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Biodiversity of *Vitis vinifera* endophytes in conventional and biodynamic vineyard

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Abstract: Plants are permanently exposed to biotic and abiotic stress and have therefore developed intricate resistance mechanisms, consequently. These include the presence of microbial endophytes, which can promote plant growth and ensure better resilience against unfavourable conditions. These microorganisms colonising plant tissues can directly affect plant growth by producing phytohormones, antioxidants, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, or indirectly by the production of siderophores and antifungal agents. To the best of our knowledge, this is the first study devoted to assessing bacterial endophyte diversity and their plant growth-promoting properties in two utterly distinct vineyards in view of agricultural management (conventional, biodynamic) in the Czech Republic. With these different agricultural approaches, we hypothesised different numerical representations of bacterial endophytes acquired from vine shoots and leaves, which was not proved ($P = 0.743$, $F = 0.129$). A total of 470 distinct bacterial endophytes were isolated from the *Vitis vinifera* plants from the conventional and biodynamic vineyard and from which over 80% were identified by the matrix-assisted laser desorption-time-of-flight mass spectrometry (MALDI-TOF MS). In both vineyards, the dominant bacterial genus was *Bacillus,* followed by *Pantoea*, *Pseudomonas* and *Staphylococcus.* Plant-promoting endophyte properties varied with respect to the season and type of vineyard. The ability to produce indole-3-acetic acid (IAA) and ACC deaminase was higher in the biodynamic vineyard, in comparison with antioxidant activity, which was found in a higher proportion in isolates from the conventional vineyard.

Keywords: 1-aminocyclopropane-1-carboxylate deaminase; antioxidant activity; grapevine; indole-3-acetic acid; matrix-assisted laser desorption-time-of-flight mass spectrometry (MALDI-TOF MS)

Vitis vinifera is one of the world's most commonly cultivated and economically most important plants. Vineyards cover 7 million ha worldwide, and annual production is around 27 million tons of table grapes (Andreolli et al. 2017). Of the 8 million hectares of vineyards worldwide, 90% are managed conventionally, 9% organically and the remaining 1% biodynamically (Soustre-Gacougnolle et al. 2018). In recent years, there has

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been an increased interest in growing crops while maintaining soil fertility. Organic farming treats the grapevines in an alternative way, replenishing organic matter in the soil, although yields tend to be lower. The main idea of biodynamic farming is that each cultivated area is a live complex and interconnected ecosystem which can regulate itself. The vine is treated exclusively with natural compounds (compost, animal dung, apatite, and plant extracts). The Ecological Agriculture Act (Meissner et al. 2019) is applied as well. On the contrary, conventional agriculture strives to achieve the highest possible productivity with the help of industrially produced fertilisers and preservatives, such as pesticides and herbicides, which have a demonstrable adverse effect on the environment (Barbosa et al. 2015).

All plants, including the grapevine, are constantly subject to environmental stimuli and stresses that can significantly affect their growth (Kolouchova et al. 2005; Dastogeer et al. 2018). Extreme conditions like drought, salinity, low and high temperatures, acidic conditions, heavy metal stress and nutrient stress limit plant growth and development. Besides external environmental conditions, plant growth can be influenced by internal factors such as microbial endophytes. The plant can host a diverse range of endophytes. Endophytes are asymptomatic microorganisms that colonise plant tissues. They can be found in roots, stems, or leaves and inhabit mainly vascular bundles, intercellular spaces and the cell cytoplasm. Bacterial endophytes may affect plant growth directly through improved nutrient uptake, growth hormone production, production of antioxidants, 1-aminocyclopropane-1-carboxylate (ACC) deaminase production, or indirectly by the production of siderophores or antifungal agents (Bulgari et al. 2009). In return, endophytes use plants as protection against adverse environmental processes (chemical, osmotic, and thermal stresses) (Khan et al. 2016). The proportion of individual endophytes in a plant depends on many factors, including the part of the plant from which they were isolated, the age of the plant, the condition of the plant or the method of cultivation (Campisano et al. 2015).

Endophytes can also play a crucial role in plant health, growth and general physiology (Campisano et al. 2015). They can be a source of new potential natural products for application in medicine, agriculture, and industry. Endophytes have even been investigated as biocontrol agents for their ability to enhance the bioremediation potential of a plant (Pancher et al. 2012). Due to their exceptional properties, interest in their closer examination has recently been growing.

