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Growth of a desiccated amoeba under low oxygen and anoxic gas conditions from laminated microbial mats at Las Salinas of Cabo Rojo, Puerto Rico

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ABSTRACT. Amoebae (30–45 µm long) were isolated from laminated microbial mats from Las Salinas of Cabo Rojo, Boquerón Forest, Cabo Rojo (near Mayagüez), Puerto Rico. Three sets of experiments were performed to monitor amoebal growth in ambient oxygen (O_2), low O_2, and anoxic (no oxygen) gas conditions. The amoeba tolerates and grows rapidly under all tested conditions. These were cultured by use of the same methods as used for *Paratetramitus jugosus*, a Vahlkampfiid monopodial amoeba (10–15 µm in size), from different geographical areas, such as Delta del Ebro, Spain, Laguna Figueroa in Baja California del Norte, Mexico, and Eel Pond in Woods Hole, Massachusetts, USA. The amoeba from Las Salinas of Cabo Rojo differs from *P. jugosus* in length (30–45 µm), width (1–2 µm), and cyst size (20–25 µm). This amoeba also exhibits an amoebomastigote stage (15–20 µm). Thus, we confirm that Las Salinas of Cabo Rojo amoebae not only tolerate but grow abundantly under ambient O_2, low O_2, and anoxic conditions.

Keywords: Laminated microbial mats, low oxygen and anoxic gas conditions, amoebae, cysts, amoebomastigote stage.

Laminated microbial mats are complex community structures defined by both physical (e.g., light and temperature) and chemical (e.g., oxygen and salinity) abiotic factors (**Franks and Stolz, 2009**). They are found in environments ranging from salt marshes and oceanic sediments to arctic dry valleys (**Brune et al., 2000**; **Franks and Stolz, 2009**). Microbial mats have well-established oxygenic and non-oxygenic modes of metabolism and respiration for phototrophy (**Brune et al., 2000**; **Franks and Stolz, 2009**). For example, primary producer photoautotrophs, such as, cyanobacteria, and chemolithoautotrophs, such as, sulphur oxidizing bacteria, inhabit lithified mats (**Golubic, 2000**; **Strother and Barghoorn, 2000**; **Margulis and Chapman, 2010**; **Campbell et al., 2019**). Bacteria and other microorganisms hold down different layers of laminated microbial mats, preserving them for long periods of time if they remain undisturbed. However, grazing and burrowing by aquatic animals can destroy mats as quickly as they form (**Golubic, 2000**).

Microorganism growth in anoxic (no oxygen) environments mostly occurs from fermentation by anaerobic respiration; however, anaerobic respiration is part of a redox potential difference between the donor substrate and oxidant, which leads to cell growth (**Plugge, 2005**). Historically, the growth of microorganisms in low oxygen and anoxic environments has been mostly related to oceanic anoxic events (**Pacton and Gorin, 2014**) and as an adaptation mechanism of microbial activity present in oxic-anoxic environments (**Brune et al., 2000**). A recent study has investigated the mineralization and the morphology of microbial mats on the shoreline in south-western Puerto Rico (**Rodriguez-Colon et al., 2019**). Yet, amoebae growth under different levels of oxygen in microbial mats has not been previously studied in this area. Here, the growth of an intertidal laminated microbial mat amoeba that grows under ambient oxygen (O_2), low O_2, and anoxic gas conditions from Las Salinas (salt flats) of Cabo Rojo, Boquerón Forest, is reported, Cabo Rojo is near Mayagüez in south-western Puerto Rico (**Figure 1**). To the best of my knowledge, this is the first study to report the growth of a desiccated amoeba in laminated microbial mats from Las Salinas of Cabo Rojo, Puerto Rico under ambient, low O_2 and anoxic conditions.

