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Potential role of *Kosakonia radicincitans* to suppress *Fusarium oxysporum* in Maize plants

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Abstract

Maize (*Zea mays* L.) is the most important grain crop and a staple meal in the diet. Globally, yields are restricted by Fusarium root rot of maize (*Zea mays* L.). The purpose of this study is to assess *Kosakonia radicincitans*' ability to combat *Fusarium oxysporum*. The *K. radicincitans* strain that was examined tested positive for hydrogen cyanide, ammonia and siderophores (31.7%). Concerning antibiotics, it was resistant and intermediate to all antibiotics except streptomycin. In addition, it restricted the mycelia of *F. oxysporoum* and exhibited a highly antagonistic effect with inhibition percentage (80.07%) *in vitro*. Scanning electron microscopy (SEM) revealed severe morphological damage to fungal hyphae, including hyphal shrivelling and spore reduction. *In vivo* this bacterial treatment was efficient in reducing disease incidence up to 8% as the chemical fungicide and comparing that to the control treatment. In addition, this treatment improved the plants' fresh weight of roots and shoots, height, and chlorophyll compared to the control. This study suggests that *K. radicincitans* can be used as a natural fungicide to combat harmful fungus. therefore, it might be a promising agent to reduce the dependence on the synthetic fungicides.

Keywords: Biocontrol, *Kosakonia radicincitans*, plant pathogens, fungicides

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1. Introduction

Worldwide, plant pathogens which include bacteria, viruses, nematodes, and fungi severely damage or destroy crops. This loss poses a significant annual risk to global food production. Plant diseases are mostly caused by fungi, which are also responsible for the global harvest failures of crops like maize and other grains [1]. One of the most significant fungal plant infections that seriously damages a variety of crops is Fusarium [2]. *Fusarium oxysporum* is a genus of filamentous fungi that includes numerous significant plant diseases for agronomy [3]. In line with [4], *Fusarium oxysporum* is a harmful fungus that impacts all stages of plant growth. To control this fungal infection, numerous strategies were used [5]. Chemical fungicides are used primarily in commercial agriculture to kill and inhibit the cells and spores of fungus, protecting agricultural plants against fungal diseases [6]. Fungicide overuse or inappropriate usage has a negative impact on beneficial biological systems, the environment, and the health of people and animals. In addition, the emergence of resistant strains of fungal phytopathogens complicates the treatment of fungal infections in plants.

To effectively control fungal infections in plants, it is important to find safe, non-toxic, and environmentally friendly alternatives to chemical and synthetic fungicides. These alternatives are known as "green strategies of fungal control [7,8]. It has been shown that some plant growth-promoting rhizobacteria (PGPR) contribute to defence mechanisms against pathogens [9] and insects [10]. Sustainable agriculture is highly interested in traits that enhance output and reduce susceptibility to biotic stresses because they offer respite from the excessive use of synthetic fungicides, pesticides and fertilizers [11]. *Kosakonia radicincitans* is a rod-shaped, PGP gram-negative bacterium that was discovered in the new genus Kosakonia of the Enterobacteriaceae family [12]. A number of Kosakonia species that can enhance plant development have been isolated from various plants [13]. [14] isolated and identified *Kosakonia radicincitans* strain DSM 16656, which has accession number OM980222.1. It could solubilize potassium and phosphate *in vitro* and tested positive for exopolysaccharides, indole-3-acetic acid production and nitrogen fixation In comparison to the control plants, According to [14], this bacterial strain enhanced the chlorophyll, grain yield, and 100-grain weight of wheat plants grown in the salt-affected soil and also reduced proline. A previous study [15] demonstrated the bacterium's ability to make siderophores taking into account the role of siderophores in the biocontrol interactions. An other study [16] showed that *Rahnella aquatilis* and *Kosakonia radicincitans*, either alone or in combination, are effective for preventing various forms of postharvest rot in apples that are kept in storage.