Several studies have recently focused on the influence of various factors (soil composition, cultivation method, locality and climate) on the number of endophytes and their impact on plant growth and the content of health-promoting substances (Vitulo et al. 2019; Stranska et al. 2021). The influence of soil management on the content of microorganisms and indicators of microbial activity in the vine plant was discussed by Freitas et al. (2011). In this study, the authors demonstrated a significant effect of organic vine growing on both microorganism content and microbial activity indicators such as fluorescein diacetate activity (FDA) and an increase in microbial biomass carbon. Pancher et al. (2012) and Schmidt et al. (2011) also compared the effect of organic and conventional farming on endophyte diversity in grapevine shoots. Both studies found a significant impact of organic vine growing on endophyte diversity in the vine plant.

Endophytic bacteria are most often isolated after surface sterilisation, followed by fragmentation of plant tissue and cultivation on agar media. 16 rRNA sequencing (Manias et al. 2020) or the matrix-assisted laser desorption-time-of-flight mass spectrometry (MALDI-TOF MS) technique could be used to identify the bacteria (Angeletti 2017).

This study was focused on the assessment of the diversity of bacterial endophytes from vineyards in the Czech Republic by comparing the conventional vineyards and biodynamic vineyards in the Mělník subregion. For further characterisation, we studied endophyte properties supporting plant growth [ACC deaminase, indole-3-acetic acid (IAA), antioxidant production].

MATERIAL AND METHODS

Biological material and sampling

Endophytic microorganisms were isolated from the leaves and shoots of *Vitis vinifera* (varieties Müller Thurgau, Pinot Gris, Pinot Noir, Riesling) from conventional and biodynamic vineyards in the Czech wine region, Mělník subregion, Czech Republic, during the growing season spring 2018 – winter 2019. The vineyards were selected based on their appropriate geographical location and applied vine-growing methods. It was confirmed that there is no conventional vineyard in its vicinity, so the vine properties, including endophytes, cannot be affected by possible drift or flushing of chemicals used to treat the conventional vineyard. The soil composition of the two vineyards was as follows: loam-sandy soil, subsoil calcareous sediments of the Mesolithic Age, clays and loess. Grapevine plants were approximately 12 years old.

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Plant material (shoots and leaves) was collected aseptically to isolate endophytes from *Vitis vinifera*. Isolates from the shoots were taken from the middle part of the stems in an approximate length of 2–3 cm. Seven samples of leaves and shoots from seven different plants were taken from each variety (Müller Thurgau, Pinot Gris, Pinot Noir, Riesling), which were put together into one representative sample. The utensils used for collecting samples (scissors, tweezers) were sterilised with a 70% ethanol before use. Plant samples were collected and immediately washed with 70% ethanol to eliminate surface microorganisms and placed in sterile Falcon test tubes with 30 mL of sterile $MgSO_4$ solution (1.2 g·L^{-1}), which were transferred to an ice bucket. Samples were processed within 24 h (Table 1).

Isolation and identification of endophytes

Endophyte isolation. After the surface sterilisation of harvested plant material, 2 g of representative sample was homogenised with 20 mL of phosphate buffer. After homogenisation, four dilution sets (1 : 10, 1 : 100, 1 : 1 000 and 1 : 10 000) were prepared from the samples, from which 100 µL was transferred to Petri dishes with agar containing 50% tryptic soy agar (TSA) medium and fungal growth inhibitor (cycloheximidine 100 µg·mL^{-1}). TSA plates were incubated in the dark for 3 days at 28 °C. Individual colonies were transferred into Petri dishes with plate count agar (PCA) medium for characterisation or TSA medium for further storage and testing.

Matrix-assisted laser desorption-time-of-flight mass spectrometry (MALDI-TOF MS). Bacterial isolates were identified by MALDI-TOF MS according to Uhlík et al. (2011) using an Autoflex Speed MALDI-TOF spectrometer and MALDI Biotyper 2.0 software (Bruker Daltonics, Bremen, Germany).

