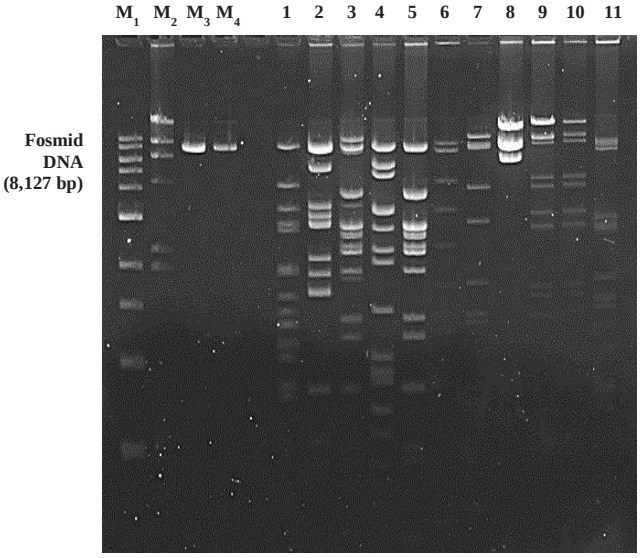

Figure 5. Agarose gel (1%) electrophoresis of the digested fosmids isolated from the 11 clones selected of the library generated in the study of Huertas et al., 2017. Marker 1Kb Ladder (lane M1), Lambda/HindIII marker (lane M2), pCC1FOS DNA uncut (lane M3), pCC1FOS DNA cut (lane M4) and recombinant fosmid digested with BamHI (lane 1-11).

To verify the hydrolysis of starch, the indicator Gram's iodine was used, where a positive result would have the presence of a yellow zone around the colony corresponding to the degradation of the starch present in the medium (Figure 6). For this test 11 clones (55%), belonging to the newly constructed metagenomic library (Table 4) and 5 (45%) clones belonging to the previous metagenomic library created by Huertas et al., 2017 were positive (Table 5).

Table 4. Enzymatic activity of clones from soil metagenomic library from Úcar, Quebradillas.

Enzymes	Positive clones	Negative clones	Percent
Amylase	11	9	55
Cellulase	3	17	15
Lipase	0	20	0

Table 5. Enzymatic activity of clones from soil metagenomic library of Huertas et al., 2017.

Enzymes	Positive clones	Negative clones	Percent
Amylase	5	6	45
Cellulase	0	11	0
Lipase	0	11	0

Clones from both libraries showed a negative result for the lipase

test (Figure 8, Table 4 and Table 5).A positive test for cellulase resulted in a clear zone around the bacterial colony (Figure 7). Three clones (15%) from the new library (Table 4) were positive. None was detected for Huertas et al, 2017, library (Table 5).

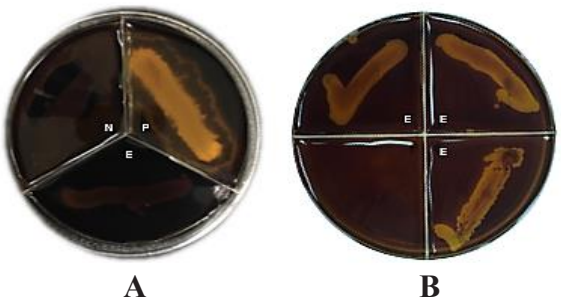

Figure 6. Evaluation of enzymatic activity for amylase. Negative control (N), positive control (P), and experimental clone (E). (A) Negative result for amylase activity with non-degradation area around the colony. (B) Positive result for amylase activity presented a yellow zone around the colony corresponding to starch hydrolysis.

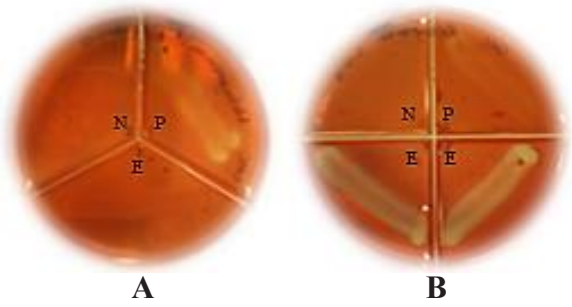

Figure 7. Evaluation of enzymatic activity for cellulase. Negative control (N), positive control (P), and experimental clone (E). (A) Negative result for cellulase enzymatic test with a non-degradation zone. (B) Positive result for cellulase activity or hydrolysis presented a clear around the bacterial colony.