Maize or corn (*Zea mays* L.) is one of the world's most important crop plants [17]. Root rot pathogens are responsible for several of the most significant plant diseases that affect multiple crops globally [18]. The objective of this study was to assess *Kosakonia radicincitans'* effectiveness against Fusarium root rot of maize, which represents a serious risk to crop.

2. Materials and Methods

2.1. Bacterial strain

The endophytic *Kosakonia radicincitans* strain, accession number OM980222.1, was previously isolated from the root nodules of faba bean (*Vicia faba*) plants grown in clay soil that has been affected by salt in Egypt [14].

2.2. Fungal Material

Fusarium oxysporum was obtained from Central food safety Lab, Faculty of Agriculture, ASU and was stored on potato dextrose agar (PDA) slants prior to use

2.3. Chemical Fungicide

To prevent soil-borne diseases, it was recommended to use 1.5 L/hectare of the fungicide Uniform 390 SE (azoxystrobin + mefenoxam), produced by the Syngenta company in Basel, Switzerland.

2.4. Detection of hydrogen cyanide (HCN)

The bacterial strain under study was examined to produce HCN following [19]. The studied strain was observed on standard nutritional (SN) agar medium supplemented with 4.4 g glycine/L, and it was thereafter incubated at 28^0C. After 48 hours of inoculation, the formation of cyanide was observed and confirmed by looking for color changes in a piece of filter paper that had been saturated with 0.5% picric acid and 2.0% sodium carbonate. The colour changed from yellow to light brown, brown, or reddish brown, indicating weak, moderate, or severe cyanogenic activity. Control plates were used without inoculation.

2.5. Assay for NH3 production

In order to assess the bacterial strain's ability to produce ammonia in peptone water, 10 ml of freshly formed culture were added to each tube, and the tubes were then incubated for 48 hours at 30°C. Following the application of 0.5 ml of Nessler's reagent to each tube, the development of a brown to yellow colour indicated a positive test for ammonia production.

2.6. Siderophores production

The technique of [21] was utilized for the quantitative estimation of siderophores and at 630 nm, the optical density was observed. The method of [22] was used to calculate the quantity of siderophores in the aliquot.

2.7 Resistance test to antibiotics

The antibiotic resistance of the selected bacterial strain was estimated using the conventional disc diffusion method as outlined by [23], in presence of ampicillin, azithromycin, colonistin, gentamicin, kanamycin, oxytetracycline, and streptomycin. Using Gram-negative bacteria's normal range for antibiotic resistance (inhibition zone width, mm), a strain is considered as resistant, intermediate, or susceptible [24].

2.8. Antifungal Activity Assay

Selected bacteria were added to potato dextrose agar (PDA) medium. About 25 millilitres of the growth media were applied to each petri dish, and they were then allowed to solidify. A five-day-old culture of the test fungus was put in the middle of the petri dish using a five-mm disc, and it was cultivated for seven days at 27°C. The growth was measured in millimeter. The control was PDA medium without of bacteria. Percentage inhibition of mycelia growth was calculated by using the formula:

% inhibition =dc– dt/dc.100

Where, dc = average increase in mycelial growth in control, dt = Average increase at each treatment [25].

2.9. Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to study the biocontrol interaction between a *Fusarium oxysporum* and the bacterial treatment. After cutting a little (3 mm) piece of hyphae at an interaction site, it was fixed in 2.5% glutaraldehyde (pH 7.4) and cultivated for 12 hours at 4°C. For five minutes, the mycelia were rinsed with.1 M phosphate buffer (pH 7.4). After 20 minutes of dehydration in ethanol solutions, the samples were dried for 24 hours at 25°C [26, 27], After the samples were gold-sputter-coated, they were examined with a Jeol JSM 5200 scanning electron microscope. The investigation was in Applied Center for Entomonematodes (ACE) as building located in the Experimental Research Station at Faculty of Agriculture, Cairo University, Giza, Egypt.