Plant growth-promoting properties of endophytes

ACC deaminase activity. The bacterial endophyte isolates were grown in 14 mL tubes with 5 mL lysogeny broth (LB) medium (aerobic cultivation, 28 °C, 24 h, 2.5 Hz). The biomass was centrifuged (5 min, 8000 × g), and the cell pellet was washed twice with Dworkin and Foster minimal salt medium (DF), resuspended in DF-ACC (DF medium without $(NH_4)_2SO_4$ supplemented with 3 mM ACC) medium, and cultured (28 °C, 24 h). A negative control (DF-ACC medium) was incubated at the same time. The supernatant (100 µL) was diluted with 900 µL of DF medium, and 60 µL of diluted supernatant with the addition of 120 µL of ninhydrin reagent was transferred to a strip of PCR tubes. The strips were incubated in boiling water for 30 min. From the strip of PCR tubes, 100 µL of the sample was transferred to a microtiter plate, and the absorbance at 570 nm was measured (Li et al. 2011).

Production of antioxidants. The bacteria were cultured in 14 mL tubes with 5 mL LB medium (28 °C, 7 days, 2.5 Hz rpm). The grown biomass was centrifuged (5 min, 8 000 × g), and the supernatant was collected for subsequent absorbance measurements. Ascorbic acid, dissolved in distilled water, was used as a standard. An aliquot of 100 µL of the sample was pipetted together with 200 µL of DPPH at a concentration of 52 mg·L^{-1} (in methanol) in the wells. Distilled water was used as a blank, and pure LB medium was used as the second blank to eliminate the effect of medium components on the determination. The plate was incubated in the dark for 15 min. The absorbance was measured at 517 nm. The resulting change from the blank was expressed as a percentage and converted to ascorbic acid equivalents used as a standard (Fidler and Kolářová 2009).

Determination of indole-3-acetic acid production. The bacteria were cultured in LB medium supplemented with 0.1% tryptophan (5 mL LB medium, 28 °C, 7 days, 2.5 Hz). After centrifugation (10 min, 9 000 × g), 1 mL of the supernatant was transferred to tubes with 2 mL of Salkowski reagent and incubated (room temperature, 25 min). 100 µL of the sample was transferred to a microtiterre plate, and the absorbance was measured at 530 nm. The auxin concentrations were estimated using a standard calibration curve of IAA (0 – 100 µg·L^{-1}) (Xin et al. 2009).

Table 1. Number of endophytic bacteria isolated from the conventional vineyard and biodynamic vineyard as dependent on the season during 2018–2019

Sampling season	Vineyard	Number of isolates	Material
Spring 2018	conventional	55	shoots and leaves
	biodynamic	87	
Summer 2018	conventional	75	shoots and leaves
	biodynamic	20	
Autumn 2018	conventional	77	shoots and leaves
	biodynamic	56	
Winter 2019	conventional	42	shoots
	biodynamic	58	

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Statistical analysis

Statistical analysis was performed in RStudio, where one-way and two-way analysis of variance (ANOVA) with Tukey's post hoc test was used for the comparisons of the colonisation parameters and plant growth-promoting properties of endophytes between the vineyards. In the case of non-normal distributions, the data were transformed to obtain the normal distributions using a natural logarithm before statistical analysis.

RESULTS

Isolation and identification of endophytes

In winter 2019, the endophytes were isolated only from shoots and in the remaining seasons (spring – autumn 2018), from shoots and leaves. During this time, a total of 470 strains of endophytic bacteria were isolated from the grapevine, of which 81% were identified [Electronic Supplementary Material (ESM), Table S1 and S2; for ESM see the electronic version]. A total of 249 endophytes were isolated from a conventional vineyard; 31% belonged to the genus *Bacillus*, 18% to *Pantoea* and 10% to *Pseudomonas*. Altogether 221 endophytes were isolated from samples originating from a biodynamic vineyard, 41% of the isolates belonging to the genus *Bacillus*, 10% to the genus *Staphylococcus* and 8% to *Pantoea*. In both vineyards, bacteria from the *Bacilli* class were the most frequently represented (36% in the conventional, 52% in the biodynamic vineyard), followed by the *Gammaproteobacteria* class (34% in the conventional, 20% in the biodynamic vineyard) (Figure 1 and 2). Overall, bacteria from the phylum *Firmicutes* were the most frequently represented in both the conventional and biodynamic vineyard, followed by the phylum *Proteobacteria* and *Actinobacteria*. The number of isolated endophytes in the vineyards depending on the season, and the percentage of endophytes in vineyards by species are shown in Figures S1 and S2 (ESM).

