International Journal of Agriculture and Crop Sciences.
Available online at www.ijagcs.com
IJACS/2014/7-15/1497-1503
ISSN 2227-670X ©2014 IJACS Journal

IJACS

# Endophytic fungi from grapevine cultivars in Canary Islands and their activity against phytopatogenic fungi

**A. Cosoveanu*, C. Gimenez-Mariño, Y. Cabrera, G. Hernandez, R. Cabrera**

Unidad de Fitopatología. Dpt. De Biología Vegetal. Universidad de La Laguna. Avda. AstrofisicoFco. Sanchez s/n. 38206 La Laguna.Tenerife.España

***Corresponding author email:*** andreeacosoveanu@gmail.com

**ABSTRACT:** In Canary Islands due to the absence of phylloxera, the vine is cultivated directly into the soil without using American vine rootstocks. The variability of endophytic fungi in cultivated plant species may be conditioned by geographical location and farm management.Our aim was to study endophytic fungi from branches of 11 wine varieties cultivated in the same plot, thus influence of geographic location and culture techniques is minimized. We evaluated the antagonistic activity of the isolated endophytes and their extracts against phytopathogenic fungi, including Botrytis cinerea, which causes severe damage to grapes.We found that the diversity of fungal endophytes genera in this plot is similar to that observed in other studies of vineyards. Although the number of different endophytes present in each variety is small, some of the fungal species were isolated from many varieties while others from one variety only, suggesting a specific relation between some endophytes and their hosts. Bionectriaochroleuca, Aureobasidiumpullulans, Chaetomiumspirochaete,Alternaria sp.Acremoniumstrictum and their subsequent extracts showed activity against targeted phytopatogenic fungi in in vitro bioassays.
***Key words:***Vitisvinifera, microorganisms, bioactivity

## INTRODUCTION

Vitisvinifera L. is an extensive crop with a highly important economic sector. In some regions with dedicated surface, the system has experienced a steady growth in the last decades. In the case of Canary Islands (Spain) the development was along with the change of the crop and wine technology. Moreover in this region phyloxera is absent, which makes the plantations of each variety to be cultivated directly into the soil without using American rootstocks which may influence the fungal diversity. Thus, between the cultivated plants on rootstocks and the ones cultivated on their own roots, physiological and biochemical differences appear: pH level, sugars quantity, potassium content, the mineral absorption or pest resistance(Renouf et al., 2010).The increment of the crop with modern and high productive systems has led to a major preoccupation for pest and diseases, introducing more and more chemical treatments. The diseases frequently encountered in vineyards are: mildew, powdery mildew and Botrytis rot. Their control is based on periodically application of chemical fungicides to reduce the damages associated to the fruit and quality loss as well as reduction in the vigor of the plant.

Seeking new methods of control seems a priority and receives a great attention in research. High efforts have been made searching active microorganisms against these grapevine diseases. It is known that species like Trichoderma spp. and Pseudomonas fluorescens may be used as biological control agents.

Despite of searching new bio control ‘tools’, their interaction mechanisms with pathogens and plants and the induction of systemic resistance are studied (Rühmann et al., 2013). Nevertheless many of these mechanisms have an array of technical problems in the posterior wine elaboration. In fact, the quality of wine depends on the protein accumulation (especially PR-pathogenesis related species) in ripe grapes which is directly related to the climate and pathological conditions of the crop.

Another research line which develops nowadays is the red of endophytic microorganisms in various plant species of medicinal plants(Chowdhary et al., 2012), for their potential use as sources of secondary metabolites of pharmacological interest, industrial or potential control agents against pest and diseases (Giménez et al., 2007).

Studies carried on the diversity of endophytes in plants of agricultural interest are becoming more frequent. To our knowledge, grapevine endophytic fungi received less attention(González and Tello, 2011;

Halleen et al., 2003; Musetti et al., 2007; Núñez-Trujillo et al., 2012; Pancher et al., 2012; Polizzotto et al., 2012).

