A survey on the species diversity of cellulose and xylan hydrolysing soil bacteria in soil samples taken from agricultural areas, by culturing methods

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Introduction

After harvest crop-plant litter generally does not decompose until the next vegetation period, and therefore represents a major issue in soil management. The intact residues on the fields retain nutrients from the soil and also may serve as overwintering surface and, accordingly infection sources for plant pathogenic fungi. In extreme cases, large amount of stalk may also hinder water in reaching the soil surface. Therefore, rapid decomposition of stalk residues is highly important, because it provides nutrient supply for both plants and soil microbes in due time and diminishes the chances of a fungal epidemy next vegetation period. The aim of this research was the isolation and characterization of cellulose and xylan (NSP, non-starch polysaccharide) hydrolyzing microbes that accelerate crop residue degradation, and can be used as post-harvest soil inoculant.

Material and methods

1. Sampling:

Soil types:
- Meadow chernozem
- Pseudogley brown forest

2. Isolation and purification media

Culture media with low nutrient content and containing 0,5% (CMC-0,2%) polysaccharide, as shown below.

- Microcrystalline cellulose
- Carboxymethyl cellulose (CMC)
- Wallpaper glue
- Mn300 . Xylan . Mannan
- Bamboo

3. Selection of positive strains with Congo red staining

1% Congo red solution, stained plates were washed with 1N NaCl solution.

4. Identification

Genomic DNA was extracted and purified by MoBio UltraClean Microbial DNA Isolation Kit.

The 16s rRNA gene was amplified and sequenced with the universal bacteria primer set 27F-1492R.[1]

The purified PCR products were sequenced by BaseClear B.V. (Einsteinweg 5, 2333 CC LEIDEN The Netherlands)

5. Screening for xylanase and cellulase activity with DNSA methods (detection of reducing sugars)

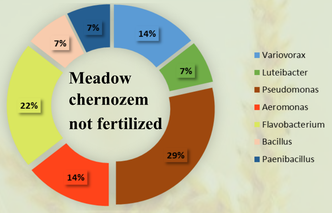
Cultures were grown in two types of broth containing CMC or ground corncob. For the DNSA method, the substrates were xylan from beechwood and CMC. [2]

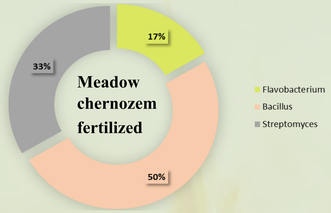
Results

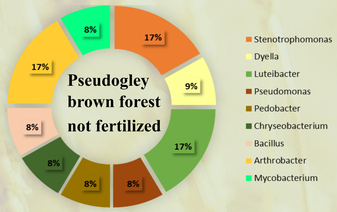
Congo red staining results

A total of 250 isolates were screened for polysaccharidase enzyme activity with Congo red staining. Light-colored halos around the colonies indicate the desired enzyme activity. Incubation time and colony morphology were also recorded. Thirty-two strains were polysaccharidase positive and selected for further investigations.

Dispersion of cellulose and xylan hydrolyzing bacterial genus

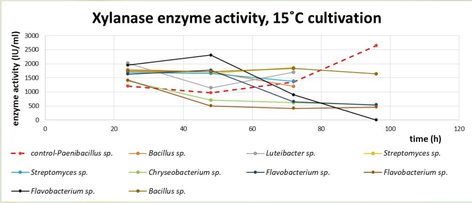


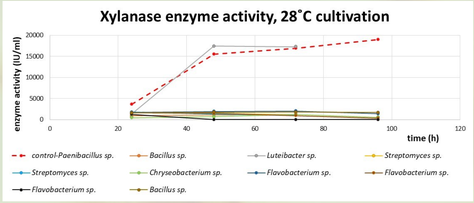


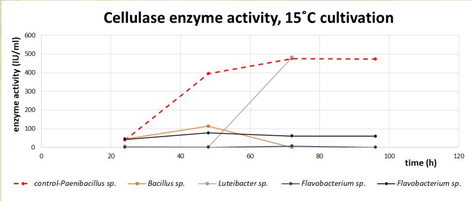


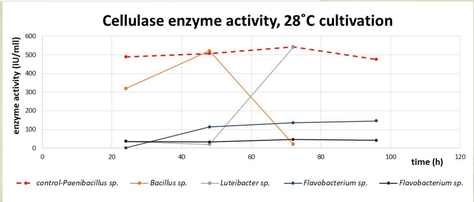
Twelve bacterial species representing 9 genera were isolated from pseudogley brown forest soil, whereas 20 species of 8 genera were found in meadow chernozem samples. Diversity in fertilized meadow chernozem was lower compared to unfertilized soil. Fertilizers had no effect on the occurence of *Flavobacterium* and *Bacillus* genera.

Results of spectrophotometry









Outstanding xylanase (48h; 17742.03 IU/ml) and cellulase enyzm activity (72h; 540.261 IU/ml) was observed for *Luteibacter sp*. In terms of cellulase activity, a *Bacillus sp*. and a *Flavobacter sp*. had promising results in cellulose degradation tests, however cellulase activity of *Bacillus sp*. showed a gradual decrease over time.

Summary

Out of the 250 isolates cultured on different xylan an cellulose substrates, 32 strains were polysaccharidase positive based on the zone of clearance in Congo red tests. Positive samples isolated from unfertilized pseudogley brown forest soil showed a more diverse bacterial soil life than samples from meadow chernozem. However, a greater number of polysaccharide producing isolates were obtained from meadow chernozem samples. The number of non-starch polysaccharide degrading isolates obtained from unfertilized meadow chernozem were double than that of the fertilized soil. Numerous strains performed excellent polysaccharidase activity a few of them showed considerable xylanase and cellulase acitvity. This observation probably relies on the Congo red screening method which produces relatively high percent of false positives for the enzyme tests. The selected promising xylanase and cellulase strains were selected for further applications.

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[1] **Lane DJ.** 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M, editors. *Nucleic Acid Techniques in Bacterial Systematics*. New York: John Wiley and Sons; 1991. pp. 115-175. [2] Szendefy J. Xilanáz enzimek előállítása szilárd fázisú fermentációval és papíripari hasznosításuk. Phd értekezés, 2005.