Above-ground parts of white grapevine *Vitis vinifera* cv. Furmint share core members of the fungal microbiome

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Summary

Grapevine (*Vitis vinifera*) is a reservoir of fungal endophytes that may affect its growth, health status and grape production. Although there is growing interest in comparing fungal communities of mainly red grape varieties across various factors using only high-throughput sequencing, the small-scale mycobiome variations in geographically close vineyards need further examination. We aimed to characterize the fungal microbiome of the above-ground tissues of *V. vinifera* cv. Furmint in different plant parts, seasons and sites using culture-dependent and culture-independent methods, and in planta fluorescent microscopic visualization techniques. Samples were collected from four sites of the Tokaj wine region in Mád and two reference sites in Eger, Hungary, across different seasons for 2 years. Fungal endophytes of young and mature leaves, flowers and grape bunches were collected at different phenological stages. Based on each technique, *Aureobasidium pullulans*, *Cladosporium* spp. and the complex species *Alternaria alternata* dominated the community at every site, season and plant organ. We found no significant difference among communities in distinct neighbouring vineyards, nor when compared with the distant reference sites. We can conclude that the different shoot parts of the Furmint grapevines harbour a common core group of fungal community in these regions.

Introduction

Most terrestrial plants form symbioses with diverse fungal endophytes, which are important members of the plant microbiome (Vandenkoornhuyse et al., 2015). Communities of fungal endophytes, which colonize plant tissues without causing any visible symptoms in the hosts, can be found in both natural and managed ecosystems (Porras-Alfaro and Bayman, 2011). Grapevine varieties (*Vitis vinifera*) also represent reservoirs of these endophytes, which may affect their growth, health status and grape production (Morgan et al., 2017). To date, information on endophyte presence and role have been mostly unknown compared with that on fungal pathogens; however, there is an intensifying focus on different microbial communities in distinct wine-growing regions. This could lead to a better understanding of their potential influences on grape variety and production year (Bokulich et al., 2014). Relatively less attention has been paid to the fungal microbiota of grapevines compared to the bacterial community and the above-ground compared with the below-ground community (Martínez-Diz et al., 2019; Deyett and Rolshausen, 2020; Liu and Howell, 2021).

Numerous studies have addressed questions on the seasonal, temporal and regional variations of fungal communities inhabiting different above-ground organs of several varieties of grapevines using high-throughput sequencing (HTS) and other methods (Pinto et al., 2014; Setati et al., 2015; Varanda et al., 2016; Jayawardena et al., 2018; Martínez-Díez et al., 2019; Deyett and Rolshausen, 2020; Swift et al., 2020). However, most of these studies compared communities of different vineyards, mainly red grape varieties, with large geographic
distances among them. Although fungal endophyte isolation can support and supplement the results from culture-independent methods (Dissanayake et al., 2018; Eichmeier et al., 2018; Jayawardena et al., 2018), there are few grapevine studies where these techniques have been applied. Moreover, the in planta visualization of the fungal microbiome of grapevines has largely been neglected. Nevertheless, studies focusing on bacterial communities have localized these endophytic microorganisms (Compton et al., 2010, 2011).

Fungal diversity differences among vineyards could play a role in forming the terroir effect (Gilbert et al., 2014); therefore, understanding the fungal community dynamics among vineyards could be useful for the wine-making process. The Tokaj wine region, a historically and economically important wine-producing territory in Hungary, is ideal for such studies (Szepesi et al., 2017). This region produces the world’s oldest botrytised ‘aszú’ wines, growing almost exclusively indigenous white varieties such as Furmint (Kovács et al., 2017). However, almost no information is available on the fungal microbiome of grapevines in the region, except for the characterization of a few potential fungal pathogens collected from here (Kovács et al., 2017; Váczy, 2017; Váczy et al., 2018). A small-scale geographical study focusing on above-ground fungal communities of white grape varieties from neighbouring vineyards could provide new information on V. vinifera, which has been investigated from several other aspects.

We aimed to test whether organs, sites and seasons affected the fungal community structure in the above-ground tissues of V. vinifera cv. Furmint. To determine this, we (i) studied the fungal microbiome using culture-dependent and -independent methods and identified the common and dominant members of the community, (ii) compared the endophytic fungal communities of different plant organs and evaluated their seasonality on a fine-scale regional variation and (iii) visualized the fungal endophytes within the plant tissues.

