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Functional and Structural Microbial Diversity in Organic and Conventional Viticulture: Organic Farming Benefits Natural Biocontrol Agents[▽]†

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Statistically significant differences in the structure and function of above-ground grapevine-associated microorganisms from organically and conventionally managed vineyards were found. *Aureobasidium pullulans*, a copper-detoxifying fungus and biocontrol agent, plays a key role in explaining these differences. The black fungus was strongly enriched in the communities of organically managed plants and yielded a higher indigenous antiphytopathogenic potential.

Contemporary farming systems undermine the wellbeing of communities in many ways. Huge regions of natural habitats, including their ecosystem services, have been destroyed; plant protection measures have caused human health problems, and they are responsible for about 30% of greenhouse gas emissions (15). An alternative to conventional agriculture is offered by organic farming, which aims to minimize its impact on the environment by using crop rotation, pathogen-resistant cultivars, limited amounts of chemical pesticides, and organic manure instead of synthetic fertilizers. However, the beneficial effect of organic agriculture on biodiversity in general and plant-associated microorganisms in particular is still controversial (6, 8, 9, 14).

Vitis vinifera L. is one of the oldest cultivated plants, with high economic importance. In viticulture, comprising an area of 7.4 million hectares of grapevine plants worldwide (FAOSTAT, 2008), the proportion of organically cultivated grapes is strongly increasing (19). Although it was shown that the taste and quality of organically produced wine are better than those of wine produced by conventional viticulture (20), plant protection using mainly copper-containing products is extremely problematic. The latter resulted in high soil contamination, endangering plant health and fruit quality, as well as in the spread of copper- and antibiotic-resistant microbes (4, 13). The impact of copper treatment and other organic plant protection methods on plant-associated microorganisms and their function in ecosystem service is still poorly understood. We hypothesize that the type of plant protection used in conventional and organic vineyards has a great impact on the microbial, and especially the fungal, community structure associated with grapevine plants.

To compare epiphytic and endophytic microbial communities of the phyllosphere of conventionally and organically managed grapevines, we sampled leaves, shoots, and undamaged grapes of the cultivar Sauvignon Blanc in vineyards in Schlossberg, Austria (46°37′N, 15°28′E; owned by the Fachschule für Weinbau und Kellerwirtschaft und Weingut Silberberg), in the last week before the harvest (on 3 October 2006 and 11 September 2007, respectively). Half of the vineyards were managed conventionally, and the other half were managed organically. The chemical plant protection agents used in the conventional plots were sulfur, paraffin oil, manganese-zinc ethylene bis(dithiocarbamate), proquinazid, iprovalicarb, folpet, pyrimethanil, mandipropamid, quinoxyfen, chlorpyrifos-methyl, boscalid, and cyazofamid. The chemicals used in the organic parcel were sulfur, copper, Myco-Sin, potassium water glass, Frutogard, and fennel oil. Four independent composite replicates for each habitat were investigated; the same microbial fraction isolated according to Berg et al. (2) was used for DNA- and cultivation-based analyses.

To perform molecular analysis, total DNA was extracted from samples using the Fast DNA Spin Kit for Soil (Qbiogene, Inc., Carlsbad, CA). Microbial fingerprinting was performed by single-strand conformation polymorphism analysis (SSCP) according to Schwieger and Tebbe (16) with different primers (16, 18). Gels were analyzed with the program GelCompare (Applied Maths, Kortrijk, Belgium) and statistically assessed (11). Gel slices containing single bands were analyzed (16). To quantify *Aureobasidium pullulans*, primers ApuIIF1 (5′-GAT CATTAAAGAGTAAGGGTGCTCA-3′) and ApuIIR1 (5′-G CTCGCCTGGGACGAATC-3′), both developed by the National Exposure Research Laboratory (Cincinnati, OH) were used. For quantification of *Sporidiobolus pararoseus*, primers Spa2f (5′-CCAATCTTTTCTTGTAATCG-3′) and Spa2r (5′-CCTTAATGAAGTTGGCTC-3′) were designed as described in the supplemental material. Primers ITS1 and ITS2 (18) were used for quantification of total fungal internal transcribed spacer 1 (ITS1) copies. The calculated copy number was corrected by PCR efficiency in a sample matrix, which was determined by measurement of serial dilutions of standard fragments in a DNase I-digested sample matrix. Each replicate was analyzed three times. Significances of differences between conventional and organic treatments were calculated by using the unpaired Student *t* test. Melting curves of Spa2f/Spa2r and ApuIIF1/ApuIIR1 reactions resulted in one homogeneous product.

