Vaccinium myrtillus L. in Baltic-Nordic Region

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Contributor: Ingrida Mazeikiene

Bilberry (Vaccinium myrtillus L.) is a natural resource and a useful wild berry in Europe. Various parts of the plant contain many benefits for human health. The adaptation and secondary metabolism of V. myrtillus plants can be synergistically affected by a community of microbial endophytes.

Vaccinium myrtillus L | enzymatic activity | endophytes

1. Introduction

Nutritional composition and antioxidant activity due to the abundance of phenolic compounds in leaves extracts are beneficial to human health [1][2]. The growing area of *V. myrtillus* is native to Europe, but this species is also found in temperate and sub-Arctic regions around the world. Bilberries are widely abundant and easily found in the forests of Lithuania, Latvia, Finland, and Norway. Well-drained, moist, acidic soils are best for this species, but it can also grow in very acidic soils (pH 4.5–6). Thus, the adaptation and secondary metabolism of *V. myrtillus* plants can be synergistically affected by microbial endophytes, the benefits and potential of which are, in many cases, unknown in wild forest plants. In addition, some endophytes have shown a good ability to colonize host plant tissues; therefore, bacteria have a beneficial effect on plant growth by providing plants with the necessary nutrients or bioactive compounds [3][4][5][6]. Many beneficial microorganisms from different plant species and environments have recently been identified that can act as sources of new bioactive compounds and can therefore be used in the medical, agricultural or food industries. Numerous microbiological and ecological studies have shown that plant endophytes and their products may be promising candidates as a biological control measure.

2. Biodiversity of Endophytic Bacteria in Bilberry Leaves

A total of 25 genetically distinct endophytic bacteria were isolated from the leaves of *V. myrtillus* according to the sequence data of the 16S rRNA gene (**Table 1**). BLAST and phylogenetic analysis of 16S rRNA gene sequences revealed that the endophytic bacterial isolates were 99.58–100% similar to the sequences available in the NCBI GenBank. The 16S rDNA nucleotide sequences were submitted to GenBank and assigned accession numbers MZ469297 to MZ469321. The genetic identity among the sequences was found to be 100% in 18 bacteria strains. The 16S rRNA gene sequences demonstrated that strains Bil-LT1_1, Bil-LT1_2, Bil-LT4_7, Bil-LV3_1, and Bil-FIN2_3 were identical to several *Bacillus* spp. species from NCBI; however, due to genetic similarity within the *Bacillus* spp. species, precise identification of the isolates should be performed for further studies. The same situation was observed for strains Bil-LT4_8 and Bil-NOR3_14, which were related to *Micrococcus* sp., and strains Bil-Bil-2_5, Bil-Bil-2_6, and Bil-NOR3_11, which were related to *Staphylococcus* sp. One strain, Bil-FIN2_7, was

related to *Lysinibacillus* spp. Three strains (Bil-LV3_4, LV3_6, and NOR3_18) with minor nucleotide polymorphisms were closely related to *Paenibacillus* spp. Strains of endophytic bacteria, such as *Rothia amarae* (Bil-LT4_1), *Paenibacillus tundrae* (Bil-LV3_3), *Kocuria kristinae* (Bil-FIN2_9, Bil-FIN2_13), *Weissella hellenica* (Bil-FIN2_10), *Micrococcus terreus* (Bil-FIN2_12), *Corynebacterium freneyi* (Bil-NOR3_13), *Pseudomonas monteilii* (Bil-NOR3_15), *Sporosarcina aquimarina* (Bil-NOR3_16), and *Paenibacillus xylanexedens* (Bil-NOR3_17), were identified in bilberry leaves.

Table 1. Comparative matches for the closest phylogenetic genotypes (according NCBI records) obtained for the culturable bacteria isolates based on profile of 16S rRNA gene.