Results
Here, we present the first data on fungal microbiomes of different above-ground tissues of the white grapevine variety Furmint, one of the most cultivated white grape varieties in Hungary. The sampling was carried out in four different vineyards in Mád (Tokaj wine region); Betsek (BET), Király (KIR), Szent Tamás (STT) and Úrágya (URA) (Fig. S1), and two reference sites in Eger wine region; Nagy-Eged (NEG) and Hangács (HAN) vineyards (see Supplementary Information Experimental Procedures). Young and mature leaves and grape bunches were examined in spring, summer and autumn for 2 years (Fig. 1), and the fungal microbiome was studied by an HTS-based method, culture-dependent methods and fluorescence-based microscopic visualization (see Supplementary Information Experimental Procedures).

High-throughput sequencing
After paired-end alignments, quality filtering, and deletion of singletons, chimeric and non-fungal sequences, a total of 4 053 313 fungal ITS sequences were generated from 140 plant samples (Supporting Information Table S1). The average read number of the samples was 28 952 and the median was 4875. The samples collected in autumn had generally higher read numbers compared to those from other seasons (Supporting Information Table S1). After exclusion of samples with low read numbers and random subsampling to 3000 reads, 853 OTUs represented the fungal community of the grapevine (Supporting Information Table S2). A vast majority of the sequences belonged to Ascomycota (92%), and the most abundant orders were Dothideales (51%), Capnodiidales (16%), Pleosporales (11%), Helotiales (9%) and Filobasidiales (6%). Within these orders, generally 1–1 dominant genus comprised the majority of the reads and shaped the community: Aureobasidium (51%), Cladosporium (16%), Alternaria (9%), Erysiphe (4%) and Filobasidium (4%) respectively (Fig. 2). Ascomycota (70%) and Basidiomycota (30%) represented almost all of the total OTUs detected (Supporting Information Table S1). The order Pleosporales represented the most OTUs (221) followed by Agaricales (105), Tremellales (51), Helotiales (47) and Capnodiidales (43). The 25 most abundant OTUs comprised more than 95% and the top 10 OTUs almost 91% of the sequences (Supporting Information Table S2). The most dominant OTU represented Aureobasidium pullulans, which was dominant in all seasons, sites and plant parts (Fig. 1). The dominant presence of Cladosporium spp. and Alternaria aff. alternata was in itself remarkable. These three OTUs represented over 70% of all reads in general, and at least 60% of the reads grouped by seasons, distinct sites and different shoot parts. To have an overview of the similarity of the samples, Principal Coordinate Analysis (PCoA) with Bray–Curtis distances was used, and the most conspicuous finding was the arrangement of the communities by sampling season (Fig. 3). To reveal the groupings of the samples according to sampling sites, plant parts, sampling years and seasons general linear model (GLM) using generalized least squares were applied for the first two extracted PCoA coordinates. Changes in samples’ Chao1 diversities were also tested the same way. Generally, sampling sites were not separated from each other based on community composition nor based on diversity (Fig. 4A and B; Supplementary Information Fig. S2A).
However, there were significant differences in fungal diversity between seasons and also between different plant parts with a decreasing fungal diversity from spring to autumn and young leaves to grapes. (Fig. 4C, D, G and H). Furthermore, the two sampling years differed significantly (Fig. 4E and F).

Culture-dependent technique

During the isolation process to acquire fungi from the internal tissues, more than 3400 samples/parts of different plant materials were surface-sterilized and laid onto PDA media. Endophytes growing out from the tissues of the same plant sample with identical colony morphology were considered to represent the same taxa and were sub-cultured and used for further analyses. Altogether, 353 isolates were obtained from different organs of the Furmint grapevine from Mát and Eger (Supporting Information Fig. S3; Table S5). Most of the collected isolates belonged to the phylum Ascomycota, representing diverse orders. Only a few basidiomycetes were found, including the yeast Curvibasidium and Peniophora species (Supporting Information Fig. S3; Table S5). Based on molecular phylogenetic identification, the majority of the clades could be identified at the species or genus level, whereas others could only be identified at higher taxonomic ranks (Supporting Information Figs S3 and S4; Table S5). Based on the analysis of the ITS sequences, the isolates represented 44 lineages introduced here as clade 1 to clade 44. The most numerous clade, representing almost one-third of the collected fungi, consisting of 122 isolates, was the complex species Alternaria aff. alternata (clade 1), followed by clade 7 (Epicoccum nigrum), clade 11 (Botrytis cinerea) and clade 13 (Aureobasidium pullulans), with 46, 34 and 32 isolates respectively (Supporting Information Fig. S3; Table S5). Most of the isolates (331) belonged to non-singleton clades, while 22 clades contained only one isolate. Pleosporales was the most represented order with 209 isolates and 10 clades (209/10), followed by Helotiales (35/2), Dothideales...
(32/1) and Capnodiales (31/2); however, all these orders, especially the last three, were dominated by only one or two clades. Among the cultivable fungal taxa *A. aff. alternata* dominated the above-ground plant tissues, and *E. nigrum, B. cinerea* and *A. pullulans* also had a considerable presence. Compared to the OTUs revealed by HTS, the dominant culturable filamentous fungi were also identified by the culture-dependent method (Supporting Information Table S5). However, ascomycetes were overrepresented by isolation-based techniques, and e.g. basidiomycete groups (Agaricales and Tremellales) frequent in the HTS analyses were not isolated.

