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RESEARCH ARTICLE

Biological Control of Fungal Brown Spot Disease in Rice Plants by *Streptomyces spp.*

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Abstract

This study used thirty isolates of streptomycetes were isolated from different locations of soil. All these isolates were subjected for antagonistic test (as biological control agents) against Brown spot fungus *Bipolaris oryzae*. About 43% of tested streptomycetes isolates gave antifungal activity against Brown spot fungus but 57% of tested organisms did not reveal any antifungal activity. The highest antifungal activity were obtained from two isolates (42 mm and 45 mm), both streptomycetes isolates were identified according to International *Streptomyces* Project (ISP) based on morphological (macroscopic and microscopic) and physiological characterization as *Streptomyces orientalis* and *Streptomyces exfoliatus*.

Keywords: *Biological control, Streptomyces orientalis, Streptomyces exfoliatus, Bipolaris oryzae, Brown spot disease.*

Introduction

Rice is very important food crops of the world. Its yield is affected by several diseases causes both quantitative and qualitative losses of rice crop. One of these diseases is fungal brown spot disease caused by Bipolaris oryzae. It causes seedling blight and damages the foliage and panicles of rice plants [1].

Bipolaris oryzae (Breda de Haan) Shoemaker Other names: Drechslera oryzae and Helminthosporium oryzae Teleomorph: Cochliobolus miyabeanus (Ito and Kuribayashi) Drechsler ex Dastur (Etymology: from bipolaris, bipolar, referring to the bipolar germination of the spores). The brown spots can be seen on the leaves (gray or whitish centers on maturity), leaf coleoptile, leaf sheaths, and panicle branches.

Blackish lesions may be appear on young roots and when optimum conditions Bipolaris oryzae development growth and can be seen over the seeds, may enter the glumes and leave blackish spots on the endosperm of rice plants [2]. Streptomyces species are abundant in soils and able to produce a group of secondary metabolites (bioactive substances) against phytopathogenic fungi, such as antibiotics and extracellular enzymes, which have a role in degradation of complex molecules especially [3].Streptomyces vinaceusdrappus was gave percentage growth inhibition of the rice phytopathogens (Curvularia oryzae 65.00; Bipolaris oryzae 53.5; Pyricularia oryzae 53.5; Fusarium oxysporium 48.84) [4].

Streptomyces sp. strain 5406 has been used in China for the past thirty five years to protect cotton crops against soil borne pathogens [5], also Streptomyces flavotricini that gave antifungal activities against fungal blast disease in rice plants (Pyricularia oryzae) and obtained inhibition zone (40 mm) [6].

The fungicides and resistant cultivars can be controlled on growth of the phytopathogenic fungi but these agents such as chemical pesticides are harmful to host plant resistance and environment. Therefore, selection antagonistic microorganisms were achieving better method in crop protection against various phytopathogenic [7].

The goal of this study is evaluate the efficacy of biocontrol using activity of streptomycetes isolates were isolated from field soil against fungal brown spot disease caused by Bipolaris oryzae under in vitro conditions.

Material and Methods

Thirty streptomycetes isolates were isolated from different locations of fields in Egypt, by collection several random rhizosphere soil samples in depth (20 -25) cm. Soil samples were collected in clean plastic bags. The samples were air dried, sieved in 4 mm mesh screen. This samples were mixed thoroughly with $CaCO_3$ (10% w/w) and incubated at 30°C for 10 days to increase the number of *Streptomyces* [8], for isolation of the streptomycetes isolates, using the serial soil dilution technique [9].

Five g of each soil sample was agitated in 95 ml of sterile distilled water in 250 ml Erlenmeyer flask. One ml samples of proper serial dilutions (10^{-2}, 10^{-3}, 10^{-4}) were spread onto the surface of solid starch- nitrate medium. Three plates (replicates) were established for each dilution and the plates were incubated at 28°C for 7 days.

Streptomyces colonies were developed and checked for purity by repeating the sub-culturing and pure colonies were preserved on agar slant of starch nitrate medium. The antagonistic activity of the 30 streptomycetes isolates were evaluated against *Bipolaris oryzae* (*Bipolaris oryzae* (van Breda de Haan) Shoemaker, anamorph. EMCC Number 819 (Designation: Mousa (129), 2001) using dual culture technique [10].

Eight mm diameter mycelia disc of each test antagonist streptomycetes isolates taken from 5-7 day old culture on starch nitrate agar was paired against 8 mm diameter mycelia disc of *Bipolaris oryzae* at opposite end on (Potato Dextrose Agar) PDA contained in 9 cm diameter Petri- dish.

The pathogen and antagonist disc were placed at equal distances from the periphery of the Petri- dish. The PDA plates inoculated only with *Bipolaris oryzae* as control. The plates were incubated at 28± 2°C. Data were obtained for percent inhibition of radial growth (PIRG) = (R1–R2)/R1 x100. Where R1 = radial growth of pathogen in control. R2 = radial growth of pathogen in dual culture experiments with antagonists.