The representation of bacterial classes in the grapevine varied according to the season. Still, no statistically significant difference was found in the total number of identified bacterial species between seasons (P = 0.786, F = 0.363). The *Bacilli* class predominated in the conventional vineyard in spring, *Gammaproteobacteria* were dominant in the remaining seasons. The *Bacilli* class predominated in the biodynamic vineyard in spring, summer and winter, and the *Gammaproteobacteria* class was dominant in autumn, similarly to the conventional vineyard. Our results are consistent with the literature, where a greater biodiversity of endophytes was found in organic farming when comparing organic and conventional plant cultivation (Freitas et al. 2011; Schmid et al. 2011; Vitulo et al. 2019).

Plant growth-promoting properties of endophytes

Production of ACC deaminase. When comparing the ability of endophytes to synthesize ACC deaminase,

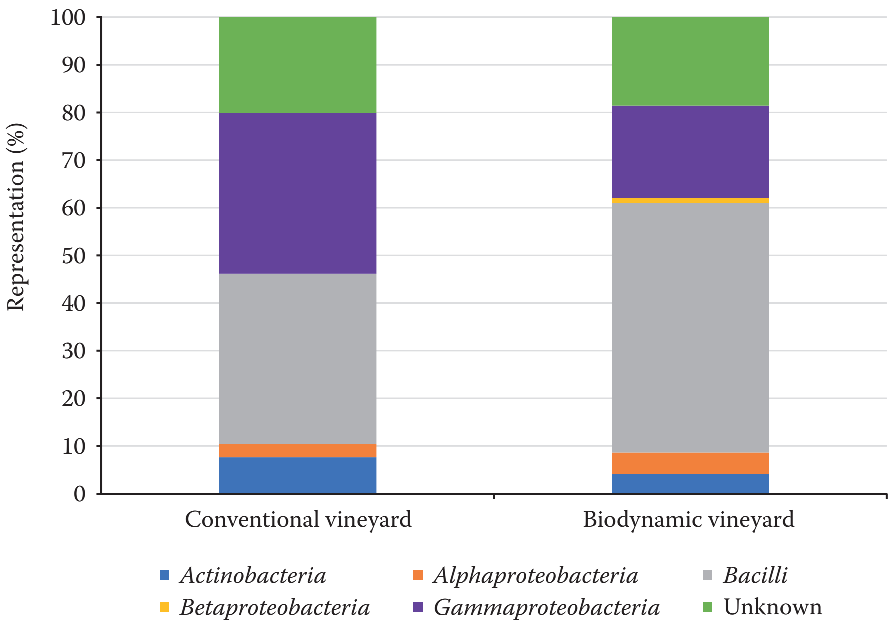

Figure 1. Isolation and identification of endophytes from conventional and biodynamic vineyard. Percentage of endophytes in vineyards by classes

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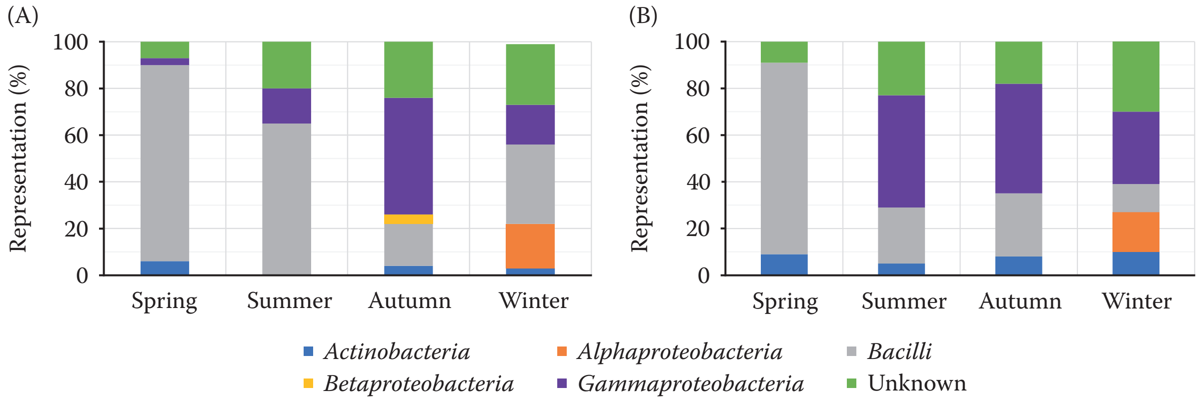

