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COLOMBIAN *VANILLA* AND ITS MICROBIOTA, I
First report of *Fusarium* taxa from both wild and cultivated species

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Vanilla is a genus of tropical orchids with some fragrant species few of which have growing economic importance as the source of the original vanilla flavour: *Vanilla planifolia*. This crop is reproduced asexually and its genetic variability is quite low, which means that it is highly vulnerable to pathogens. Among them, *Fusarium* is important around the world. Here I report for the first time the presence of various *Fusarium* taxa (*F. oxysporum* f. sp. *loti*, *F. oxysporum* f. sp. *melonis*, *F. solani*, and *Fusarium* sp.), as foliar endosymbionts in Colombian *Vanilla*. Species sampled were: *Vanilla planifolia*, *V. odorata*, and a wild species not yet identified. Additionally, *Colletotrichum boninense*, an anthracnose-producing fungus, was also found as an endophyte in *Vanilla* leaves. The Colombian government is promoting alternative crops to coffee, and *Vanilla* is a promising candidate. It is important to study the diversity and interactions between *Vanilla* and *Fusarium*, in order to both understand the pathogenesis and contribute to control of a potential threat to *Vanilla* cultivations.

La vainilla es una orquídea aromática nativa de América que pertenece al género *Vanilla* y se constituye en la única especie de las orquídeas con un producto comestible (*V. planifolia*). Este cultivo se reproduce asexualmente y posee una angosta base genética, razón por la cual es vulnerable a los patógenos. Entre éstos se destacan los virus y varias especies del género *Fusarium*. En este manuscrito se reporta por primera vez la presencia de *Fusarium oxysporum* f. sp. *loti*, *F. oxysporum* f. sp. *melonis*, *F. solani* y una cepa de *Fusarium* no identificada, como endófitos foliares en *Vanilla planifolia*, *V. odorata* y una vainilla silvestre no identificada. Adicionalmente, una cepa de *Colletotrichum boninense*, un hongo productor de antracnosis, fue aislada también. El gobierno de Colombia está promoviendo los cultivos diferentes al café y la vainilla se presenta como una interesante alternativa, razón por la cual es importante conocer los posibles patógenos que tendrá que enfrentar esta planta cuando su cultivo sea más extensivo.

Key words: Colombia, endosymbiont, *Fusarium*, pathogen, vanilla

INTRODUCTION

Vanilla is a genus of tropical orchids in which some aromatic species are present, being *Vanilla planifolia* Jacks. ex Andrews (RBG 2013) the most important one, since it is the original source of vanilla flavour. More than 8,000 tons of vanilla were produced by 2011 (FaoStat 2013), being Indonesia, Madagascar, and China main producers. In Colombia, vanilla is considered by agricultural authorities a promising candidate to partially replace coffee (main traditional Colombian crop), and its cultivation is being promoted (Moreno and Díez 2011). Since it is a clonally propagated crop, it has a narrow genetic base and is highly vulnerable to biotic stress produced by pathogenic microorganisms, mainly viruses and fungi. Among fungi, *Phytophthora*, *Colletotrichum* and *Fusarium* are important pathogens that cause significant damage to vanilla crop. *Fusarium* is an asexual group of fungi with many species being severe pathogens of cultivated plants as banana, cacao, cassava, cotton, passion fruit, pine, sugarcane, and vanilla (Ploetz 2006). Infraspecific taxa include species complex (e.g. *Fusarium solani* and *F. oxysporum*), and contain hundreds of races and "forma specialis" with differential abilities to colonise plant hosts (Summerell *et al.* 2003). In fact, there is a f. sp. *vanillae* that is pathogenic to *V. planifolia*, but since it is difficult to morphologically differentiate "forma specialis", it appears necessary clearly establishing what *Fusarium* taxa are present if you want to booster vanilla cultivation. The purpose of this short paper is reporting the presence of various *Fusarium* taxa in Colombian *Vanilla*, using foliar samples from both wild and cultivated species.

METHODS

Plant tissues from healthy leaves of *Vanilla planifolia*, *V. odorata*, and a wild vanilla not yet identified, were sampled for studying their microbiota. Samples were taken to the lab and surface-sterilised by immersion in tap water and commercial soap, 70% ethanol during 1 min., commercial hypochlorite 50% during 3 min., and ethanol 70% during 30 seconds. Then tissues were washed in distilled water and cut into 2 mm × 2 mm squares that were plated in Petri dishes served with potato dextrose agar (PDA, 20 g of potato dextrose agar Scharlau® in 1 L of water) within a biosecurity cabinet. Samples were kept at room temperature and observed on a daily basis (Gamboa and Bayman 2001, Gamboa *et al.* 2002). Growing mycelia were transferred to separated Petri dishes containing PDA as they emerged, and observed for selecting prospective *Fusarium* species, i.e. those with initial white mycelium that become red, pink, and violet pigmented. Strains were transferred to liquid potato dextrose medium and kept two weeks at room temperature. Mycelia

were lyophilised and sent to the Molecular Biology Laboratory at Pontificia Universidad Javeriana-Cali for DNA extraction. Sequencing was performed at Macrogen (Korea). Observation of conidia under optical microscope and ITS sequencing were methods used for further confirmation of *Fusarium* species.

