Research article

Recognition of potential beneficial fungi associated with Chontaduro (*Bactris gasipaes* H.B.K.) rhizosphere in the Pacific region of Valle del Cauca, Colombia

Reconocimiento de hongos con potencial benéfico asociados a la rizósfera de chontaduro (*Bactris gasipaes* H.B.K.) en la región Pacífico del Valle del Cauca, Colombia

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Abstract

The peach palm or chontaduro (*Bactris gasipaes* H.B.K.) is the principal crop of the rainforest agroecosytems of the Colombian Pacific coast. This region is poorly referenced in the scientific literature despite its high biodiversity and agroecological importance. The aim of this study was to isolate and identify up to the level of genus, fungi from the rhizosphere of *B. gasipaes* in two areas with different crop management, Citronela and Sabanetas, both in Valle del Cauca. Roots and rhizospherical soil were sampled at three times of varying rainfall from 2006 to 2007. It is hypothesized that the size and diversity of the fungi population are negatively influenced by rainfall, as this is the dominant climatic variable in the study region. Results showed that in Citronela fungi populations were stable during the first two sampling, independent of rainfall between the sampling dates. In Sabaletas, rainfall was higher than in Citronela, which was associated with anaerobic conditions in the rhizosphere that limited fungal growth. The third sampling had higher fungal populations and diversity of fungal genera at both sites which coincides with the period of high rainfall and palm fruit production, conditions that favor the release of exudates from the roots. These factors could have favored secretion of root exudates which in turn, could favor the growth of beneficial microorganisms in the rhizosphere. The genera of fungi isolated were: *Trichoderma* in both sites, *Fusarium* and *Rhizopus* in Citronela only, and *Penicillium* y *Thielaviopsis* in Sabaletas only. These fungi could be beneficial and useful in programs of integrated pest management for palm peach production. Thus, the results could serve as a base for future studies in the Pacific region.

Key words: Buenaventura, *Fusarium*, humid tropics, pejibaye, *Penicillium*, *Rhizopus*, soil fungi, *Thielaviopsis*, *Trichoderma*.

Resumen

El chontaduro o pejibaye (*Bactris gasipaes* H.B.K) es el principal cultivo de los agroecosistemas de la selva húmeda tropical de la costa pacífica colombiana. Esta región no aparece referenciada en la literatura científica a pesar de su alta biodiversidad e importancia agroecológica. El objetivo de este estudio fue aislar y caracterizar morfológicamente hasta género, hongos presentes en la rizósfera de *B. gasipaes* en dos sistemas de producción diferentes, localizados en Citronela y Sabaletas, Valle del Cauca, Colombia. Durante 6 meses se tomaron muestras de raíces y suelo rizosférico en tres épocas con distinta precipitación pluvial. Se planteó como hipótesis que el tamaño y diversidad de la población de hongos son influenciados negativamente por la precipitación, ya que esta es la variable climática preponderante en la región del estudio. Los resultados mostraron que en la localidad de Citronela las poblaciones de hongos permanecieron estables durante los dos primeros muestreos, independiente de los cambios ocurridos en el régimen de lluvias entre épocas. En Sabaletas, durante estos mismos periodos, los registros de lluvias fueron más altos, lo que pudo generar condiciones anaeróbias en la rizósfera y limitar la expresión poblacional fúngica. Para el tercer muestreo, en ambas localidades aumentó la población y diversidad de géneros de hongos, coincidiendo con la época de alta precipitación pluvial y llenado de frutos en las palmas, condiciones que aparentemente favorecen la liberación de exudados en las raíces y consecuentemente el desarrollo de las poblaciones microbianas benéficas de la rizósfera. Los géneros de hongos aislados en este estudio fueron: *Trichoderma* en ambas localidades, *Fusarium* y *Rhizopus* sólo en Citronela, *Penicillium* y *Thielaviopsis* sólo en Sabaletas. Estos microorganismos fungosos podrían presentar potencial benéfico para ser utilizados en programas de manejo integrado dentro del sistema de producción del chontaduro en la región Pacífico de Colombia.

Palabras clave: Buenaventura, *Fusarium*, hongos del suelo, pejibaye, *Penicillium*, *Rhizopus*, *Thielaviopsis*, *Trichoderma*, trópicos húmedos.

