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Identification of culturable microbial functional groups isolated from the rhizosphere of four species of mangroves and their biotechnological potential

Miguel A. Ramírez-Elías[a], Ronald Ferrera-Cerrato[a], Alejandro Alarcón[a,*], Juan J. Almaraz[a], Gustavo Ramírez-Valverde[b], Luz E. de-Bashan[c], Fernando J. Esparza-García[d], Oscar García-Barradas[e]

[a] Área de Microbiología, Postgrado de Edafologia, Colegio de Postgraduados, Carretera Mexico-Texcoco km 36.5, Montecillo 56230, Estado de Mexico. Mexico
[b] Departamento de Estadística, Colegio de Postgraduados, Carretera México Texcoco km 36.5, Montecillo 56230, Estado de México, Mexico
[c] Grupo de Microbiología Ambiental, Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Avenida I.P.N. 195, Col. Playa Palo de Santa Rita Sur, La Paz, B.C.S. 23096, Mexico
[d] Laboratorio de Ecología Bioquímica, CINVESTAV, México, D.F., Mexico
[e] Unidad de Servicios de Apoyo en Resolución Analítica (SARA), Universidad Veracruzana, Xalapa, Veracruz, Mexico

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ABSTRACT

This study identified microbial functional groups like total culturable bacteria, potential N_2-fixing free living bacteria, N_2-fixing hydrocarbonoclastic bacteria, N-assimilating hydrocarbonoclastic bacteria, total fungi, actinobacteria, P-solubilizers, lipolytic microorganisms, and starch, cellulose, pectin and protein degrading microorganisms, isolated from the rhizosphere of four species of mangroves (Red, Black, White, and Button) from the natural protected area at the Terminos Lagoon, Campeche, México. Overall, microbial populations showed significant differences ($P < 0.05$) among the four mangrove species. The rhizosphere of White mangrove showed better chemical and textural soil properties, and harbored the highest microbial populations when compared to the remaining mangrove species. The principal component analysis indicated that two components accounted the 85.3% of the total variation. The most significant textural and chemical soil properties were the major components, CP1 (organic matter and total organic carbon) and CP2 (sand and clay). Microbial populations correlated ($P < 0.05$, Pearson coefficient) with sand and clay particles, and with some soil chemical properties such as organic matter. The total nitrogen and organic carbon significantly correlated with cellulose degraders, while phosphorus with N_2-fixing bacteria, total fungi, and with pectin and starch degraders.

1. Introduction

There are reported approximately 73 species of mangroves worldwide (Spalding et al., 2010). Many coastal lagoons in the tropics and subtropics support dense mangrove forests. These are some of the most productive areas in the marine and estuarine environments supporting large plants and animal communities, many of which are economically and ecologically important (Jones, 1992; Toledo et al., 1995). Mangroves in Mexico are distributed in shoreline lagoons and estuaries in the Gulf of Mexico and in the Pacific Ocean, covering 655,667 ha. Campeche is the Mexican state with the highest mangrove coverage (196,552 ha), which is mainly found in the natural protected area of the Terminos Lagoon, and in the biosphere reserve Los Petenes (Spalding et al., 2010). Mangrove ecosystems in Campeche consist of four species: *Rhizophora mangle* L. (Red Mangrove), *Avicennia germinans* (L.) Stern (Black Mangrove), *Laguncularia racemosa* (L.) Gaertn. f. (White Mangrove) and *Conocarpus erectus* L. (Button Mangrove) (Day et al., 1987, 1996).

Mangrove ecosystems are characterized by the accumulation of lignocellulose residues that are subjected to mineralization throughout microbial activities (Alongi et al., 1989; Holguin et al., 2001). Plant residue decomposition generates detritus that is the baseline for the food chain in mangrove ecosystems for several organisms such as crustaceans, mollusks, insect larvae, nematodes, and commercial species of shrimps, scallops, oysters and fish (Odum and Heald, 1975a,b; Aburto-Oropeza et al., 2008;

* Corresponding author at: Área de Microbiología, Postgrado de Edafología, Colegio de Postgraduados, Carretera México-Texcoco km 36.5, Montecillo 56230, Estado de Mexico, Mexico. Tel.: +52 59595 202 87; fax: +52 59595 202 87.
E-mail address: aalarconcp@gmail.com (A. Alarcón).

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Duke et al., 2007). Nevertheless, detritus is not only important for species living in the mangrove ecosystems since near to 25% of this material is transported to open seas, mangroves are also considered as nutrient exporting ecosystems (Clough, 1991). Conversely, mangroves are nutrient deficient ecosystems, especially for nitrogen and phosphorus (Holguin et al., 1992; Alongi et al., 1993), but paradoxically, mangroves are highly productive. Thus, microbial activity contributes significantly on nutrient availability in mangroves ecosystems in which biological nitrogen fixation and P-solubilization have ecological relevance (Sengupta and Chaudhuri, 1990; Alongi et al., 1993; Rivera-Monroy and Twilley, 1996; Vázquez et al., 2000; Holguin et al., 2001; Flores-Mireles et al., 2007; Vovides et al., 2011a,b).