Because of *A. alternata* and related species that belong to *Alternaria* sect. *Alternaria*, where the ITS region is not adequate for species delimitation (Woudenberg *et al.*, 2015), 24 isolates originating from different sites, plant parts and seasons were selected for further identification, and the partial RPB2 region was sequenced.
The phylogenetic analysis resulted in at least six well-supported different lineages within *Alternaria* sect. *Alternaria* (Supporting Information Fig. S5). The isolates represented different clades and morphospecies within *A. alternata* and two lineages in the *A. arborescens* species complex (Supporting Information Fig. S5).

Visualization

The *in planta* fungal colonization of each collected sample could be detected by fluorescent microscopy (Fig. 5). Using different excitation wavelengths and barrier filters for epifluorescence microscopy, the specificity and applicability of WGA-AlexaFluor488 dye were tested, and we found only weak autofluorescence of the plant tissues in the background at an excitation of 488 nm (Fig. 5A–C). Fungal colonization seemed to be restricted to the intercellulars of the leaves. The hyphal structures did not show typical intracellular growth; in most cases, hyphae formed relatively long branches in the intrafoliar space (Fig. 5E). Although we did not aim for any quantitative measurement, the results of the visualization showed that the colonization of the leaves and young grape clusters was weakest at the beginning of the growing season and strongest in autumn. In particular, in the six young leaves collected in early spring of 2019, almost no colonization or presence of fungal structures could be detected.

We detected only septate hyphae within the plant tissues (Fig. 5H). In case of some fungal lineages that were found to be dominant by both culture- and non-culture-dependent techniques, morphological identification of a particular taxonomic level may be feasible based on specific structures of the fungi. The most common fungal structures were longitudinal hyphae, which generally formed arthroconidia (Fig. 5D–E). Although we cannot strongly conclude much about these species, these structures might be formed by *Aureobasidium pullulans* based on its known morphological features and significant abundance in the grapevine (Fig. 5D–E). In numerous samples, obclavate conidia with both transverse and longitudinal septations were present (Fig. 5G), which are characteristic of *Alternaria* species.

Discussion

This study reports the first observations of the fungal community within different above-ground tissues of one grapevine variety in different seasons and vineyards located in a limited geographic proximity. We also present the first information on the fungal microbiome of white grapevine *V. vinifera* cv., Furmint, historically cultivated in the Tokaj wine region of Hungary. To date, only the occurrence of grapevine trunk diseases (GTDs) and pathogens involved in botryosphaeria dieback (Kovács et al., 2017; Váczy, 2017; Váczy et al., 2018), as well as the yeast population of the berries (Naumov et al., 2002; Sipiczki, 2016) have been investigated in this area. These data provide important information as most diversity studies applying HTS focusing on fungal communities of grapevines generally present mycobiomes of different red varieties (Pinto et al., 2014; Setati et al., 2015; Varanda et al., 2016).
et al., 2016; Jayawardena et al., 2018; Martinez-Diz et al., 2019; Deyett and Rolshausen, 2020), and only a few studies present data from white varieties (e.g. Bokulich et al., 2014, 2016).

