Asian Journal of Research in Botany

5(3): 47-56, 2021; Article no.AJRIB.63291

In vitro Enzymatic and Antifungal Activity of Rhizobacteria against *Fusarium oxysporum* f. sp. *lycopersici*

K. J. Ayantola[1*] and E. D. Fagbohun[2]

[1]Department of Science Laboratory Technology, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria.
[2]Department of Microbiology, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author KJA designed the study, Performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EDF and KJA managed the analyses of the study. Author KJA managed the literature Searches. Both authors read and approved the final manuscript.

Article Information

Editor(s):
(1) Dr. Vinayaka K. S, Kumadvathi First Grade College, India.
Reviewers:
(1) Dr. S. Radhika, Pioneer Kumaraswamy College, India.
(2) Dr. Wassim Yezli, Ibn Khaldoun University, Algeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/63291

Original Research Article

Received 20 September 2020
Accepted 27 November 2020
Published 19 March 2021

ABSTRACT

Aim: This study was carried out to investigate the enzymatic activity of rhizobacteria in the rhizosphere of tomato plant in a farm at Elemi, Ado Ekiti Nigeria as well as to explore the possibility for control of *Fusarium oxysporum,* the causal agent of tomato wilt, using their antagonistic traits.
Place and Period of Study: The study was carried out in the Department of Microbiology, Faculty of Science, Ekiti State University, Ado Ekiti, Nigeria in August 2018.
Methodology: In this study, rhizobacteria were isolated from rhizosphere of healthy tomato plant in the fields. Using pour plate method, isolation was carried out from rhizosphere soil of tomato plant collected from a field located at Elemi farm, Ado Ekiti Nigeria. Standard methods of Enzyme assay was employed to determine the ability of isolated rhizobacteria to produce hydrolytic enzymes require for biocontrol of phytopathogenic fungi. Antagonistic assay was performed using dual culture method on Potato Dextrose Agar (PDA) Plates. Isolates were tentatively identified with help of Bergy's manual for identification.

**Corresponding author: E-mail: ayantola86@gmail.com;*

Results Five bacteria isolates coded as *BaC1*, *BaC2*, *BaL*, *BaS,* and *CiF* were identified from preliminary screening. Evaluation for hydrolytic enzyme activities such as cellulase, protease, chitinase and glucanase showed that the isolates have the ability to produce enzymes but *BaC1* has the highest enzymatic activity in all except in protease, while the *CiF* has the lowest activity in the entire enzyme assay carried out. In antagonistic activity, *BaC1* shows a maximum inhibition of 33.0% against *Fusarium oxysporum* f. sp. *lycopersici B*, after five days of incubation followed by *BaS* with an inhibition of 29.7%, *BaL* with an inhibition of 29.5%, and *CiF* with least inhibition of 15.9% against *Fusarium oxysporum* f. sp. *lycopersici* A. Based on cultural and biochemical characteristics, the isolates were identified as *Bacillus cereus1 (Bac1), Bacillus cereus2 (Bac2), Bacillus licheniformis (Bal), Bacillus subtilis (Bas).*

Conclusion: Thus, this present study concludes that these rhizobacteria isolates could serve as a good alternative biocontrol agent's inoculant in the integrated management of soil borne diseases of tomato.

Keywords: Rhizobacteria; Fusarium oxysporum; biocontrol agent; hydrolytic enzymes; bacillus; rhizosphere.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the world's most widely cultivated vegetable crops for consumption as fresh fruits and various types of processed products. Low yield of tomato is attributed to its susceptibility to several pathogenic fungi, bacteria, viruses and nematodes which are major constraints to tomato cultivation [1]. Fusarium wilt caused by the soil borne fungus, *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) is one of the most devastating diseases of tomato. Wilt of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici* (*Fol*) and crown and root rot of tomato caused by *Fusarium oxysporum* f.sp. *radicis-lycopersici* (*Forl*) have been reported in at least 32 countries [2]. These diseases occur both in green house and field and result in significant crop losses [3,4].

Management of *Fusarium* wilt is mainly through chemical, soil fumigation and resistant cultivars. The broad-spectrum biocides used to fumigate soil before planting, particularly methyl bromide, are environmentally damaging. The most cost effective, environmentally safe method of control is the use of resistant cultivars, when these are available. For example, all the varieties of tomato grown in glasshouses for fresh fruit production are resistant to the common races of *F. oxysporum* f. sp. *lycopersici*. Breeding for resistance can be very difficult when no dominant gene is known (e.g. carnation, cyclamen, flax) or if the host is dioecious (palm trees). In addition, new races of the pathogen can develop which overcome host resistance. The difficulty in controlling Fusarium wilt has stimulated research in biological control of Fusarium wilt independently of the recent concern for environmental protection [5].

