Rhizosphere 6 (2018) 67–73

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

Plant growth promotion traits of rhizosphere yeasts and their response to soil characteristics and crop cycle in maize agroecosystems

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ARTICLE INFO

Keywords:
Agroecology
Maize
Soil
Rhizosphere
Yeasts

ABSTRACT

Yeasts are common soil inhabitants, but information about their ecology is limited. Here we examined the abundance of rhizosphere yeasts in six conventional maize agroecosystems in two different geographic areas in Mexico differing in soil characteristics and agricultural practices. In order to examine the plant growth promotion potential of maize rhizosphere yeasts a collection of yeast species was obtained, which were identified taxonomically in terms of sequencing of the D1D2 domain. Main results showed that yeasts were present in all maize fields during the complete growing cycle, though highest during flowering. The abundance of rhizosphere yeasts responded negatively to soil pH and amount of Mg. The maize rhizosphere yeast collection obtained included eight species from six genera with the Ascomycota species *Meyerozyma guillermondii* and *Candida railenensis* as the most frequent. Four out of the eight yeast species solubilised $Ca_3(PO_4)_2$, whereas none of the yeasts solubilised $FePO_4$. Maize plant growth was promoted after inoculation with *Cryptococcus flavus* and *Solicoccozyma aeria* in terms of shoot dry weight and with *C. railenensis* in terms of root dry weight, but only in combination with mineral P fertilisation. In conclusion, rhizosphere yeasts with plant growth promotion traits are common in maize agroecosystems, where soil physico-chemical characteristics and plant growth stage seem to determine their abundance.

1. Introduction

The rhizosphere is a biological hot spot in the soil where interactions between roots and soil biota take place, which regulate plant and soil nutrient dynamics (Lynch and de Leij, 2012). Rhizosphere microorganisms play an important role in soil fertility since they are involved in the cycling of nutrients like phosphorus and nitrogen, which are required for plant growth (Philippot et al., 2013). Plant growth promoting traits of soil microorganisms include nitrogen fixation by diazotrophic bacteria and phosphate solubilisation by bacteria and fungi (Larsen et al., 2014).

Yeasts are a polyphyletic fungal group belonging to the Ascomycota and Basidiomycota (Kurtzman et al., 2011). Many yeasts are common soil inhabitants (Botha, 2006) in both natural (Yurkov et al., 2012) and agricultural ecosystems (Sláviková and Vadkertiová, 2003). Some of the most frequently isolated soil yeasts belong to the genera *Candida, Cryptococcus, Rhodotorula, Lipomyces, Sporobolomyces, Trichosporon, Pichia, Saccharomyces, Debaryomyces, Aureobasidium* and *Williopsis* (Yurkov et al., 2012; Sláviková and Vadkertiová, 2003).

Though yeasts are abundant in soil (Xu et al., 2012) limited information is available about their ecology and the role of soil yeasts in agroecosystems is largely unknown (Botha, 2006). However, known functional traits of soil yeasts include organic matter decomposition, phosphate solubilisation, root growth promotion, soil aggregation and biocontrol of root pathogens, and yeasts also serve as prey for other soil biota (Botha, 2011). More specifically, plant growth promotion by soil yeasts has been reported in rice (Amprayn et al., 2012), sugar beet (El-Tarabily, 2004) and maize (Sarabia et al., 2017a; Nakayan et al., 2013; Gollner et al., 2006; Nassar et al., 2005), which has been linked to P solubilisation (Nakayan et al., 2013), increased root growth induced by indole acetic acid (Nassar et al., 2005) and improved P uptake (Sarabia et al., 2017b).

Integration of soil microorganisms, including yeasts, in agroecosystems is pivotal for the development of strategies to improve crop nutrient use efficiency for key plant nutrients like phosphorus, which is often limiting plant growth due to its low mobility in soil (Richardson

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https://doi.org/10.1016/j.rhisph.2018.04.002
Received 7 March 2018; Received in revised form 12 April 2018; Accepted 13 April 2018
Available online 14 April 2018

et al., 2011). However, in order to integrate plant beneficial soil microorganisms in agroecosystems, profound knowledge about their ecology is required. This includes information about their abundance, functional traits, interactions with other soil biota and how they respond to the soil environment and agricultural practice (Larsen et al., 2014).

Maize is worldwide an important cereal crop for human consumption and animal fodder (Bennetzen and Hake, 2009) and in Mexico maize is the main basic food crop. In the present study, we examined the response of maize rhizosphere yeasts to soil physico-chemical characteristics and plant growth stage in conventional maize agroecosystems in Mexico as well as their *in-vitro* P solubilisation traits and plant growth promotion potential under different mineral P fertilization scenarios.

2. Materials and methods

2.1. Study areas

Six different rain-fed maize fields in Mexico were selected according to agricultural practice and geographic site. Field 1 (20° 38.32′N 101° 28.11′W 1733 m above sea level (masl)), field 2 (20° 39.36′N 101° 24.22′W 1719 masl) and field 3 (20° 45.11′N 101° 23.51′W 1747 masl) were located in Irapuato, Guanajuato and field 4 (19° 26.80′N 98° 46.30′W 2989 masl), field 5 (19° 26.81′N 98° 46. 24′W 2994 masl) and field 6 (18° 28.79′ N 98° 47. 88′W 2512 masl) in Estado de Mexico (State of Mexico). All six maize fields had been cultivated with monoculture maize for more than ten years before sampling of rhizosphere soil in the autumn 2013. Characteristics of the six maize agroecosystems are presented in Table 1.