To isolate grape-associated microorganisms, serial dilutions

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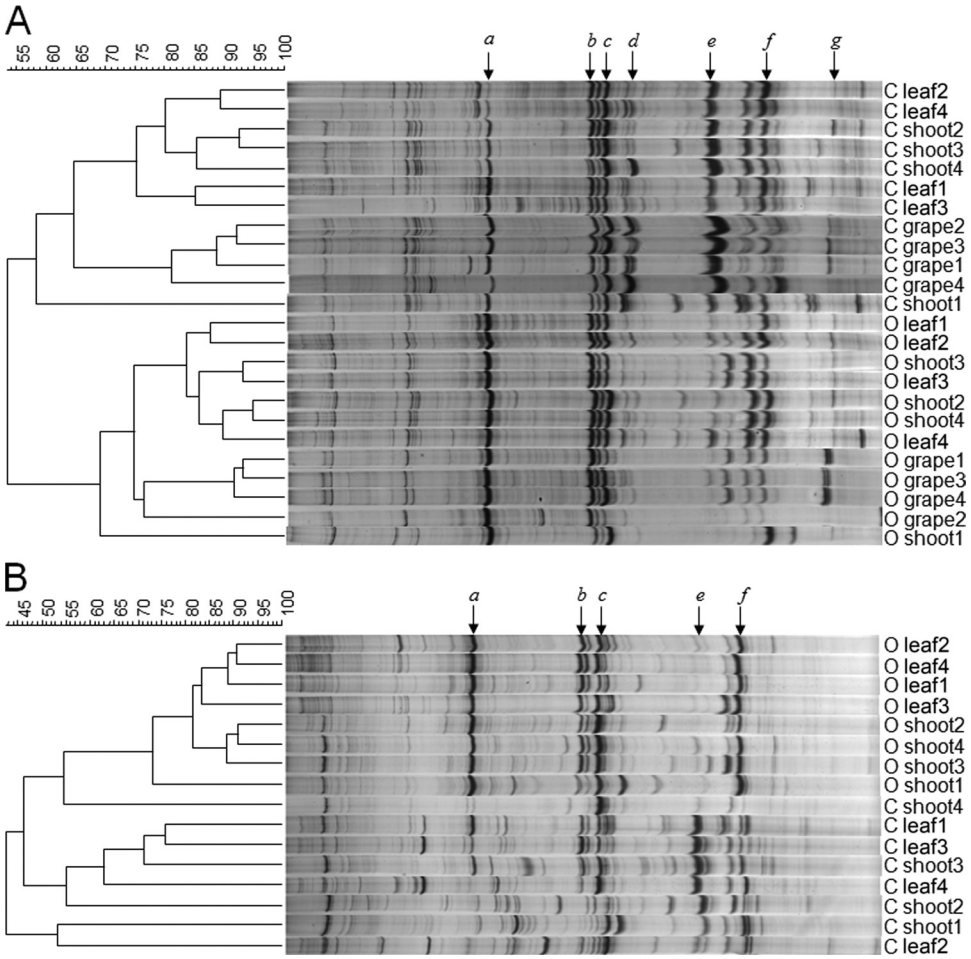

FIG. 1. Clustering of the SSCP profile of the fungal community of grapevine plants showing a clear difference between conventionally (C) and organically (O) managed plants. Samples from shoots, leaves, and grapes were taken 1 week prior to harvest. Community DNA was extracted from the respective plant parts, followed by amplification of the fungal ITS region using general primers. The PCR product was subjected to SSCP analysis. Band patterns were compared and clustered by the unweighted-pair group method using average linkages. Sequences of single bands were obtained and compared to those in the GenBank database using the BLAST algorithm, giving hits for *A. pullulans* (*a*), *Cladosporium* sp. (*b*), *A. tenuissima* (*c*), *Alternaria* sp. (*d*), *S. pararoseus* (*e*), *Epicoccum nigrum* (*f*), and *Cryptococcus flavescens* (*g*). (A) Community profile of ectosphere samples of leaves, shoots, and grapes. (B) Community profile of endosphere samples of leaves and shoots. For sequences, refer to EMBL accession numbers FN430614 to FN430640. Data shown are for samples from 2007.

of the primary cell suspensions were plated on R2A, Sabouraud dextrose agar (Roth, Karlsruhe, Germany) containing 100 μg ml^{-1} chloramphenicol, and synthetic low-nutrient agar (2). From each habitat and replicate, 18 yeast and 15 filamentous fungal isolates were selected randomly and tested for antagonistic activity (1).

