Mycosystema
菌 物 学 报 15 January 2008, 27(1): 75-84

jwxt@im.ac.cn
ISSN1672-6472 CN11-5180Q
©2008 Institute of Microbiology, CAS, all rights reserved.

Fungal endophytes in green coffee seeds

Fernando E. VEGA[1*] Francisco POSADA[2] M. Catherine AIME[3] Stephen W. PETERSON[4] Stephen A. REHNER[5]

[1]*Sustainable Perennial Crops Laboratory, U. S. Department of Agriculture, Agricultural Research Service, Building 011A, BARC-W, Beltsville, Maryland 20705, USA*

[2]*Centro Nacional de Investigaciones de Café, CENICAFÉ, Chinchiná, Caldas, Colombia*

[3]*Department of Plant Pathology and Crop Physiology, Louisiana State University AgCenter, 302 Life Sciences Bldg., Baton Rouge, LA 70803, USA*

[4]*Microbial Genomics and Bioprocessing Research Unit, National Center for Agricultural Utilization Research, U. S. Department of Agriculture, Agricultural Research Service, 1815 N. University St., Peoria, IL 61604, USA*

[5]*Systematic Mycology and Microbiology Laboratory, U. S. Department of Agriculture, Agricultural Research Service, Building 011A, BARC-W, Beltsville, Maryland 20705, USA*

Abstract: Green coffee seeds from Colombia, Guatemala, India, Kenya, Papua New Guinea, Puerto Rico and Vietnam were sampled for the presence of fungal endophytes. Stions of surface sterilized seeds were plated on yeast malt agar, and fungal growth was isolated for subsequent DNA extraction and sequencing. Several fungal genera were isolated, including *Acremonium*, *Aspergillus*, *Eurotium*, *Fusarium*, *Gibberella*, *Penicillium*, *Pseudozyma* and an undescribed clavicipitaceous species. The biological activities that these fungi might be playing in coffee seeds remain unknown, but in other plants some of the genera isolated have been reported to protect against plant pathogens.

Key words: *Acremonium*, *Aspergillus*, Clavicipitaceae, *Coffea*, endophytic, *Penicillium*, *Pseudozyma*

[*]Corresponding author. E-mail: Fernando.Vega@ars.usda.gov
Received: 29-08-2007, accepted: 08-10-2007

1 INTRODUCTION

Coffee (*Coffea arabica* L.), a native of Africa, is grown in more than 50 countries throughout the tropics by more than 20 million coffee farming families (Cardenas 2001). The wide distribution of coffee throughout the tropics has exposed this plant to the fungal diversity within each region where it grows. Fungi associated with coffee have been extensively studied only from a plant pathogen perspective, including infection of the seed and berry. Still, hidden from most observers, the plant harbors a myriad of fungi within its internal tissues, fungi that do not cause any adverse symptoms in the plant and are referred to as fungal endophytes.

Our sampling for fungal endophytes has revealed more than 250 unique sequences (phylotypes) in coffee plants sampled from Mexico, Hawaii, Colombia, and Puerto Rico (Vega *et al.* unpublished). One particular area of interest is whether endophytes are present in "green coffee". The processing of coffee after harvest involves dry or wet fermentation, followed by mechanical or sun drying and hulling. Once processing is completed, the coffee is ready to be roasted and is sold in the market as "green coffee".

After sampling surface-sterilized coffee seeds extracted from ripe coffee berries, Vega *et al.* (2006) reported the presence of three endophytic *Penicillium* species in Hawaii: *P. brevicompactum* Dierckx, *P. olsonii* Bainier & Sartory, and *P.* sp. (near *P. roseopurpureum*); and one in Mexico: *P. crustosum* Thom. Other *Penicillium* and several *Aspergillus* species have been reported in both non-sterilized and surface-sterilized green coffee beans* (Mislivec, Bruce & Gibson 1983; Batista *et al.* 2003; Reynaud *et al.* 2003). Several bacterial endophytes have also been reported in coffee beans extracted from ripe berries originating in Colombia, Hawaii and Mexico, including species of *Bacillus*, *Burkholderia*, *Clavibacter*, *Curtobacterium*, *Escherichia*, *Micrococcus*, *Pantoea*, *Pseudomonas*, *Serratia* and *Stenotrophomonas* (Vega *et al.* 2005). The goal of this study was to determine whether fungal endophytes are present in green coffee harvested from various countries.