In this study endophytic fungi were isolated from 11 varieties of vine of ten years old, cultivated in the same parcel, in the locality of Puntallana, La Palma Island (Canary Islands), with the same agronomical practices and influenced by the same climatological and edaphic conditions, thus minimizing the effect of the agricultural practices which may influence the diversity of endophytic fungi in crop(Wilberforce et al., 2003). Subsequently their antagonistic andfungicide extracts´activity against three species of phytopathogenic fungi were assayed (Alternariaalternata(Fries) Keissler,Fusariumoxysporum f. splycopersici(Sacc.) W.C. Snyder and H.N. Hans.andBotrytis cinereaPers.:Fr).

## MATERIALS AND METHODS

### *Isolation*

The parcel where the varieties of vine are cultivated it is found in El Granel (Puntallana), northeast of La Palma Island, at an altitude of 500 m. Healthy plants have been selected for each of the studied cultivars: Albillo, Almuñeco, Baboso negro, Malvasía, Lanzarote, Malvasíarosada, Moscatel, Negramoll, Listánblanco, Listán negro, Tintilla, Torronté and Verdello. Samples have been collected in March, 2013; from each variety stems were kept in paper bags at 4ºC until transported to the laboratory and within 24 hours were processed.

A surface sterilization method (Núñez-Trujillo et al., 2012)was used in order to suppress epiphytic microorganisms from the plant samples. After this process, sterile blotting sheet was used to remove water from plant fragments, which were excised in pieces of 2 cm and cut longitudinally with a sterile scalpel. Segments were placed in PDA Petri plates. To assure that surface sterilization was successful before cutting all fragments were rolled on a Petri plate as control, this way discarding all the potential contaminant fungi. Plates with the plant segments were incubated at 25°C in the dark for 20 days and observed daily. When fungal outgrowth from the plant tissues occurred observations on emerged fungi were made. Only the fungi with different morphological characteristics were subcultured. Eventually, when an endophyte was acquired in pure culture it was preserved (-20º C in glycerol 20%) and identified.

### *Identification, DNA extraction and amplification*

Prior to taxonomic identification, a preliminary visual inspection was made in order to avoid the selection of identical strains arising from the same plant stand. Among these, morphological identification was carried out based on macroscopic and microscopic observations. Microscopic slide preparations of fungi were obtained from 5 or 10 days-old colonies and stained with cotton blue.

Genomic fungal DNA was extracted using commercial kit Maxwell 16 Mouse Tail DNA purification kit. The Promega kit is designed for automated DNA extraction from plant tissue samples using the Maxwell™ 16 platform (Promega). The protocol was performed according to the manufacturers' instructions. Samples were amplified and sequenced by the Genomics Service of the University of La Laguna (Tenerife, Spain). Amplification of the internal transcribed spacer (ITS) region was carried out using the universal eukaryotic primers of ITS1 (5′TCCGTAGGTGAACCTGCGG 3′) and ITS4 (5′ TCCTCCGCTTATTGATATGC 3′).

Sequence-based identifications were realized by searching with FASTA algorithms the Genbank database of fungal nucleotide sequences. When the homology between a strain sequence and a sequence in GenBank was greater than 97%, the match was accepted at the species level. Each result was compared to its correspondent microscopic preparation.

### *Antagonism bioassays*

Target fungal organisms and endophytes were cultured at $25^0$C in PDA. Dual culture technique was used as screening method to find endophytic fungi that produce metabolites which inhibit B. cinerea, F. oxysporumf.splycopersici and A. alternata growth in vitro. PDA plates were incubated at 25ºC in darkness and after 7 days observations were interpreted (Table 3).

Fermentation and extraction

Antagonism was used as preliminary bioassay to select eight active endophytic isolates. In order to obtain a high quantity of mycelia, endophytic fungi were cultivated on rice. Each Erlenmeyer contained 60 grams of rice and 30 ml of $H_2O$. Subsequently, ten plugs of the endophytic fungus were introduced and let to incubate (T= $25^{\circ}$C, darkness). After three weeks the mycelia was proceeded to the extraction using Ethyl acetate, in three steps, leaving the solvent to extract for 24 hours each time. The extracts were combined and solvent was removed at 50ºC using rotary evaporator at low pressure (260 mbar).

