Quantification and Identification of culturable DNase producing bacteria from leachates as influenced by crop species

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INTRODUCTION

- The continued release and use of genetically engineered (GE) crops has become a source of hot debate as they are thought to pose potential risks to the environment including introgression of genes with novel traits into soil microbes via natural transformation and perhaps even into other plants via transformed soil microbes (Hart et al., 2009).
- Plants influence soil functions through the release of root exudates which consist a wide range of carbon compounds including extracellular DNA (eDNA) that can serve as a source of nutrients and genetic information to other organisms. Therefore eDNA cycle is an interesting area in assessing the perceived unintended effects of GE crops.
- Persistence of eDNA in the environment is greatly hindered by the presence of extracellular DNases and especially from microbial origin (Nielsen et al., 2007) and thus DNase activity contributes to soil function as a component of nutrient cycling in particular (N and P).
- There is however little information on how crop species affect culturable DNase producing soil bacteria in agricultural soils.

OBJECTIVE

- To quantify and identify culturable soil DNase producing bacteria from leachate samples as influenced by crop species.

METHODS

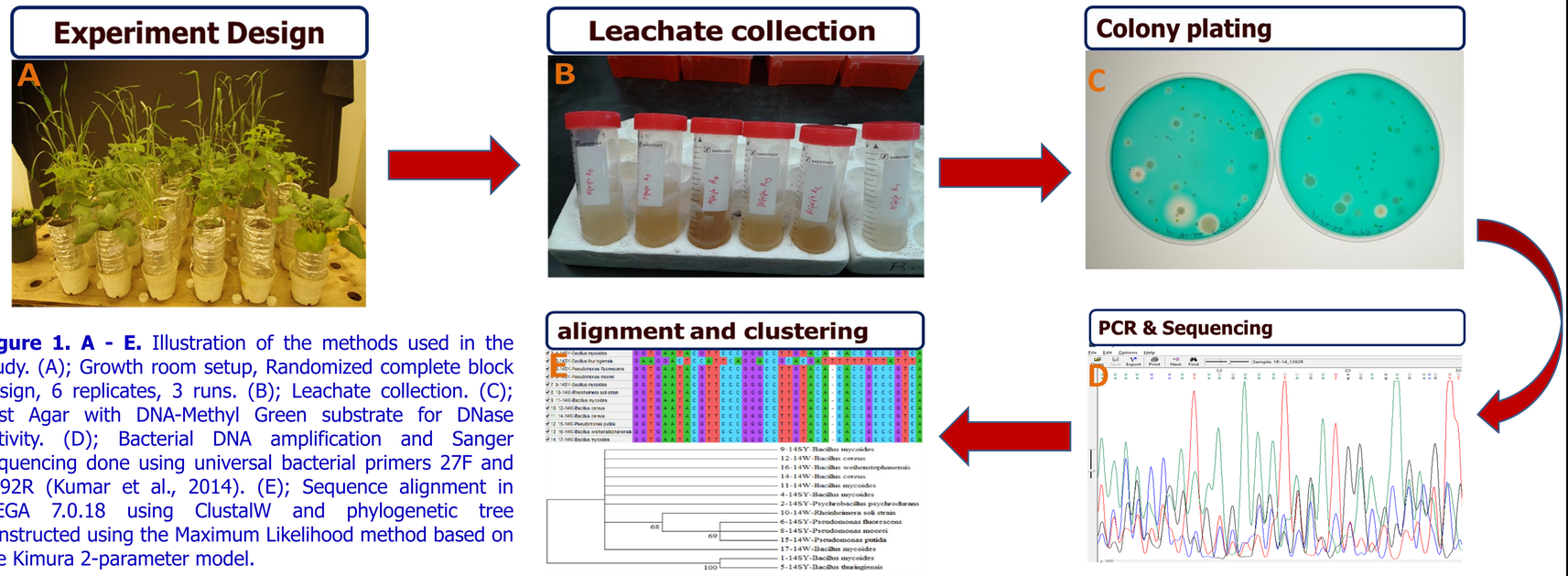

Figure 1. A - E. Illustration of the methods used in the study. (A); Growth room setup, Randomized complete block design, 6 replicates, 3 runs. (B); Leachate collection. (C); Test Agar with DNA-Methyl Green substrate for DNase activity. (D); Bacterial DNA amplification and Sanger sequencing done using universal bacterial primers 27F and 1492R (Kumar et al., 2014). (E); Sequence alignment in MEGA 7.0.18 using ClustalW and phylogenetic tree constructed using the Maximum Likelihood method based on the Kimura 2-parameter model.