2 MATERIALS AND METHODS

2.1 Coffee samples

We sampled green coffee from Colombia, Guatemala, India, Kenya, Papua New Guinea, Puerto Rico, and Vietnam. All samples were obtained from country exhibitors attending Specialty Coffee Association of America meetings, except for the sample from Puerto Rico which we obtained during a visit to the island, and the Vietnam sample, which we obtained from a vendor on Ebay.

2.2 Endophyte isolation

Seeds were surface sterilized by successively dipping in 0.5% sodium hypochlorite for two

* Even though it is botanically a drupe, coffee seeds are referred to as "beans".

minutes, 70% alcohol for two minutes, rinsing in sterile autoclaved water and then drying on sterile towel paper. Seeds were cut into stions and then plated on yeast malt agar (YMA; Sigma Aldrich Co., St. Louis, MO) to which 0.1% stock antibiotics was added as described in Vega *et al.* (2005). Plates were kept at room temperature and any fungal growth on the seed stions was subcultured on individual Petri plates for subsequent DNA extraction. Countries of origin of recovered isolates in this study are provided in Table 1. Voucher cultures are maintained at the Sustainable Perennial Crops Laboratory (Beltsville, MD). Duplicates of some cultures are maintained at the culture collection of the U.S. Fungus Collections (USDA, ARS, BPI, Beltsville, MD).

Table1 Identification of fungal endophytes isolated from green coffee seeds from Colombia, Guatemala, India, Kenya, Papua New Guinea, Puerto Rico, and Vietnam

Fungal endophyte	GenBank[#]	Country
Acremonium sp.	DQ778915	Puerto Rico
Aspergillus fumigatus	DQ778906	India
Aspergillus fumigatus	[1]	Vietnam
Aspergillus niger	DQ778907	Vietnam
Aspergillus niger	[1]	Kenya
Aspergillus pseudodeflectus	DQ778908	Papua New Guinea
Aspergillus pseudodeflectus	[1]	Vietnam
Aspergillus sumatrense	DQ861286	Puerto Rico
Aspergillus tubingiensis	DQ778910	Colombia
Aspergillus tubingiensis	[1]	Kenya
Clavicipitaceae sp. 1	DQ778911[2]	Puerto Rico
Clavicipitaceae sp. 2	DQ778912	Puerto Rico
Eurotium (Aspergillus) ruber	DQ778909	India
Fusarium solani complex	DQ778913	Vietnam
Gibberella sp.	DQ778914	Colombia
Penicillium sp., subgenus *Biverticillium*	DQ779816	India
Penicillium crustosum	DQ778917	Guatemala
Penicillium olsonii	DQ778918	Colombia
Pseudozyma sp.	DQ778919	Puerto Rico

Note: [1]Sequence is identical to the one deposited above; [2]A TEF sequence (GenBank DQ862130) was obtained for this isolate. Partial sequences were also generated for clavicipitaceous isolate 59, which like ITS, are nearly identical among the isolates.