Introduction

Chontaduro (*Bactris gasipaes* H.B.K.) is one of the most important crops for the Colombian Pacific zone economy. This specie is cultivated in alluvial plains and warm humid forest hills together with other musa crops and borojo (*Borojoa patinoi* Cuatrec.), mainly for self-consumption and local comercialization (Escobar *et al.*, 1996).

Chontaduro palm tree is affected by a large range of pathogen microorganisms, which under high humidity in the soil and the environment, can cause significant phytosanitary problems with important economic losses. Among the diseases associated with protist, fungi and bacteria are: bud rot or "Pudrición del cogollo" (for its name in Spanish) caused by *Phytophthora palmivora* or *Erwinia chrysanthemi,* the leaf black spot caused by *Colletotrichum* spp., the leaf spot caused by *Pestalotia* spp., the brown spot caused by *Mycosphaerella* spp., the ring spot caused by *Drechslera setariae,* frayed leaf or "hoja deshilachada" (for its name in Spanish) caused by *Lasiodiplodia theobromae* and stem rot and leaf burn caused by *Erwinia* spp. (Orduz and Rangel, 2002; Arroyo *et al.*, 2004).

Traditional management of this crop is not suitable to identify diseases, symptoms and causing agents at an early stage (Mora *et al.*, 1997; Arroyo *et al.*, 2004), which results in an indiscriminate use of fungicides by the farmers. Farmers ignore that those products have a high spectrum that negatively affect natural soil biota, including beneficial microbes' populations (Alabouvette *et al.*, 2004). For the farmers, the indiscriminate use of chemical products represents a high cost for both their economy and health, and the environment (Baker and Dickman, 1995; Whipps, 2001).

In addition to the above mentioned, the fact that some pesticides have reduced their effect on harmful microorganisms, due to resistance mechanisms and selective pressure, means that the dosages must be exceeded which is harmful for the human, animals, plants and environment (Pramauro, 1990). Nowadays, due to the need of finding compatible alternatives with the environment, the interest in biological control and strategies of integrated disease management in economically important crops has raised (Whipps, 2001).

Biocontrol is the reduction of pathogen inoculum by the action of one or more organisms different to man (Baker y Dickman, 1995). The success of this system depends on the isolation of antagonistic microorganisms from different habitats that can confront the causal agent of a determined disease. The highest success with this system has been seen on soil ecosystems (Olalde y Aguilera, 1998).

Barea *et al.* (2005) considered that the interaction between plant root and microbial communities promotes the development of a dynamic environment, the rhizosphere, which is defined as the soil portion adjacent to the plant root system that is affected by the exudates of such roots (Cooke, 1979; Manoharachary *et al.,* 2006). Both, the exudates and the soil organic matter, give the necessary strength for the development of an active microbial community around the roots, this is known as rhizospheric effect (Whipps, 2001; Manoharachary *et al.,* 2006). Rhizosphere community is composed mainly by non-pathogenic microorganisms (Alexander, 1994). In this zone, it is possible to find 10^6 fungi, 10^7 actinobacteria, 10^9 bacteria and 10^3 protozoan per gram of soil (Dix y Webster, 1995). They can affect positively plant growth and development, nutrition, defense against diseases, tolerance to heavy metals, and resistance to the degradation of xenobiotics caused by chemical products from a natural or synthetic origin that are present in the environment (Barea *et al.*, 2005).

The presence of *Trichoderma* genus in the rhizosphere is common for diverse plant species in which it acts as antagonist, mycoparasite, nutrient and space competitor with pathogen microorganisms and/or inducer of plant resistance (Yedidia and Chet, 1999; Baker and Dickman, 1995; Barea *et al.*, 2005; Harman, 2006). According to Whipps (2001) some *Trichoderma* species dominate the fungi group exhibiting antagonist properties. Meanwhile, *Penicillium* is an important primary active agent for the organic matter decomposition, it is characterized by its high contribution to the regulation of pathogen populations by inducing plant resistance (De Cal *et al.*, 1997) and, the production of antibiotic substances such as penicillin and fungitoxic extrolites like griseofulvin, dechlorogriseofulvin and curvulinic acid (Raper and Thom, 1930; Nicoletti *et al.*, 2007).