Figure 2. Isolation and identification of endophytes from conventional and biodynamic vineyard. Percentage of endophytes in vineyards by season; (A) biodynamic vineyard; (B) conventional vineyard

statistically significant differences were found between the monitored vineyards both in the type of vineyard ($P = 0.005$, $F = 8.152$) as well as in the season when the samples were collected ($P < 0.001$, $F = 76.809$). In a conventional vineyard, the ability to produce ACC deaminase was found in 15% of isolates, and in a biodynamic vineyard, in 25% of isolates. As seen from Figure 3, the highest proportion of bacteria with ACC deaminase activity was isolated from samples from a biodynamic vineyard in winter (62%), in contrast to a conventional vineyard, where the maximum representation was found in spring (27%).

Antioxidant activity. Similarly to ACC deaminase activity, antioxidant activity showed statistically significant differences for the type of vineyard ($P = 0.013$, $F = 6.222$) as well as for the sampling season ($P < 0.001$, $F = 266.950$). Antioxidant activity of bacteria was divided into low (below 10 $mg_{AA}·L^{-1}$; AA – equivalents of ascorbic acid), medium (above 10 $mg_{AA}·L^{-1}$) and high (above 20 $mg_{AA}·L^{-1}$) according to the concentration of AA. All summer and autumn isolates had the ability to show antioxidant activity at concentrations above 10 $mg_{AA}·L^{-1}$ (Figure 4). In a conventional vineyard, 68% of isolates showed medium/high (concentration above 10 $mg_{AA}·L^{-1}$) activity in spring and winter, in a biodynamic vineyard, it was 64%. The highest detected antioxidant activity produced by the *Pantoea agglomerans* was found in autumn samples from both vineyards. The antioxidant activities were 26.5 $mg_{AA}·L^{-1}$ in a conventional vineyard and 28.6 $mg_{AA}·L^{-1}$ in a biodynamic one.

Production of indole-3-acetic acid (IAA). As with antioxidant activity, all endophytes isolated in summer and autumn had a medium/high (production of IAA above 1 $\mu g·L^{-1}$) ability to produce IAA (Figure 5).

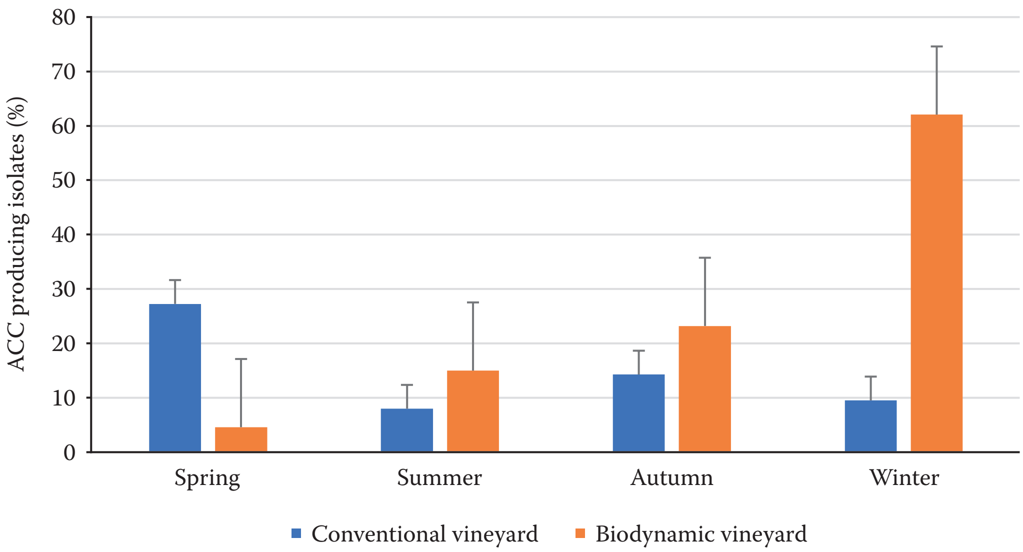

Figure 3. 1-aminocyclopropane-1-carboxylate (ACC) deaminase production determined in endophytes isolated from the conventional vineyard and biodynamic vineyard as dependent on the season during 2018–2019