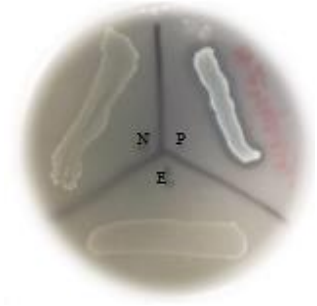

Figure 8. Evaluation of enzymatic activity for lipase. Negative control (N), positive control (P), and experimental clone (E). A clear zone around the bacterial colony represented a positive result for the test.

DISCUSSION

The versatility and diversity of soil niche makes it of great microbiological importance, since it is the main source of new natural products such as antibiotics and enzymes (Zipper, Buta, LaEmmle, Brunner, Bernhagen & Vitzthum, 2003). For this reason, we constructed a metagenomic library by extracting environmental DNA from the soil. The library contained DNA from a variety of organisms and communities present in the soil.

The biotic and abiotic factors surrounding the soil make each collected sample unique, even though the two samples that were collected were from soil. Based on our results, it is important to use fresh soil in order to avoid pH changes. The soil sample collected in Camuy showed a pH decrease from 8 to 6 when stored at 4 or -80 ºC.

It is known that during the DNA extraction a co-extraction of humic acids could occur (Steffan, Goksoyr, Bej, & Atlas, 1988; Tebbe & Vahjen, 1993; Zipper et al., 2003; Olson & Morrow, 2012; Sar, Pal, & Dam, 2018). The presence of humic acids in the sample interferes with digestions, amplifications, reduces the efficiency of vector cloning, among other procedures that could be performed with the recovered DNA (Tebbe & Vahjen, 1993; Olson & Morrow, 2012; Sar, Pal, & Dam, 2018). Also, the presence of humic acids can be observed in an agarose electrophoresis (Tebbe & Vahjen, 1993). In our study the levels of humic acids were low and could not be detected in an agarose gel electrophoresis, showing that our eDNA samples were of good quality to create the library. The eDNA extracted from the soil sample collected at Ucar was used to construct the library because of it's high concentration and purity. Overall, the extraction method to recover the eDNA was successful and efficient, showing quality DNA suitable for the metagenomic library construction.

The ligation of the eDNA with the pCC1FOS produced a high quantity of clones (5,649) that were further analyzed. Most of the clones that were evaluated, presented inserts of eDNA and some of those clones were positive for the amylase (55% new matageomic library; 45 % previous library) and cellulase (15% new library) enzymatic activity tested. Further analysis such as sequencing, should be done, to characterize the enzymes and to provide insight into the genomic context of the genes encoding the function and phylogeny of that organisms (Williamson et al., 2005). Sequencing of the total metagenomic DNA can provide information about several aspects of the sample. It can not only reveal the identity of species present but also can provide insight into the metabolic activities and functional roles of the microbes present in a specific population (Coughlan, Cotter, Hill, & Alvarez-Ordoñez, 2015). More studies can include the evaluation of enzymatic tests of more complex substrates. Studying enzymatic activity can open the way into bioremediation, which is the use of microbial metabolism to eliminate environmental

pollutants. This provides a safe and economic alternative for the protection of our environment (Pieper & Reineke, 2000).

Finally, an important study to conduct is the analysis of antibiotic resistance. This is a problem that is becoming a major global health issue. Studies have shown an increase in antibiotic resistance in pathogenic bacteria, and over time, many bacteria of clinical importante is expected to developed resistance to antibiotics (Munir et al., 2010). Therefore, actions must be taken to reduce this problem. One example is the development of research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The problem of antibiotic resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain (Nascimento, Locatelli, Freitas, & Silva, 2000). Because of the constant changes in microbiota, metagenomics researches must continue improving and discovering new proteins and other treatment options.