Some non-pathogenic species of *Fusarium* compete against other pathogenic species from the same genus mainly for carbon, iron and nitrogen (Whipps, 2001; Alabouvette *et al.*, 2004). Additionally, this genus is characterized for its saprophytic activity (Barnett y Hunter, 1972). This function is also complete by *Rhizopus*, in addition to the decomposition of soluble compounds of the soil organic matter like free aminoacids, organic acids and sugars (Agrios, 2005; Luo and Zhou, 2006). In Colombia, Buriticá (1999) cites *Thielaviopsis* as a banana and plantain pathogen, however, Mora *et al.* (1997) considered it as a saprophyte because it was found growing over rotten chontaduro´s fruits. To understand the function of agroecosystems from its microbiological component, the interpretation of biomass and microbial activity values is needed to develop management strategies on the crop systems (Smith *et al.*, 1993) and, in this way, contribute to the improvement of agricultural practices and biodiversity conservation methods (Morgan *et al.*, 2005). The development of research studies to recognize beneficial microorganisms populations in the rhizosphere of chontaduro grown on the humid tropics, contributes to secure the inclusion of bioprospection elements in the biological component of the integrated management of this crop. This will help reducing the use of chemical products by farmers that follow traditional agricultural practices.

The aim of this work was to recognize the population of some fungi which can have a beneficial activity in the rhizosphere of chontaduro palms located on agroecosystems and little farms in the towns of Citronela and Sabaletas, Buenaventura, Valle del Cauca, Colombia. The specific objectives were: (1) isolation and quantification of fungi populations present in the rhizosphere of chontaduro which can be grown and, have a potential beneficial effect (antagonist, entomopathogens and saprophytes); (2) characterization, at the genus level, of the isolated fungi population to establish its biodiversity; (3) preserve the isolated fungi to create a collection for a future use in biocontrol studies and pest integrated management programs that are associated with chontaduro agroecosystems in the Pacific region of Valle del Cauca.

Materials and methods

Field phase

This work was done between November 2006 and April 2007 in the towns of Citronela and Sabaletas, in the rural area of Buenaventura (3° 52' 46" N, 77° 04' 12" O), Valle del Cauca (Colombia), where the main crop was chontaduro under contrasting production systems.

Citronela has an average temperature of 25.8 °C and 6408 mm of rainfall. It is located on a hills landscape in the alluvial plains of the Dagua river. pH in soil is 4.7, texture is clay, bulk density of 0.60 gr/cm and gravimetric moisture percentage of 75.68% (Eslava, 1994). Associated to chontaduro palms there were Borojo (*Borojoa patinoi* Cuatrec.) and cherimoya (*Annona cherimola* Mill.) bushes. Sabaletas has an average temperature of 26.5 °C and annual rainfall of 6500 mm. This place, together with Citronela, is located at 7 MASL in the lower alluvial zone of the Anchicaya river. Soils have a pH of 5.21, texture is silty clay loam, bulk density is 0.89 g/cm^3 and the percentage of soil humidity is 63.97% (Eslava, 1994). As associated crops there are borojo and Brazilian guava (*Psidium araca* Raddi).

Crop management of chontaduro was different in both places. In Citronela, fertilizers were applied to the soil and chemical products were sprayed. Associated weeds were cut and left to decompose in the field. Contrastingly, in Sabaletas, the crop was managed in the traditional system without addiction of fertilizers or weed elimination, thus they were growing in association with the crop. Historical series of precipitation show a bimodal regime for the Colombian Pacific Region with two picks of low precipitation in January and July and, two picks of high precipitation in April and October (Eslava, 1994). Based on these weather features, two samplings were done on the high precipitation picks (November 2006 and April 2007) and one was done on the low precipitation pick (January 2007).

In both farms of study and the three times of sampling, 500g of rhizospheric soil and root samples were collected randomly in a zigzag path, each sample was composed of subsamples from 15 productive palms. Sampling depth was determined according to the root system development of each plant taking into account the fasciculated roots of chontaduro and the quaternary roots that uptake nutrients (Trujillo, 1981).

