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Analysis of rhizospheric bacterial community in soils affected by the formation of calcrete

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ABSTRACT

Crust of carbonate minerals, named calcrete, underlies the arable soils of Southeast Bulgaria. The basic microorganism groups from soil, developed on top of a massive calcrete and in the rhizosphere of *Cichorium intybus* (Common chicory) were estimated by culturable methods. In this plant's rhizosphere, microorganisms from some physiological groups were found in numbers 1 to 3 orders of magnitude higher, compared to non-rhizosphere samples of massive calcrete. The dominant microorganisms in this plant's rhizosphere are represented by genera *Bacillus*. 16S rRNA gene nucleotide sequences were used for identification of four strains, obtained from the rhizospheres of *C. intybus*. The sequences obtained were compared to those of *Bacillus* species held in GenBank. The strains CI R1 and CI R3 were found to belong to the species *Bacillus subtilis* and the strains CI R2 and CI R4 to the species *Bacillus amyloliquefaciens*. Numerous studies confirm that these species are good facilitators of plant growth in nutrient deficient carbonate environment. Study of the growth-promoting potential of these bacterial species, for economically important plants cultivated in this region, is currently in progress.

1. Introduction

In warm and dry areas, where the annual rainfall is less than 1000 mm m^{-2}, the chemical composition of the naturall soil waters favors deposition of carbonate minerals, forming carbonate soils or hards rock crusts named calcrete or caliche. The main constituents of the calcrete are the minerals calcite and dolomite, although a variety of other minerals such as gypsum or chalcedony may occur in minor amounts. Calcrete occurs in a variety of forms, e.g. as calcareous sands in aeolian transported cover materials, as carbonate powders and rhizoliths, as well as nodular concretions, mottled horizons and discrete hard banks (McQueen et al., 1999). Important factors in the formation of calcrete are climate, topography, vegetation, presence of carbonate and oxalic phases, carbonate content in the waters, texture, porosity and permeability of the hosting substrate, action of microorganisms as well as exposure time (Ehrlich, 2001; Reith et al., 2009).

Calcretes of a variety of textures and compositions are present in the Thracian plane in Southeast Bulgaria (Dimitrov et al., 2009a,b). Most of the arable lands in Thrace are underlain by indurate pedogenic calcrete of various thickness. According WRB soil classification most of the soils in the Thracian plane are classified as calcic vertisols (IUSS Working Group WRB, 2006).

Alonso-Zarza and Jones (2007) described various interactions between the microorganisms and plants growing in carbonate environment under subaerial conditions. From one hand, plants and microorganisms participate in biomineralisation of secondary Ca-carbonates, from the other hand, beneficial rhizobacteria plays a very important role for plant growth, especially in calcrete.

The rhizosphere is inhabited by many systematic groups of microorganisms such as: *Acetobacter*, *Agrobacterium*, *Alcaligenes*, *Azoarcus*, *Azomonas*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Clostridium*, *Comamonas*, *Derxia*, *Enterobacter*, *Erwinia*, *Herbaspirillum*, *Klebsiella*, *Microbacterium*, *Micrococcus*, *Paenibacillus*, *Pseudomonas*, *Rhizobium*, *Variovorax*, *Xanthobacter* etc. (Andrade et al., 1997; Duineveld et al., 2001; Joseph et al., 2007; Park et al., 2005; Vazquez et al., 2000). A number of authors (Andrade et al., 1997; Krishnaveni, 2010) reported that among the genera of bacteria isolated from the rhizosphere samples, *Bacillus* and *Pseudomonas* were the most predominant.

The importance of specific functions of rhizobacteria has been recognized for many decades (e.g. Lugtenberg and Kamilova, 2009). The impact of micoorganisms on plants could be neutral, positive, or negative. The impact of rhizobacteria on plant nutrition, resistance to root diseases, and a variety of other environmental stressors, has been known for a long time (e.g. Avis et al., 2008; Balandreau and Knowles, 1978; Barea et al., 1976; García et al., 2004; Idriss et al., 2002; Leifert et al., 1995; Zhang et al., 2002 etc.).

Plant growth-promoting rhizobacteria (PGPR) show direct and indirect mechanisms of plant growth stimulation (Ryu et al., 2005), such as: increasing of the fixation and administration of the atmospheric

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nitrogen; transformation of hard to dissolve phosphoric compounds into easily assimilated ones (Vazquez et al., 2000); production of siderophores, chelation of iron into a biologically absorbable form; microbic synthesis of physiologically active substances (phytohormones — auxins, cytokinins, gibberellins, vitamins, aminoacids, and others); synthesis of exopolysaccharides; biocontrol of infected patogenic plants, through synthesis of substances with antibiotic and fungi-toxic action (Leifert et al., 1995; Munimbazi and Bullerman, 1998); change of membrane permeability of root cells, and an increase in the absorbtion capabilities of the plant roots.

In last few decades a long array of bacteria, including species of — *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Pseudomonas* and *Serratia* have been reported to enhance plant growth (Bai et al., 2002; Joseph et al., 2007; Liu et al., 2006).

The influence of PGPR on the plant nutrition in carbonate soils is very significant, because these soils are poor of available biogenic elements (C, N, and P), and have alkaline properties (pH 8–9), which inhibit the assimilation of various nutrients and microelements, in particular iron. Because of its poor permeability, the calcrete layer prevents the infiltration of soil water and the delivery of oxygen to the root plants.