Isolate	Accession Number in NCBI	Identity Accessions, According NCBI	Sequence Length, bp (Identity, %)
Bil-LT1_1	MZ469297	*Bacillus halotolerans* MK517597.1 *B. mojavensis* MF040286.1 *B. velezensis* MT634548.1 *B. axarquiensis* GU568194.1 *B. subtilis* AB526464.1	1437 (100)
Bil-LT1_2	MZ469298	*Bacillus simplex* LK391525.1 *Peribacillus butanolivorans* CP050509.1	1431 (100)
Bil-LT4_1	MZ469299	*Rothia amarae* MG905369.1	1400 (99.79)
Bil-LT4_3	MZ469300	Bacterium strain MTL8-4 MH151301.1	1439 (99.58)
Bil-LT4_7	MZ469301	*Bacillus zhangzhouensis* MN826587.1 *B. pumilus* CP054310.1 *B. safensis* KJ542766.1 *B. stratosphericus* KY203662.1	1420 (100)
Bil-LT4_8	MZ469302	*Micrococcus* sp. MG132043.1 *M. luteus* AJ409096.1	1398 (100)
Bil-LV3_1	MZ469303	*Bacillus* sp. strain MK736127.1 *B. aryabhattai* MN515130.1 *B. megaterium* MF988696.1	1427 (100)
Bil-LV3_3	MZ469304	*Paenibacillus tundrae* HF545335.1	1431 (100)
Bil-LV3_4	MZ469305	*Paenibacillus* sp. MK290403.1	1435 (99.65)
Bil-LV3_6	MZ469306	*Paenibacillus* sp. MG758020.1	1450 (99.86)
Bil-FIN2_3	MZ469307	*Bacillus cereus* MN068934.1 *B. thuringiensis* CP050183.1	1439 (100)

Isolate	Accession Number in NCBI	Identity Accessions, According NCBI	Sequence Length, bp (Identity, %)
Bil-FIN-2_5	MZ469308	*Staphylococcus warneri* CP038242.1 *S. pasteuri* MW433878.1	1437 (100)
Bil-FIN2_6	MZ469309	*Staphylococcus warneri* CP038242.1 *S. pasteuri* MW433878.1	1437 (100)
Bil-FIN2_7	MZ469310	*Lysinibacillus macrolides* MH542661.1 *L. xylanilyticus* KP644237.1 *L. fusiformis* FJ641020.1	1427 (100)
Bil-FIN2_9	MZ469311	*Kocuria kristinae* KX055834.1	1384 (100)
Bil-FIN2_10	MZ469312	*Weissella hellenica* CP042399.1	1447 (100)
Bil-FIN2_12	MZ469313	*Micrococcus terreus* KJ781899.1	1385 (100)
Bil-FIN2_13	MZ469314	*Kocuria kristinae* KX055834.1	1384 (100)
Bil-NOR3_11	MZ469315	*Staphylococcus sp.* KM253075.1	1431 (100)
Bil-NOR3_13	MZ469316	*Corynebacterium freneyi* EF462412.1	1393 (99.86)
Bil-NOR3_14	MZ469317	*Micrococcus* sp. KX350143.1 *M. luteus* MN826463.1	1385 (100)
Bil-NOR3_15	MZ469318	*Pseudomonas monteilii* CP013997.1	1422 (100)
Bil-NOR3_16	MZ469319	*Sporosarcina aquimarina* MK726086.1	1433 (100)
Bil-NOR3_17	MZ469320	*Paenibacillus xylanexedens* CP018620.1	1436 (99.79)
Bil-NOR3_18	MZ469321	*Paenibacillus* sp. KR055031.1	1427 (99.86)

Lithuania, m bilberry e families blueberry *occaceae* strains were identified in one or more cases.