Laboratory phase Isolates were obtained following the protocol of Mosquera-Espinosa (2001). For that, serial dilutions in base 10 were done taking 10 of the rhizospheric soil samples to resuspend them in 90 ml of sterile deionizated water. Such suspension was mix by vortexing for 10 minutes and was used as the starting solution to make the serial dilutions till 10^{-6}. Fungi were obtained by sowing 50 µl of each serial dilution on individual Petri dishes with potato-dextrose- agar acidulated (PDAA) with 25% lactic acid (pH 6.5). Two petri dishes were used for each dilution; they were considered repetitions which were incubated in Buenaventura weather conditions for 48 hours at 26 °C and 85% relative humidity. Dilutions 10^{-2} and 10^{-3} were selected to quantify colony forming units per gram of humid soil (cfu/g of humid soil).

An average colony quantification values for each dilution was obtained and transformed to logarithm base 10 to express the fungi population in cfu/g of humid soil (Benson, 2001). Subsequently, fungi were purified in PDAA and incubated again at room temperature and humidity.

Fungi characterization of each isolate was done based on macroscopic (colony descriptions) and microscopic (conidia, conidiophores, hyphae, chlamydospores) characteristics, among other structures in order to classify them till genus. Therefore microcultures were done following Benson's methodology (2001) to get a better formation and visualization of the fungal structures and make an accurate description. Mounts were incubated at room temperature and both, the observations under light microscope and macroscopic description of colonies, were done 96 h after inoculation. Identification was done using fungi taxonomical keys (Agrios, 2005; Paulin-Mahady *et al.*, 2002; Benson, 2001; Barnett and Hunter, 1972).

Isolated fungi were preserved following these techniques: (1) PDAA + fungal growth for 5 days in sterile water; (2) storage at 4 °C in PDAA petri dishes + fungal growth for 5 days (Smith and Onions, 1983); and (3) storage at -20 °C in filter paper for fugal growth (Aricapa and Correa, 1994).

Results and discussion

Fungal population level present in the rhizosphere In Citronela, the first and second sampling (low precipitation) showed stable fungal populations values of 1.0 x 10^3 cfu/g in humid soil. For the third sampling (high precipitation) populations were increased in both dilutions 10^{-2} and 10^{-3}, with 2.0×10^3 and 1.0×10^4 cfu/g of humid soil, respectively. For the last sampling, the population increase was confirmed with the presence of colonies in the 10^{-3} dilution (Figure 1). During the times of sampling, rainfall values in a daily average were: 8.23 mm, 18.79 mm and 19.39 mm, respectively.

In Sabaletas, for the first two samplings there were no fungal population expression in the evaluated dilutions; however, in the third sampling, fungal population was registered for both dilutions, with 3.0×10^3 cfu/g of humid soil in 10^{-2} and 1.0×10^4 cfu/g in humid soil for 10^{-3} (Figure 2). Average daily rainfall for this location in the three sampling times was 22.73 mm, 16.43 mm and 26.6 mm, respectively.

According to Dix and Webster (1995) rhizospheric mycoflora composition depends on environmental factors, soil characteristics, plant species present in the ecosystem and physiological conditions of the plants. Nevertheless, in this study fungal population results were associated mainly to the rainfall of each study zone (Table 1). Therefore, in Citronela, during the two first samplings population levels were lower (10^6 fungi/g of rhizospheric soil) than the ones considered by the same authors.

In Sabaletas, during the same sampling periods there were no registries of fungi, which correspond to the high rainfall levels. In this case, it is possible that the excessive humidity in soil favored an anaerobic environment which is unfavorable for fungal development (Smith *et al.*, 1993).

In both locations fungal population levels were increased in the third sampling, this could be associated with favorable changes in the environment like temperature, humidity and soil aeration that occurred before February when rainfall diminished (Wieland *et al.*, 2001; Smith *et al.*, 1993). This sampling coincided with the fruit filling time in both locations, this is a physiological condition that could favor root exudates release which, together with organic matter in the rhizosphere, stimulates active microbial population development (Whipps, 2001; Manoharachary *et al.*, 2006) and, therefore, induce the effective establishment of fungal populations

Fungal genus diversity with potential benefit present in the rhizosphere

Trichoderma was the fungal genus that predominated in the chontaduro rhizosphere under this study. Fifteen isolated were obtained and preserved. In Citronela, this fungus was presented in three isolates in each of the three