**Diversity**

Although various techniques are commonly used to assess OTU or amplicon sequence variant (Deyett and Rolshausen, 2019; Deyett and Rolshausen, 2020) numbers in fungal communities of grapevines using different primer sets (Pinto et al., 2014) and sequencing techniques (Kemaghan et al., 2017), our 853 OTUs of the filtered samples correspond to those of similar studies, in which the OTU number ranges between 200 and 2200 (e.g. Pinto et al., 2014; Jayawardena et al., 2018; Martinez-Diz et al., 2019, 2020; Fan et al., 2020; Liu and Howell, 2021). Random subsampling of the samples to a
few thousand reads is not unusual as seen in various analyses of fungal microbiomes of different above-ground grapevine plant materials (Bokulich et al., 2014, 2016; Pinto et al., 2014; Fan et al., 2020). The relatively low number of fungal sequences in the internal plant tissues can be caused by the sampling of only the above-ground tissues, where the fungal abundance and diversity of the organs, especially the leaves, is generally below that of other plant parts. Based on several studies focusing on the mycobiome of roots, rhizosphere and different shoot tissues, the fungal dominance and diversity reported to be higher in below-ground than that in above-ground parts of several Vitis varieties (Zarraonaindia et al., 2015; Deyett and Rolshausen, 2020; Liu and Howell, 2021). Although there might be clear correlations and differences in ITS-based diversities, we should bear in mind the substantial biases in fungal diversity due to highly variant rDNA copy numbers, different cell and growing characteristics, and the number of nuclei in a specific region (Lofgren et al., 2018).

**General microbiome, dominant members of the community**

Grapevine hosts a diverse spectrum of fungal endophytes, and the vast majority of these fungi belong to various orders of the phylum Ascomycota, as detected by different techniques (Dissanayake et al., 2018; Jayawardena et al., 2018; Liu and Howell, 2021). Using both culture-dependent and independent methods, studies showed that healthy-looking grapevine plants live together with a core community consisting of *Alternaria*...
Alternaria alternata (and related Alternaria species), Aureobasidium pullulans, Epicoccum nigrum, and sometimes Botrytis cinerea along with Cladosporium, Fusarium, Penicillium, Phoma/Didymella and other species (González and Tello, 2011; Brum et al., 2012; Pancher et al., 2012; Setati et al., 2012; Bokulich et al., 2014; Pinto et al., 2014; Setati et al., 2015; Kernaghan et al., 2017; Dissanayake et al., 2018; Jayawardena et al., 2018; Mezzasalma et al., 2018; Wei et al., 2018; Deyett and Rolshausen, 2020; Liu and Howell, 2021). In the current study, the most abundant OTUs were represented mainly by the taxa mentioned above; however, some lineages considered as dominant by other studies were not present, such as Mycosphaerella, which is usually a member of the core community (Deyett and Rolshausen, 2020; Liu and Howell, 2021). The isolation technique confirmed the dominant presence of the most common lineages found by molecular investigations. Species representing six of the 10 most common OTUs, such as A. alternata and close relatives, A. pullulans, B. cinerea, Cladosporium sp1, Cladosporium sp2 and E. nigrum were present in high numbers, and O. korrae was also isolated. One of the primary importance of the isolation technique was finding numerous isolates belonging to the Alternaria alternata within Alternaria sect. Alternaria, which could not be separated into different species by only the ITS2 or the whole ITS region (Woudenberg et al., 2013, 2015). The obligate biotrophic pathogen of grape powdery mildew Erysiphe (Uncinula) necator and two yeasts, Filobasidium magnum and F. wieringae belonging to Filobasidiaceae (Basidiomycota) could not be cultured.

The top three OTUs were A. pullulans, Cladosporium sp. and A. aff. alternata representing over 70% of the fungal sequences obtained from grapevine, and they were also found to be common in different studies in grapevine fungal microbiomes. Aureobasidium pullulans is a cosmopolitan dimorphic fungus known as black yeast due to its melanin production, representing one of the most abundant members of the grapevine fungal microbiome worldwide (González and Tello, 2011; Kernaghan et al., 2017; Deyett and Rolshausen, 2020). Although our knowledge has been limited to the accurate identity, taxonomy and function of Cladosporium species related to grapevines, its dominance within plant tissues has been found in several studies (Zhang et al., 2017; Dissanayake et al., 2018; Deyett and Rolshausen, 2019, 2020).