2.3 DNA extraction

Fungal endophytes were grown in potato dextrose broth (Difco, Becton Dickinson, Sparks, MD) at 125r/min on an Innova 4000 Incubator Shaker (New Brunswick Scientific Co., Inc., Edison, NJ) at 25℃ for one week. Tissue was then harvested, lyophilized, and stored at -80℃. For DNA extraction, ca. 50mg lyophilized mycelium was placed in a 2mL microcentrifuge tube with ca. 0.2mL 1.0mm zirconia-glass beads (Cat # 1107911-0z, BIOSPEC, Bartlesville, OK). The mycelium was crushed with a plastic pestle and further ground in a FastPrep-120 sample grinder (Q-BIOgene, Irvine, CA) for 3sec at the maximum speed setting of 6. The powdered mycelium was suspended in 700μL detergent solution (2mol/L NaCl, 0.4% w/v deoxycholic acid-sodium salt, 1% w/v polyoxyethylene 20 cetyl ether) and then agitated for 14sec in the Fast-Prep at maximum speed. Vials were incubated 5min at 55℃ in a heat block and then centrifuged at 10,600r/min for 5min followed by emulsion with 700μL chloroform/isopropyl and centrifugation at 10,600r/min for 5min. The aqueous phase was transferred to a clean tube to which an equal volume of 6mol guanidinium thiocyanate was added. 15μL of silica powder were gently mixed with the solution while incubating at room temperature for 5min, followed by 3sec centrifugation, after which the supernatant was discarded. The glass powder was rinsed twice by suspending in 750μL ethanol buffer (10mmol/L Tris-HCl, pH 8.0, 0.1mmol/L EDTA, 50% ethanol) with a disposable transfer pipette, then collected by centrifugation. The supernatant was discarded, and the glass powder pellet was dried on a heat block at 55℃ for 5-10min. The glass powder was re-hydrated with 105μL ultra-pure water and the genomic DNA eluted by incubating on a heat block at 55℃ for 5-10min. Following vortexing and centrifugation, 100μL of the aqueous supernatant was transferred to a clean tube. In a few instances, DNA extractions were made directly from fungal cultures grown on potato dextrose agar (Difco, Becton Dickinson, Sparks, MD). Approximately 2-4mm^2 of mycelium was aseptically removed from the growing edge of the colony and extracted with the UltraClean Plant DNA Isolation Kit (MoBio Laboratories, Inc., Solana Beach, CA) as per the manufacturer's instructions.

2.4 DNA sequencing

Primers ITS1-F (fungal-specific) (Gardes & Bruns 1993) and ITS4 (White *et al*. 1990) were used for both amplification and sequencing of the internal transcribed spacer region (ITS) of the nuclear rDNA repeat for each isolate. PCRs were done in 25μL reaction volumes with 12.5μL of PCR Master Mix (Promega Corp., Madison, WI), 1.25μL each of 10μmol/L primers, and 10μL of diluted (10- to 100-fold) DNA template. Amplification was done with an initial denaturation step of 5min at 94℃; 35 cycles of 30sec at 94℃, 45sec at 50℃, and 45sec at 72℃; and a final extension of 7min at 72℃. The PCR products were purified with Montage PCR Centrifugal Filter Devices (Millipore Corp., Billerica, MA) according to the manufacturer's protocol. The elongation factor-1

alpha (TEF) was also amplified for clavicipitaceous isolates 57 and 59 as described by Rehner and Buckley (2005). Purified PCR products were sequenced with BigDye Terminator sequencing enzyme v.3.1 (Applied Biosystems, Foster City, CA) in the reaction: 2μL of diluted BigDye in a 1:3 dilution of BigDye: dilution buffer (400mmol/L Tris pH8.0, 10mmol/L $MgCl_2$); 0.3μL of 10μmol/L primer; 10-20ng of cleaned PCR template; and H_2O to 5μL total reaction volume. Cycle sequencing parameters consisted of a 2min denaturation step at 94℃, then 35 cycles of 94℃ for 39sec, 50℃ for 15sec, and 60℃ for 4min. Sequencing reactions were cleaned by ethanol precipitation and sequenced on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequencing reactions were edited and contiguous sequences for each isolate were assembled in Sequencher v.4.1.4 (Gene Codes Corp., Ann Arbor, MI). DNA sequences were deposited in GenBank (Table 1).

2.5 Identification of isolates

Initial identification of isolates was obtained by BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/). *Penicillium* and *Aspergillus* species were further identified using additional sequences (Peterson, unpublished) and exact matches to the sequences from ex type cultures were considered reliable identifications. Where genealogical concordance multilocus phylogenetic studies have been published, those concepts were used to determine the identity of the isolate (Taylor *et al.* 2000). For other isolates, if the results indicated 98%-100% concordance to a known species, it was identified as the same genus (e.g., *Gibberella* sp., *Fusarium* sp., *Pseudozyma* sp.). Blasts with low concordance (85%-92%) were considered from the same family or order.