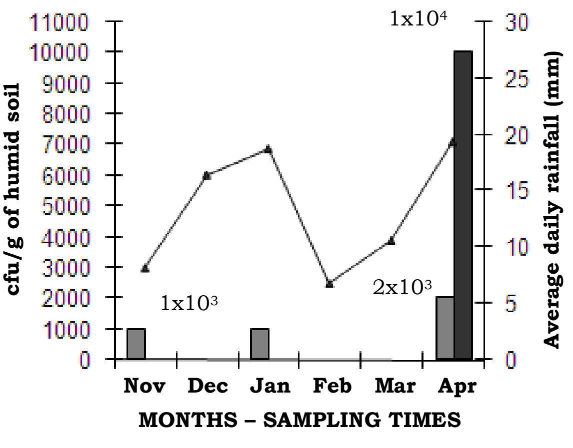

Figure 1. Population levels of fungi associated to the rhizosphere of chontaduro (*Bactris gasipaes* H.B.K) palms and monthly rainfall in Citronela, Buenaventura, Colombia.

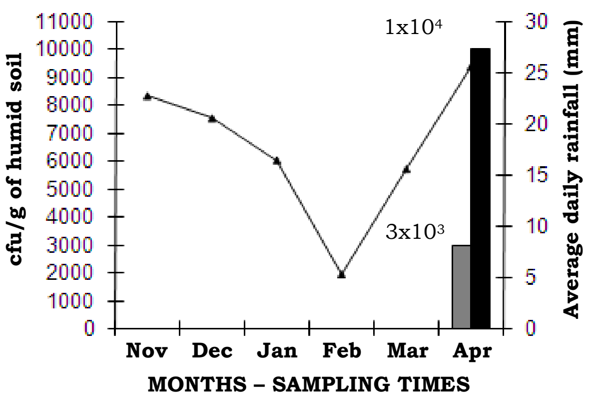

Figure 2. Population levels of fungi associated to the rhizosphere of chontaduro (*Bactris gasipaes* H.B.K) palms and monthly rainfall in Sabaletas, Buenaventura, Colombia.

sampling times; whereas in Sabaleta, only six isolates were observed in the last sampling, because this was the only sampling time where fungal populations were observed. Additionally, for the third sampling, in Citronela there were found *Fusarium* and *Rhizopus*, each one with one isolate; in Sabaletas there were *Penicilium* and *Thielaviopsis* with 3 and 1 isolate, respectively (Figures 3 and 4).

The prevalence of *Trichoderma* in chontaduro rhizosphere in Citronela, even under contrasting rainfall conditions, suggests a synergistic interaction fungi-host. This is the product of an interaction between plant roots, their exudates and the microbial community stimuli that favors a dynamic environment in the rhizosphere (Barea *et al.*, 2005). These results suggest that the rhizosphere of some plants is dominated for non-pathogenic fungal species, as it is suggested by Alexander (1994), being *Trichoderma* one of the main genera (Whipps, 2001; Manoharachary *et al.*, 2006).

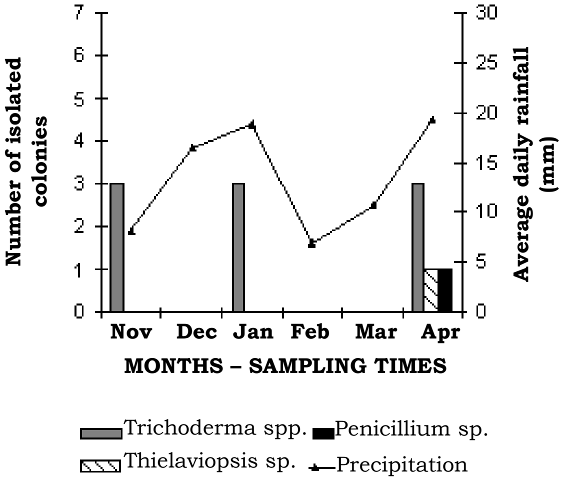

Figure 3. Fungal genera with potential benefit isolated in the sampling times in Citronela, Buenaventura, Valle del Cauca, Colombia.