Las Salinas of Cabo Rojo formed from a shift of tectonic plates approximately 190 million years ago mostly from Jurassic to Eocene volcanic and plutonic rocks (**Cheadle, 2015**). The shift created

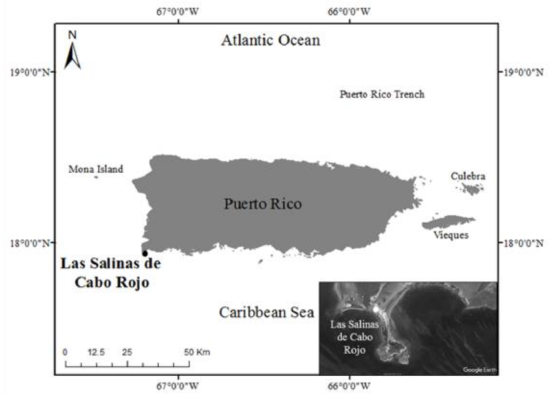

Figure 1. The location of Las Salinas of Cabo Rojo, Boquerón Forest, Cabo Rojo, Puerto Rico (Data source: NOAA); B is a Google Earth satellite image (© 2016 Google Inc.).

Figure 2. The laminated microbial mats for this study are from the Candelaria Inlet (shown by the white arrow).

the Sierra Bermeja, the oldest mountain range in Puerto Rico that is located to the northeast, and Los Morrillos, found to the south, allowing the formation of the Las Salinas of Cabo Rojo (**Weaver and Schwagerl, 2009**). Today, Las Salinas of Cabo Rojo has rocky shorelines composed of limestone and volcaniclastic rocks with alluvial deposits, beaches, wetlands, and mangrove shorelines (**Morelock et al., 2000**). Las Salinas of Cabo Rojo are a sub-tropical dry forest consisting of many ecosystems, such as dry forest, hypersaline lagoons, salt flats, marine lagoons, mangrove forest, seagrass meadow, and coral reefs (**C.P.S.A., 2020**).

The Salinas of Cabo Rojo were flooded in the winter between 2005 and 2006. It is not clear in precisely which months the flooding occurred, but Dr. Carlos Ríos Velázquez (oral communication, 2009) mentioned that the area suffered from severe flooding both in 2005 and 2006. The laminated mats were estimated at the time to be approximately 60–65 years old. This preservation of the mats has been possible because Las Salinas of Cabo Rojo is part of the protected Cabo Rojo National Wildlife Refuge where the mats have been undisturbed by grazing and burrowing animals.

Amoebae (30-45 µm) were collected from laminated microbial mats in the Candelaria Inlet in November 2009 (**Figure 2**). The samples had been drying for approximately one year before the laboratory experiments were performed. The samples were isolated into monoprotist cultures in the laboratory. The amoebae were cultured by the same methods as *Paratetramitus jugosus* collected from laminated microbial mats at Laguna Figueroa, Baja California del Norte, Mexico, and from mud at Eel Pond, Woods Hole, Cape Cod, Massachusetts, USA (**Margulis et al., 1990**; **Read et al., 1983**; **Santiago-Ramírez, 2011**). Three sets of plates of desiccated microbial mat samples were inoculated in the sterilizing hood. Approximately 1 mm^3 of mat sediment sample was cut and placed directly in the centre of each thin, translucent, sterile agar plate containing manganese acetate media. Immediately after placement, 1 ml of distilled water was added to the dry sample by dropper. This suspended the organisms and initiated feeding, growth, and reproduction (**Margulis et al., 1990**).

Sets of inoculated manganese acetate plates were then incubated under three different conditions. The control, a Brewer's jar without any gas pack, was subject to ambient air (20% O_2). The second and third sets were placed in jars under low O_2 and anoxic gas conditions, respectively. All the jars were incubated at ambient temperature (25ºC). The other ambient conditions (e.g., temperature, diurnal light cycle) were maintained constant for all three sets of jars. Anoxia in the jars was maintained by a GasPak® EZ hydrogen (H_2) + carbon dioxide (CO_2) with palladium catalyst and a GasPak® EZ anaerobe container system with indicator. All the plates were inoculated immediately following the preparation of the manganese acetate medium. Sterilized water also was used to flood and then wet the sample when the plates were inoculated.

The desiccation of an amoeba (30–45 µm) was found in intertidal laminated microbial mats (**Figure 3**). This amoeba forms cysts (20–25 µm) and survives desiccation (**Figures 3-4**) (**Margulis et al., 1990**; **Page, 1983**). Furthermore, the mat amoeba grows well under low O_2 (**Figures 4-5A**) and anoxic conditions (**Figure 5C**) and exhibits an amoebomastigote stage (**Figure 5B**).