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LA INVESTIGACIÓN EN EL CAMPUS/ RESEARCH ON CAMPUS

Resumen de otras investigaciones realizadas por nuestros estudiantes en el Recinto durante el año académico 2017-2018 en los campos de farmacología y bioquímica.

Summary of other research conducted on campus by our students during the academic year 2017-2018 in the fields of pharmacology and biochemistry.

FARMACOLOGÍA-BIOQUÍMICA/ PHARMACOLOGY-BIOCHEMISTRY

1-A Evaluation of the potential synergistic effect of curcuminoids and bexarotene for induction of apoptosis of multiple myeloma cells

Verónica Medina, Gustavo Colón, Karimar Vélez, Kevin Ongay, and Karen Woolcock Rodríguez

Multiple myeloma (MM) is a plasma cell malignancy, commonly treated with immunomodulators, proteasome inhibitors, and steroids, but that becomes resistant to the pharmacological actions of these drugs. Accordingly, is important to evaluate alternate approaches to improve patient prognosis. Natural products derived from *Curcuma longa* and the third-generation retinoid compound, bexarotene, are known to increase apoptosis. Therefore, it was examined if the co-administration of curcumin or bisdemethoxycurcumin (1, 10, 25, 50 and 100 mM) with bexarotene (10 mM) increase the amount of MM cells (RPMI-8226) that are able to activate apoptosis through the analysis of Caspase-3/7 activation using flow cytometry. Our results show that at high concentrations (100 mM) curcumin was more efficient inducing net apoptosis (36.2%) when compared to bisdemethoxycurcumin (6.4%), and to bexarotene (3.1%). In addition, it was observed that bexarotene (10 mM) seems to potentiate the ability of curcumin (25 mM) to induce apoptosis. This observation is significant, since one of the major drawbacks of curcuminoids is their limited absorption and the low physiological concentrations that are achieved after oral administration. However, this synergistic effect was not observed with bisdemethoxycurcumin, demonstrating that active curcuminoids have different pharmacological profiles.

1-B Analysis of the effect of curcuminoids with and without bexarotene on the cell cycle phases of multiple myeloma cells

Aixamarie López, Glorymar Nieves, Frances Seín, Yolanda Martínez and Karen Woolcock Rodríguez

Curcumin, alone or in combination with other agents, has been used for the prevention and treatment of various forms of cancer in humans, including colorectar, pancreatic, breast, prostate, multiple myeloma, lung and oral cancer. Another group of compounds capable of inducing reduction of cell proliferation are the retinoids that have been shown to inhibit cell growth, induce cell differentiation and enhance apoptosis of cancer cells. The aim of this study was to analyze the ability of curcuminoids with or without bexarotene to arrest the cell cycle of the RPMI 8226 cells at G0/G1 phase and to evaluate the potential synergystic effect of curcuminoids and bexarotene. Our results suggest that co-administration of bexarotene (10 µM) can potentiate the ability of curcumin and bisdemethoxycurcumin at low concentration (10 µM) to arrest cells in G0/G1 stage. However, the synergetic effect of bexarotene is stage-dependent, since when co-administered with curcuminoids allowed the cell population to progress through S stage and G2/M stage of the cycle. Also, it is concentration-dependent, since at high concentrations (50-100 µM) of curcuminoids, bexarotene did not increase the population of cells arrested in G0/G1 phase.

1-C Optimization for the detection and semi-quantification of NF-kβ by western blot using stain free gel system and total protein normalization

Jonás Tapia, Amanda Feliciano, Alejandra Agosto, María Martínez and Karen Woolcock Rodríguez