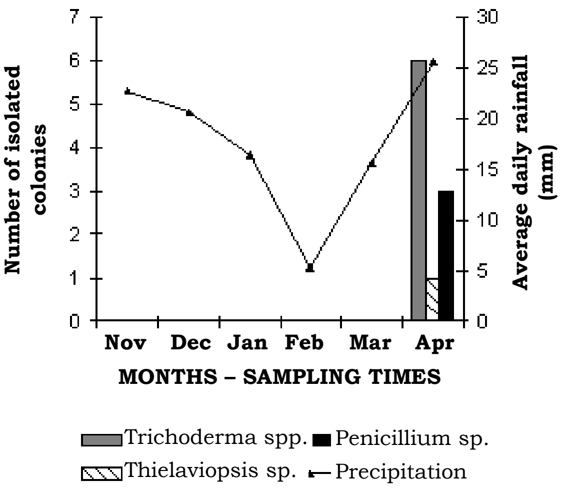

Figura 4. Fungal genera with potential benefit isolated in the sampling times in Sabaletas, Buenaventura, Valle del Cauca, Colombia.

During the time of the third sampling, when both locations had the highest fungal diversity, is possible that physiological changes due to fruit filling had affected in a positive way the quality and amount of root exudates released by the roots (Souza-Mota *et al.*, 2003; Drigo *et al.*, 2009). According to Bardgett (2005) and Manoharachary *et al.* (2006) these compounds have a selective effect on the microorganisms present in the rhizospheric zone, which favors the establishment and activity of the fungi found. In the same way, this condition can stimulate the presence of microbial communities, mainly of arbuscular mychorrizal fungi, which have been found in association with chontaduro palms grown on the same production systems in the Pacific region of Colombia (Molineros-Hurtado, 2007).

The fungal genuses identified in this study have been found to be associated to the rhizosphere of other plant species of agricultural, food and environmental interest (Souza-Mota *et al.*, 2003; Tariq *et al.*, 2008). It is possible that in the studied chontaduro production systems, these fungi have a beneficial role since disease symptoms were not found, contrarily, their beneficial action is translated in plant growth, development and protection against phytosanitary problems (Orduz and Rangel, 2002; Barea *et al.*, 2005; Yedidia and Chet, 1999; Baker and Dickman, 1995).

It is possible that *Penicillium* population contributes in a similar way as the *Trichoderma* population, taking into account its capacity to produce useful antibiotics for control of soil pathogens (Baker and Dickman, 1995). In the case of *Fusarium*, not all the species are pathogenic, since there are evidences of their antagonist or saprophytic activity in the rhizosphere (Barnett and Hunter, 1972; Whipps, 2001). Additionally, *Rhizopus*

Table 1. Daily average rainfall between November 2006 and April 2007 in Citronela and Sabaletas locations, Buenaventura, Valle del Cauca, Colombia.

Location	Months					
	November	December	January	February	March	April
	Rainfall (mm/day)					
Citronela	8.23	16.48	18.79	6.86	10.65	19.39
Sabaletas	22.73	20.54	16.43	5.28	15.59	25.60

(Agrios, 2005) and *Thielaviopsis* (Mora *et al.*, 1997) present similar activities to the ones of *Fusarium*, plus their participation on nutrient cycling activities (Alexander, 1994) on the chontaduro agroecosystem.

Preservation of the isolated fungi

All the isolated microorganisms were preserved by the three techniques described in the methodology section. This allowed the creation of a strain collection (bank) that can be amplified and used in future studies of microbial diversity and bioprospection that are emphasized in biological components. Therefore, they can be integrated on phytosanitary management strategies for plagues and diseases associated to chontaduro crops in the Pacific agroecosystems in Colombia.

Conclusions

- Population levels of the identified fungal genera were variable according to rainfall intensity in the locations of this study. Anaerobic conditions that occurred for water excess in the rhizosphere affected beneficial fungi populations under the levels reported on the literature.
- Fruit filling in chontaduro plants coincides with a higher population of potentially beneficial fungi in the chontaduro rhizosphere.

Although in both locations of this study the management practices were contrasting, *Trichoderma* genus was predominant. In Citronela, *Fusarium* and *Rhizopus* genus and, in Sabaletas, *Penicillium* and *Thielaviopsis* genus were isolated, these present a beneficial activity that coincide with a good sanity observed in chontaduro palms during the evaluation time.

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