2.2. Soil physico-chemical characteristics

Before rhizosphere soil sampling, representative soil samples between rows were collected in a zig-zag design for physico-chemical parameters, soil type, pH, P, N, K, Ca, Mg, Na, Fe, Zn, Mn, Cu, organic matter content, clay, carbonates and base saturation according to the soil fertility analyses service provided by INIFAP (Instituto de Investigaciones Forestales, Agrícolas y Pecuarias, Celaya, Mexico) (Supplementary material Table 1).

2.3. Rhizosphere soil sampling

In each of the six fields, an X sampling design was employed with the four extreme points and the center as the sampling points resulting in five samples. In each field the outermost 3 m of the four sides were excluded to avoid neighbour effects. Sampling of maize plants was performed at three plant growth stages, including vegetative (v6, six leaves stage, July 2013), flowering (both female and male reproductive organs visible, September 2013) and senescence growth stages (entire aboveground plant system completely dry, November 2013). From each of the five sampling points from all three vegetative growth phases in all six fields complete 30 × 30 × 30 cm blocks of soil were excavated with the plant stem as the centre and transferred to laboratory facilities for subsequent sample processing and analyses. Excessively wet soil blocks were left to dry for some days outside to facilitate soil mixing and sampling. Stones, large debris and macro fauna were removed from the soil, which thereafter was homogeneously mixed. Approximately 100 g soil from each of the five soil blocks was mixed in a composite sample, which was divided in two subsamples each with approximately 250 g soil. Soil samples were kept in the refrigerator (4 °C) until further processing, but with a maximum of two days storage after sampling.

Table 1
General features of the maize fields examined.

Feature	Study sites	
Field	1, 2, 3	4, 5, 6
State	Guanajuato	State of Mexico
City	Irapuato	Texcoco
Altitude (m above sea level)	1700–1750	2500–3000
Climate	Dry temperate	Subhumid temperate
Farming system	Rainfed maize monoculture	Rainfed maize monoculture
Fertilisation type and level (kg N ha^{-1})	Synthetic and organic (30–200)	Synthetic and organic (150)
Seed variety	Hybrid	Land race
Cultivation	Tractor	Tractor, animal
Pest, disease and weed control	Chemical, mechanic	Chemical, manual
Maize yield (ton ha^{-1})	10	2

2.4. Quantification and isolation of yeasts

From each of the six maize fields two rhizosphere soil (80 g) samples obtained from the composite soil sample were individually suspended in 720 ml sterile millipore water. The soil suspensions were subjected to serial dilutions (10^{-1}–10^{-10}). From each dilution, 0.1 ml sample was plated in duplicate on sterile Saboraud media with chloramphenicol (65 g sabouraud agar and 0.1 g chloramphenicol in 1 l Milipore water) and incubated at 28 °C for 3–5 days, after which yeasts colonies were counted and the abundance of yeasts in soil expressed as colony forming units (CFU) g^{-1} soil dry weight. Ten colonies were randomly isolated from plates representing the three plant growth stages from both geographic sites, transferred to potato dextrose agar (PDA) (39 g of PDA in one liter of Milipore water) and stored at 4 °C for further species identification.

2.5. Taxonomic identification of yeasts

Pure yeast isolates were grouped into morphotypes according to color and texture based on fresh cultures on PDA. Genomic DNA was extracted from solid cultures on Saboraud medium after 4 days of incubation at 28 °C. The cells were collected and placed in 1.5 mL microcentrifuge tubes containing 450 µL of NTES lysis buffer (250 mM NaCl, 200 mM Tris–HCl pH 8.5, 25 mM EDTA, 0.5% SDS and 0.01% β-mercaptoethanol). The cells were broken by vortexing the suspension for 2 min at maximum speed. A volume of phenol-chloroform (25:25, pH 8.0) was added, vortexed for 1 min and centrifuged to extract the organic phase. After RNAse A (Invitrogen™) treatment, a volume of phenol-chloroform was added and the DNA was precipitated with 0.5 volumes of isopropanol at − 20 °C. Pellets were washed with ethanol (70% v/v) and resuspended in 20 µL of sterile deionized water.

The primer pair NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) (O'Donnell, 1993) were used to amplify the D1/D2 domain of the 26 S ribosomal DNA gene. The amplifications were carried out in 30 µL reaction volume containing 25 ng of template DNA, 1 µM of each primer and Platinum® PCR SuperMix (Invitrogen, USA). PCR reactions were done using a Veriti® Thermal Cycler (Thermo Fisher Scientific) instrument with the following amplification schedule: one initial denaturing cycle at 94 °C for 6 min, followed by 35 cycles of denaturing at 94 °C for 1 min, alignment at 62 °C for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 7 min. Both isolated DNA integrity and amplification products were analyzed in 1% agarose gels stained with SYBR® safe (Thermo Fisher Scientific). The amplification products (600 pb) were purified and sequenced by Macrogen Inc. (Rockvill, MD, USA). The obtained sequences were submitted to GenBank with accession numbers KY952838 to KY952876.

The sequences of the D1D2 domain of the yeasts were individually analyzed within the database of the NCBI GenBank by means of the algorithm BLASTn. Sequences displaying a similarity of 98% or higher with study sequences were selected for creating a local database from