The zone of inhibition was recorded as the distance in mm between the fungal pathogen and the area of antagonist growth. Morphological (macroscopic and microscopic) and physiological characterization of the *Streptomyces* species were tested according to the standard protocol of the International *Streptomyces* Project (ISP) [11, 12].

The color of aerial mycelium and macroscopic characters were noted using cultures developed on solid starch nitrate medium (ISP 1) incubated for 7 and 14 days at 28°C, Malt Extract-Yeast Extract Agar (ISP 2), Glycerol Asparagine Agar (ISP 5) and Glucose nitrate agar medium.

The color of substrate mycelium was checked using cultures grown at 28°C for 7, 14 and 21 days on (ISP 1) and the presence of soluble pigments were noted on (ISP 2, 5) medium. The macroscopic and microscopic characters of the *Streptomyces* species were assessed as described by [11] as colour of aerial mycelium and colour of substrate mycelium. While, spore ornamentation examined by Transmission Electronic Microscope (TEM).

The physiological activities used with other characters to identify the species of streptomycetes isolates using many tests as carbon sources utilization using 8 carbon source (D-glucose, D-fructose, sucrose, D-mannitol, arabinose, xylose, raffinose and inositol), production of melanin pigments, gelatin liquefaction for (production of proteolytic enzymes), coagulation and peptonization of milk (production of proteolytic enzymes), cellulose decomposition and starch hydrolysis.

Results and Discussion

Thirty streptomycetes isolates were purified and maintained on starch nitrate agar. The antifungal activity of these isolates different from each other, 43% of tested streptomycetes isolates were active against *Bipolaris oryzae*.

While, 57% of tested organisms were not revealed any antifungal activity against *Bipolaris oryzae* .The highest antifungal activities were obtained from the culture of isolates (No. 3b and No.1b) (The results present in Figure 1 and Table 1). Both isolates were chosen for further experimental studies.

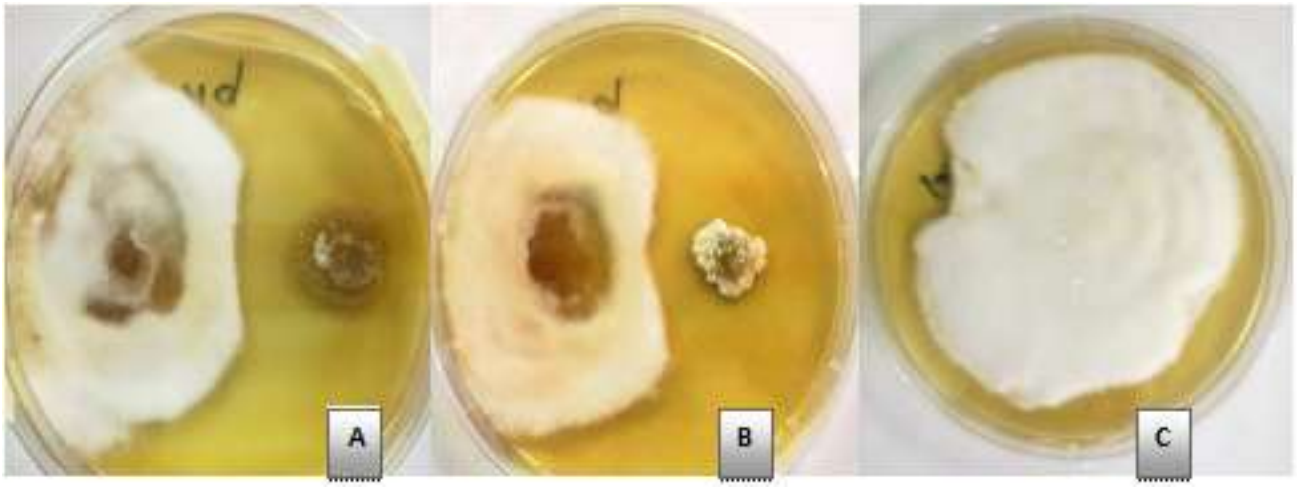

Fig. 1: A- Antagonism test No. 3b isolate against *Bipolaris oryzae* (Dual culture) 7 days old culture on PDA medium, B- Antagonism test No. 1b isolate against *Bipolaris oryzae* (Dual culture) 7 days old culture on PDA medium, C- *Bipolaris oryzae* 7 days old culture on PDA medium as control

Table 1: The highest antifungal activities of streptomycetes isolates against *Bipolaris oryzae* on PDA medium.