2.10. Efficacy of K. radicincitans (in vivo)

In a greenhouse at Agricultural Research Center (ARC), Giza, Egypt, a pot experiment was carried out during the summer season of 2022 to evaluate the effect of selected antagonistic bacteria against Root rot caused by *Fusarium oxysporoum*. *Zea mays* plant grains (C.V. Single cross173) were grown in 30 cm diameter pots that were filled with 5 kg of sterilized silt clay soil with a pH of 7.5, E.C. of 3.1 dSm^{-1}, organic matter of 1.7%, and available nitrogen of 17 ppm. Pots were arranged in randomized complete block design; the following treatments were practiced:

1) Uninoculated control (infected plants)

2) Chemical Fungicide

3) *Kosakonia radicincitans*

There were three pots (replicates) for each treatment, each holding five plants. The grains were submerged for two hours in 10 ml of the antagonistic bacterial culture (10^8 cells/ml). The grains in the control treatment were immersed in 10 ml of distilled sterile water. The plants were infected with 30 ml with *Fusarium oxysporoum* three weeks after they were sown (10^6 conidia /ml). Four weeks after fungal infection, Root rot incidence was calculated by the method of [28].

2.10.1. Plant growth parameter

Plants from each treatment were measured for height, removed from the pot, washed with distilled water, wiped with tissue paper, and the fresh weights were calculated.

2.10.2. Estimation of total chlorophyll content

Using a portable chlorophyll meter (SPAD-502), the amount of chlorophyll was measured 45 days after planting [29].

2.10.3. Soil analysis

The main soil properties of the experiment were determined as follow, electrical conductivity (EC) and soil organic matter (SOM) were measured in the saturated soil paste extract, and the pH was measured with a pH meter in soil suspension (1: 2.5) as explained by [30]. The modified Kjeldahal technique was used to measure the amount of nitrogen that was available by [31].

2.11. Statistical analysis

The general linear models approach from [32] was used to statistically analyse the findings that were produced. Duncan's multiple range tests were used to statistically assess the differences and determine how significant they were.

3. Results

3.1. Plant growth promotion assay

Plant growth promoting traits of bacterial strain of *Kosakonia radicincitans* were tested for production of Hydrogen cyanide (HCN), ammonia and siderophore as shown in Table &Figure (1). Numerous endophytic bacterial strains create HCN, a volatile secondary metabolite that protects plants from fungus and other diseases [33]. The plates' visual examination revealed that the selected bacterial strain had cyanogenic potential with a reddish brown color of Whatman filter paper no. 1 (soaked in a solution of 2% sodium carbonate and 0.1% picric acid). This hydrogen cyanide synthesis ensures that the PGPR strain is used as a biocontrol agent in agriculture, as demonstrated by [34,35].

PGPR generates ammonia, which has several biological functions, such as reducing disease and infection symptoms and inhibiting the growth of plant pathogens [36,37]. Additionally, the bacterial strain that produces ammonia supplies nitrogen to the host plants and supports biomass production [38]. In this study, the tested bacterial strain was positive for ammonia production suggesting its potential use as bio-control agent.

Concerning siderophore production the bacterial strain found to be positive (31.7 %). These findings agree with those of [39], who demonstrated that *K. radicincitans* exhibited a number of characteristics that promoted plant development such as phosphate solubilization, nitrogen fixation, siderophore, and indoleacetic acid synthesis. Under aerobic conditions with a pH between neutral and alkaline, the majority of the iron is found as the almost insoluble Fe (III) mineral, Fe $(OH)_3$ [40]. Plant development can be promoted by the rhizosphere bacteria's excretion of siderophores, which can either improve the plant's intake of iron or suppress plant diseases and other detrimental microbes as previously indicated by [41].

