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## Bacillus mycoides: novel tools for studying the mechanisms of its interaction with plants

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# 2

## Draft genome sequences of seven *Bacillus mycoides* strains, isolated from potato endosphere and soil

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### **This chapter is partly based on:**

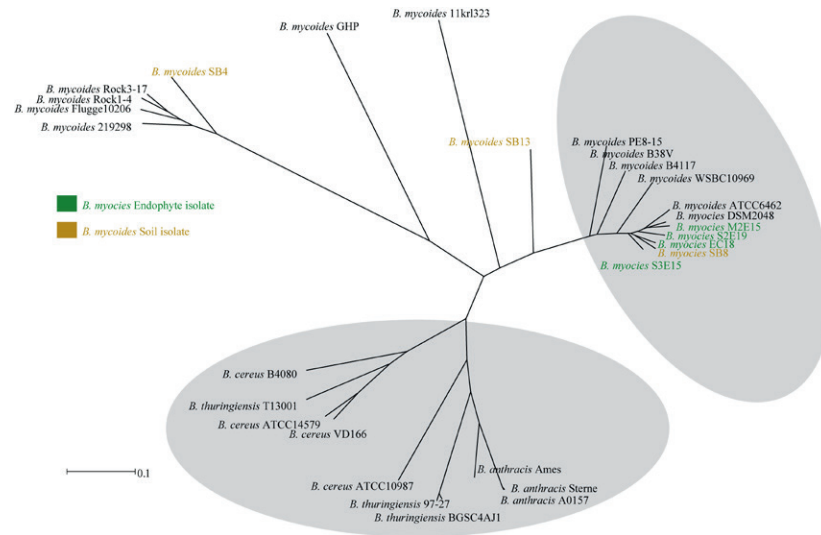
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& Kuipers, O. P. (2016). Draft genome sequence of *Bacillus mycoides* M2E15, a strain isolated from the endosphere of potato. *Genome announcements*, 4(1), e00031-16.



*Bacillus mycooides* is a rod-shaped and spore-forming gram-positive bacterium which is associated with the *Bacillus cereus* group. On agar plates, it forms unique rhizoid colonies resulting from cells that are linked end-to-end. The strong intra-cellular connections yield a stable network that favors interactions with other soil inhabitants, such as microbes and plant roots (Turchi et al., 2012). There are several studies showing that *B. mycooides* is able to promote the plant growth (Karagöz et al., 2012), fix N<sub>2</sub> (Ambrosini et al., 2016), and control several plant diseases (Paul et al., 1995; Bargabus et al., 2002; Neher et al., 2009). We found some *B. mycooides* strains to be present in the endosphere of potato without causing visible signs of disease. In order to understand the genetic make-up of this bacterium, we present the draft genome of several *B. mycooides* strains, which was isolated from potato endosphere or soil (Wijster, the Netherlands).

Strains were grown overnight in Luria-Bertani (LB) broth at 30 °C, 200 rpm. Bacterial cells were harvested at the exponential growth phase and lysed with lysozyme. After RNase treatment, proteins were removed by proteinase K digestion. DNA was extracted from the lysate by phenol-chloroform treatment and recovered by isopropanol precipitation. The extracted DNA was checked for purity by Nanodrop (Thermo Fisher Scientific) and 1% agarose gel, then quantified by Quantus™ Fluorometer (Promega). The high-quality DNA was sequenced using the MiSeq sequencing system of Illumina, yielding 250 bp paired-end reads with a mean library size of 400 bp. *De novo* assembly was performed using Velvet (Zerbino and Birney, 2008). Prediction of protein-encoding regions and automatic functional annotation was performed using the Rapid Annotations using Subsystem Technology (RAST) server (Aziz et al., 2008). Moreover, the bacteriocin identification tool Bagel3 was used (van Heel et al., 2013).

The whole genome alignment of the seven strains was performed with GEGENEES tool (Ågren et al. 2012). Phylogram was generated with Neighbour joining method and is shown in Figure 1. The resulting phylogenetic tree has two main branches that group the 29 genomes into two species clades: *B. mycooides* and other *B. cereus*-group members. For *B. mycooides*, we selected the 7 strains based on their origin of isolation and plant colonization ability: four strains isolated from the endosphere that could be endophytic again (EC18, M2E15, S2E19, and S3E15), and three strains isolated from soil which could not be endophytic (SB4, SB8, and SB13). The phylogenetic tree showed that the four endophytic strains are clustered together, indicating that the endophytic strains have some common features. Intriguingly, the soil strain SB8 was also grouped in this putative endophytic clade.



**Figure 1.** Phylogenetic analysis based on the whole genome sequences of the 7 *B. mycoides* isolates and other representatives of the *B. cereus*-group: *B. cereus*, *B. anthracis*, and *B. thuringiensis*.

**Table 1.** Genome features and GenBank accession numbers of the strains.

Species	Strain	Source of isolation	Accession No.
<i>Bacillus mycoides</i>	EC18	Endosphere	MRWW00000000.1
<i>Bacillus mycoides</i>	S2E19	Endosphere	MRWV00000000.1
<i>Bacillus mycoides</i>	M2E15	Endosphere	LLWA00000000.1
<i>Bacillus mycoides</i>	S3E15	Endosphere	MRWU00000000.1
<i>Bacillus mycoides</i>	SB4	Soil	MRZX00000000.1
<i>Bacillus mycoides</i>	SB8	Soil	MRWS00000000.1
<i>Bacillus mycoides</i>	SB13	Soil	MRWT00000000.1

**Nucleotide sequence accession number.** The genome sequences of the 7 *B. mycoides* strains have been deposited as whole genome shotgun projects at GenBank under the accession numbers listed in Table 1.

## ACKNOWLEDGMENTS

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