### *Dilution agar assays*

Tests were carried out to determine the biological activity of extracts using biometric agar dilution method. The extracts were incorporated into PDA as follows: 0.1, 0.5 and 1 mg $ml^{-1}$. In those cases where this

concentration range was not enough to calculate the EC50 due to the high activity, concentrations of 0.05, 0.01, 0.005 and 0.001 mg $ml^{-1}$ were used. The final percentage of ethanol in the media was adjusted to a concentration of 1% (v/v). Plates containing the solvent (ethanol) were used as negative control. Each pathogen was spot - inoculated at eight equidistant points to PDA media amended with the fungal extracts at tested concentrations.Three replicates were used per treatment. For each extract and concentration, inhibition of radial growth compared with the control was calculated after 72 hours of incubation at 25°C, in the dark. The radial growth was measured with an image - processing softwareImageJ -Wayne Rasband (NIH).

Results were expressed as effective concentration EC50 (the concentration which reduced mycelia growth by 50%) determined by Probit analysis of radial growth inhibition values (%) against the log 10 values of the extract concentrations (IBM SPPS Statistics v.21 Software).

## RESULTS AND DISCUSSION

The endophytic fungi (EF) species are shown in Table 1. As for the wine varieties, Albillo and Verdello have the highest diversity of endophytes (Table 2).

Three of twelve species have been found in different grapevine cultivars. B. parva and A. pullulans were isolated from seven and six grapes varieties respectively, while P. medicaginis only from two. The rest of the endophytic species were isolated from one variety only. Moreover, these differences cannot be the subject of different cultivation practices, variations of the climate or soil, as before stated that all the grapevine cultivars pertain to the same conditions. The variety of fungal species encountered in each grapevine cultivar is indicating that it may be a fungal specificity to plant host(González-Coloma et al., In press).

The obtained results concerning the number and the species of the endophytic fungi isolated in our study are similar to others authors, except the Eutypa genus. Though one might have expected a higher diversity studying 11 grapevine cultivars, we may suppose that the relative small number of isolates, comparing to other previous studies, is due to the homogeneity of plant cultivation condition and/or to the relative small number of samples.

The fact that B. parva and A. pullulans were present in seven and six respectively of 11 studied cultivars makes us think at horizontal transmission of the previously mentioned species. A. strictum has been isolated as endophyte in various plants (unshown data). Its mycoparasitism upon HelminthosporiumsolaniMcAlpine was demonstrated, showing that during parasitism the aspect of H. solani colony changes (concentrically circles and varying color) being black when not parasitized (Rivera-Varas et al., 2007).

B. parvaand B. ribis are closely related species which present difficulties to identification through morphological characteristics. Thus, combination between these and molecular technics lead to a precise identification. B. parva was found as saprophyte, pathogen and endophyte(Casieri et al., 2009)in a big number of plants, especially in woody plants. Inside vine plants was described as associated to various types of cancer, stem dieback and excoriosis(Phillips, 2002).

A. pullulans (black yeastlike species), presented as ubiquist, oligotrophic, and found inclusive in extreme environments, with a high salinity(Gunde-Cimermana et al., 2000), was proved to be very interesting from the industrial point of view as high producer of pullulan polysaccharide(Thirumavalavan et al., 2009). It was isolated as endophyte and epiphyte in grapevine plants and its activity against post-harvest diseases provoked by B. cinerea and Penicilliumexpansum Link was checked (Martini et al., 2009).Moreover, it was confirmed that induces the formation of stilbene phytoalexins in vine plants (Rühmann et al., 2013). In antagonism assays presents one of the highest values of bio activity. On the other side, the percentage of inhibition in agar dilution is inferior among other isolates.

Species ofBionectria have been isolated before from other plant species and the genus is known to produce metabolites with antimicrobial and antifungal activity (Samaga et al., 2014).

The antagonism activity (Table 3) shows 5 isolates, Bionectriaochroleuca, Aureobasidiumpullulans, Chaetomiumspirochaete,Alternaria sp.Acremoniumstrictum which inhibited the development of the 3 phytopathogenic fungi used in bioassays.

