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Bio - priming and Antagonistic Potentials of *Senna obtusifolia* Endophytic Bacteria

Inuwa, A .B[1*]., Abubakar, A. S[2]. Ibrahim, M. A[2]. and Sulaiman, H. U[1].

[1]Department of Microbiology, Faculty of Life Sciences, Bayero University Kano.

[2]Department of Agronomy, Faculty of Agriculture, Bayero University, Kano.

*Correspondence author: abinuwa.mcb@buk.edu.ng. Phone: +2348095153202.

Abstract

Endophytic microorganisms have continued to gain prominence as rich sources of useful compounds such as plant growth promoting chemicals, bioactive compounds among others. The present study aimed at evaluating the tomato seeds bio-priming and, antagonistic potentials of endophytic bacteria isolated from *Senna obtusifolia*. Endophytic bacteria harboured in the roots and leaves of *S. obstusifolia* were isolated using a combination of cultural, biochemical and microscopic techniques. The isolates were evaluated for possible applications as growth-promoting agents of tomato seeds and also, as antagonistic agents to the notorious plant pathogenic fungus *Fusarium oxysporum*. Diverse genera of bacteria were isolated from the plant and these, prominently include, *Bacillus* spp; *Staphylococcus aureus; Escherichia coli; Enterobacter* spp; *Rhizobium* spp and *Pseudomonas* spp. Although, tomato seeds bio-primed with *Enterobacter* spp germinated before all others, the germination period (4 days) was statistically the same ($P< 0.05$) as that yielded by the control (4.5). Similarly, tomato seeds treated with *S. aureus* yielded the highest number of leaves (2.5) and, this was also statistically the same as that yielded by the control ($P < 0.05$). All the isolates used in the evaluation of antagonistic activity yielded significantly larger ($P > 0.05$) zone of inhibition than the control (11.0 mm). Among these, *Bacillus* spp yielded the largest zone (21.6 mm). The study revealed that *S. obstusifolia* harbours endophytic bacteria that could inhibit the growth of the plant pathogen, *F. oxysporum*.

Keywords: *Senna obtusifolia*, endophytic bacteria, bio-priming potentials, antagonistic potentials, *Fusarium oxysporum*

INTRODUCTION

Plant-microbe interactions that enhance growth and development of plant as well as health promotion have been the subject of considerable interest to researchers. Plants constitute vast and diverse niches for endophytic organisms. Among the microorganisms, endophytic bacteria occupy internal tissues of plants without causing damage to their hosts (Hallmann *et al*. 1997). An endophyte is a bacterial or fungal microorganism that spends whole or part of its life cycle inter-and/or intra-cellularly inside the healthy tissues of the host plant without causing harm (Berg *et al*., 2005 and Sturz, 1997). Several reports asserted the presence of endophytes in a vast number of plants tissues (Hallman, *et al*., 1997). Plants roots is reported to have the higher number of endophytes, though substantive number also occur in stems, leaves, seeds, fruits, tubers, ovules, and also inside legume nodules (Rosenblueth and Martinez-Romero, 2004; Hallmann *et al.,* 1997; Sturz *et al.,* 1997). Bacterial endophytes were isolated from, sorghum and prairie plants (Zinniel *et al.,* 2002), wheat (Zinniel *et al.,* 2002; Germida *et al.,* 1998), Carrots (Zinniel *et al.,* 2002), and many more.

Endophytes have been found to under appropriate conditions accelerate seedling emergence and plant establishment (Chanway, 1997) and promote plant growth (Bent and Chanway, 1998). Bacterial endophytes have been shown to also prevent disease development through endophyte-mediated de novo synthesis of novel compounds and antifungal metabolites. Investigation of the biodiversity of endophytic strains for novel metabolites may identity new drugs for effective treatment of diseases in humans, plants and animals (Strobel *et al*., 2004). Most data is found on rhizosphere with much less available on endophytes even though they likely deploy the same mechanisms for promoting plant growth and health (Berg, 2005).

To our knowledge, there are no previous reports of endophytic bacteria isolated from *Senna obtusifolia* in Nigeria and beyond. The objective of the current study is therefore to evaluate the potentials of endophytic bacteria isolated from *S. obstufolia* in the promotion of tomato seeds germination and also, their antagonistic potentials against a plant pathogenic fungus, *F. oxysporum*.

MATERIAL AND METHODS

Sample Collection, Preparation and Inoculation

The experiment was conducted at the Department of Microbiology Research Laboratory, Bayero University Kano. The sample of *S. obtusifolia* was collected randomly from healthy wild grown plants along the street of Bayero University, Kano ($11^{0}58^{1}$N and $8^{0}25^{1}$E). The root and leaf of each plant was cut and washed under running tap water to remove adhering soil particles and other foreign materials clung to it. It was then surfaced sterilized using 70% ethanol for 1 minute followed by 3% sodium hypochlorite for 3 minutes and rinsed five times with sterile distilled water (Inuwa *et al.,* 2018).