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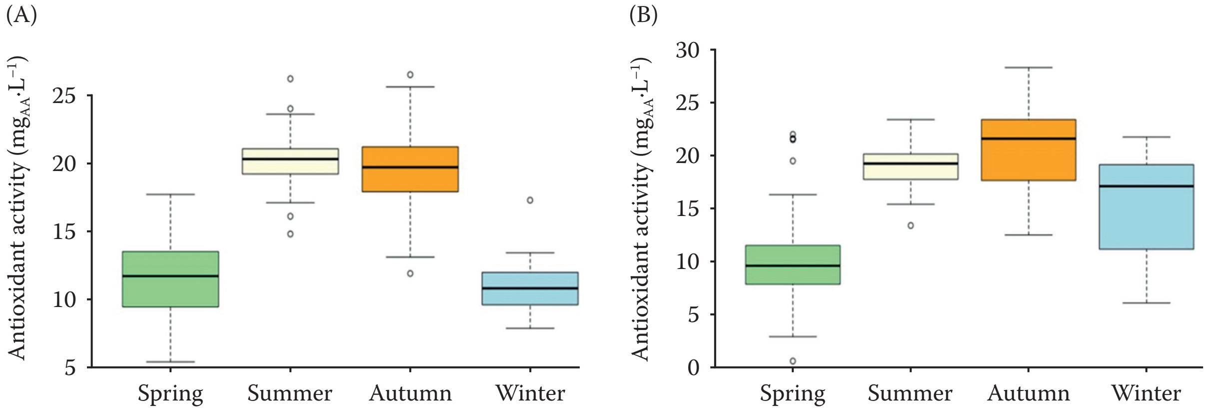

Figure 4. Box plot showing the production of antioxidants by endophytes in individual seasons, (A) in a conventional and (B) a biodynamic vineyard

Centre lines – medians; box limits – the 25th and 75th percentiles (whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles); dots – outliners

When the obtained results were compared, statistically significant differences for the type of vineyard ($P < 0.001$, $F = 13.884$) as well as for the sampling season ($P < 0.001$, $F = 43.737$) were observed. The highest difference in IAA production between the two vineyards was recorded in spring. During this period, bacteria of the genus *Bacillus* predominated in both the conventional and biodynamic vineyards (Table S1 and S2, ESM). In the conventional vineyard, the percentage of bacteria and their diversity in spring were lower compared to the biodynamic vineyard, which could cause a difference in IAA production. In spring isolates from conventional vineyards, only 5% of endophytes produced IAA above 1 $\mu g·L^{-1}$, but in winter, the percentage of bacteria with this ability increased to 81%. In the biodynamic vineyard, the percentage of bacteria with medium/high IAA production did not differ much in spring and winter (84% versus 93%).

DISCUSSION

Isolation and identification of endophytes

Bacteria can enter the plant from both the soil and the air. Soil is one of the wealthiest microbial sources, and bacteria from the phyla *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* are most

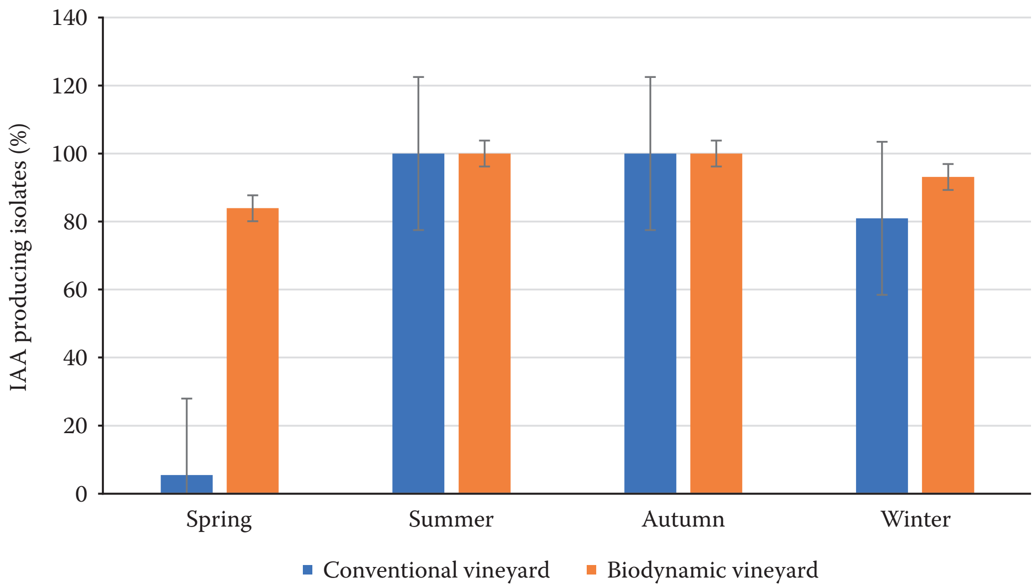

Figure 5. Production of indole-3-acetic acid (IAA) (above 1 $\mu g·L^{-1}$) determined in endophytes isolated from the conventional vineyard and biodynamic vineyard as dependent on the season during 2018–2019

