Food Research International 109 (2018) 497–505

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Original Paper

Microbiota of edible *Liometopum apiculatum* ant larvae reveals potential functions related to their nutritional value

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ARTICLE INFO

Keywords:
Ant
Larvae
Bacteriocytes
MALDI-biotyping
Microbiota
16S rRNA gene

ABSTRACT

Edible insects, due to their high nutritive value, are currently considered as a potential renewable source for food and feed production. *Liometopum apiculatum* ants are widely distributed in arid and semi-arid ecosystems and their larvae (escamoles) are considered as a delicacy, however the microbial importance in *L. apiculatum* nutritional ecology is unknown. The aim of this research was to characterize the microorganisms associated with both *L. apiculatum* larvae and the reproductive adult ants using the *16S rRNA* gene sequencing and culturomics approaches. The obligate endosymbionts were also investigated through microscopic analysis. The most abundant Phylum identified by sequencing in the larvae was Firmicutes while in adult ants was Proteobacteria. Interestingly, the culturomics results showed 15 genera corresponding to the bacteria identified by sequencing analysis. Particularly, it was observed a large population of nitrogen-fixing bacteria, which could be linked with the high protein content in escamoles. Endosymbionts were detected in bacteoriocytes, these bacteria are related with vitamins and essential amino acids biosynthesis, and both compounds contributing to the high nutritional value of escamoles. This is the first report of the microorganisms present in the *escamolera* ant ensuring their safety as food and opening new areas of nutritional ecological and food processing.

1. Introduction

The world population will increase to 9 billion people by 2050, which will demand an increase of food production and currently innovative solutions to meet Food Security are needed. Insects are part of the diet of at least two billion people worldwide and from about one million of known insects, 1900 species are currently used as food (FAO, 2013). Moreover, insects are ecologically sustainable, they use less natural resources, emit less green-house gases than conventional livestock, and produce protein more efficiently compared with animals (van Huis, 2011). In many countries, insects are considered as delicacy and entomophagy (practice of eating insects) has been extended in Western societies (Jansson & Berggren, 2015; van Huis, 2011). These data suggest that insects are a good alternative to conventional livestock farming (Jansson & Berggren, 2015). However, little is known regarding the microbial relevance in the insects ecology, a critical point in relation to their nutritional quality and food safety (Douglas, 2009; Vandeweyer, Crauwels, Lievens, & Van Campenhout, 2017).

The larvae of *L. apiculatum* ants, known in Mexico as “escamoles”, are consumed since pre-Hispanic times. Due to their nutritional and organoleptic characteristics, escamoles are recognized as a Mexican delicatessen (Ladrón de Guevara, Padilla, Garcia, Pino, & Ramos-Elorduy, 1995; Melo-Ruiz, Quirino-Barreda, Calvo-Carrillo, Sánchez-Herrera, & Sandoval-Trujillo, 2013). Escamoles have high protein content (42%) with a high ratio of essential amino acids; they are rich in essential lipids such as oleic (67.66%), linoleic (2.61%), and arachidonic (0.16%) acids. Vitamins (A and E), tiamin, riboflavin, and niacin are also present in these larvae (Melo-Ruiz, Quirno-Barreda, Díaz-Garcí, & Gazga-Urioste, 2016). This richness of nutrients in escamoles is interesting because the ants live in arid and semiarid zones with food shortages, thus it is possible that the *escamolera* ant is associated with diverse microorganisms to address specific imbalances of some nutrients. However, to our knowledge, there is no information regarding the microorganisms associated with *L. apiculatum*.

In other insects, the valuable nutritional properties have been attributed to a high efficiency of their endosymbionts (microorganisms

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https://doi.org/10.1016/j.foodres.2018.04.049
Received 8 February 2018; Received in revised form 10 April 2018; Accepted 21 April 2018
Available online 24 April 2018

living inside the host) that assist insects in the food management through the production of a broad array of enzymes responsible of plant lignocellulose degradation, reductive acetogenesis, and nitrogen recycling and fixation (Brune, 2014; Engel, Martinson, & Moran, 2012; López-Sánchez et al., 2009; Warnecke et al., 2007). The gut endosymbionts are mainly responsible for the production of metabolites such as essential amino acids, vitamins and fatty acids that compensate the severe nutritional deficits of insects´ diet (Brune, 2014; Burnum et al., 2010; Fan, Thompson, Dubois, Moseley, & Wernegreen, 2013; López-Sánchez et al., 2009; Sapountzis et al., 2015; Warnecke et al., 2007). While ectosymbionts (microorganisms living outside the host) mainly produce antimicrobial compounds as part of the host defence system (Barke et al., 2010; Santos, Dillon, Dillon, Reynolds, & Samuels, 2004).