Multiple myeloma (MM) is a currently incurable hematologic malignancy in which there is infiltration of malignant plasma cells in the bone marrow. Recently, it has become clear that Nuclear Factor-kβ (NF-kβ) signaling has a critical role in cancer development and progression. However, curcumin (CUR) has shown to suppress the activation of NF-kβ in multiple myeloma cells. In this research, the optimization of experimental conditions for the detection of NF-kβ was studied. Exposure of MM cells to curcuminoids at different concentraions with or without bexarotene (BXT) was done to subsequently evaluate the translocation of NF-kβ from the nucleus to the cytoplasm using western blot analysis and immunodetection by chemiluminescence. The presence of p50 (50 kDa) and p105 (105 kDa) subunits of NF-kβ were detected on the cytoplasm and nuclear extract of cells treated with DMSO 0.05%. The signal obtained for p50 was used to determine the linear dynamic range (LDR), in which changes of protein expression levels can be detected. For the cytoplasm extracts the LDR was within 5 to 20 µg of total loaded protein. These results confirm that the NF-kβ expressed in treated cells could be measured by a semi-quantitative western blot.

1-D Morphology analysis and study of epithelial to mesenchymal transition of PANC-1 cells

Natalia Acevedo, Cristopher Marrero, Kevin Ongay, Joshua Ortiz, Joaneliz Torres and Karen Woolcock Rodríguez

Pancreatic ductal adenocarcinoma is a highly aggressive type of cancer that still has a poor prognosis. This is mainly due to the high metastatic nature of the disease. A widely accepted theory for cancer progression and invasion is that epithelial cells undergo a mesenchymal transition. This process is associated with the repression of E-cadherin expression by EMT regulators such as Snail. Therefore, it is important to evaluate if natural products derived from *Curcuma longa* and ginger can reduce EMT. E-cadherin expression was evaluated through episcopic and diascopic microscopy to determine if natural compounds derived from *Curcuma longa* (curcumin and bisdemethoxycurcumin) or ginger derivates (shogaol and zingerone) can increase E-cadherin expression in PANC-1 cells. Also, the synthetic compounds gemcitabine and E-Hop 16 were evaluated. The expression was detected in cells without treatment, cells exposed to the pharmacological treatments and controls. Curcumin produced a slight increase of 6.9% of E-Cadherin expression in the PANC-1 cells. Optimizations of staining procedure are needed to further evaluate the expression pattern.

1-E Inhibition of proliferation of human pancreatic carcinoma cells by phenolic compounds obtained from ginger

Yara Peraza, Juan Curbelo, Marycarmen Iguina, Glorymar Nieves, Zuleida Hernández, Aixamarie López and Karen Woolcock Rodríguez

Pancreatic cancer is one of the most lethal types of cancer. The symptoms that a pancreatic cancer patient presents are vague or in many cases absent. The condition is often diagnosed at a late stage when the treatment options are very limited. There are no effective treatments currently available for this type of cancer. Therefore, there is an immediate need for the identificatin, development and characterization of novel therapeutic agents. The objective of this investigation was to study the effect of different natural compounds derived from ginger (zingerone, 6-shogaol), turmeric (curcumin, bisdemethoxycurcumin) and synthetic compound (EHop-16), in the proliferation of the human pancreatic adenocarcinoma cell line, PANC-1. For this, the cells were exposed to natural and synthetic treatments

(Ehop-16 and gemcitabine) at different concentrations (1, 10, 25, 20 and 100 µM) for 48 hours under standard culture conditions using DMSO as a control. The effect on the cell proliferation was evaluated by the MTT assay. The results obtained demonstrate that gemcitabine, the current pharmacological treatment for adenocarcinoma pancreatic cancer reduced cell viability to only 70%. At high concentration (100 µM) 6-shogaol, curcumin, bisdemethoxycurcumin and EHop-16 reduced the viability of PANC-1 cell to 11.8% ± 1.8, 55.1 % ±15.8, 42.7% ± 11.3 and 12.6% respectively. When the total cells per well were reduced to 1,250, treatment with 6-shogaol (10 µM) significantly reduced PANC-1 cell viability to 18.6% ± 5.6. Since this value is within physiological concentrations, the incorporation of ginger as a treatment may be a reasonable option either in an early stage of the disease or when the tumor is resected.