The surface-sterilized samples were grinded separately using sterile pestle and mortar. Serial dilution of each of the root and leaf was performed up to 10^{-3} dilution. 1ml each of the diluents (10^{-1}, 10^{-2}, and 10^{-3}) was separately poured into Nutrient (NA), Yeast sucrose agar (YESA), Nutrient broth yeast extract (NBY), Brain heart infusion (BHI) and Mackonkey agar which were initially autoclaved at 121^{0}C, 15psi for 15min. These were incubated at 30^{0}Cfor 24hrs in order to recover the maximum possible colonies of bacterial endophytes. Morphologically different colonies were selected and sub-cultured into fresh media to obtain pure cultures.

Characterization of the Endophytic Bacterial Isolates

Cell morphology was determined using Gram staining method (Bathlomelow, 1962). Similarly, indole production, methyl-red, Voges proskauer, citrate utilization, urea hydrolysis, oxidase, sugar fermentation, starch hydrolysis, catalase test and coagulase test were carried out according to the procedures described by Cheesbrough (2004) and Cappuccino and Sherman (2000). All incubations of the bacterial cultures were done in an incubator at 30°C.

Evaluation of the Bio-Priming Potentials of the Endophytic Bacterial Isolates

The isolates were first sub-cultured in Luria bertani medium (LB) and, incubated at 30^{0}C for 24 hours before use. Tomato seeds obtained from the Department of Agronomy, Bayero University, Kano were surface sterilized using 70% ethanol, followed by 3% sodium hypochlorite and washed 5 times with sterile distilled water and subsequently soaked in the 24 hour old LB cultures of the bacterial isolates for 24 hours. The culture fluid was then decanted aseptically and the seeds left to drying for 24 hours. Ten sterile seeds each were placed in a Petri dish containing a layer of cotton wool pre-moistened with sterile distilled water. The eight isolates served as the experimental treatments and were laid out in completely randomized design (CRD) and replicated three times (Ji *et al.,* 2014). The Seeds were observed at daily intervals and moistened with sterile distilled water whenever necessary. This lasted for a period of 14 days.

Antagonistic Potentials of the Endophytic Bacterial Isolates against *Fusarium oxysporum*

A needle full mycelia growth of soil borne plant pathogenic fungus, *Fusarium oxysporum* was placed on one side of Petri plates of potato dextrose agar (PDA) and each bacterial isolate streaked on the other side of the plate, a minimum separation of 35 mm was maintained between the bacterial isolate and the fungus. Each test bacterium was allocated two separate Petri plates. The cultures were incubated at room temperature for 7 days. Zones of inhibition were measured and used to assess the antagonistic effects of the bacterial isolates against *F. oxysporum*. A plate containing *F. oxysporum* alone was prepared and maintained as the control (Ji *et al.*, 2014).

Statistical Analysis

Data generated on germination days, percentage germination, seedling length, number of leaves and seedling weight as well as inhibitory effect were recorded and subjected to analysis of variance (ANOVA) using SAS statistical software. Means were separated using Student-Newman-Keuls test (SNK).

RESULTS

The result obtained from this study revealed the presence of eight bacterial isolates in the roots and six in the leaves of *S. obtusifolia*.

The result of evaluation of the Bio-priming potentials of the Endophytic bacteria shows that *Enterobacter* spp has the highest bio-priming potentials is presented in Table 1.

Similarly, the result of the antagonistic potentials of endophytic bacterial isolates against *Fusarium oxysporum* is presented in Table 2.

Table 1: Bio - Priming Potentials of the Endophytic Bacteria on Tomato Seeds

Isolates	FW	LP	NL	PG	FDG
Staphylococcus aureus	0.025	3^{bc}	2.5^{a}	55^{abc}	5.5^{bcd}
Enterobacter spp.	0.025	3.85^{ab}	$2a^{b}$	90^{a}	4^{d}
Bacillus spp.	0.02	2.5^{c}	1.35^{b}	30^{c}	7^{ab}
Micrococcus spp.	0.03	4^{a}	$2a^{b}$	80^{ab}	5^{cd}
Proteus spp	0.02	2.5^{c}	1.3^{b}	25^{c}	8^{a}
*Rhizobi*um spp.	0.03	4.25^{a}	2.15^{ab}	55^{abc}	6.5^{abc}
Acinetobacter spp.	0.02	2.35^{c}	1.35^{b}	45^{bc}	7^{ab}
Psuedomonas spp.	0.02	$3.3a^{bc}$	2^{ab}	40^{c}	8^{a}
Control	0.035	4.25^{a}	2.3^{a}	90^{a}	4.5^{d}
SE±	0.007	0.427	0.402	17.0	0.656

Means having the same superscript within the same column are statistically the same at 5% level of significance.
Key: FW = fresh weight of seedling, LP= length of seedling, NL= number of leaves, PG= percentage germination and FDG = first day to germination.