Fingerprints from the fungal community were obtained by ITS PCR and SSCP, and their statistical analysis resulted in a clear separation of samples from the conventional and organic management techniques (Fig. 1). *P* values showing statistically significant differences between organic and conventional samples were <0.001, both for ectosphere samples and grape berries (Fig. 1A) and for endosphere samples (Fig. 1B). Altogether, the fingerprints from organically managed plants were more homogeneous and showed a higher similarity to each other than those from conventionally managed plants. From SSCP gels, differing and dominant bands were identified by sequencing. The strongest band (Fig. 1A and B) in samples from the conventional treatment corresponded to *S. pararoseus*, a yeast already described as associated with grapes (12, 20). Conversely, bands for *A. pullulans* were stronger from organic treatment samples. *A. pullulans* is a cosmopolitan yeastlike (black) fungus and is ubiquitously associated with plants, including grapevines (5, 8). *Cladosporium* sp. and *Alternaria tenuissima* were common species found in all of our samples. DNA from a different *Alternaria* that could not be identified to the species level was detected in ectosphere and to a greater extent in grape samples only from conventionally managed plants.

Due to the dominance of *A. pullulans* and *S. pararoseus* bands and their discriminative character in fingerprints of fungal communities, both fungi and total fungal ITS copy numbers were determined using quantitative PCR. In general, the fungal ITS copy number was higher in organic than in conventional agriculture samples (data not shown). The relative quantification of *A. pullulans* ITS copy numbers showed a higher

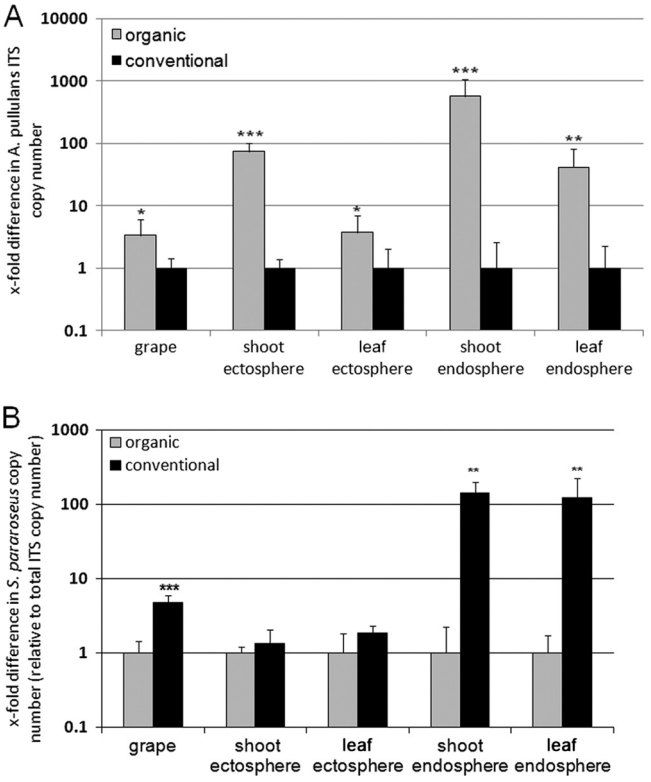

FIG. 2. ITS copy numbers per gram of fresh weight determined by quantitative PCR. (A) Specific primers were used for determination of *A. pullulans* ITS1 copy numbers in samples from organically and conventionally managed plants. (B) ITS2 copy numbers of *S. pararoseus* determined using specific primers relative to total fungal ITS copy numbers determined with general fungal primers. The relative copy number of samples from conventionally managed plants is shown in relation to the copy number of samples from organically managed plants. Error bars indicate standard deviations, and asterisks indicate significances of differences with respect to management type (*, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$). Data shown are for samples from 2007.