Subsequently to antagonism preliminary assays, dilution agar method has been used to check the activity of fungal extracts. The highest activity has been obtained with Chaetomiumspirochaeteextract against B. cinerea (EC:0.008 mg $ml^{-1}$) (Table 4).

EC50 values show 6 cases under 1mg $ml^{-1}$, and even less as C. spirochaeteagainst B. cinerea (0.008 mg $ml^{-1}$). A. pullulans showed in antagonism elevated values with all 3 phytopathogenic fungi while its extract presented a smaller activity in agar dilution assays. C. spirochaete presented in antagonism low bioactivity against B. cinerea and a high one against F. oxysporum and A. alternatarespectively, but in agar dilution maintained its activity against F.oxysporum, increased against B. cinerea, and decreased in the case of A. alternata. One probability that may explain these facts is that the quantity of the produced compounds in PDA, during the antagonism, would not be the same as in the case of pure culture extracts thus is not high enough to show the same effect. Another case may be that while interacting fungi respond chemically different than when

are maintained separately, as in the case of ParaconiothyriumvariabileRiccioni, Damm, Verkley&Crous and FusariumoxysporumSchltdl.(Bertrand et al., 2013).

Members of Ascomycota are ubiquitous in woody plants;Phoma, Fusarium,Penicillium, Alternaria, Botryospaheria, are the dominant genera found as endophytes in trees like citrus, sour cherry, panax ginseng and vine(Hortova and Novotny, 2011; Park et al., 2012). Nevertheless their role as pathogens is well known. Various explanations have been found for this issue: gene expression- Colletotrichum magna which was reported as an avirulent strain after a mutation at a single locus(Lana et al., 2011), isolation timing - the fungus may be saprophytic, latent pathogen or it may display asymptomatic biotrophy followed by a rapid necrotrophic phase as in the case of Colletotrichumhigginsianum(Schulz et al., 1999). Different strains may colonize the same plant tissue and have different virulence levels of expression(Lana et al., 2011). Related to the problem we have isolated Phomamedicaginiswhich is for the first time, of our knowledge, found as endophyte in Vitisvinifera. Being a necrotrophic pathogen for grasses, especially for Medicago sp., is part of the complex genus Phoma which raises problems to taxonomists due to the asexual nature of most species and the high morphological variability(Tsuge et al., 2013). A study upon Phomaviticola isolated as endophyte in Vitisvinifera has brought into light different taxa with various levels of pathogenicity(Mostert et al., 2000). Regarding the establishment of P. medicaginis inside V. vinifera and the lack of expressed pathogenicity, despite of the host specificity, the presence of resveratrol, detected in the stem of V. vinifera(Wang et al., 2010)has shownhyphal growth inhibition of P. medicaginis(Hipskind and Paiva, 2000).

Another controverted fungus is Eutypa sp. Known as the causal fungal agent of Eutypa dieback in Vitisvinifera, slowly developing disease symptoms appear in vineyard several years after infection. At least five species of eutipiaceae [including Eutypalata(Pers.) Tul. & C. Tul.] have been isolated in eastern Spain(Luque et al., 2012). Eutypa and B.ribis were isolated in our study from one cultivar only, Malvasia Lanzarote and Listan Negro respectively. B.parva, saprophyte but also dieback fungi, has been found in seven of 11 studied grapevine cultivars as endophyte. Due to theslow development of the diseases and vineyard management influencing the levels of carbohydrates which appear to be important in vine's defense mechanism, we cannot establish if these fungi are pathogens on the way or endophytes. Bionectriaochroleucaand its related taxa have been found by far as endophytes and pathogens of various plants, less inVitisvinifera. In our study it has a scant occurrence: only one vine cultivar as host. Similar host preference seem to showC.spirochaete, A.strictum and P.solitum.