No. Of streptomycetes isolates	Diameter inhibition zone (mm)	*(PIRG)
1b	42	49.3%
3b	45	53.5%

*PIRG more than 75% considered as strong inhibition, 50 - 75% as moderate inhibition, 25 - 50% as low inhibition and 10 - 25% as slight inhibition

The results of antagonistic test that obtained from this study were in an agreement with many investigators used the antagonistic activities of streptomycetes against the microorganisms, [6] found that 64% of tested actinomycetes isolates that isolated from soil were active against *Pyricularia grisea* but 36% of tested organisms did not reveal any antifungal activity against *Pyricularia grisea*, also *Streptomyces globisporus* in dual cultures on solid media, inhibited mycelia growth of several plant pathogenic fungi, especially that of *Magnaporthe oryzae*, *Bipolaris maydis* and *Cryphonectria parasitica* [7].The morphological and cultural (macroscopic, microscopic), physiological characterization of (No. 1b and No. 3b) isolates were tested for identification and taxonomic studies. Both isolates proved that they were aerobic, spore forming, Gram positive, form extensive branching substrate mycelia that are not able to fragment into bacillary or coccoid forms. Comparing the aforementioned results with those described for *Streptomyces*, it was concluded that they are similar and thus, (No. 1b and No. 3b) isolates were identified as *Streptomyces*. Table 2 shows morphological and physiological characterization and Table 3, 4 show cultural properties of both streptomycetes isolates on different medium that was done according to (ISP) [11, 12].

Table 2: Physiological characterization of (No. 1b and No. 3b) isolates.

Physiological tests	Results	
	1b	**3b**
Soluble pigments	ND*[1]	ND
Spore chains	Straight	Straight
Spore surface	Smooth	Smooth
Carbon source utilization		
D- glucose	+5*[2]	+4
D- fructose	+5	+4
Sucrose	-ve	+3
D- mannitol	+4	+3
Raffinose	-ve	+4
Inositol	+3	+4
Arabinose	+4	+2
Xylose	+4	+2
Gelatin liquefaction	+ve	+ve
Coagulation and peptonization of milk	+ve	+ve
Decompose of cellulose	+ve	+ve
Starch- hydrolysis	+ve	+ve
Produce melanin pigments	ND	ND

*[1]ND: No produced; *[2]+5 excellent, +4 very good, +3 good and +2 moderate growth, -ve No growth

Table 3: Cultural properties of (1b) isolate on different medium.

Type of Medium	Type of growth	Color of colony			Growth intensity
		Aerial mycelium	Reverse side of colony	Diffusible pigments	
Starch- nitrate agar	flat- good sporulation	off white greyish	yellow- yellow brownish	non pigmented	*+ 4
Glycerol- asparagin agar	flat- good sporulation	off white	yellow whitish	non pigmented	+4
Malt-yeast extract agar	thin- good sporulation	white	off white- yellow	non pigmented	+3
Glucose- nitrate agar	thin- good spoulation	white	white yellowish	non pigmented	+3

Table 4: Cultural properties of (3b) isolate on different medium

Type of Medium	Type of growth	Color of colony			Growth intensity
		Aerial mycelium	Reverse side of colony	Diffusible pigments	
Starch- nitrate agar	elevated- good sporulation	pink	yellow brownish	non pigmented	*+ 4
Glycerol- asparagin agar	elevated- good sporulation	yellow	yellow	non pigmented	+3
Malt-yeast extract agar	elevated- good sporulation	yellow whitish	dark yellow	non pigmented	+4
Glucose- nitrate agar	thin- good spoulation	yellow	off white yellowish	non pigmented	+3

* (+4) very good growth, (+3) good growth

According to the morphological and physiological characteristics above and comparing these characteristics with those used in working key, suggested by Küster (1972) [13] (Key for classification and identification of *Streptomyces* species included in International *Streptomyces* Project (ISP)), identified (1b) isolate as *Streptomyces orientalis* and (3b) isolate as *Streptomyces exfoliatus*.

These results are in an agreement with various investigations used the International *Streptomyces* Project (ISP) for characterization and identification of *Streptomyces* isolates such as [6, 14]. Many reports refer to the role of actinomycetes, specially *Streptomyces* strains, in plant protection and are antagonists against most of the phytopathogenic fungi. The results of antifungal activity were in an agreement with several workers as [15] whom reported that *Streptomyces exfoliatus* produced antifungal activity against the many of tested fungi and the measure of inhibition zones were 30.6 mm, 25 mm, 35 mm, 31 mm against *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum* and *Candida albicans*. While, *Streptomyces exfolaitus* (SG7) showed the highest antagonism against *Fusarium culmorum* and strains of *Rhizoctonia solani* [16], also *Streptomyces orientalis* was obtained from several marine sediment samples gave antifungal activity against *Helminthosporium oryzae*, *Pyricularia oryzae*, *Rhizocotonia solani* and *Colletotrichum falcatum* [17].

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