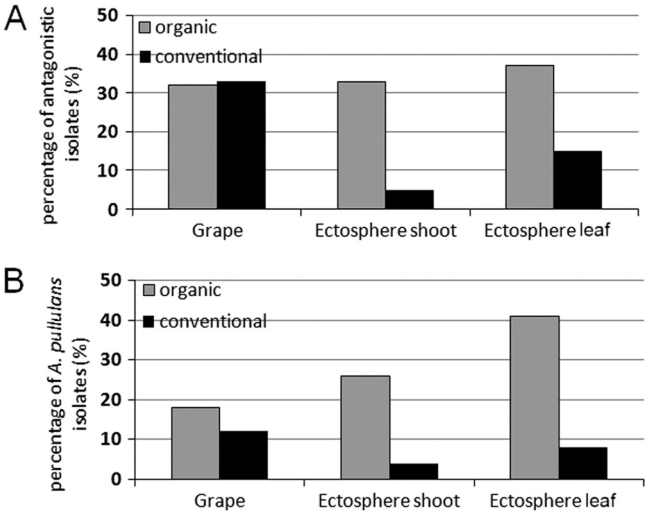

FIG. 3. Correlation of the antagonistic potential of different plant parts and plant protection management systems with the relative abundance of *A. pullulans*. Yeasts and filamentous fungi were isolated from samples of organically (gray bars) and conventionally (black bars) managed Sauvignon Blanc grapevines. (A) Antagonistic potential of isolates assayed by using dual-culture plate assays determining the percentage of isolates with antagonistic activity against *B. cinerea*. (B) Percentage of *A. pullulans* isolates among isolates determined by morphological characterization. Each value was calculated from a total of 132 isolates (72 yeast and 60 filamentous fungal isolates). Data are for samples from 2007.

abundance of this species in organically than in conventionally managed plants (Fig. 2A). This result was statistically significant for all investigated samples from both years, with only one exception (grapes in 2006, $P = 0.43$). Significantly larger relative amounts of *S. pararoseus* DNA were present in the grapes and endosphere samples of conventionally managed plants (Fig. 2B). A tendency toward higher *S. pararoseus* ITS copy numbers was seen in the ectosphere samples of conventionally managed plants. Results were confirmed by analyzing samples from 2006 (data not shown).

Above-ground microhabitats of grapes were highly colonized by microorganisms; up to 5.7 $\log_{10}$ CFU were calculated for bacteria, 5.2 $\log_{10}$ CFU were calculated for yeasts, and 5.3 $\log_{10}$ CFU were calculated for fungi. Statistically significant differences in CFU counts between samples of organically and conventionally managed plants were found; e.g., on/in organically managed plants, higher abundances of filamentous fungi and yeasts were isolated. The antiphytopathogenic potential of fungal isolates, estimated by *in vitro* antagonism toward *Botrytis cinerea* (1), on organically managed plants was greater than that on conventionally managed plants (Fig. 3A). As 33 out of 34 *A. pullulans* isolates showed antagonistic activity against *B. cinerea in vitro* (data not shown), we assessed the contribution of the fungus to the total indigenous antagonistic potential. In addition to results of quantitative PCR, cultivation of *A. pullulans* yielded a higher abundance in samples from organically managed plants than in those from conventionally treated plants (Fig. 3B). All of our results support a key role for the yeastlike fungus *A. pullulans* in explaining the structural and functional differences between the two agricultural systems. Interestingly, *Aureobasidium* can utilize inorganic sulfur (10) and is able to absorb, and in this way detoxify, copper (7). These properties can explain the enrichment under organic farming conditions, which was also reported for apples under storage conditions (8). Furthermore, *A. pullulans* is a well-studied potent antagonist of several fungal pathogens; mechanisms of *A. pullulans* antagonism against fungi include competition for nutrients and space and production of cell wall-degrading enzymes (5). Interestingly, typical flavor components of wine were detected as being produced by *A. pullulans* (17), which can explain the better taste of organically produced wine (20).

In addition, two well-known dominant groups of plant-associated bacteria were analyzed to compare bacterial fingerprints: *Pseudomonas* and *Firmicutes* (1, 3). Although cultivable cell numbers of bacteria differed between the two treatments, no significant difference between the community profiles of the two bacterial groups was found. Results led to the conclusion that vineyard management has no influence on the bacterial, at least the *Pseudomonas* and *Firmicutes*, community. This can be explained by the fact that mainly antifungal substances are used for plant protection in both management systems.