Despite A.strictum and P.medicaginis, the other taxa have been isolated previously in vineyards(Núñez-Trujillo et al., 2012). Nevertheless, Alternaria sp., one of the most dominant genus as endophyte in perennial, woody and vegetables (authors data not shown), has been isolated in this study from 2 grapevine cultivarsonly. A.pullulans is the second fungus in terms of host preference found in this study, its occurrence in Vitisvinifera has been mentioned previously (Martini et al., 2009).

While host preference remains to be further researched, interactions between fungi have resulted into a series of paradoxical answers. Antagonism has been found to increase with genetic difference (Miller, 2011). Nevertheless we have experimented Alternaria sp. isolated as endophyte having a strong inhibitory activity against pathogen A. alternata. A.pullulanshas been reported to produce the biopolymer pullulan, siderophores, aureobasidins an oligopeptide fungicide and enzymes [N-acetyl-β-d-glucosaminidase, Nagase and β-1-3-glucanase] possibly involved in antagonistic activity(Prasongsuk et al., 2013). In the present study its activity against all three pathogens in dual assay is high, yet its use as extract does not bring favorable results (highest EC50=1.18 mg $ml^{-1}$ against F. oxysporum). Previously reported data show this yeast as an effective antagonist of postharvest fungal pathogens and the produced enzymes are supposed to play a role in the interactions(Wachowska et al., 2013). A.pullulansstrain AS 55.2 is already patented as biological agent against fusarium head blight (Schisler et al., 2009). B.ochroleuca is another example of strong antagonist. In our experiments it showed a high activity in dilution agar assays also. Previously it has been reported as an important agent of inhibition for Aspergillus sp., F.oxysporum and various bacteria(Samaga et al., 2014). Well known for its mycoparasitism of B.cinerea, A.strictum`s activity comes reinforced by our assays (EC50> 0.48 mg $ml^{-1}$). In dual assays its activity is high against B. cinerea and A. alternatarespectively.Acremonium has been detected on many saprotrophic fungi, several plant pathogenic fungi and on the mycoparasiteMycogoneperniciosa(Magnus) Delacr.andH.solani whose growth it inhibited(Narayanasamy, 2013).

Our results suggest specific host-endophyte relations, as the 75% of the isolated species were specific for each grape variety.

Some of the endophytic species need further studies to confirm their role as endophytes or latent pathogens (to be taken into consideration: Eutypa sp. and P. medicaginis).

Some of the isolated endophytes showed high in vitro activity against phytopathogenic fungi. The presence of these inside V.vinifera plants may contribute to natural defense against pathogens. It would be of great interest to check whether those grapevine cultivars which host active endophytes against B. cinerea would still present their resistance in field conditions. An array of field experiments based on inoculation of

active endophytes to test the maintenance of the resistance against grapevine diseases in plant would give responses to the symbiotic processes between endophyte and plant.

## REFERENCES


Bertrand S, Schumpp O, Bohni N, Monod M, Gindro K, Wolfender JL. 2013. De novo production of metabolites by fungal co-culture of Trichophyton rubrum and Bionectria ochroleuca. J. Nat. Prod. 76(6):1157-1165.

Casieri L, Hofstetter V, Viret O, Gindro K. 2009. Fungal co,mmunities living in the wood of different cultivars of young Vitis vinifera plants. Phytopathologia Mediterranea 48:73-83.

Chowdhary K, Kaushik N, Coloma AG, Raimundo CM. 2012. Endophytic fungi and their metabolites isolated from Indian medicinal plant. Phytochem. Rev. 11(4):467-485.

Giménez C, Cabrera R, Reina M, González-Coloma A. 2007. Fungal endophytes and their rol in plant protection. Current Organic Chemistry 11:707-720.

González V, Tello ML. 2011. The endophytic mycota associated with Vitis vinifera in central Spain. Fungal Diversity 47(1):29-42.

González-Coloma A, Cosoveanu A, Cabrera R, Giménez C, Kaushik N. In press. Endophytic fungi and their bioprospection. In: Deshmukh K (Ed.), Progress in Mycological Research. Science Publishers (An Imprint of Edenbridge Ltd, British Channel Islands), Enfield, New Hampshire.