3 RESULTS AND DISCUSSION

We have isolated and identified 15 different endophytic fungi belonging to 8 taxa from green coffee seeds originating in seven countries. These include 5 *Aspergillus* species, 3 *Penicillium* species, 2 Clavicipitaceae, and 1 species in each of the following genera: *Acremonium*, *Eurotium*, *Fusarium*, *Gibberella*, and *Pseudozyma* (Table 1). Out of 19 endophytic fungi, 5 were isolated from green coffee seeds originating in Puerto Rico, 4 from Vietnam, 3 from Colombia, 3 from India, 2 from Kenya, 1 from Papua New Guinea, and 1 from Guatemala.

Due to the nature of coffee processing, which is done in the open in the same areas where the coffee berry is harvested, it is not surprising that a high number of fungi have been isolated from green coffee seeds from various countries: Brazil (Mislivec, Bruce & Gibson 1983; Tsubochi *et al.* 1984; de Carvalho *et al.* 1989; Silva *et al.* 2000; Batista *et al.* 2003; Martins, Martins & Gimeno 2003; Reynaud *et al.* 2003), Cameroon (Tagne & Mathur 2003), Indonesia and Mexico (Tsubochi *et al.* 1984), and India (Tharappan & Ahmad 2006). Fungi have also been isolated from coffee berries from Brazil (Silva *et al.* 2000; Taniwaki *et al.* 2003) and Mexico (Roussos *et al.* 1995). The question is

whether these fungi occurring on the seed and berry surfaces are the same as those that occur inside the seed, and whether the latter are truly endophytic or just present due to fungal infection of the seed.

Batista *et al.* (2003) and Mislivec, Bruce & Gibson (1983) reported *Penicillium* and *Aspergillus* species in both non-sterilized and surface-sterilized green coffee beans, but they did not refer to any of these as endophytes. The authors assumed that detection in surface-sterilized seeds was due to invasion from the surface of the seed which is certainly a possibility, although if this were the case they should not have detected a drastic reduction in incidence when they surface-sterilized the seeds. The levels of incidence for *Aspergillus* fell from 96% in non-surface sterilized seeds to 47% in surface-sterilized seeds, and from 42% to 24% for *Penicillium* (Batista *et al*. 2003). Assuming detection inside the seed is correlated with fungal presence on the surface, they should have found a similar percent incidence in both substrates. Similar reductions were reported for seven *Aspergillus* species (*A. niger* Tiegh., *A. ochraceus* G. Wilh., *A. glaucus* (L.) Link, *A. flavus* Link, *A. tamari* Kita, *A. wentii* Wehmer, and *A. versicolor* (Vuill.) Tirab.) and five *Penicillium* species (*P. frequentans* Westling, *P. citrinum* Thom, *P. brevicompactum* Dierckx, *P. cyclopium* Westling, and *P. expansum* Link) after seeds from 31 countries were surface-disinfected (Mislivec, Bruce & Gibson 1983).

We detected five *Aspergillus* species (*A. tubingensis* Mosseray, *A. fumigatus* Fresen., *A. pseudodeflectus* Samson & Mouch., *A. niger* Tiegh., and *A. ruber* Thom & Church), and three *Penicillium* species (*P. olsonii* Bainier & Sartory, *P. crustosum* Thom, and *P.* sp., subgenus *Biverticillium*) from green coffee seeds. Some *Aspergillus* and *Penicillium* species are known to produce the toxic metabolite ochratoxin A (OTA; Frisvad & Samson 1991; Frisvad *et al.* 2004). Of the *Aspergillus* isolates detected in this study, only *A. niger* (Abarca *et al.* 2001; Accensi *et al.* 2001) and *A. tubingensis* (Medina *et al.* 2005; Perrone *et al.* 2006; Varga & Kozakiewicz 2006) have been shown to produce OTA. We did not assess whether our isolates produced OTA. Vega *et al.* (2006) reported endophytic *P. crustosum* isolated from coffee seeds extracted from ripe coffee berries in Mexico, and endophytic *P. olsonii* from leaves and various parts of the coffee berry (seed, skin, crown, peduncle) collected in Hawaii that produced low levels of OTA (Vega *et al.* 2006).