Biological control by antagonistic organism has been studied extensively and rhizobacterial strains are potential biocontrol agents for the control of root and foliar diseases [6]. It has been proved that microorganisms isolated from roots or rhizosphere of a specific crop adapted better to that crop and provided effective control of diseases than organisms isolated from other plant species. The natural control of several phytopathogens is based on the presence of suppressive soils where several biocontrol microorganisms' rhizosphere soil tomato plants and screen them in relation with the control of *Fusarium oxysporum*, which can cause tomato root rot disease [6].

Rhizobacteria are Plant beneficial microorganisms known to antagonize phytopathogens through competition for niches (e.g. iron through siderophores synthesis); parasitism, that may involve production of hydrolytic enzymes such as chitinase, β-1,3 glucanase, protease and cellulase, that lyse pathogen cell walls, inhibit the pathogens by secreting anti-microbial compounds and induce systemic resistance in host plants [7]. Rhizobacteria are most widely studied as plant growth-promoting bacteria (PGPB), associated with plant rhizosphere and are present in all agricultural ecosystems [8]. Antagonistic bacteria are considered ideal biological control agents (BCA) because of the rapid growth, easy handling, and aggressive colonization of the rhizosphere [9]. The use of PGPR specifically as biocontrol agents of soil borne fungal plant pathogens as an alternative or complementary

strategy to physical and chemical disease management have been investigated for over a century [10]. PGPR indirectly enhance plant growth via suppression of phytopathogens by producing chemicals that inhibit the growth of plant pathogens. Thus this research was carried out to study the enzymatic activity of rhizobacteria isolated from tomato roots and the possibility of controlling *Fusarium oxysporum* f.sp. *lycopersici* the causative agent of fusarium wilt

2. METHODOLOGY

2.1 Isolation of Rhizobacteria

The rhizosphere soil sample was collected from agricultural zone Elemi area of Ado-Ekiti Nigeria. This was transported in a sterile polythene bag for further analysis in the laboratory. The soil sample was treated to remove the presence of any vegetative cells whereby one gram of soil sample was weighed into 9ml of distilled water. The mixture was taken to a water bath and heated at 80^0C for 20 minutes. Serial dilution of 1ml of soil suspension was carried out until dilutions 10^{-5} were obtained. One ml each of aliquots of 10^{-1}, 10^{-2}, 10^{-3}, $10^{-4}$and 10^{-5} was inoculated into Nutrient Agar (NA) plates in duplicates. The plates were swirled gently to obtain homogeneous mixture. This was allowed to solidify and incubated for 24 hours at 37° C. After the incubation period, plates with growth were counted and recorded. Distinct colonies on each of the plates were aseptically sub-cultured onto freshly prepared solidified Nutrient agar by streaking and were incubated at 37 ° C for 24hours until pure cultures were obtained. The pure isolates were then observed for their cultural and morphological characteristics. They were then stored in freshly prepared nutrient agar slants at 4 ° C until ready to use. These pure bacterial isolates were then subjected to biochemical tests for presumptive identification of the bacterial isolates.

2.2 Collection of Fungal Isolates

The fungi *Fusarium oxysporum* f.sp. *lycopersici* (A and B) used in this study were obtained from the stock culture kept by Dr. O. A. Borisade of the Department of Crop, Horticulture and Landscape Design Ekiti State University, Ado-Ekiti. The two strains were grown on potato-dextrose agar (PDA, Himedia, India) in 9-cm petri dishes and incubated in a growth chamber at 25±2 ° C for 5 days.

2.3 Enzyme Assay

2.3.1 Cellulase assay

Substrate**:** 1% Carboxymethyl cellulose (CMC) was prepared in 0.5M sodium acetate buffer of pH 5.5. About 0.2 ml of the enzyme solution was added to 0.2 ml of the substrate solution and incubated at 37 °C for 30 minutes. One ml of 3.5 DNSA was added to the solution and heated for 5 minutes in a boiling water bath. The solution was allowed to cool and 10 ml of distilled water was added. The same procedure was carried out on the substrate without the addition of the enzyme solution but instead distilled water was added. The absorbance was read at 540nm [11]. The absorbance at 540nm obtained was extrapolated from the glucose standard curve to obtain the amount of glucose liberated.