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often present. The above-ground part of the plant consists mainly of leaves, the predominant phyllosphere phyla are *Actinobacteria*, *Firmicutes* and *Proteobacteria* (Bulgarelli et al. 2013). The highest density of endophytes is in the roots, and their incidence decreases acropetally (towards the apex). Therefore, the incidence and diversity of these bacteria in flowers, fruits and seeds are very low compared to other parts of the grapevine plant, despite their ability to move to the above-ground parts of the plant (Compant et al. 2011).

Although no statistically significant difference between the vineyards in the number of endophytic bacteria detected ($P = 0.743$, $F = 0.129$) was observed, we found that the proportion of major bacteria isolated from grapevine leaves was very similar in both vineyards (the genus *Bacillus* was the most frequently represented, then *Pantoea* and *Staphylococcus*). The spectrum of endophytes was different, which may be due to different agricultural practices that may have an impact on soil microorganisms as potential plant colonisers. Our results correspond to the literature when Suman et al. (2016) examined the representation of endophytes in 17 plants, they found the above-mentioned phyla as dominant (Suman et al. 2016).

Changes observed in biodiversity could be caused by the growth temperature optima of individual bacteria, by changes in the plant physiology in the growth phases, or by alternating external temperatures (Shen and Fulthorpe 2015). Some studies also reported the dependence of the proportion of endophytes in the grapevine on the season (Bulgarelli et al. 2013; Baldan et al. 2014). The diversity of microorganisms may also have been caused by changes in the composition of soil bacteria, the developmental stage of the plant or agronomic practices. Several studies have confirmed the effects of pesticides or chemical fertilizers fertilisers on the soil bacterial community and the phyllosphere community (Prashar and Shah 2016; Arora et al. 2019).

Our tested biodynamic vineyard belongs to organic agriculture, which seeks to optimise soil microbial communities and their diversity. Adding organic supplements and using humus should have a beneficial effect on soil microorganisms due to their increased diversity (Xia et al. 2015). Hole et al. (2005) summarised 14 papers that addressed the differences in microbial communities between organic and conventional systems. This study found that the content of microbial biomass on organic farms was higher by 10–26%. In our study, 249 bacteria (4 classes, 17 genera, 40 species) were isolated from a conventional vineyard. Fewer endophytes were isolated from the biodynamic vineyard (221), but they were richer in genera and species (5 classes, 20 genera, 49 species). The genera *Clavibacter*, *Rothia* and *Salmonella* were isolated only from the conventional vineyard, while the genera *Bravibacillus*, *Burkholderia*, *Erwinia*, *Methylobacterium*, *Ralstonia* and *Sphingobium* were obtained only from the biodynamic vineyard.

According to Campisano et al. (2015), the reduced taxonomic diversity does not necessarily mean the loss of significant properties used in agriculture. This study shows that 25 genera were isolated from the wild grapevine and only 6 from the domesticated grapevine. Still, endophytes from bred grape vine showed better plant growth-promoting properties and better biocontrol activity (Campisano et al. 2015). The genus *Bacillus* was the most abundant in both vineyards (conventional vineyard: 39%, biodynamic one: 50%), and most of these bacteria were isolated in spring and summer. This genus also predominated in other studies on grapevine endophytes, e.g. in West et al. (2010), it was 26%, and in Baldan et al. (2014), it covered 30% of all examined endophytes. After the genus *Bacillus*, a large part of the isolates was the genus *Pantoea* (conventional: 23%, biodynamic: 10%), which was most common in the grapevine in summer and autumn. *Pantoea agglomerans* could be used as a biocontrol agent as it has the potential of secretion of antibacterial agents and the ability to activate induced systemic plant resistance. *Pantoea agglomerans* was recently successfully applied as an agent against fungal diseases in apple trees and pear trees caused by *Erwinia amylovora* (Bulgari et al. 2009).