RESULTS

Five morphospecies were isolated in PDA that showed initial characteristics of *Fusarium* species since they grew up a mycelium white in colour and exhibited pigment depositions. After further growing, visual examination under microscope and DNA amplification and sequencing using ITS4 and ITS5 primers, four species were confirmed as members of the *Fusarium* genus, and the fifth one was identified as *Colletotrichum boninense*. Species found were *F. oxysporum* f. sp. *loti* from *Vanilla odorata* leaves, *F. oxysporum* f. sp. *melonis* and *F. solani* from leaves of cultivated *V. planifolia,* and *Fusarium* sp. from a wild unidentified vanilla.

Consensus sequences using ITS4 and ITS5 primers were as follows:

Colletotrichum boninense

TCTCCGTTGGTGAACCAGCGGAGGGATCATTACTGAGTTACCGCTCTATAAC-
CCTTTGTGAACATACCTACAACTGTTGCTTCGGCGGGTAGGCCGTCCCCT-
GAAAAGGACGCCTCCCGGCCCGGACCGGACCCCCCGCGGGACCGGACCCG-
GCGCCCGCCGGAGGATAACCAAACTCTATTGTAACGACGTTTCTTCTGAGT-
GGCATAAGCAAAATAATCAAAACTTTTAACAACGGATCTCTTGGTTCTGGCATC-
GATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT-
GAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGCATTCTGGCGGGCAT-
GCCTGTTCGAGCGTCATTTCAACCCTCAAGCTCTGCTTGGTGTTGGGGCTCTACG-
GTCGACGTAGGCCCTCAAAGGTAGTGGCGGACCCTCCCGGAGCCTCCTTTGCG-
TAGTAACATTTCGTCTCGCACTGGGATCCGGAGGGACTCTTGCCGTAAAAC-
CCCCCAATTTTCCAAAGGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAA

Fusarium oxysporum f. sp. *loti*

TCTCCGTTGGGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCCAAAC-
CCCTGTGAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACG-
GGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAG-
TAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC-
GATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT-
GAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT-
GCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGT-
TAATTCGCGTTCCTCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAG-
TAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCT-
GAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAA

Fusarium oxysporum f. sp. *melonis*

TCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTTACAACTCCCAAAC-
CCCTGTGAACATACCACTTGTTGCCTCGGCGGATCAGCCCGCTCCCGGTAAAACG-
GGACGGCCCGCCAGAGGACCCCTAAACTCTGTTTCTATATGTAACTTCTGAG-
TAAAACCATAAATAAATCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCATC-
GATGAAGAACGCAGCAAAATGCGATAAGTAATGTGAATTGCAGAATTCAGT-
GAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCGGGCAT-
GCCTGTTCGAGCGTCATTTCAACCCTCAAGCACAGCTTGGTGTTGGGACTCGCGT-
TAATTCGCGTTCCCCAAATTGATTGGCGGTCACGTCGAGCTTCCATAGCGTAGTAG-
TAAAACCCTCGTTACTGGTAATCGTCGCGGCCACGCCGTTAAACCCCAACTTCT-
GAATGTTGACCTCGGATCAGGTAGGAATACCCGCTGAACTTAAGCATA

Fusarium solani

TCTCCGTTGGTGAACCAGCGGAGGGATCATTACCGAGTTATACAACTCAT-
CAACCCTGTGAACATACCTATAACGTTGCCTCGGCGGGAACAGACGGCCCCG-
TAACACGGGCCGCCCCCGCCAGAGGACCCCCTAACTCTGTTTCTATAATGTTTCT-
TCTGAGTAAACAAGCAAATAAATTAAAACTTTCAACAACGGATCTCTTGGCTCT-
GGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAAT-
TCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGCCAGTATTCTGGCG-
GGCATGCCTGTTCGAGCGTCATTACAACCCTCAGGCCCCCGGGCCTGGCGTT-
GGGGATCGGCGGAAGCCCCCTGCGGGCACAACGCCGTCCCCCAAATACAGT-
GGCGGTCCCGCCGCAGCTTCCATTGCGTAGTAGCTAACACCTCGCAACTGGA-
GAGCGGCGCGGCCACGCCGTAAAACACCCAACTTCTGAATGTTGACCTCGAAT-
CAGGTAGGAATACCCGCTGAACTTAAGC

Fusarium sp.