RESULTS

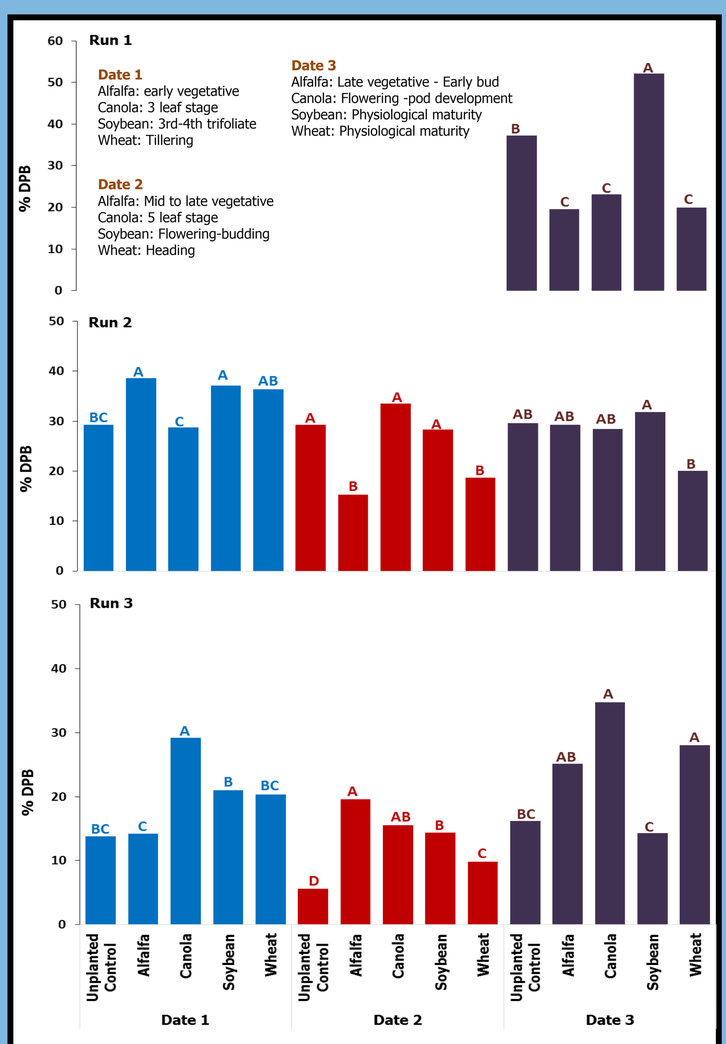

Figure 2. Proportions of culturable DNase producing bacteria (DPB) in leachate samples in response to crop species. Mixed model ANOVAs were conducted within dates and different letters above bars indicate significant differences between means as determined by Fisher's protected least significant difference (alpha = 0.05).

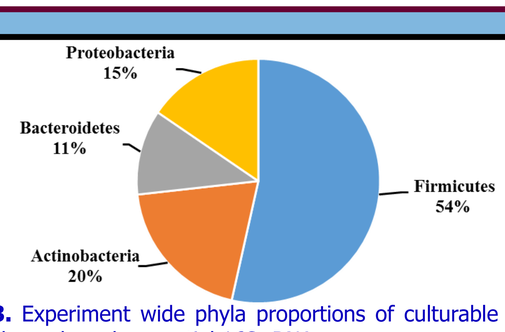

Figure 3. Experiment wide phyla proportions of culturable DPB isolates from leachates based on partial 16S rRNA gene sequences.

- Compared with unplanted control, crop species altered ($P < 0.05$) the proportion of culturable DNase producing bacteria (DPB) populations of the soil which differed across the sampling dates and runs.
- Soybean leachate consistently contained higher proportions of DPB populations in the 1st and 2nd runs while in run 3 Canola cultured higher proportions (Fig. 2).
- Proportions of culturable DPB in leachate among treatment means ranged between 5.57 to 52.08%. An indoor study using transgenic white poplars reported 62.5 to 100% of total culturables to be nuclease producing bacteria (Balestrazzi et al., 2007).
- Bacterial isolates were classified into four phyla groups, 11 genera and the highest proportion of culturable DPB (54%) (Fig. 3) were firmicutes with 7 different Bacillus species (Fig. 4).
- Isolates clustered according to phyla groups with exception of isolate **1-14SY**-Bacillus_mycoides, **5-14SY**-Bacillus_thuringiensis and **30-14SL**-Rhizobium_huautlense (Fig. 4). It is possible isolate **30-14SL** didn't cluster within its phyla group due to low identity (85%), for the other two no apparent explanation could be found at this point.