Table 1: Hydrogen cyanide, Ammonia and Siderophore production by the bacterial strain *in vitro*

Strain	HCN production	Ammonia production	% of Siderophore Unit
K. radicincitans	+	+	31.7

Large numbers of different microorganisms can be found in the rhizosphere. Some of them affect susceptible bacterial communities in the soil by producing antibiotics. Thus, the biocontrol agent's resistance to antibiotics is a desired property. It improves the bacteria's chances of proliferating, growing, and remaining in the soil [42]. Selected strain of *Kosakonia radicincitans* was studied for their sensitivity towards various antibiotics like Ampicillin, Azithromycin, Colistin, Gentamicin, Kanamycin, Oxytetracycline and Streptomycin on YEM plates. Based on the inhibition area values, the results shown in Table (2) and Figure (1) showed that, in comparison to the other antibiotics, particularly ampicillin and colistin, which have no zones of inhibition, streptomycin has a larger zone of inhibition, followed by oxytetracycline and azithromycin, respectively.

Table 2: *K. radicincitans's* zone of inhibition (in mm) on YEM plates

Strain	Zone-inhibition diameter (mm)						
	Ampicillin	Azithromycin	Colistin	Gentamicin	Kanamycin	Oxytetracycline	Streptomycin
K. radicincitans	0 R	15 I	0 R	8 R	10 R	18 I	20 S

R: Resistant I: Intermediate S: Sensitive

Fig. 1: Plant growth promotion assay of *Kosakonia radicincitans*
1. Cyanogensis assay 2. Ammonia production 3. Antibiotic susceptibility

3.2. Antifungal activity of the treatments in vitro

The antagonistic activity was tested *in vitro* by assaying the ability to inhibit the mycelia growth of *Fusarium oxysporoum* using the selected strain of *Kosakonia radicincitans* as indicated in fig. (2). It is cleared that the tested selected bacterial strain restricted the mycelia of *Fusarium oxysporoum* and exhibited a highly antagonistic effect with inhibition percentage (80.07%). The antagonistic activity of *Kosakonia radicincitans* against *Fusarium oxysporoum* could be attributed to production of antifungal metabolites (HCN and ammonia) as confirmed by [43] who proved that Rhizobial bacteria secrete secondary metabolites such antibiotics, HCN, and siderophores, which contribute to their antagonistic impact. Furthermore, strains that tested positive for HCN production were shown to be effective against sugarcane pathogens, as described in [44]. Moreover, it was suggested in [45] that HCN gas stops the development of pathogens by disrupting their respiratory system. [46] proved that siderophores directly stimulate the biosynthesis of other antimicrobial compounds and suppress the growth of pathogenic as *F. oxysporum*.

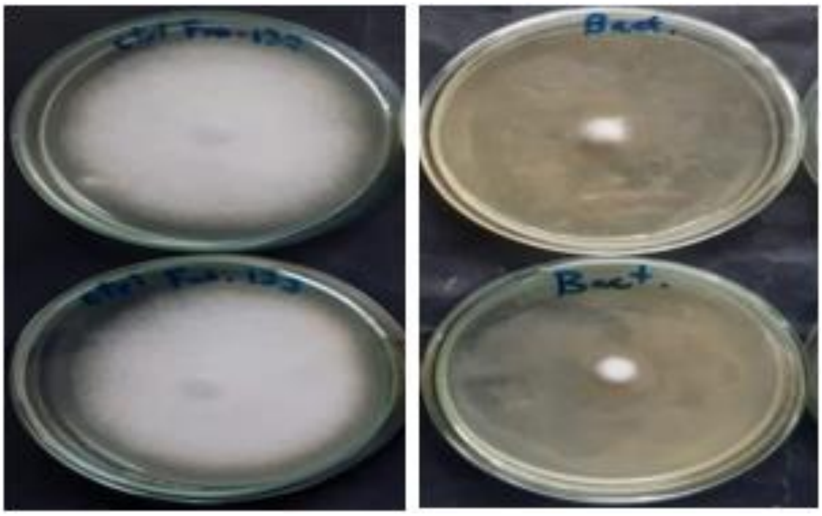

Fig. 2: Antagonistic effect of *K. radicincitans* against *F. oxysporoum*.