1-F Evaluation of the potential of phenolic compounds from ginger, curcuminoids and E-Hop to induce apoptosis in adenocarcinoma of the pancreas

Natalia Acevedo, Cristopher Marrero, Kevin Ongay, Joshua Ortiz, Joaneliz Torres and Karen Woolcock Rodríguez

Pancreatic ductal adenocarcinoma a highly aggressive type of cancer is commonly treated with a wide range of chemotherapeutic drugs, but a major drawback is that these cells are highly resistant to the pharmacological action of these drugs. Therefore, it is important to evaluate alternate approaches to improve patient prognosis. Natural products derived from *Curcuma longa* and ginger, are known to induce apoptosis. In this project we evaluated if exposing PANC-1 cell to natural compounds derived from curcumin or ginger can induce apoptosis and improve the expression of the epithelial cell marker E-cadherin. Also, the effect of synthetic compounds such as gemcitabine, the currently approved pharmacological therapy, and E-hop 16 a novel compound were evaluated. Our results show that the highest induction of caspase activity after 24 hours of treatments was produced by curcumin followed by Ehop-16 and shogaol. Also, it was observed that when the cells were treated for 24 hours the only compound that reflects net caspase activation and net apoptosis at low concentration (10µM) were shogaol and gemcitabine. Our results suggest that both curcumin and ginger may represent an option to help to induce adenocarcinoma cell death.

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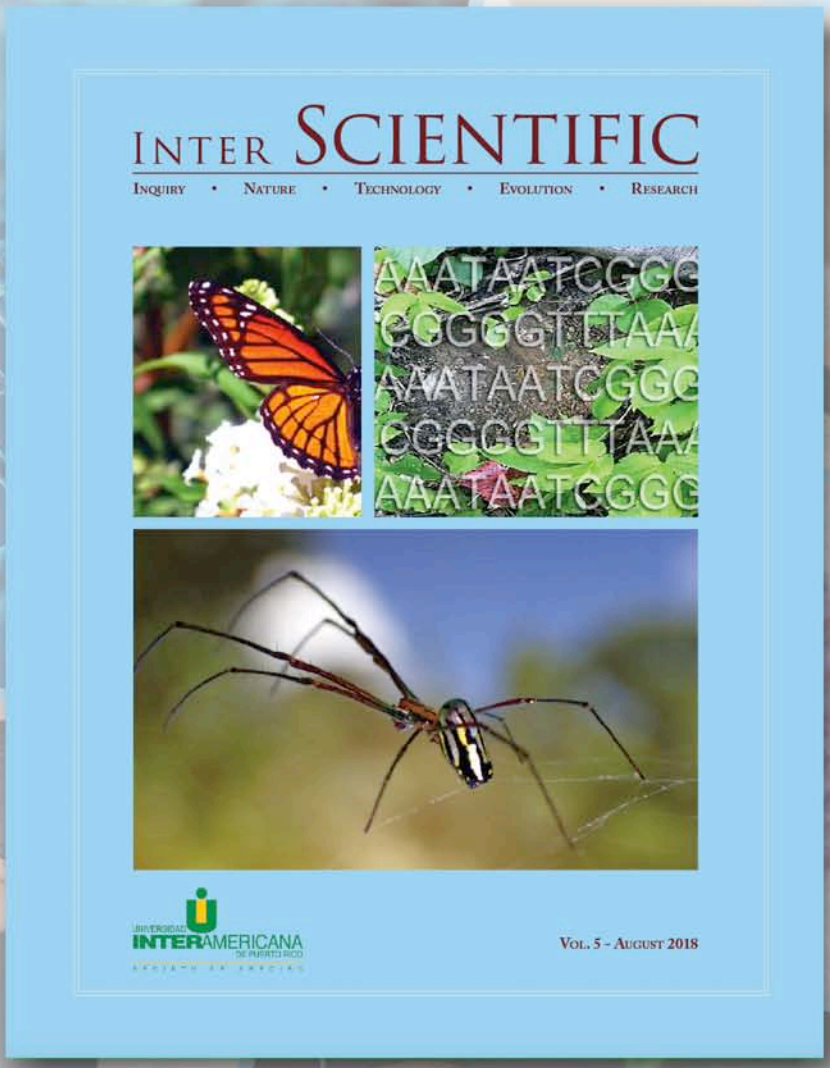

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