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Screening rhizobacteria for biological control of *Fusarium* root and crown rot of sorghum in Ethiopia

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Abstract

Fusarium oxysporum Schlectend causes root and crown rot in several crops including sorghum that results in low grain yield in Ethiopia and other East African countries. Seventy-eight bacterial isolates were obtained and subsequently tested both *in vitro* and in the greenhouse. Of the 78 isolates tested, 23 displayed between 30 and 66.3% inhibition of *in vitro* mycelial growth of *F. oxysporum* and also showed significant root colonization ability on sorghum seedlings. These isolates were further tested for their biocontrol ability against *F. oxysporum* in the greenhouse. Four isolates viz. KBE5-7, KBE5-1, KBE2-5 and NAE5-5 resulted in 100% disease suppression and no symptoms of root and crown rot were observed compared to the control. The complete suppression of *F. oxysporum* by these isolates was also confirmed by root plating on Fusarium-selective medium. The most effective isolates were identified by means of the API system as members of the Genus *Bacillus* including *B. cereus*, *B. subtilis*, *B. circulans*, *B. licheniformis* and *B. stearothermophilus*. Two other isolates, which colonized the sorghum rhizosphere and resulted in more than 70% disease suppression, have been identified as *Chromobacterium violaceum*. The study demonstrated effective biological control by the rhizobacterial isolates tested, thereby indicating the possibility of application of rhizobacteria for control of soilborne diseases of sorghum in Ethiopia and other countries.

Keywords: Plant growth promoting rhizobacteria; *Fusarium oxysporum*; *Bacillus cereus*; *B. subtilis*; *B. circulans*; *B. licheniformis*; *B. stearothermophilus*; *Chromobacterium violaceum*; Biocontrol; Sorghum; Root colonization; *Fusarium* root and crown rot

1. Introduction

Sorghum (*Sorghum bicolor* (L.) Moench) is ranked second among the five most important crops in East Africa (Food Agricultural Organization, 1999) and it is a staple food crop in arid and semi-arid areas in Ethiopia. The crop grows under a wide range of ecological conditions and is drought tolerant. Production is, however, very low in this country because of, amongst other factors, diseases caused by phytopathogenic fungi. Symptoms such as seedling death and root rot a few weeks after planting are commonly observed in the major sorghum fields.

Several members of the Genus *Fusarium* cause root diseases in sorghum leading to serious yield losses. Among the major pathogens in this group are *Fusarium oxysporum* Schlectend, *F. moniliforme* J. Sheld, *F. graminearum* Schwabe and *F. tricinctum* (Corda) Sacc. (Forbes et al., 1986). Most of the fungal pathogens reported on sorghum are found predominantly in Ethiopia and other East African countries (Huluka and Else, 1992).

In Ethiopia, repeated attempts have been made to control *Fusarium* root rot in sorghum with fungicidal treatments using, amongst others, benomyl. However none of the fungicides used have been successful mainly because of their phytotoxicity (Benhamou, 1992). Chemical control of sorghum diseases is also unaffordable in most developing countries. The use of broad-spectrum

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fungicides further results in imbalances within the microbial community creating unfavorable conditions for the activity of beneficial organisms (Villajuan-Abgona et al., 1996).

Soilborne diseases have been controlled more recently by means of certain beneficial bacteria that are indigenous to the rhizosphere of plants (Thomshaw, 1996). The rhizosphere, representing the thin layer of soil surrounding plant roots and the soil occupied by the roots, supports large and metabolically active groups of bacteria (Villacieros et al., 2003) known as plant growth promoting rhizobacteria (PGPR) (Kloepper et al., 1980). PGPR are known to rapidly colonize the rhizosphere and suppress deleterious microorganisms as well as soilborne pathogens at the root surface (Rangajaran et al., 2003). These organisms can also be beneficial to the plant by stimulating growth (Bloemberg and Lugtenberg, 2001).

Currently there is very limited knowledge regarding the biological suppression of *Fusarium* root and crown rot in sorghum by the application of PGPR in Ethiopia. The aim of this study is therefore to isolate bacteria from the sorghum rhizosphere and screen the organisms for *in vitro* and *in vivo* antagonistic activity against *F. oxysporum*, one of the major causes of root rot in Ethiopia. This study may contribute to the introduction of PGPR systems in biological control of phytopathogenic fungi in sorghum and other crops in Ethiopia.