3.3. Hyphal Morphology as affected by bacterial treatment

A scanning electron microscope (SEM) was used to examine the hyphal morphology of *Fusarium oxysporoum* both before and after treatment with *Kosakonia radicincitans.* The appearance and morphology of the fungus changed after using the bacterial treatment. The control mycelia were thick, elongated, continuous, intact, and smooth. The bacterial treatment significantly decreased the number of spores, and the mycelia were rougher and thinner than the control mycelia. (Fig. 3). This may be explained by the bacterial treatment's damage to the fungal cells, which led to cytoplasmic leakage and hyphae shrinking, as indicated by [47]. [48] demonstrated that the yeast *C. laurentii* and the bacteria *K. radicincitans* were chosen for usage in a combination because of their strong ability to suppress the *Penicillium expansum* fungus and significantly reduce the mycotoxin.

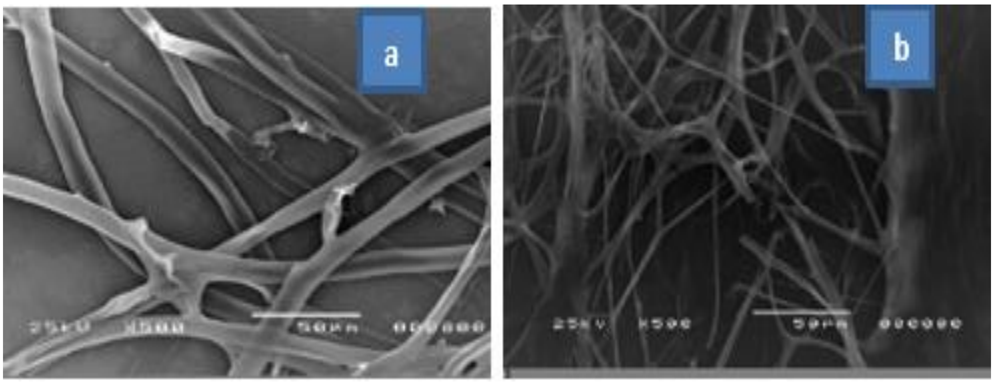

Fig. 3: Scanning electron microscope images of the hyphae (a) *Fusarium oxysporum* (control); (b) *Fusarium oxysporum* treated with *Kosakonia radicincitans*

3.4. Disease incidence percentage

Significant reduction in disease incidence was observed on plants treated with *K. radicincitans* and chemical fungicide as compared with control (Fig.4). Both fungicide treatment and bacterial treatment were the most effective to induce systemic resistance and reduced disease incidence to 8% as compared to the control treatment. These findings agree with those of [49], who demonstrated the effectiveness of organisms like *Kosakonia radicincitans* and *Rahnella aquatilis*, either alone or in combination, in controlling various forms of postharvest rot in storage.