The amoeba from Las Salinas of Cabo Rojo is extremely similar in life history to *P. jugosus* (e.g., **Read et al., 1983**; **Margulis et al., 1990**) and to the

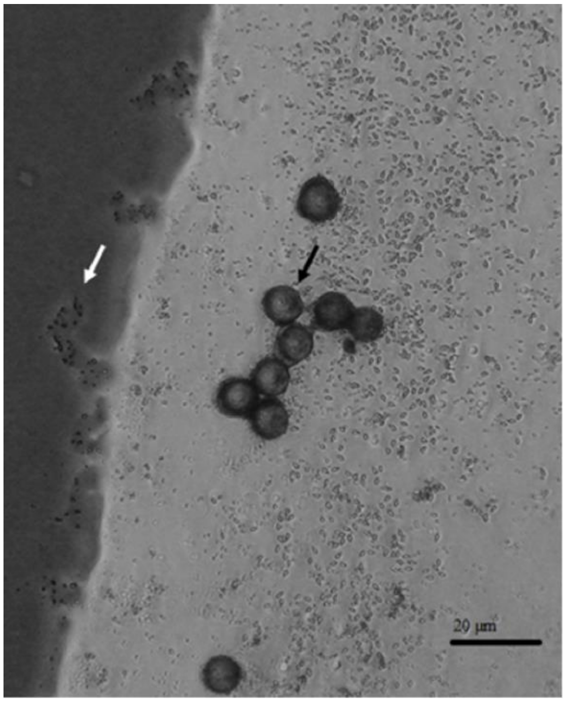

Figure 3. Amoebae (shown by the white arrow) and cysts (shown by the black arrow) in control samples. An interface is shown between wet and dry conditions (from left to right).

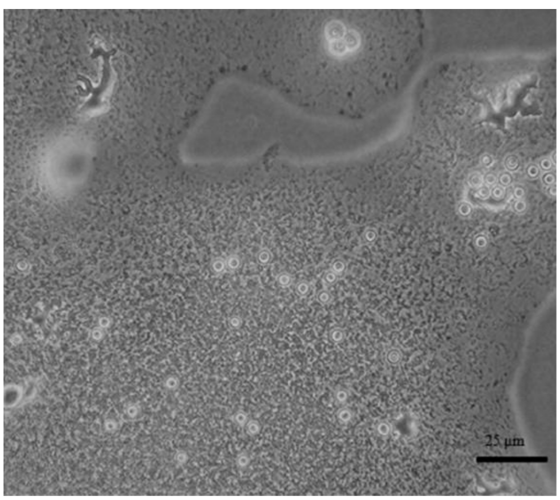

Figure 4. Amoebae and cysts in low oxygen gas conditions, 200x.

amoebae first described in California by **Wherry** (**1913**) and at Woods Hole, Massachusetts by **Hogue** (**1914**). The amoeba (30–45 µm) differs from *P. jugosus* in being polypodial, and very thin,

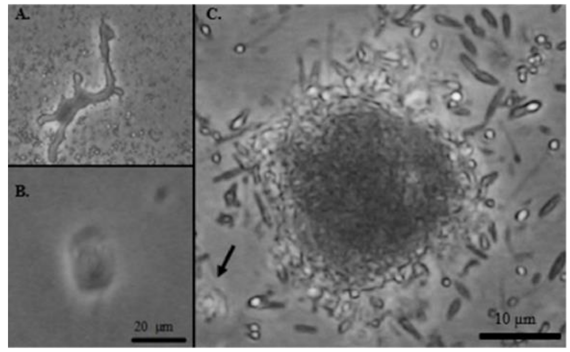

Figure 5. A is an amoeba in low oxygen gas conditions; B shows an amoebomastigote from control samples, C is the anoxia sample with bacillus spores and an amoeba (shown by the black arrow).

with a smooth glycocalyx (**Figures 3, 4, 5A**). For comparison, preliminary studies were made of similar amoeba from different field sites in Europe, the United States, Mexico and the Caribbean. The amoeba resembles the genus *Heteroamoeba* from the Family Vahlkampfiidae, the same family which includes *P. jugosus*. However, further studies are needed for the classification of this amoeba. This research confirms that the amoebae from the Las Salinas of Cabo Rojo laminated microbial mats grow well under ambient O_2, low O_2, and anoxic conditions. The results further our understanding in how amoebae respond to environmental changes, such as different levels O_2 on the south-western shoreline of Puerto Rico.

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