Two *Penicillium* species (*P. brevicompactum* and *P. canadense* Smith syn. *P. arenicola* Chalabuda) have been reported in surface-sterilized seeds of *Pinus roxburghii* Sargent (Mittal & Sharma 1982). Other *Penicillium* species have been shown to protect seeds against pathogenic fungi. For example, the presence of *Penicillium cyaneum*, *P. implicatum*, or *P. damascenum* Baghd. as epiphytes in seeds of *Picea glehnii* (Fr. Schm) Masters protect the seedlings against damping-off caused by *Pythium vexans* de Bary (Yamaji *et al.* 2001, 2005). *P. oxalicum* has been shown to protect plants against *Fusarium oxysporum* f. sp. *lycopersici* (De Cal, Pascual & Melgarejo 1997; De Cal, Garcia-Lepe & Melgarejo 2000). The antagonistic effects of *Penicillium* on other fungi suggest that

in a natural setting, some of the endophytes found in coffee seeds could serve to protect from pathogenesis caused by other fungi. This hypothesis would be very difficult to test because it would require *a priori* knowledge of what seeds are infected by a particular endophyte and which seeds are endophyte-free. In addition, it would require the presence of only the desired endophyte species.

In this study, we also detected an *Acremonium* sp. in green seeds from Puerto Rico. Vega *et al.* (unpublished) isolated *Acremonium* sp. from coffee roots in Hawaii and *A. alternatum* Link: Fries from the skin of coffee berries collected in Colombia. Bargmann & Shönbeck (1992) have reported reduced incidence of wilt symptoms caused by *F. oxysporum* f. sp. *lycopersici* when tomato roots are infected with endophytic *Acremonium kiliense* Grütz, yet one more instance suggesting that fungal endophytes in green coffee might possibly have a positive role.

The anamorphic basidiomycetous yeast-like fungus *Pseudozyma* Bandoni, isolated in green coffee from Puerto Rico, has also been isolated from various tropical fruits (Trindade *et al.* 2002) and as an epiphyte in various plants (Wang, Jia & Bai 2006; Allen, Burpee & Buck 2006). *Pseudozyma flocculosa* (Traquair, L.A. Shaw & Jarvis) Boekhout & Traquair has antifungal activity against powdery mildew (Avis & Bélanger 2002) and *P. fusiformata* acts against many yeasts (Golubev, Kulakovskaia & Golubeva 2001). It would be interesting to determine what role, if any, *Pseudozyma* might play in coffee seeds.

Clavicipitaceous isolates 57 and 59, which were nearly identical at both ITS and TEF and thus likely conspecific, were the most problematic taxonomic identifications among the endophytes isolated. Although provisionally identified as *Beauveria* based on BLAST similarity criteria, morphological examination of these isolates revealed that both produce an *Acremonium*-type anamorph unlike *Beauveria*. When subjected to phylogenetic analysis with *Beauveria* and other clavicipitalean taxa (Rehner, unpublished), isolates 57 and 59 were positioned apart from *Beauveria* but in a clade that contained entomopathogenic taxa (e.g., *Ascopolyporus*, *Cordyceps*, *Gibbelula*, *Torrubiella*).

The role of fungal endophytes in green coffee is an area that deserves further study. It is likely that fungal endophytes are providing a vital role to the seed, otherwise, if they were simply infecting the seed these would exhibit symptoms such as musty odor, off-color and moldy off-flavor, which was not the case with any of the seeds we tested. Most of the seeds used in this study were obtained at Specialty Coffee Association of America meetings which are where coffee producers bring their best seeds with the objective of finding buyers; therefore, the green coffee at these meetings is always top quality. Future studies in our laboratory will assess a large variety of green coffee samples for the presence of other fungal endophytes.