The purpose of this study was to provide information on the composition of cultural microbial community in soil affected by formation of calcrete. The structure of rhizospheric microbial communities in roots of naturally occurring *Cichorium intybus* (Common chicory), collected from two localities in the Thracian plane, is studied and the dominant rhizobacteria for these plants identified. Both plants grow on top of massive calcrete, and appear resilient to the nutrient deficient environment, dominated by the abundance of carbonate minerals.

This study has provided the basis for further investigation, aimed at the selection of microbes and developing of artificial bacterial additives for plant growth facilitation in areas, affected by formation of calcrete.

2. Materials and methods

2.1. Study site

The sampling sites for this study are located about 1 km to northwest of the village Scalitsa (WGS84, Lat/Lon: 42.275224° N, 26.242694° E) and about 2 km to west of the village Miladinovtsi (WGS84, Lat/Lon: 42.281044° N, 26.313292° E), Southeast Bulgaria.They are on a flatland with an average elevation of about 170 m above sea level. The local rivers cut the flatland to an elevation of approximately 140 m. The calcrete hardpan is highly impervious to surface waters and controls the drainage paths on the hills. It is underlain by permeable sands and conglomerates of neogene age (Fig. 1).

At both sampling sites exist small quarries for calcrete extraction. The calcrete layer was mined and used locally for primitive production of quicklime for the masonry. The Scalitsa and Miladinovtsi sites were never cultivated and contain only indigenous plant communities.

In these sites the soil is classified as calcic vertisols. The petrocalcic horizon occurs 25–35 cm under the surface and its thickness is between 1.0 and 3.0 m. This layer contains various amounts of calcite and dolomite. It is a magnesium rich variety (Table 1). From each locality two plants were harvested several meters apart from the quarry's benches. The roots of *C. intybus* from Scalitsa locality were taken from depth of 1.5 m under the surface and those from Miladinovtsi locality from depth of 0.35–0.45 m. It is observed in the quarry sections, that only the roots of *C. intybus* penetrate the calcrete hardpan to a depth of 3.0–5.0 m (Fig. 2).

2.2. Study methods

2.2.1. Chemical analysis of soil

In the course of the project, the soils of the two sampling sites as well as elsewhere in the region were subjected to instrumental *in situ* and lab based analysis of a number of geochemical parameters. In this study nine soil samples were collected from three adjacent spots and three depths (0–20 cm, 20–40 cm and 40–60 cm) from the Scalitsa site. Six soil samples were taken from two adjacent spots and the same three depths from the Miladinovtsi site. The soil samples were collected from adjacent spots due to the soil heterogeneity. The chemical analyses data for each depth at the Scalitsa and the Miladinovtsi sites are averaged in Table 2.

The pH values were found by an instrumental method for a routine determination of pH using a glass electrode in a 1:5 (V/V) suspension of soil in water (ISO 10390). Total carbon concentrations were found by dry combustion via an elemental analyzer (ISO 10694). The soil carbon was oxidized to carbon dioxide (CO_2) by heating the soil to at least

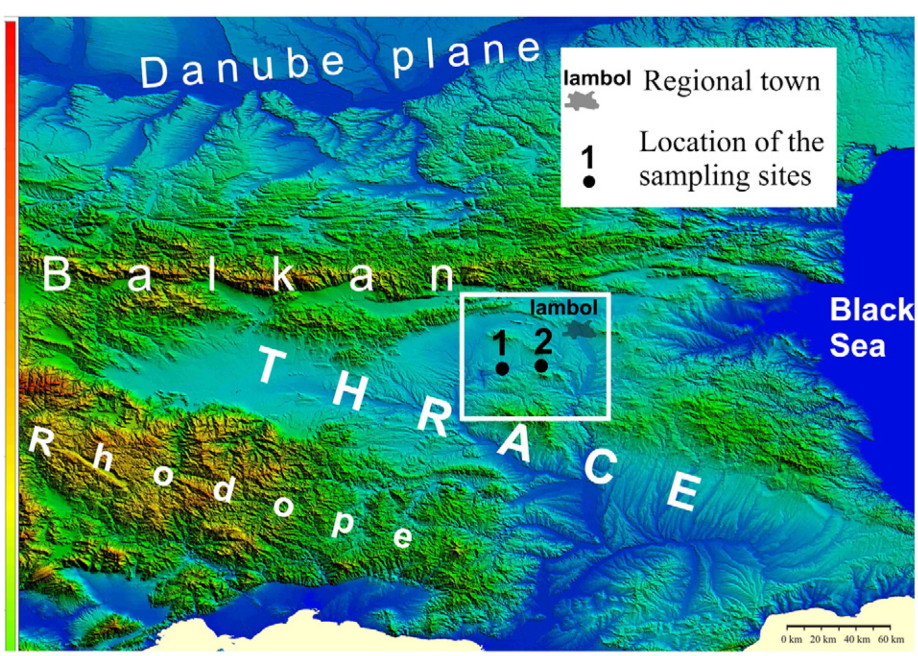

Fig. 1. Digital elevation model of the eastern Balkan Peninsular derived from SRTM 90. On the model is shown part the Thrace intermountain basin, where the sampling sites are located.