Metagenomic approach has revolutionized the understanding of the microbiome of several organisms. Recent studies have used the *16S rRNA* gene sequencing to explore ants intestinal microbiota composition (Kautz, Rubin, Russell, & Moreaua, 2013). Data have shown an extensive relationship with nitrogen-fixing bacteria (Russell et al., 2009; Sapountzis et al., 2015). However, the advances in sequencing technologies have generated gaps corresponding to the unidentified sequences (Lagier et al., 2015). Hence, pure strains remain the key for solving the role of these bacterial endosymbionts and the resurgence of culture in microbiology through the recently area named culturomics, which also is used to validate the metagenomic data (Hamad et al., 2017; Lagier et al., 2016). Culturomics uses the Matrix Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) for the identification of large number of isolated bacteria improving the characterization of the insect microbiota (Tandina et al., 2016). Other efforts have been carried out through microscopic and proteomic approaches, which have helped to understand the functional contribution of *Blochmannia*, an intracellular endosymbiotic bacteria of *Camponotus* or carpenter ant (Fan et al., 2013; Stoll, Feldhaar, Fraunholz, & Gross, 2010). All those works have hypothesized that microorganisms are a system of nutrient supply for ants, which are linked to the essential amino acids biosynthesis, beginning with the recycling of waste nitrogen or the atmospheric nitrogen fixation. This microbial importance in insect nutritional ecology is a key tool to preserve their nutritional quality by the development of appropriate conservation and management strategies. However, most of the works with insects are focused in a narrow range of model organisms, such as bees and termites.

Hence, the aim of this research was to characterize the gut endosymbionts associated with the larvae and reproductive adult ants of *L. apiculatum* through amplicon-based metagenomics and culturomics approaches. The work was complemented with microscopic analyses to gather the knowledge about the location and possible function of the *escamolera* ant endosymbionts.

2. Materials and methods

2.1. Collection site and conditions for obtaining bacterial samples

L. apiculatum larvae and adult ants were collected at Pocitos-Charcas, San Luis Potosí, Mexico (latitude 23°09′41.60″ N and longitude 100°58′08.94″ W). Ecosystem was characterized by the scarcity of flora, being cacti and agave the predominant species (Fig. 1). Biological triplicates of larvae and adult ants were collected from three different colonies at distances of approximately 5 km from each other. Each replicate was prepared from representative sample (pool) with at least 50 larvae (18–25 days of age) and 50 adult ants (60–70 days of age). Samples were collected directly from their nest, placed into plastic bags and transported in ice to the laboratory (Suen et al., 2010).

Samples were surface sterilized through continuous washes with 70% ethanol solution, 15% sodium hypochlorite (5.25% solution), sterile water, and sterile phosphate-buffered saline (PBS) pH 7.4, The final PBS wash was plated on the growth media as a negative control. The complete larvae and the guts of adult ants, aseptically dissected,

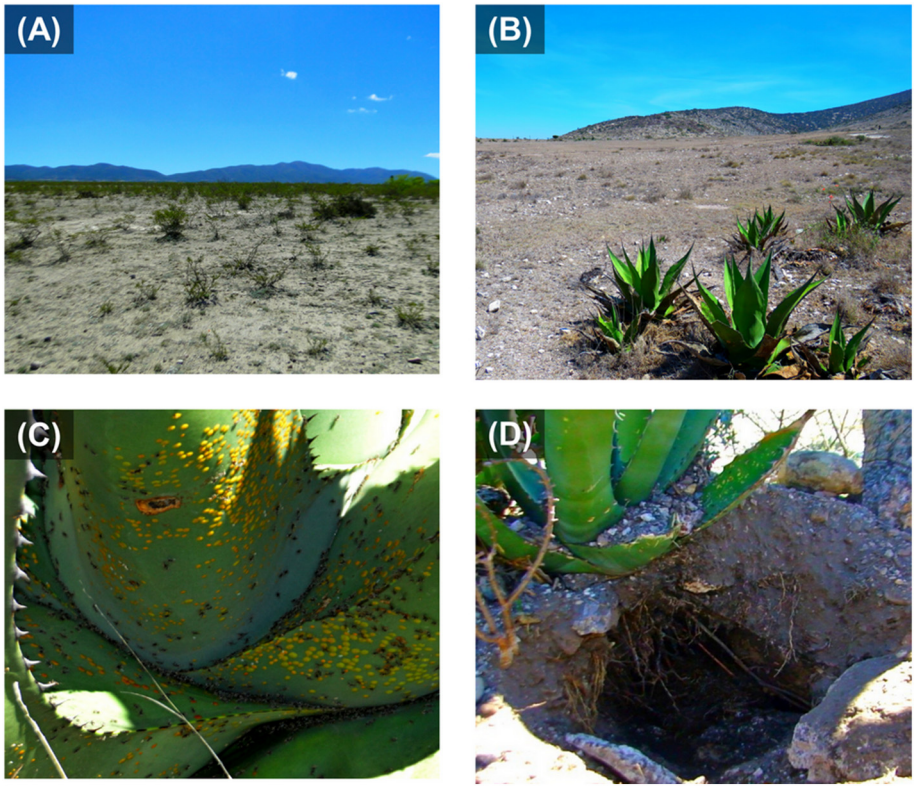

Fig. 1. Images of *L. apiculatum* ant ecosystem. A) and B) general images of the *L. apiculatum* niche of growth. C) Nest of the ants in the roots of the agave plants. D) Agave plant where adult ant interacts with another insects.