In our study, we showed that plant protection in conventional and organic vineyards influenced grape-associated microorganisms. The most interesting fact was that not only the structure but also the function of the fungal community was affected. In/on organically managed grapevine plants, the number of *in vitro* antagonists was enhanced due to an enrichment of *A. pullulans*. Despite the fact that this is not direct evidence for a biocontrol effect on grapes, there are hints at this beneficial interaction. *A. pullulans* was described as a potent *ad planta* antagonist (5), and biocontrol products against *Botrytis* based on this fungus are already on the market (Botector; bio-ferm, Tulln, Austria). Biological control comprises the application of naturally occurring antagonists as biocontrol agents, as well as management of the indigenous antagonistic potential (3). Our study was an unexpected but interesting example of the latter and showed that basic knowledge of the structure and function of plant-associated microbial communities is essential for the development of environmentally friendly strategies for plant protection.

Nucleotide sequence accession numbers. The sequences obtained in this study were submitted to EMBL and assigned accession no. FN430614 to FN430640.

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REFERENCES

1. **Berg, G., et al.** 2002. Plant-dependent genotypic and phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticillium* host plants. Appl. Environ. Microbiol. **68:**3328–3338.
2. **Berg, G., et al.** 2005. Impact of plant species and site on rhizosphere-associated fungi antagonistic to *Verticillium dahliae* Kleb. Appl. Environ. Microbiol. **71:**4203–4213.
3. **Berg, G.** 2009. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. J. Appl. Microbiol. Biotechnol. **84:**11–18.
4. **Berg, J., A. Tom-Petersen, and O. Nybroe.** 2005. Copper amendment of agricultural soil selects for bacterial antibiotic resistance in the field. Lett. Appl. Microbiol. **40:**146–151.
5. **Chi, Z., et al.** 2009. Bioproducts from *Aureobasidium pullulans*, a biotechnologically important yeast. Appl. Microbiol. Biotechnol. **82:**793–804.
6. **Crowder, D. W., T. D. Northfield, M. R. Strand, and W. E. Snyder.** 2010. Organic agriculture promotes evenness and natural pest control. Nature **466:**109–112.
7. **Gadd, G. M., and L. de Rome.** 1988. Biosorption of copper by fungal melanin. Appl. Microbiol. Biotechnol. **29:**610–617.
8. **Granado, J., et al.** 2008. Culturable fungi of stored 'Golden Delicious' apple fruits: a one-season comparison study of organic and integrated production systems in Switzerland. Microb. Ecol. **56:**720–732.
9. **Hole, D. G., et al.** 2005. Does organic farming benefit biodiversity? Biol. Conserv. **122:**113–130.
10. **Killham, K., N. D. Lindley, and M. Wainwright.** 1981. Inorganic sulfur oxidation by *Aureobasidium pullulans*. Appl. Environ. Microbiol. **42:**629–631.
11. **Kropf, S., H. Heuer, M. Grüning, and K. Smalla.** 2004. Significance test for comparing complex microbial community fingerprints using pairwise similarity measures. J. Microbiol. Methods **57:**187–195.
12. **Li, S. S., et al.** 2010. Yeast species associated with wine grapes in China. Int. J. Food Microbiol. **138:**85–90.
13. **Pietrzak, U., and D. C. McPhail.** 2004. Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. Geoderma **122:** 151–166.
14. **Ruimy, R., et al.** 2010. Organic and conventional fruits and vegetables contain equivalent counts of Gram-negative bacteria expressing resistance to antibacterial agents. Environ. Microbiol. **12:**608–615.
15. **Sachs, J., et al.** 2010. Monitoring the world's agriculture. Nature **466:**558–560.
16. **Schwieger, F., and C. C. Tebbe.** 1998. A new approach to utilize PCR-single strand conformation polymorphism for 16S rRNA gene-based microbial community analysis. Appl. Environ. Microbiol. **64:**4870–4876.
17. **Verginer, M., E. Leitner, and G. Berg.** 2010. Production of volatile metabolites by grape-associated microorganisms. J. Agric. Food Chem. **58:**8344–8350.
18. **White, T. J., T. Bruns, S. Lee, and J. W. Taylor.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p. 315–322. *In* M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White (ed.), PCR protocols: a guide to methods and applications. Academic Press, Inc., New York, NY.
19. **Willer, H.** 2008. Europe: statistics, support schemes and research, p. 134–142. *In* H. Willer and M. Yussefi (ed.), The world of organic agriculture—statistics and emerging trends 2007. International Federation of Organic Agriculture Movements, Bonn, Germany.
20. **Yıldırım, H. K., Y. D. Akçay, Y. Güvenç, and E. Y. Sözmen.** 2004. Protection capacity against low-density lipoprotein oxidation and antioxidant potential of some organic and non-organic wines. Int. J. Food Sci. Nutr. **5:**351–362.