2. Materials and methods

2.1. Soil sample collection and analysis

In December 2004, a total of nine soil samples were collected from the rhizosphere of sorghum in two fields in Ethiopia, namely Meeson and Jijiga. Both areas are located in the Eastern part of the country where sorghum is commonly produced as a staple crop. These sites were selected because the soils have been previously cropped for at least 20 years with sorghum, maize and teff (*Eragrostis teff*), an indigenous crop of the grass family commonly used as a staple crop in the Northern and Central Ethiopia. Farming practices in these fields include fungicide sprays with benomyl and metalaxyl. After harvest, the remaining stubble is ploughed into the soil. Five collection sites in Meeson and four collection sites in Jijiga were selected and carefully marked before sample collection. Sorghum roots were uprooted and 1 kg rhizosphere soil was carefully transferred into sterile plastic bags before they were transported to the Microbiology Laboratory, Alemaya University, Ethiopia for isolation of bacteria. The soil particle size varies among the nine samples. This ranges from 18 to 61.2% for coarse sand, 11.5 to 37.7% for silt, and 21.8 to 61.1% for clay. All the soil samples in general have a larger proportion of Calcium (Ca) ranging from 838 to 8830 mg/kg followed by magnesium (Mg), potassium (K), and sodium (Na) ranging from 191 to 1079 mg/kg, 88 to 583 mg/kg, and 20 to 88 mg/kg, respectively. The least detected inorganic component was phosphorous (P) and this ranged from 0.1 to 1 mg/kg.

2.2. Isolation of bacteria

Each soil sample was mixed before 1 g was transferred to 9 ml quarter strength sterile Ringer's (Merck, Halfway house, South Africa) solution and was serially diluted. A 0.1 ml aliquot of the serially diluted suspension was spread-plated on King's B medium (King et al., 1954) and nutrient agar (NA) medium (Biolab, Wadesville, South Africa) in triplicate. The spread-plate cultures were incubated for 24 h. at 28 °C. Ten to fifteen representative colonies, with different morphological appearances, were selected from the countable plates and re-streaked on a new plate but of the same media to obtain pure colonies. A total of 142 isolates originally obtained in this manner were maintained on agar slants and transported to the Plant Pathology Laboratory at the Department of Microbiology and Plant Pathology, University of Pretoria, South Africa. Because many isolates were morphologically indistinguishable in culture, preliminary characterization procedures included the following tests: Gregorson's KOH (Gregorson, 1978), cytochrome oxidase (Kovacs, 1956), oxidation fermentation (Huge and Leifson, 1953), catalase and motility tests. A total of 78 isolates were selected from the original 142 isolates. Soils were chemically analysed for their content of inorganic elements in the soil analysis laboratory of the Department of Plant Production and Soil Sciences, University of Pretoria, South Africa.

2.3. Bacterial inoculum preparation

Isolates were grown in nutrient broth (BioLab, Wadesville, South Africa) on a rotary shaker (LABOTECH) at 28 °C and 180 rpm for 24 h. The suspension was centrifuged (Avanti TM J-25 Beckman centrifuge) in 50 ml capacity sterile plastic tubes at 5000 rpm for 10 min. The pellets were re-suspended in quarter strength sterile Ringer's (Merck) solution to give a final concentration of 10^8 cfu/ml (OD = 0.5) at 550 nm using the viable plate count method and optical density measurement.

2.4. In vitro antagonistic activity

The *in vitro* inhibition of mycelial growth of *F. oxysporum* by the bacterial isolates was tested using the dual culture technique as described by Paulitz et al. (1992) and Landa et al. (1997). Three 50 µl drops from the 10^8 cfu/ml suspension were equidistantly placed on the margins of potato dextrose agar (PDA) (BioLab) plates and incubated at 28 °C for 24 h. A 4 mm agar disc from fresh PDA cultures of *F. oxysporum* was placed at the centre of the PDA plate for each bacterial isolate and incubated at 27 ± 1 °C for seven days. The radii of the fungal colony towards and away from the bacterial colony were mea-