TGATATGCTTAAGTTCAGCGGGTATTCCTACCTGATCCGAGGTCAACATTCA-
GAAGTTGGGGGTTTAACGGCTTGGCCGCGCCGCGTACCAGTTGCGAGGGTTT-
TACTACTACGCAATGGAAGCTGCAGCGAGACCGCCACTAGATTTCGGGGCCG-
GCTTGCCGCAAGGGCTCGCCGATCCCCAACACCAAACCCGAGGGCTTGAG-
GGTTGAAATGACGCTCGAACAGGCATGCCCGCCAGAATACTGGCGGGCG-
CAATGTGCGTTCAAAGATTCGATGATTCACTGAATTCTGCAATTCACATTACT-
TATCGCATTTTGCTGCGTTCTTCATCGATGCCAGAACCAAGAGATCCGTTGTT-
GAAAGTTTTGATTTATTTATGGTTTTACTCAGAAGTTACATATAGAAACAGAGTT-
TAGGGGTCCTCTGGCGGGCCGTCCCGTTTTACCGGGAGCGGGCTGATCCGCCGAG-
GCAACAATTGGTATGTTCACAGGGGTTTGGGAGTTGTAAACTCGGTAATGATC-
CCTCCGCTGGTTCACCAACGGAGACCTTGTTA

DISCUSSION

This is the first official report of *Fusarium* species isolated from leaves of both wild and cultivated Colombian vanillas. The relevance of this finding is that *Fusarium* is one of the main pathogenic microorganisms affecting vanilla

crop all around the world (Tombe and Liew 2010, P. Bayman, pers. comm. 2013). Establishing the presence of these pathogens well in advance vanilla becomes an important crop in Colombia, will allow to know which species are present and what kind of damage they produce in Colombian environmental conditions. Government of this country has the intention of promoting cultivation of alternative crops to coffee, the traditional main national product, since international prices are dropping and diversification is important for both establishing agroecological productive settings and helping poor farmers in this emergent economy. Vanilla is a promising candidate.

Fusarium species found appears to be naturally associated to Colombian vanillas since not only cultivated plants were sampled but wild species too. Given that cultivated vanillas in Colombia are mainly purchased in Costa Rica, it is normal that the world's most common reported pathogen – *F. oxysporum* – (Adame-García *et al.* 2011, Pinaria *et al.* 2010, Tombe and Liew 2010) was found in them. Since *F. oxysporum* f. sp. *loti* was isolated from healthy *Vanilla odorata* leaves interesting ecological questions arise, as for example if there are natural antagonistic microorganisms that inhibit pathogenic properties of that fungus in that plant. As an alternative, it is possible to think that this fungal "forma specialis" is in fact an endophytic partner that lives asymptomatically within vanilla tissues although it is able to induce pathogenesis in other species. Furthermore, natural resistance of the plant to this microorganism is another interesting possibility, and hybridisation of cultivated and wild species could serve to improve resistance to pathogens in cultivated vanilla.

Fusarium oxysporum f. sp. *melonis* and *F. solani* were isolated from healthy leaves of cultivated *V. planifolia*, which could be explained as they being endophytic fungi or latent pathogens (Carroll 1988). The role of a resting pathogen is supported, at least for *F. solani*, because that species has already been reported as an important pathogen in *V. planifolia* (Tombe and Liew 2010). Presence of f. sp. *melonis* must be interpreted here as the first record of this taxon as an endophyte in vanilla, but further studies will be needed for certainly establishing its role in the complex scenario plant-microorganism interaction.

An additional *Fusarium* strain was isolated from an unidentified wild healthy vanilla, and again a latent pathogen or endophytic role must be attributed to it. Before giving this taxon a new name based on differences in DNA sequences that do not allow to fit it with any known species, it is more important to keep this strain alive, to characterise it both morphologically and genetically, and to test if it holds properties as a prospective antagonist to pathogenic *Fusarium* strains. Then, a decision can be made about naming it a new taxon. It is important to keep in mind that *Fusarium* is a species complex with hundreds of races and "forma specialis" named (Summerell *et al.* 2003), and in many cases morphological differentiation of them is not possible. In

those cases, the criterion used for taxa identification relies only on infection capabilities or even host affinity.

Worth to say that *Fusarium oxysporum* f. sp. *vanillae* was not found in this study yet, although characterisation of many fungal endosymbionts from Colombian vanillas continues (Gamboa-Gaitán, in prep.), including samples from sick plants. The absence of this taxon can be due to the fact that only healthy plants were sampled, but the inability to morphologically distinguishing different "forma specialis" of *Fusarium* represents a real problem to date. In fact, nucleotide databases used show tiny differences (1%) between f. sp. *melonis* and f. sp. *vasinfectum* in the NCBI database (USA), and the same difference with f. sp. *lycopersici* in the European Nucleotaide Archive (ENA). If wrong identifications of races, patovars, subspecies, "forma specialis", etc. were used in these nucleotide databases, we are facing a real confusing scene.

Further work in this field includes sampling of diseased vanillas, isolation of prospective pathogens, and confirmation of Koch postulates. Results from this research will allow to be prepared for facing real pathogens of *Vanilla* when this crop becomes broadly cultivated in Colombia.

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