KEY FINDINGS

- Crop species altered proportion of culturable DPB, however trends were not always consistent among the runs and sampling dates. To the best of our knowledge this is the first report of crop species effect on DPB.
- Most culturable DPB were classified as members of the Bacillus genera belonging to the phylum Firmicutes.
- The results suggest that crop species have a great influence on below-ground DNase producers abundance. These results are based on culturable bacteria in leachate samples which constitutes a small fraction of the total soil bacterial community. Work on soil samples is ongoing.

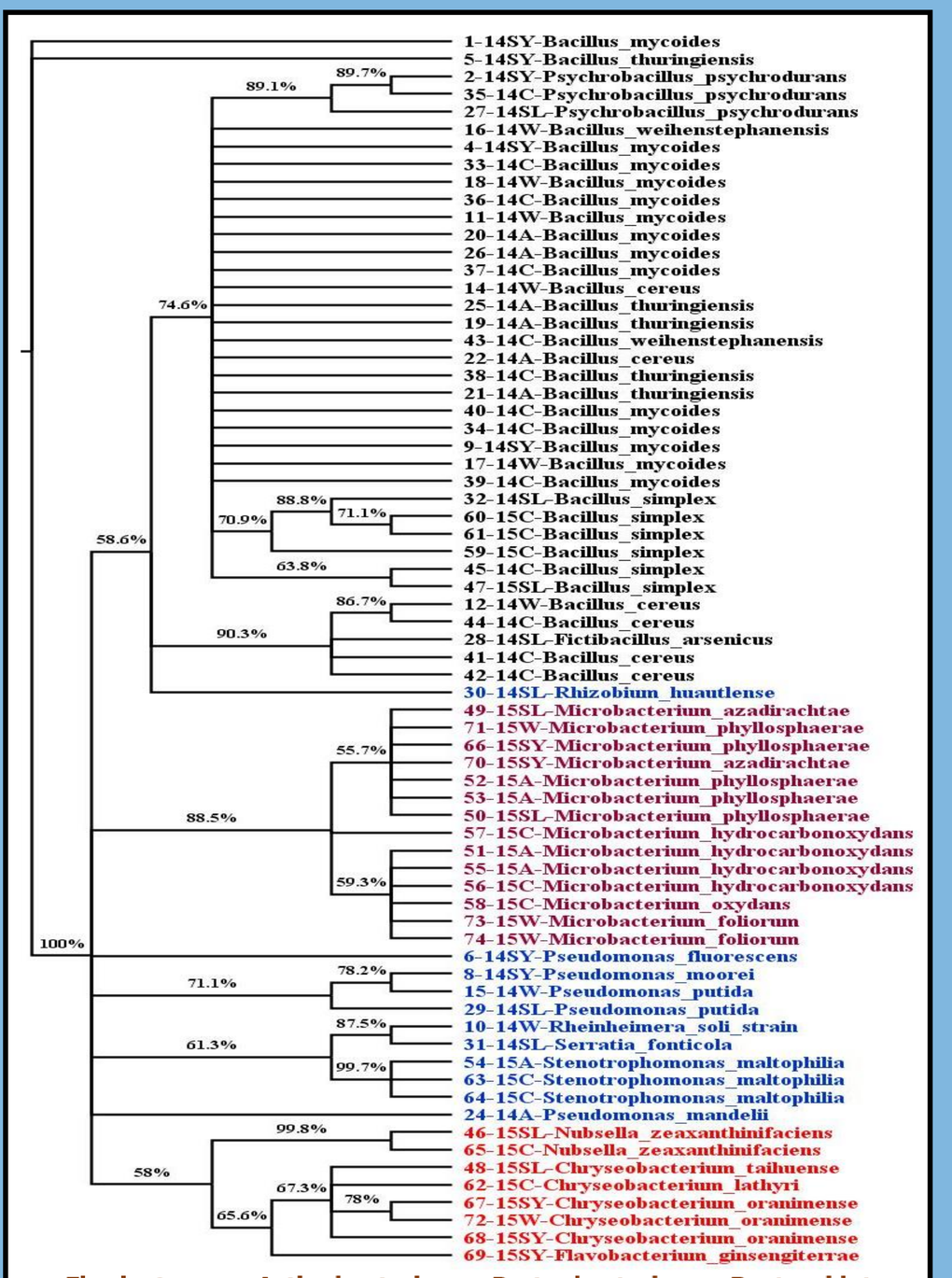

Figure 4. Maximum likelihood tree showing relatedness of 71 DNase producing bacterial isolates recovered from leachates based on partial 16S rRNA gene sequences. Bootstrap values are shown when >50 based on 1000 replicates

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