In this study, most isolates, and a significant number of the reads in the HTS analysis belonged to the genus Alternaria, which has been found among the dominant members of the grapevine community in different regions and varieties (González and Tello, 2011; Pancher et al., 2012; Varanda et al., 2016; Kernaghan et al., 2017). Polizzotto et al. (2012), using a combination of morphological, molecular and chemical analyses, also reported that grapevines are associated with a dominant and unique Alternaria endophyte community. As a genus, Alternaria is a biologically, morphologically and ecologically diverse group of fungi producing multi-celled conidia (Simmons, 2007; Lawrence et al., 2016). Many of its species are considered to be cosmopolitan saprobes, endophytes, pathogens, or the causal agents of postharvest rots of numerous agronomic plants (Ragazzi et al., 2001; Polizzotto et al., 2012; Armitage et al., 2015). The complex A. alternata species comprises 35 morphospecies (Woudenberg et al., 2015) often called small-spored Alternaria, with species such as A. alternata, A. arborescens and A. gaisen. Our findings showed that at least six lineages of A. alternata and A. arborescens species complex are present in grapevines masked by identical ITS sequences. Isolation of these fungi is important to assess the structure of the community of even the closely related Alternaria species within the plant because major differences in their strategy and effects on the host might be present. Since endophytic fungi belonging to this complex species may play an essential role in the biocontrol of plant pathogens such as Plasmopara viticola (Musseti et al., 2006, 2007), the accurate identification of members of this dominant group is of vital importance.

**Season, plant parts and site**

Seasonal changes in the endophytic community of grapevines were found in the bacterial microbiome (Bulgari et al., 2009; Campisano et al., 2017); however, with fungal endophytes, these seasonal changes are not entirely understood. Pinto et al. (2014) reported significant alterations in the fungal community structure of leaves from July to August in Portugal. Martinez-Diz et al. (2020) investigated wood-inhabiting fungi of grapevines at three sampling times, November, February and May, in Spanish vineyards, and they found a correlation between fungal diversity and sampling time. Fungal richness and diversity were lower from February to May. Deyett and Rolshausen (2019) also found temporal variation in the sap microbiome of grapevines in California. Significant variation in fungal richness and diversity were also recorded at each grapevine habitat over time in Australian vineyards (Liu and Howell, 2021), where differences were observed in fungal community composition over time regardless of grapevine habitat. Our results using endophyte isolation, microscopy and HTS pointed to fewer isolate numbers, structures and reads in spring when the colonization and probably the whole fungal biomass are likely less dominant. According to these
findings, communities in spring differed from those in other seasons. The community structure showed significant variations across seasons and years based on alpha diversity and PCoA coordinates (Figs 3 and 4E–H). We also found substantial variation in the community among sampling years, similar to that by Deyett and Rolshausen (2019).

Investigation of potential variations among the different shoot parts of the grapevine has been addressed in several studies (Wei et al., 2018; Deyett and Rolshausen, 2020; Fan et al., 2020); however, more information is needed on the different phenological stages of generative parts and leaves. Here, we found significant differences in community diversity. As discussed above, the young leaves, mainly in spring, were inhabited by fewer fungi. The community structure of the mycobiome of both the leaves and grape clusters were more similar (Fig. 4C and D).

Our geographical sampling setup that included four nearby vineyards in Mát and two reference sites indicated the presence of a core fungal microbiome and similar community structures (Fig. 4A and B, Supporting Information Fig. S2A). Therefore, the terroir effect is not likely driven by the mycobiome of the above-ground plant tissues. Similarly, Martinez-Diz et al. (2020) found no significant differences between the diversity of fungal communities of grapevine wood samples in different sampling plots. Liu and Howell (2021) also reported that geographic location only slightly affected the microbial diversity and composition, except for fungal diversity associated with roots; however, regional and small-scale variation in the below-ground fungal microbiome is relatively common (Zarraonaindia et al., 2015; Knight et al., 2019; Deyett and Rolshausen, 2020). Based on our study and that by others, we may assume that small-scale geographical differences are usually not evident in the mycobiome of the shoot; nevertheless, the fungal microbiome of the must from the same cultivars (including the white variety, Chardonnay) can be significantly different among vineyards (Bokulich et al., 2014, 2016).

Visualization

In the present study, we carried out fungal visualization within plant tissues using fluorescence microscopy. Although endophytic bacterial communities of the grapevine are often visualized (e.g. Compant et al., 2010, 2011; Pacifico et al., 2019), we are not aware of studies focusing on the fungal microbiome of grapevine using microscopy to prove the presence and structure of endophytic fungi. Here, we used a relatively simple technique of fluorescent staining of the cell wall of fungi for in planta visualization, which is generally used for visualization of fungal endophytes (Andrade-Linares et al., 2011; Knapp et al., 2019). Our microscopic observations suggest that the colonization of plant tissues is weaker in spring and young organs becoming relatively more severe towards the end of the growing season. These findings were in putative correlation with the results of the isolations, especially with the low read-numbered samples from spring and young leaves in the HTS dataset. The sole presence of septate hyphae within the plant is also consistent with Ascomycota and Basidiomycota dominated communities of the plants found using further approaches.