Gunde-Cimermana N, Zalarb P, de Hoogc S, Plemenitasd A. 2000. Hypersaline waters in salterns - natural ecological niches for halophilic black yeasts. FEMS Microbiol. Ecol. 32(3):235-240.

Halleen F, Crous PW, Petrini O. 2003. Fungi associated with healthy grapevine cuttings in nurseries, with special reference to pathogens involved in the decline of young vines. Australas. Plant Pathol. 32(1):47.

Hipskind JD, Paiva NL. 2000. Constitutive accumulation of a resveratrol-glucoside in transgenic alfalfa increases resistance to Phoma medicaginis. Mol. Plant Microbe Interact. 13(5):551-562.

Hortova B, Novotny D. 2011. Endophytic fungi in branches of sour cherry trees: a preliminary study. Czech. Mycol. 63(1):77-82.

Lana TG, Azevedo JL, Pomella AWV, Monteiro RTR, Silva CB, Araújo WL. 2011. Endophytic and pathogenic isolates of the cacao fungal pathogen Moniliophthora perniciosa (Tricholomataceae) are indistinguishable based on genetic and physiological analysis. Genet.Mol.Res. 10(1):326-334.

Luque J, Garcia-Figueres F, Legorburu FJ, Muruamendiaraz A, Armengol J, Trouillas F,P. 2012. Species of Diatrypaceae associated with grapevine trunk diseases in Eastern Spain. Phytopathologia Mediterranea 51(3):528-540.

Martini M, Musetti R, Grisan S, Polizzotto R, Borselli S, Pavan F, Osler R. 2009. DNA-dependent detection of the grapevine fungal endophytes aureobasidium pullulans and epicoccum nigrum. Plant Dis. 93(10):993-998.

Miller JD. 2011. Foliar Endophytes of Spruce Species Found in the Acadian Forest: Basis and Potential for Improving the Tolerance of the Forest to Spruce Budworm. In: Pirttila AM, Frank AC (Eds.) Endophytes of Forest Trees - Biology and Applications. Springer, London, pp. 237-250.

Mostert L, Crous PW, Petrini O. 2000. Endophytic fungi associated with shoots and leaves of Vitis vinifera, with specific reference to the Phomopsis viticola complex. Sydowia 52(1):46-58.

Musetti R, Polizzotto R, Vecchione A, Borselli S, Zulini L, D'Ambrosio M, Toppi LSd, Pertot I. 2007. Antifungal activity of diketopiperazines extracted from Alternaria alternata against Plasmopara viticola: An ultrastructural study. Micron 38(6):643-650.

Narayanasamy P. 2013. Biological Management of Diseases of Crops - Volume 1: Characteristics of Biological Control Agents. Springer, New York.

Núñez-Trujillo G, Cabrera R, Burgos-Reyes RL, Da Silva E, Giménez C, Cosoveanu A, Brito N. 2012. Endophytic fungi from Vitis vinifera L. isolated in Canary Islands and Azores as potential biocontrol agents of Botrytis cinerea Pers.:Fr. Journal of Horticulture, Forestry and Biotechnology 16(1):1-6.

Pancher M, Ceol M, Corneo PE, Longa CMO, Yousaf S, Pertot I, Campisano A. 2012. Fungal endophytic communities in grapevines (Vitis vinifera L.) Respond to crop management. Appl. Environ. Microbiol. 78(12):4308-4317.

Park SU, Lim HS, Park KC, Park YH, Bae H. 2012. Fungal Endophytes from Three Cultivars of Panax ginseng Meyer Cultivated in Korea. J. Ginseng Res. 36(1):107-113.

Phillips AJL. 2002. Botryosphaeria species associated with diseases of grapevines in Portugal. Phytopathologia Mediterranea 41:3-18.

Polizzotto R, Andersen B, Martini M, Grisan S, Assante G, Musetti R. 2012. A polyphasic approach for the characterization of endophytic Alternaria strains isolated from grapevines. J. Microbiol. Methods 88(1):162-171.