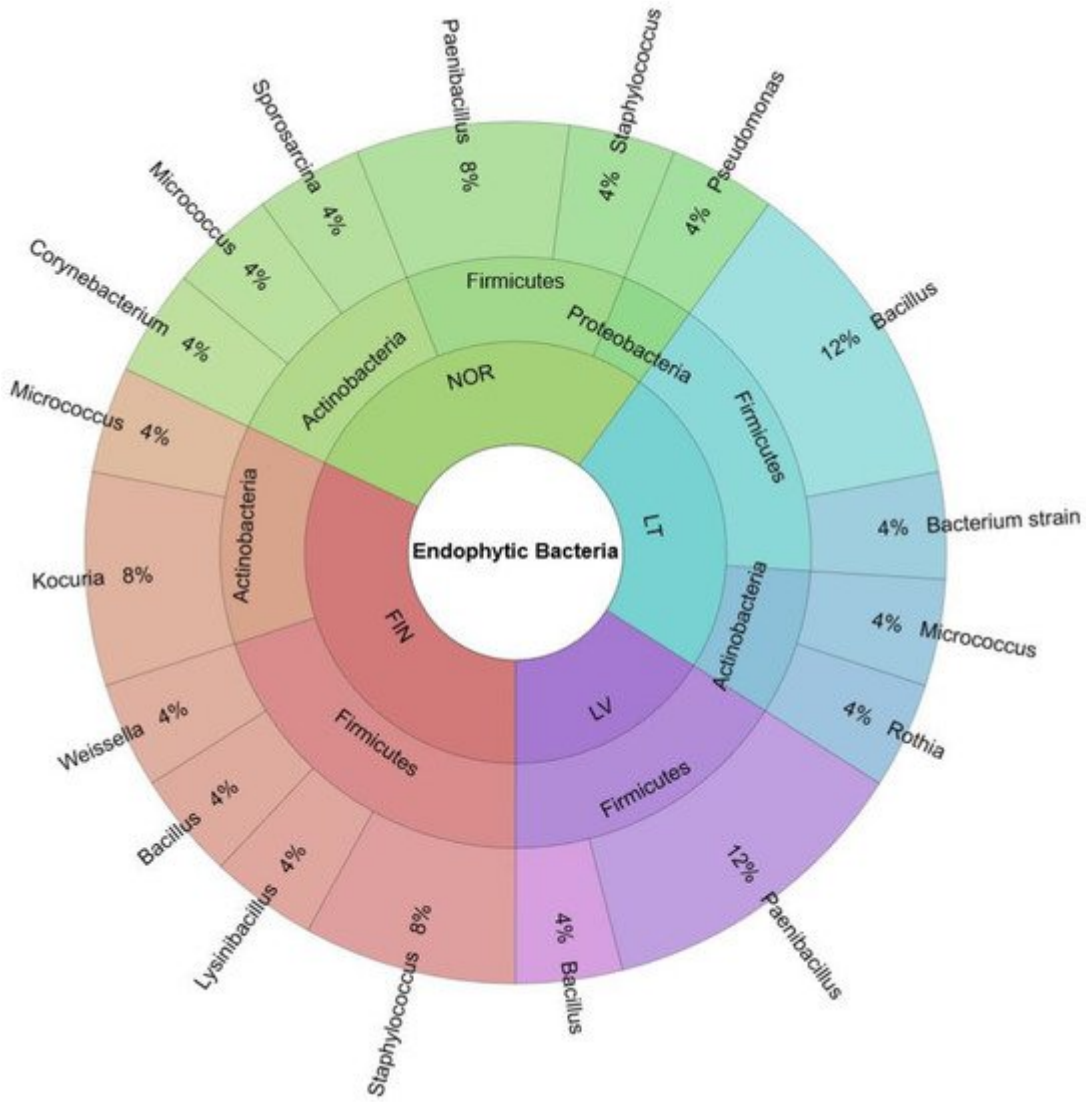

Figure 1. Biodiversity of endophytic bacteria in bilberry leaves of the Baltic-Nordic region's forests—Lithuania (LT), Latvia (LV), Finland (FIN) and Norway (NOR).

Endophytic bacteria isolated from Norwegian bilberry leaves belonged to *Firmicutes* 1%, *Actinobacteria* 12%, and *Proteobacteria* 4% phyla. Meanwhile, bacteria in Finnish and Lithuanian leaf samples belonged to *Firmicutes* (20% and 16%, respectively) and *Actinobacteria* (12% and 8%, respectively). Only *Firmicutes* phylum bacteria were isolated from the Latvian samples. Bacteria of the *Bacillaceae* family were isolated and identified in the bilberry leaves of Lithuania, Latvia, and Finland, but were not found in plant samples from Norway. Bacteria belonging to six different families isolated from leaves samples were collected from the northern countries—Finland and Norway (**Figure 2**).

3. Enzymatic-Genetic Features of Endophytic Bacteria in Bilberry Leaves

Enzymatic activity was tested in all bacterial endophytes isolated from bilberry leaves (**Table 2**). The results showed that amylase was detected in 44% of the tested isolates, proteases in 56%, and catalases in 88%. The

study showed that seven bacterial endophytes isolated from bilberry leaves were able to produce amylase, protease, and catalase. All bacterial endophytes tested had at least one enzymatic activity, except for one bacterial strain, *Weissella hellenica* Bil-FIN2_10, which lacked amylase, protease, and catalase activity.

