

Dissertation abstract

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Title: The occurrence of relatively anaerobic, spore-forming cellulolytic bacteria in soils of different agricultural uses

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Abstract of the doctoral thesis

The ecology of cellulolytic bacteria in agricultural soil remains relatively unknown. There are still only a few studies concerning the abundance and diversity of this bacterial group in agriculturally managed soils. The presented research aims to determine the influence of different plant cultivation systems and manure fertilization on the relative abundance and diversity of facultatively anaerobic spore-forming cellulolytic bacteria (FASCB) and facultatively anaerobic spore-forming potentially cellulolytic bacteria (FAPSCB).

The study site is a nearly 100-year-old fertilization experiment, one of the oldest continuously active field experiments in Europe. The relative abundance of FASCB and potential FASCB was assessed using classical microbiological methods - most probable numbers (MPN) and Illumina MiSeq 16S rRNA sequencing. The highest MPN of the studied bacterial group was recorded in soil subjected to crop rotation without legumes (ARP) fertilized with manure (382 colony-forming units (CFU) g⁻¹). Through bioinformatic analysis, the highest Shannon-Wiener index values and the greatest number of operational taxonomic units (OTUs) were found in ARP-FYM, while the lowest values of these parameters were recorded in soil originating from CRWL without manure fertilization. At the order level, the dominant taxa in all samples were Brevibacillales (13.1–43.4%), Paenibacillales (5.3–36.9%), and Bacillales (4.0–0.9%). Meanwhile, Brevibacillaceae (13.1–43.4%), Paenibacillaceae (8.2–36.9%), and Clostridiaceae (5.4–11.9%) dominated at the family level in all examined samples. The families Aneurinibacillaceae and Hungateiclostridiaceae exhibited higher

relative abundances in manure-fertilized soil. In summary, the results obtained in this doctoral thesis demonstrate that the influence of crop cultivation on FASCB was negligible, while the actual factor shaping the FASCB community was the use of manure in crop rotation.

To confirm the presence of FAPCB in the studied soil and determine the activity of their cellulolytic enzymes, dozens of spore-forming bacterial strains were isolated, one of which was selected for further research. Through 16S rRNA gene sequencing, the selected bacterial strain was identified as belonging to the genus *Bacillus* (strain 8E1A). *Bacillus* sp. 8E1A showed carboxymethylcellulase (CMCase) production with visualization using Congo red (the size of the clear zone was 25mm). Furthermore, the activity of CMCase, filter paperase (FPase), and microcrystalline cellulose Avicel hydrolase (avicelase) was investigated.

In the cultivation of the strain, three different cellulose sources were utilized: carboxymethyl cellulose (CMC), filter paper (FP), and microcrystalline cellulose Avicel. The highest CMCase (0.617 U mL^{-1}), FPase (0.903 U mL^{-1}), and avicelase (0.645 U mL^{-1}) activities by *Bacillus* sp. 8E1A were observed using CMC (after 216 h of incubation), Avicel cellulose (after 144 h of incubation), and CMC (after 144 h of incubation), respectively. Subsequently, cellulase activity was measured at different temperatures and pH values. The optimal temperature for CMCase (0.535 U mL^{-1}) and avicelase (0.666 U mL^{-1}) activities was $70 \text{ }^{\circ}\text{C}$. However, the highest FPase activity (0.868 U mL^{-1}) was recorded at $60 \text{ }^{\circ}\text{C}$. The highest CMCase and avicelase activities were noted at pH 7.0 (0.520 and 0.507 U mL^{-1} , respectively), and the optimal FPase activity was found at pH 6.0 (0.895 U mL^{-1}). These results indicate that the cellulases produced by *Bacillus* sp. 8E1A potentially could be utilized for the degradation of lignocellulosic waste under industrial conditions.

Keywords: crop management, Firmicutes, fertilization, cellulases