2.3.2 Protease assay

Substrate**:** About 1% casein was prepared in 0.05 citrate phosphate buffer of pH 7.5. The solution was the heat-denatured at 100°C for 15 minutes in a water bath. Exact 1 ml of 1% casein each was pipetted into different test tubes. The test tubes were incubated at 37 ° C for 15 mins. Exactly 0.2 ml of the enzyme solution was added to each test tubes and allowed stand for one hour inside a water bath. Three ml of 10% TCA was added to each test tube to terminate the reaction. The tubes were centrifuged at 3000 rpm and the supernatant was read at 280 nm for unprecipitated protein hydrolysate using a UV spectrophotometer [12].

2.3.3 Glucanase assay

Carboxymethyl cellulose (CMCase) or Endo-β-1, 4- glucanase activity was determined according to the method of Mandels and Weber [13] whereby 0.5 ml of 1% Carboxymethyl cellulose (CMC) (Sigma) in 0.1 ml citrate buffer pH 5.6 was placed in each test tubes and 1.0 ml of culture filtrate added. The test tubes were incubated at 40 ° C in a water bath with a shaker (Uniscope SM 101) for 30 minutes. The reaction was terminated by adding 2.0 ml of 3, 5-dinitrosalicylic acid (DNS) reagent to the reaction mixture, boiled for 5 min [11]. The absorbance of the appropriately diluted reaction mixture was read at 540 nm using a spectrophotometer.

2.3.4 Chitinase assay

Chitinase activity was measured spectrophotometrically in a reaction mixture

containing enzyme, 0.5 mg [3M]-chitin, 0.6 mM sodium azide, and 25 mM sodium citrate (pH 5.0) in a total volume of 1 ml. The reaction was stopped after 60 minutes of incubation by the addition of 0.05 ml of 100% (w/v) TCA. After centrifugation for 5 min at 14.000 g in a centrifuge, the supernatant was measured [12].

2.4 Antagonistic Activity of Isolated Bacteria Against *Fusarium oxysporum*

The antagonistic activity was carried out *in vitro* to determine if the isolated bacteria have any antagonistic effect against the *Fusarium oxysporum.* The antagonistic test was carried out on PDA plates without addition of any antibiotic to the PDA medium. The spore suspension was prepared from the stocked culture of *Fusarium oxysporum* and was vortexed to allow the spore to loose from the agar plug. Thereafter two loopful of spore suspension was aseptically placed on the center of the petri dish of 90mm diameter containing PDA. The inoculating loop was then flamed to red hot and a loopful of bacteria was streaked in between the edge of the plate and the fungal suspension at the center. The plates were incubated at a temperature of 37^0C for five days. A control plate was set up which contained only a test pathogen (*Fusarium oxysporum* f.sp. *lycopersici*). The bio-interaction between the rhizobacteria and *Fusarium* was observed on day 5 [14]. Percent growth inhibition was calculated, using the equation:

$$I = (\frac{C-T}{T})x100$$

Where,
I = percent growth inhibition
C = growth in control
T = growth in treatment.

2.5 Statistical Analysis

Statistical analyses of all data were performed using Microsoft excel 2010 package. The enzymatic activities were plotted using cone type of column in excel.

3. RESULTS AND DISCUSSION

3.1 Isolation

Table 1 shows the biochemical properties of the isolated rhizobacteria. Table 2 shows the total bacterial counts isolated from the rhizosphere sample. The minimum bacterial count of the soil sample was 1.0x104 CFU/ml while the maximum bacteria count was 1.12x103 CFU/ml. A total of five bacteria isolates coded as *BaC1*, *BaC2*, *BaL*, *BaS,* and *CiF* were identified from preliminary screening. Isolates were characterized and confirmed to belong to different genera by their biochemical characteristics. They were identified as *Bacillus licheniformis BaL*, *Bacillus subtilis BaS, Bacillus cereus BaC1, BaC2,* and *Citrobacter freundii CiF*. Isolates were tentatively identified with help of Bergey's Manual of Systematic Bacteriology (8).

The bacterial genera isolated in this study had been detected from the rhizosphere of several plants including tomato plant root for instance *Bacillus subtilis* was isolated from the rhizosphere soil of cowpea in the experimental field of the International Institutes of Tropical Agriculture (IITA) in the northern Guinea savanna, in Shika, Kaduna State, Nigeria [15]. *B. subtilis* with biocontrol activity against *Fusarium oxysporum* has been isolated from cotton rhizospheric soil [16]. The result in this study is also similar to the work of Aliu and Oyeyiola [17] who isolated *Bacillus cereus* and *B. subtilis* from the rhizosphere of groundnut plants.