Prasongsuk S, Ployngam S, Wacharasindhu S, Lotrakul P, Punnapayak H. 2013. Effects of sugar and amino acid supplementation on Aureobasidium pullulans NRRL 58536 antifungal activity against four Aspergillus species. Appl.Biochem.Biotechnol. 97(17):7821-7830.

Renouf V, Tregoat O, Roby JP, Van Leeuwen C. 2010. Soils, rootstocks and grapevine varieties in prestigious Bordeaux vineyards and their impact on yield and quality -. Journal International Des Sciences De La Vigne Et Du Vin = International Journal of Vine and Wine Sciences 44(3):127-134.

Rivera-Varas VV, Freeman TA, Gudmestad NC, Secor GA. 2007. Mycoparasitism of Helminthosporium solani by Acremonium strictum. Phytopathology 97(10):1331-1337.

Rühmann S, Pfeiffer J, Brunner P, Szankowski I, Fischer TC, Forkmann G, Treutter D. 2013. Induction of stilbene phytoalexins in grapevine (Vitis vinifera) and transgenic stilbene synthase-apple plants (Malus domestica) by a culture filtrate of Aureobasidium pullulans. Plant Physiology and Biochemistry 72(0):62-71.

Samaga PV, Rai VR, Rai KML. 2014. Bionectria ochroleuca NOTL33 - An endophytic fungus from Nothapodytes foetida producing antimicrobial and free radical scavenging metabolites. Ann. Microbiol. 64(1):275-285.

Schisler DA, Khan NI, Boehm MJ, Slininger PJ. 2009. Choline-utilizing microbial strains for biologically controlling fusarium head blight -. 2014(10/1/2014):.

Schulz B, Römmert A, Dammann U, Aust H, Strack D. 1999. The endophyte-host interaction: A balanced antagonism? Mycol. Res. 103(10):1275-1283.

Thirumavalavan K, Manikkadan TR, Dhanasekar R. 2009. Pullulan production from coconut by-products by aureobasidium pullulans. African Journal of Biotechnology 8(2):254-258.

Tsuge T, Harimoto Y, Akimitsu K, Ohtani K, Kodama M, Akagi Y, Egusa M, Yamamoto M, Otani H. 2013. Host-selective toxins produced by the plant pathogenic fungus Alternaria alternata. FEMS Microbiol. Rev. 37(1):44-66.

Wachowska U, Stasiulewicz-Paluch AD, Konopka I, Kucharska K, Borowska J. 2013. Aureobasidium pullulans used as a biological control agent under field conditions affects the microbial quality of winter wheat grain. World Academy of Science, Engineering and Technology 7:7-24.

Wang W, Tang K, Yang HR, Wen PF, Zhang P, Wang HL, Huang WD. 2010. Distribution of resveratrol and stilbene synthase in young grape plants (Vitis vinifera L. cv. Cabernet Sauvignon) and the effect of UV-C on its accumulation. Plant Physiol. Biochem. 48(2-3):142-152.

Wilberforce EM, Boddy L, Griffiths R, Griffith GW. 2003. Agricultural management affects communities of culturable root-endophytic fungi in temperate grasslands. Soil Biol. Biochem. 35(8):1143-1154.

Table 1. Identification of endophytes isolated from grapevine

| Isolates | GenBakAccession number | Max identity(%) | Highest BLAST affinities |
|---|---|---|---|
| HV1 | FJ755241 | 99 | BotryosphaeriaparvaPennycook& Samuels |
| HV40 | AY004336 | 97 | BotryosphaeriaribisGrossenb. &Duggar |
| HV2 | KF181220 | 99 | Phomamedicaginis Mal. et Roum |
| HV6 | HQ115728 | 97 | Bionectriaochroleuca (Schwein.) Schroers& Samuels |
| HV9 | JN642222 | 99 | PenicilliumsolitumWestling |
| HV15 | KC241878 | 98 | Aureobasidiumpullulans (de Bary) G. Arnaud |
| HV16 | JQ922158 | 99 | Eutypa sp. |
| HV24 | JN209921 | 99 | ChaetomiumspirochaetePalliser |
| HV28 | AY138482 | 98 | Acremoniumstrictum W. Gams |
| HV26 | | | Alternariasp. |
| HV27 | | | No identificated |
| HV32 | | | No identificated |