Acknowledgements: Thanks to Ann Simpkins (USDA, ARS, Beltsville, MD) for outstanding laboratory support. We thank Pat Dowd and Matt Greenstone (USDA) for comments on a previous version of this manuscript. The use of trade, firm, or corporation names in this publication is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

[REFERENCES]

Abarca MK, Accensi F, Bragulat MR, Cabañes FJ, 2001. Current importance of ochratoxin A-producing *Aspergillus* spp. *Journal of Food Protection*, **64**: 903-906

Accensi F, Abarca MK, Cano J, Figuera L, Cabañes FJ, 2001. Distribution of ochratoxin A producing strains in the *A. niger* aggregate. *Antonie van Leeuwenhoek*, **79**: 365-370

Allen TW, Burpee LL, Buck JW, 2006. Variable adhesion and diurnal population patterns of epiphytic yeasts on creeping bentgrass. *Canadian Journal of Microbiology*, **52**: 404-410

Avis TJ, Bélanger RR, 2002. Mechanisms and means of detection of biocontrol activity of *Pseudozyma* yeasts against plant-pathogenic fungi. *FEMS Yeast Research*, **2**: 5-8

Bargmann C, Schönbeck F, 1992. *Acremonium kiliense* as inducer of resistance to wild diseases on tomatoes. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*, **99**: 266-272

Batista LR, Chalfoun SM, Prado G, Schwan RF, Wheals AE, 2003. Toxigenic fungi associated with processed (green) coffee beans (*Coffea arabica* L.). *International Journal of Food Microbiology*, **85**: 293-300

Cardenas J, 2001. The world coffee crisis. World Coffee Conference, London, UK, May 2001. Available at http://www.ico.org/event_pdfs/cardenas.pdf

De Cal A, Pascual S, Melgarejo P, 1997. Involvement of resistance induction by *Penicillium oxalicum* in the biocontrol of tomato wilt. *Plant Pathology*, **46**: 72-79

De Cal A, Garcia-Lepe R, Melgarejo P, 2000. Induced resistance by *Penicillium oxalicum* against *Fusarium oxysporum* f. sp. *lycopersici*: Histological studies of infected and induced tomato stems. *Phytopathology*, **90**: 260-268

(de) Carvalho VD, Chalfoun SM, Chagas Rezende SJ, 1989. Relação entre classificação do café pela bebida e composição físico química, química e microflora do grão beneficiado. In: 15 Congreso Brasileiro de Pesquisas Caffeiras, 15, Maringa, Parana. 25-26

Frisvad JC, Samson RA, 1991. Mycotoxins produced by species of *Penicillium* and *Aspergillus* occurring in cereals. In: Chelkowski J (ed.) Cereal grain. Mycotoxins, fungi and quality in drying and storage. *Developments in Food Science*, **26**: 441-476

Frisvad JC, Frank JM, Houbraken JAMP, Kuijpers AFA, Samson RA, 2004. New ochratoxin A producing species of *Aspergillus* stion Circumdati. *Studies in Mycology*, **50**: 23-43

Gardes M, Bruns TD, 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of

mycorrhizae and rusts. *Molecular Ecology*, **2**: 113-118

Golubev VI, Kulakovskaia TV, Golubeva EV, 2001. *Pseudozyma fusiformata* BKM Y-2821 – a producer of antifungal glycolipid. *Mikrobiologia*, **70**: 642-646 (in Russian)

Martins ML, Martins HM, Gimeno A, 2003. Incidence of microflora and of ochratoxin A in green coffee beans (*Coffea arabica*). *Food Additives and Contaminants*, **20**: 1127-1131

Medina A, Mateo R, López-Ocaña L, Valle-Algarra FM, Jiménez M, 2005. Study of Spanish grape mycobiota and ochratoxin A production by isolates of *Aspergillus tubingensis* and other members of *Aspergillus* stion *Nigri*. *Applied and Environmental Microbiology*, **71**: 4696-4702

Mislivec PB, Bruce VR, Gibson R, 1983. Incidence of toxigenic and other molds in green coffee beans. *Journal of Food Protectio*n, **46**: 969-973

Mittal RK, Sharma MR, 1982. Studies on the mycoflora and its control on the seeds of some forest trees. IV. *Pinus roxburghii*. *Indian Journal of Mycological Plant Pathology*, **12**: 198-205