We were able to visualize the most common members of the mycobiome community with characteristic structural features. Aureobasidium pullulans has three distinctive forms: long-branched-septate filaments, large chlamydospores and smaller yeast-like cells (Chi et al., 2009). Alternaria species commonly produce typical septate conidia (Simmons, 2007). The confirmed presence and localisation of specific endophytic taxa within different plant organs using in planta fluorescence in situ hybridisation (FISH) may enable us to answer important functional questions (Compant et al., 2011), which could also be used for fungi. Using WGA to assess general colonization and implementation of in planta RNA FISH for visualization of living fungi, which may be used simultaneously for more taxa (Vági et al., 2014), could assist us in the provision of important information on the fungal microbiome of the grapevine.

Conclusion

Here, we investigated the above-ground fungal microbiome of the white grapevine variety Furmint in different seasons, phenological stages, plant tissues and vineyards within a fine geographical scale. We also present the first report on the mycobiome of V. vinifera in the Tokaj wine region, Hungary. We found no major differences among communities in distinct neighbouring vineyards, even compared to that of the reference sites in Eger. Our findings indicate that the shoot of the Furmint grapevines has a similar suit of fungal community, and the terroir effect is not likely driven by the fungal microbiome of the plant tissues. For potential biocontrol agents and further applied utilisations, our future focus should be on Aureobasidium pullulans, Cladosporium spp. and the complex Alternaria aff. alternata dominating the community at every site, season and plant organ. The isolation technique used was found to be important in uncovering different Alternaria species, which may have different functions and roles in the plants, and we have revealed that visualization through microscopy can also be a useful tool in studying the grapevine mycobiome.
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**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:
Appendix 1. Supporting Information Experimental Procedures

Fig. S1. The four sampling sites at the vineyards: Betsek (BET), Király (KIR), Szent Tamás (STT) and Úrágya (URA) in Mát, Hungary.

Fig. S2. Effects of (A) sampling site, (B) plant part, (C) year and (D) season on community composition (extracted 2nd PCoA coordinates of the samples) Sites: Betsek (BET), Király (KIR), Szent Tamás (STT), Úrágya (URA) vineyards in Mát, and Hangács (HAN), Nagy-Eged (NEG) vineyards in Eger; plant parts: grape berry (Grape), small juvenile leaves (Young leaf), mature leaves (Old leaf), and woody parts of the grape cluster (Rachis). Values indicate the estimated marginal means ±95% confidence intervals calculated from the general linear models.

Fig. S3. Phylogenetic tree of isolates collected from *Vitis vinifera cf*. Furmint. The 50% majority rule consensus phylogram was inferred from the Bayesian analysis of the ITS sequences. Bayesian posterior probabilities (≥90) are shown as percentages of branches. VVFIS_340 served as the outgroup. The scale bar indicates 0.5 expected changes per site per branch.

Fig. S4. Maximum Likelihood (RAxML) phylogenetic tree of ITS sequences of representative isolates in each clades and reference sequences from GenBank. ML bootstrap support values ≥70% are shown above branches. VVFIS_340 served as the outgroup. The scale bar indicates 0.1 expected changes per site per branch.

Fig. S5. Maximum likelihood (RAxML) phylogenetic tree of representative strains of *Alternaria* Sect. Alternaria and RPB2 sequences of 24 *Alternaria* aff. *Alternata* isolates from the clade 1 collected from *Vitis vinifera cf*. *Furmint*. Isolates collected from grapevine are shown in bold, GenBank accession number are indicated before the isolate names. The lineages are named and labelled sensu Woudenberg et al. (2015). ML bootstrap support values (≥70) are shown at the branches. *Alternaria alternantherae* CBS 124392 served as the outgroup. The scale bar indicates 0.01 expected changes per site per branch.

Table S1. Steps of the bioinformatics pipeline with indication of number of sequences and OTUs and after each step

Table S2. The OTU table containing subsampled reads per sample used for analyses

Table S3. The BLAST table showing identity of the most abundant sequences of each OTU

Table S4. Diversity indices and rarefaction curves of the samples

Table S5. Details of isolates collected from different tissues of Furmint grapevine