Plant growth-promoting properties of endophytes

Production of ACC deaminase (1-aminocyclopropane-1-carboxylic acid deaminase). Endophytes can enhance the plant's resistance to drought or temperature extremes. One possible mechanism is the production of ACC deaminase, a thiol enzyme commonly found in many soil microorganisms. The higher percentage of bacteria producing ACC deaminase in a conventional vineyard in spring could be due to abiotic stress caused by sunlight (UV radiation) which was recorded above the long-term average during this period (Czech Hydrometeorological Institute 2022) and in this case the producers of this enzyme were mainly bacteria of the genus *Bacillus*. The higher deaminase activity detected in the samples taken in winter from the biodynamic vineyard could be the higher number of rainfalls in this period, when the grape vines could be infested with fungal pathogens.

Antioxidant activity. Antioxidants could accumulate during biotic and abiotic stresses, such as UV radiation, low temperatures, mechanical damage, low nutrient intake or the presence of pathogens (Sakihama et al. 2002). The high ability to produce antioxidants was found during summer and autumn. During these seasons, the plant is more strongly exposed to UV radiation, and consequently, it needs a higher content of antioxidants. In summer and autumn, the grapevine was mostly inhabited by bacteria of the genera *Bacillus*, *Pantoea* and *Pseudomonas*, which are producers of molecules with good antimicrobial and antioxidant activity (Akinsanya et al. 2015).

Determination of indole-3-acetic acid (IAA) production. IAA is the primary endogenous auxin that plays a vital role in regulating plant root growth. Production of phytohormones (particularly IAA) by bacteria may directly increase the growth and yield of plants. Studies show that there is also a difference in the production of IAA between endophytes isolated from the roots (higher production) and the aerial parts of the plant (Sorokan et al. 2021).

The highest determined concentration of IAA (24 $\mu g \cdot L^{-1}$) belonged to *Pseudomonas koreensis* isolated in summer from a conventional vineyard, in the case of a biodynamic vineyard, the highest concentration (14 $\mu g \cdot L^{-1}$) was produced in summer by *Bacillus megaterium*. IAA levels decrease in plants exposed to stress such as virus infection or abiotic stress (Niculcea et al. 2013; Cui et al. 2016). This may explain the low IAA values in spring in the conventional vineyard, where the plants were exposed to 30% slower total precipitation. Phetcharat and Duangpaeng (2012), and Melo et al. (2016) monitored IAA production by endophytic and rhizospheric bacteria depending on the type of agriculture, with no apparent differences. The genus *Pantoea* is one of the good producers of IAA (Sergeeva et al. 2007), which is why its incidence was high in summer and autumn, when the plant went through stages of growth and maturation, which presents favourable conditions of this microorganism and plant interactions.

CONCLUSION

The diversity of the bacteria in both the conventional and biodynamic vineyard was highly influenced by the season of the sample collection, which is in connection with different and, during some season's unfavourable ambient conditions. Although the effect of the cultivation method on the number of microbial endophytes was not statistically significant ($P = 0.743$), statistically significant differences were found in most of the studied plant growth-promoting properties.

Altogether 88% of bacteria in the conventional vineyard had the ability to produce antioxidants above 10 $mg \cdot L^{-1}$ in comparison with 74% in the biodynamic vineyard. The endophyte *Pantoea agglomerans* grew the highest antioxidant activity in both vineyards (26.5 $mg \cdot L^{-1}$ in a conventional vineyard, 28.6 $mg \cdot L^{-1}$ in a biodynamic one). The best producers of the phytohormone IAA were *Pseudomonas koreensis* in the conventional vineyard (24 $\mu g \cdot L^{-1}$) and *Bacillus megaterium* in the biodynamic vineyard (14 $\mu g \cdot L^{-1}$). In the conventional vineyard, 76% of bacteria had the ability to produce IAA above 5 $\mu g \cdot L^{-1}$, in the biodynamic vineyard, the ratio was 92%. The ability to produce ACC deaminase was higher in isolates from the biodynamic vineyard (25%) than in the conventional vineyard (14%).

Although no statistically significant differences in the endophyte composition were found between conventional and biodynamic vineyard, the method of growing grapevines in a biodynamic vineyard is significantly more environmentally friendly due to the limited use of chemicals for plant treatment and possible subsequent contamination of soil and water with their residues (Maykish et al. 2021; Masotti et al. 2022).

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