Perrone G, Mulè G, Susca A, Battilani P, Pietri A, Logrieco A, 2006. Ochratoxin A production and amplified fragment length polymorphisms analysis of *Aspergillus carbonarius*, *Aspergillus tubingensis*, and *Aspergillus niger* strains isolated from grapes in Italy. *Applied and Environmental Microbiology*, **72**: 680-685

Rehner SA, Buckley EP, 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-α sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. *Mycologia*, **97**: 84-98

Reynaud DT, Pimentel IC, Homechim M, Kania CE, Dykstra C, 2003. Fungos isolados dos grãos do café (*Coffea arabica* L. – Rubiaceae) variedade Mundo Novo. *Estudios de Biologia Curitiba*, **25**: 49-54

Roussos S, de los Angeles Aquiáhuatl M, del Refugio Trejo-Hernández M, Gaime Perraud I, Favela W, Ramakrishna M, Raimbault M, Viniegra-González G, 1995. Biotechnological management of coffee pulp – isolation, screening, characterization, selection of caffeine-degrading fungi and natural microflora present in coffee pulp and husk. *Applied Microbiology and Biotechnology*, **42**: 756-762

Silva CF, Schwan RF, Sousa Dias E, Wheals AE, 2000. Microbial diversity during maturation and natural processing of coffee cherries of *Coffea arabica* in Brazil. *International Journal of Food Microbiology*, **60**: 251-260

Taniwaki MH, Pitt JI, Teixeira AA, Lamanaka BT, 2003. The source of ochratoxin A in Brazilian coffee and its formation in relation to processing methods. *International Journal of Food Microbiology*, **82**: 173-179

Tagne A, Mathur SB, 2003. Fungi associated with seeds of *Coffea arabica*. *Plant Genetic Resources Newsletter*, **135**: 44-46

Taylor JW, Jacobson DJ, Kroken S, Kasuga T, Geiser DM, Hibbett DS, Fisher MC, 2000. Phylogenetic species recognition and species concepts in fungi. *Fungal Genetics and Biology*, **31**: 21-32

Tharappan B, Ahmad R, 2006. Fungal colonization and biochemical changes in coffee beans undergoing monsooning. *Food Chemistry*, **94**: 247-252

Trindade RC, Resende MA, Silva CM, Rosa CA, 2002. Yeasts associated with fresh and frozen pulps of Brazilian tropical fruits. *Systematics and Applied Microbiology*, **25**: 294-300

Tsubochi H, Yamamoto K, Hisada K, Sakabe Y, 1984. A survey of occurrence of mycotoxins and toxigenic fungi in imported green coffee beans. *Proceedings of the Japanese Association of Mycotoxicology*, **19**: 16-21

Varga J, Kozakiewicz Z, 2006. Ochratoxin A in grapes and grape-derived products. *Trends in Food Science and Technology*, **17**: 72-81

Vega FE, Pava-Ripoll M, Posada F, Buyer JS, 2005. Endophytic bacteria in *Coffea arabica* L. *Journal of Basic Microbiology*, **45**: 371-380

Vega FE, Posada F, Peterson SW, Gianfagna T, Chaves F, 2006. *Penicillium* species endophytic in coffee plants and ochratoxin A production. *Mycologia*, **98**: 37-48

Wang QM, Jia JH, Bai FY, 2006. *Pseudozyma hubeiensis* sp. nov. and *Pseudozyma shanxiensis* sp. nov., novel ustilaginomycetous anamorphic yeast species from plant leaves. *International Journal of Systematic Evolution and Microbiology*, **56**: 289-293

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds.) *PCR Protocols: A Guide to Methods and Applications*. San Diego: Academic Press. 315-322

Yamaji K, Fukushi Y, Hashidoko Y, Yoshida T, Tahara S, 2001. *Penicillium* fungi from *Picea glehnii* seeds protect the seedlings from damping-off. *New Phytologist*, **152**: 521-531

Yamaji K, Hashidoko Y, Fukushi Y, Tahara S, 2005. Chemical response of *Picea glehnii* seed-epiphytic *Penicillium* species to *Pythium vexans* under *in vitro* competitive conditions for mycelial growth. *Journal of Chemical Ecology*, **31**: 805-817