Table 2: Antagonistic Potentials of Endophytic Bacterial Isolates against *Fusarium oxysporum*

Isolates	Average zone of inhibition(mm)
Bacillus spp	$21.^{6a}$
Pseudomonas spp	15.0^{b}
Staphylococcus aureus	17.5^{ab}
Enterobacter spp	18.5^{ab}
Rhizobium spp	14.0^{b}
Control	11.0
SE±	0.122

Means having the same superscript within the same column are statistically the same at 5%level of significance.

DISCUSSION

Distribution of the Endophytic Bacteria in the Roots and Leaves of *Senna obstufolia*

The higher number of the isolates in the roots is not by coincidences as a number of researchers reported plant roots to have the highest number of endophytes and substantive number to have been present in stems, leaves, seeds, fruits, tubers, ovules, and also, inside legume nodules (Rosenblueth and Martinez-Romero, 2004; Hallmann *et al.,* 1997; Sturz *et al.,* 1997). In the present study, *Psuedomonas* spp, *Staphylococcus aureus*, *Bacillus* spp and *Acinetobacter* spp were isolated from both roots and leaves of *S. obtusifolia*. Bacteria belonging to the genera *Bacillus* and *Pseudomonas* have been indicated as easy to culture, and cultivation dependent studies revealed the group as frequently occurring endophytes (Seghers *et al*., 2004). However, while the roots of the plant were found to also found to harbour *Escherchia coli, Rhizobium* spp, *Proteus* spp, and *Micrococcus* spp , the leaves were found to contain only *Enterobacter* spp in addition to those found inside both roots and leaves. *Enterobacter* spp has been identified as an endophyte of several plants such as *Citrus sinensis*, soybean and other crop plants (Araújo *et al*., 2002; Zinniel *et al*., 2002; Kuklinsky-Sobral *et al*., 2004).

Bio-Priming Potentials of the Endophytic Bacterial Isolates

The result of bio-priming potentials the endophytic bacterial isolates (**Table 1**) showed tomato seeds treated with *Enterobacter* spp. to have germinated first and also, yielded the highest percentage germination, though statistically the same as the control (P < 0.05). Rogers (2012) reported 55% greater total biomass, increased root growth of poplar and increased root to shoot ratio when inoculated with *Enterobacter* spp 638. This indicates that significant growth promotion may be yielded by this bacterium when inoculated on the tomato seeds at higher dose. Seeds treated with *Rhizobium* spp., *Psudomonas* spp., *Staphylococcus aureus, Micrococcus* spp. and *Enterobacter* spp. yielded the highest number of leaves. However, the values were statistically the same with one another and the control (P < 0.05).

Antagonistic Potentials of the Endophytic Bacterial Isolates against *Fusarium oxysporum*

The representative endophytic bacteria yielded varying degree of inhibitory activity against *F. oxysporum*. The result as presented in Table 2 showed that, all the means were statistically greater ($P < 0.05$) than the control, indicating the ability of the test endophytic bacteria to inhibit the growth of *F. oxysporum*. The greatest zone of inhibition was yielded by *Bacillus* spp (21.6 mm). However, the zone was not statistically different (P < 0.05) from those yielded by *Enterobcter* spp (18.5 mm) and *S. aureus* (17.5 mm). The smallest zone of inhibition was yielded by *Rhizobium* spp (14.0 mm). Inuwa *et al.,* (2018) also reported *S. aureus* and *Bacillus subtilis* as endophytic bacteria of *Cymbopogon citratus* and, yielding the highest zones of inhibition of 21.3 and 20.2 mm respectively against *F. oxysporum*. Ji *et al.* (2014) also reported the antagonistic activity of 12 endophytic diazotrophic bacteria isolated from Korean rice cultivars on mycelial growth of isolates of *F. oxysporum*. The report indicated that four species belonging to the genus *Bacillus* and related genus *Paenibacillus* out of the seven organisms tested yielded the highest antagonistic activity. The result of the present study also agrees with the work of Kim *et al.* (2008) who reported the antagonistic effects of 7 out of 20 *Bacillus* spp isolated from manure and cotton waste composts against soil borne fungi, *F. oxysporum, Rhizoctonia solani, Phytophthora casici* and *Sclerotinia sclerotium*. In general, the current study agrees with previous related ones on the the antagonistic activity of Endophytic *Bacillus* spp isolated from different plants against *F. oxysporum*.

CONCLUSION

In conclusion, the internal tissues of *S. obtusifolia* contain diverse genera of endophytic bacteria. The bacteria offer promising potentials as biocontrol agents of plant pathogenic fungus *F. oxysporum*. However, the screening of the isolates for possible production of bioactive compounds will be of immense significance in explaining the mechanism behind their biocontrol potentials.

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