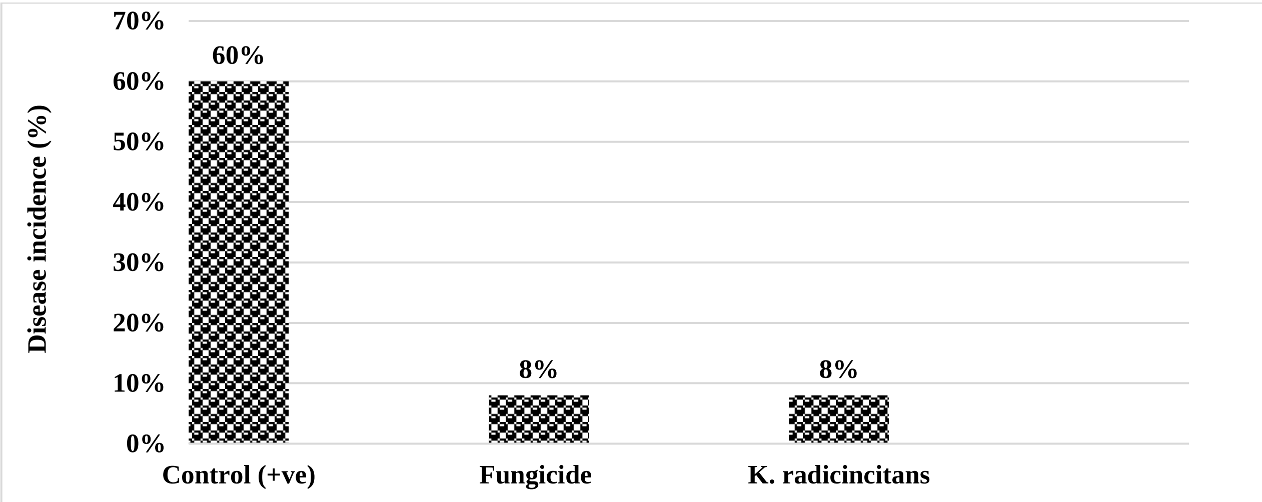

Fig. 4: Effect of bacterial treatment on disease incidence in *Zea mays* plants infected with *Fusarium oxysporoum* under greenhouse conditions

3.5. Zea mays plants and control of root rot

A strategy used in environmentally friendly and sustainable agriculture is the use of microorganisms like bacteria to prevent fungal infections and promote crop development. Promotion effect on plant growth was observed when *Zea mays* plants were treated with bacterial treatment. The obtained data revealed that plant biomass was in general greater and significantly higher than that of control. *K. radicincitans* significantly increased plant height, root and shoot fresh weight of *Zea mays* plants as compared to the control infected plants (Table 3). These results are in line with those of [50], who indicated that *Kosakonia* sp. MGR1 may effectively enhance *Arachis hypogaea* L. growth (root and shoot lengths, plant height).

3.6. Photosynthetic characteristics

The efficacy of bacterial treatment was tested to induce chlorophyll in *Zea mays* plant leaves. Results showed that chlorophyll was significantly improved greatly by the bacterial treatment as compared to the control (Table 3). These finding may be due to secondary metabolites produced by the bacterial bioagent. [51] proved that PGPR inoculation resulted in enhancement of the photosynthetic rate in plants. In this respect [14] showed that maximum content of chlorophyll in wheat leaves was obtained using bacterial isolate of *Kosakonia radicincitans* as compared to control or *Azotobacter* sp.

Table 3: Plant height, root fresh wt., shoot fresh wt., and Chlorophyll contents as affected by bacterial treatment

Treatments	Plant height (cm)	Root fresh wt. (g)	Shoot fresh wt. (g)	Total chlorophyll
Control	59.2[b]	16.8[b]	10.30[c]	31.4[b]
Fungicide	73[a]	29[a]	21.75[a]	34.5[a]
K. radicincitans	72.5[a]	28.5[a]	21.5[b]	34.43[a]

Means in the same column followed by the same letters are not significantly different (P<0.05), according to Duncan's test.

4. Conclusions

The objective of PGPR-based biocontrol is to offer sustainable and alternative methods of managing diseases. The use of the PGPB strain of *Kosakonia radicincitans* was effective in restricting *F. oxysporoum* and had a highly antagonistic impact *in vitro*. Moreover, it had obvious effect on fungal morphology where SEM (scanning electron microscopy) showed that the fungal hyphae had significant morphological damage. *In vivo* bacterial treatment and the chemical fungicide were both same efficient to reduce disease incidence. Therefore, *K. radicincitans* may be utilized as a natural fungicide to manage pathogenic fungi. and it might be a promising agent to reduce the dependence on synthetic fungicides

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