Some microbial groups also play significant roles in the carbon cycling by degrading starch, cellulose, lignin, or lipids; and other microorganisms participate in both nitrogen and phosphorus cycles in soil (Bouillon et al., 2008). Therefore, microbial activity considerably contributes on changes of soil chemical properties as well as on plant nutrition by facilitating organic matter mineralization, and increasing nutrient availability and plant uptake (Schloter et al., 2003). Plant growth promoting rhizobacteria (PGPR) are useful biological elements for reforestation and rehabilitation of mangrove ecosystems. In addition, PGPR may serve as important components in bioremediation of contaminated water, sediments, and soil (Alongi, 1994; Bashan and Holguin, 1998, 2002; Holguin et al., 2001; de-Bashan et al., 2012). Mangrove ecosystems are also subjected to anthropogenic pressures such as oil spill contamination in which the presence of hydrocarbonoclastic microorganisms may play a significant role on either detoxifying or decontaminating the impacted water and sediments (Alexander, 1994; Taketani et al., 2009). Thus, the identification of microbial strains able to degrade organic contaminants may be relevant for directing them as part of biotechnological approaches for mangrove rehabilitation (Sivaramakrishnan et al., 2006; Lageiro et al., 2007; Salihu et al., 2012; de-Bashan et al., 2012). We tested the hypothesis that mangrove ecosystem at the natural protected area of the Terminos Lagoon, Campeche (México) harbors functional microbial populations with biotechnological purposes, and that these microbial populations correlate with textural and chemical properties of the rhizosphere soil collected from four predominant mangrove species.

Thus, this study identified culturable microbial functional groups in the rhizosphere of four species of mangroves (Red, Black, White, and Button) that are predominant at the natural protected area of the Terminos Lagoon. The identified microbial groups were total bacteria, potential N_2-fixing free living bacteria, N_2-fixing hydrocarbonoclastic bacteria, N-assimilating hydrocarbonoclastic bacteria, total fungi, actinobacteria, P-solubilizing bacteria, and lipolytic and cellulolytic microorganisms, as well as pectin, starch and protein degraders.

2. Materials and methods

2.1. Site of study and samplings

Rhizosphere soil was collected from the four dominant mangrove species *R. mangle* L. (Red mangrove – RedM-), *A. germinans* (L.) Stern (Black mangrove – BlackM-), *L. racemosa* (L.) Gaertn. f. (White mangrove – WhiteM-) and *C. erectus* L. (Button mangrove – ButtonM-) present at the natural protected area of the Terminos Lagoon, Campeche, México. Soil samples were collected during both rain (September, 2010) and dry season (June, 2011). The sampling site is located along the coastal area between the Botanical Garden of the Universidad Autonoma del Carmen and the Estero Pargo (18° 38′ North and 91° 46′ West) (Fig. 1).

Rhizosphere soil samples were taken at 20 cm of depth from the root environment of each mangrove species, and placed in sterilized glass jars and kept at 4 °C for further microbial or chemical and physical analysis (Paetz and Wilke, 2005). Soil sampling consisted on a randomized collection of two samples of rhizosphere soil (1 kg each, approximately) from three mangrove trees for each mangrove species. Soil samples were used for either analyzing soil texture and chemical properties, or determining target microbial populations.

2.2. Determination of physical and chemical properties of the rhizosphere soil

Soil samples from each type of mangrove were dried and sieved (<2 mm) for determining pH (1:2 H_2O) and electrical conductivity (EC) (1:5 H_2O) (APHA, 1998), organic matter (OM) and total organic carbon (TOC) content (Walkley and Black, 1934). Total content of nitrogen (N), phosphorus (P), and calcium carbonate ($CaCO_3$) were also determined (APHA, 1998; Allison and Moodie, 1965; Olsen and Dean, 1965), as well as soil texture (Bouyoucos, 1962).

2.3. Identification of culturable microbial functional groups

Culturable microorganisms were quantified by the dilution and plate count technique using specific culture media. Total culturable bacteria (TotB), total fungi (TotF), and actinobacteria (ACMY) were determined using nutrient agar, potato dextrose agar, and CZAPEK agar adjusted to pH 8 (Merck®), respectively. Potential P-Solubilizing (PSol) bacteria were quantified by means of Pikovskaya medium amended with tricalcium phosphate (Pikovskaya, 1948). Since potential N_2-fixing free living bacteria (NFB) were determined by using a nitrogen-free medium (Rennie, 1981); this culture medium was modified by applying either crude oil (80 mg L^{-1}) or ammonium nitrate (2 mg L^{-1}) plus crude oil for enumerating potential either N_2-fixing hydrocarbonoclastic bacteria (NFHB) or N-assimilating hydrocarbonoclastic bacteria (NAHB), respectively. Plates were incubated at 28 ± 1 °C for 3–5 days, and bacteria enumeration was performed expressing the results from six replicates, as colony forming units (CFU g^{-1} dry soil). Additionally, populations of lipolytic (LIPOL) and cellulolytic (CELLUL) microorganisms as well as degraders of pectin (PEC), starch (AMYL) and protein (PROT) were also determined (Levine, 1953; Sierra, 1957; Wollum, 1982; Sumaya et al., 1993). Plates were incubated at 28 ± 1 °C for 7 days, and bacteria enumeration was performed expressing the results from six replicates, as colony forming units (CFU g^{-1} dry soil).

2.4. Experimental design and statistical analysis

Average data from six replicates were used for estimating microbial populations (CFU g^{-1} soil), but data from microbial enumeration were transformed to logarithmic units and subjected to an analysis of variance (ANOVA) and to a mean comparison test (Tukey, $\alpha = 0.05$); however, data in graphs are presented using the actual average values. Soil textural and chemical properties were analyzed by means of the principal component analysis (PCA) and main data are showed by means of a dispersion diagram for principal components CP1 and CP2. Also, Pearson correlation coefficients ($P < 0.05$) were performed for microbial populations, and soil textural and chemical properties. Statistical analysis was conducted with the software InfoStat®.