Table 2. Enzymatic activity and the presence of the genes *acdS* (1-aminocyclopropane-1-carboxylate deaminase (ACCD)) and *AcPh* (acid phosphatase) in endophytic bacteria isolated from bilberry leaves of the Baltic-Nordic region.

Endophytic Bacteria Strains in Different Geographic Locations	Amylolytic Activity, mm	Proteolytic Activity, mm	Catalase Reaction	Gene *acdS*	Gene *AcPho*
Bacillus sp. Bil-LT1_1	11.9 ± 0.1	10.0 ± 0.1	+	+	-
Bacillus sp. Bil-LT1_2			+	-	-
Rothia amarae Bil-LT4_1	12.0 ± 0.2	11.8 ± 0.2	+	-	-
Bacterium strain Bil-LT4_3		9.8 ± 0.1	+	-	-
acillus sp. Bil-LT4_7	9.0 ± 0.2	10.2 ± 01	+	-	-
Micrococcus sp. Bil-LT4_8		9.3 ± 0.1	+	-	-
Bacillus sp. Bil-LV3_1	12.5 ± 0.1	14.2 ± 0.2	+	-	-
Paenibacillus tundrae Bil-LV3_3	10.1 ± 0.2	11.9 ± 0.2	+	-	-
Paenibacillus sp. Bil-LV3_4	12.1 ± 0.1	10.2 ± 0.1	+	-	-
Paenibacillus sp. Bil-LV3_6		10.2 ± 0.1	-	-	-
Bacillus sp. Bil-FIN2_3	12.3 ± 0.2	14.3 ± 0.3	-	+	+
Staphylococcus sp. Bil-FIN2_5			+	-	-
Staphylococcus sp. Bil-FIN2_6	11.9 ± 0.2		+	-	-
Lysinibacillus sp. Bil-FIN2_7			+	-	-
Kocuria kristinae Bil-FIN2_9			+	+	-
Weissella hellenica Bil-FIN2_10			-	-	-
Micrococcus terreus Bil-FIN2_12		9.5 ± 0.1	+	-	+

Endophytic Bacteria Strains in Different Geographic Locations	Amylolytic Activity, mm	Proteolytic Activity, mm	Catalase Reaction	Gene *acdS*	Gene *AcPho*
Kocuria kristinae Bil-FIN2_13		8.8 ± 0.1	+	-	+
Paenibacillus sp. Bil-NOR3_17		10.1 ± 0.2	+	-	-
Paenibacillus sp. Bil-NOR3_18			+	-	-
Corynebacterium sp. Bil-NOR3_13	12.2 ± 0.1		+	-	-
Micrococcus sp. Bil-NOR3_14	10.3 ± 0.2	14.5 ± 0.2	+	-	-
Staphylococcus sp. Bil-NOR3_11	10.1 ± 0.1		+	-	-
Pseudomonas monteilii Bil-NOR3_15			+	-	-
Sporosarcina aquimarina Bil-NOR3_16			+	-	-

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Bil-FIN2_13, gave an amplified fragment of the expected size (~734 bp) for the *AcPho* gene, indicating the potential ability of these isolates to produce acid phosphatase.

The results of enzymatic activity (the halo zones on agar in millimeters) are expressed as a mean ± standard deviation, "+" positive and "-" negative enzymatic reaction and "+" presence or "-" absence of gene.

4. Summary

A community of 25 microorganisms was found in the leaves of bilberry (*Vaccinium myrtillus* L.). Isolates with proteolytic and amylases activity indicated the possible expression of these enzymes and their potential role in the degradation of starch and protein organic matter in the ecosystem. Ninety-six percent of endophytic bacteria strains of *V. myrtillus* L. had positive enzymatic activity and 20% had functional plant growth-promoting traits. The accumulation of this new collection of microorganisms and the primary genetic-enzymatic analysis opens opportunities for the study of some isolates against pathogenic organisms and for reducing the effects of salinity stress on other plants.

References

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