Table 2. Fungi species isolated from grapevine cultivars

| Isolated | Endophytic species | Grapevine cultivar | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | [1] | [2] | [3] | [4] | [5] | [6] | [7] | [8] | [9] | [10] | [11] |
| VH1 | Botryosphaeriaparva | x | x | | x | | | x | x | | x | x |
| HV2 | Phomamedicaginis | x | | | | | | x | | | | |
| HV6 | Bionectriaochroleuca | | | | | x | | | | | | |
| HV9 | Penicilliumsolitum | | | | | | | | | x | | |
| HV15 | Aureobasidiumpullulans | x | x | x | | x | | | x | | | x |
| HV16 | Eutypa sp. | | | | x | | | | | | | |
| HV24 | Chaetomiumspirochaete | x | | | | | | | | | | |
| HV28 | Acremoniumstrictum | | | | | | x | | | | | |
| HV26 | Alternariasp. | | | | | | x | | | | | x |
| HV27 | ---- | | | | | | x | | | | | |
| HV32 | ---- | | | | | | | | | | | x |
| HV40 | Botryosphaeriaribis | | | | | | | | x | | | |

[1]Albillo, [2]Almuñeco, [3]Baboso Negro, [4]Malvasia Lanzarote, [5]Moscatel, [6]Negramol, [7]Listan Blanco, [8]Listan Negro, [9]Tintilla, [10]Torronte, [11]Verdello

Table 3. Results of antagonism assays (*)

| Isolate species | F. oxysporum | B. cinerea | A. alternata |
|---|---|---|---|
| HV1 Botryosphaeriaparva | 0 | 0 | 1 |
| HV2 Phomamedicaginis | 1 | 1 | 1 |
| HV6 Bionectriaochroleuca | 2 | 2 | 2 |
| HV9Penicilliumsolitum | 0 | 0 | 0 |
| HV15 Aureobasidiumpullulans | 2 | 2 | 2 |
| HV16 Eutypa sp. | 0 | 0 | 0 |
| HV24 Chaetomiumspirochaete | 2 | 0 | 2 |
| HV28 Acremoniumstrictum | 0 | 2 | 2 |
| HV40 Botryosphaeriaribis | 1 | 0 | 1 |
| HV26 Alternaria sp. | 2 | 2 | 2 |
| HV27 | 2 | 2 | 2 |
| HV32 | 2 | 1 | 2 |

(*) 0. No apparent interaction.
1. Mycelia grow until touching each other, and in area where the contact is produced morphological changes occur. Slight inhibition of both the interacting fungi with narrow demarcation line (1-2 mm).

Table 4. Results of inhibition assays in agar dilution method. EC50 mg $ml^{-1}$ (confidence intervals)

| | | Fungal target species | | |
|---|---|---|---|---|
| Isolate | Species | F. oxysporum | B. cinerea | A. alternata |
| HV6 | Bionectriaochroleuca | 3,55 (1,6-24,89) | 0,09 (0,073-0,11) | 0,74 (0,64-0,87) |
| HV15 | Aureobasidiumpullulans | 1,18 (0,91-1,67) | 1,52 (1,12-3,95) | 2,35 (1,62-6,43) |
| HV24 | Chaetomiumspirochaete | 0,018 (0,013-0,024) | 0.008 (0,0- 0.02) | 0,067 (0,048-0,095) |
| HV26 | Alternaria sp. | 0,996 (0,665--1,65) | 0,14 (0,05-0,94) | 0,313 (0,243-0,416) |
| HV28 | Acremoniumstrictum | 0,74 (0,65-0,86) | 0,42 (0,36-0,48) | 1,89 (1,21-4,14) |
| HV27 | ---------- | 0,55 (0,44-0,70) | 0,29 (0,20-0,39) | 0,28 (0,22-0,36 |
| HV32 | ---------- | 1,03 (0,87-1,20) | 3,56 (2,30-7,32) | 4,13 (2,0-116) |