One of the rhizobacteria identified in this study was *Bacillus licheniformis.* It's presence in the plant rhizosphere indicates production of metabolites such enzymes and antibiotics makes it one of the plant growth-promoting bacteria and is very relevant in agricultural biotechnology. The isolation of *B. licheniformis* from rhizosphere soil in this study is similar to the earlier work reported by Rojas *et al*, [18] while working on the synergism between *Phyllobacterium* sp (N2 fixer) and *Bacillus licheniformis* (P-solubilizer) isolated from semiarid mangrove rhizosphere. Kamiloval *et al* [19] also reported the presence of chitinolytic *B. licheniformis* from rhizosphere soil and its potential in antifungal biocontrol [19]. *B. licheniformis* has been detected in plant rhizosphere from different soil and climatic condition; semiarid mangrove Kamiloval *et al,* [18], saline desert [20], temperate soil [21], Rice plant rhizosphere in River valley [22]. In this study *Citrobacter freundii CiF* was isolated from tomato plant rhizosphere soil. It has been isolated from both alkaline and acidic soils and known to promote plant growth and health as reported in the previous studies [23]. *Citrobacter freundii* is associated with the rhizosphere of other plants such as sugarcane [22], rice plant [24], and rhizosphere of para grass under saline condition [25].

Table 1. Biochemical characteristics of the isolated rhizobacteria

Tests	BaC1	BaC2	BaL	BaS	CiF
Gram Reaction	+	+	+	+	-
Catalase	+	+	+	+	+
Motility	+	+	+	+	+
Indole	-	-	-	+	-
Citrate	+	+	+	+	+
Oxidase	-	-	-	-	-
Methyl red	-	-	-	+	+
VP	+	+	+	-	-
Lactase	-	-	+	-	+
Mannose	-	-	+	-	+
Glucose	+	+	+	+	+
Sucrose	Variable	Variable	+	+	+
Urease	-	-	-	+	+

Key: + =Positive reaction, - = Negative reaction

Table 2. Total bacterial count (CFU/ml) of soil sample

Plate No	Dilutions factor	Colony count	CFU/ml
D_1	10^{-1}	58	$5.8x10^{2}$
D_1	10^{-1}	112	$1.12x10^{3}$
D_2	10^{-2}	52	$5.2x10^{3}$
D_2	10^{-2}	70	$7.0x10^{3}$
D_3	10^{-3}	2	$3.0x10^{3}$
D_3	10^{-3}	2	$3.0x10^{3}$
D_4	10^{-4}	1	$1.0x10^{4}$
D_4	10^{-4}	5	$5.0x10^{4}$
D_5	10^{-5}	40	$4.0x10^{6}$
D_5	10^{-5}	27	$2.7x10^{6}$

3.2 Enzyme Assay

Fig. 1 shows the cellulase enzymatic activity of the isolated rhizobacteria. The first identified bacterium *Bacillus cereus BaC1* showed the highest cellulase activity with 0.020527µgm/ml followed by *Bacillus subtilis* with 0.01862 µgm/ml, and the lowest cellulase activity was found in *Citrobacter freundii* with 0.00327 µgm/ml. Cellulase enzyme is produced naturally by a wide range of rhizobacteria and one the major enzymes of interest because of its ability to degrade fungi cell wall [26]. Secretion of cellulase by rhizobacteria is an integral part of the activity of biocontrol agents. The production of the cellulase enzyme by these rhizobacteria in the rhizosphere of tomato plants is very essential in the maintenance of healthy growth and development of good fruits. Although at varying quantities, all the rhizobacteria isolated in this study are very significant in disease control because of their ability to secrete cellulolytic enzymes that will contribute to breaking down of the cell wall component of *Fusarium oxysporum*.

The ability of organisms to secrete this type of metabolite around the root zone implies that they will have the capacity to breakdown the cellulose present in the cell wall of phytopathogenic fungi. This study is in line with the previous work of Zerihun *et al*., [27], who isolated cellulase producing plants promoting bacteria from the rhizosphere of Tef crops. Protease activity for the isolated rhizobacteria is shown in Fig. 2. The highest production recorded in *Bacillus cereus* BaC1 0.022525 µgm/ml followed by *Bacillus subtilis* with 0.020527 µgm/ml the least production of protease activity was recorded in *Citrobacter freundii* with 0.005177µgm/ml. Protease producing rhizobacteria are very important in the management of diseases in agriculture practices. Protease is one of the hydrolytic enzymes for characterizing biocontrol agents. Although bacteria present in the rhizosphere produce arrays of metabolites, they work synergistically to achieve overall control performance. The results obtained in this study are similar to the work of Berg *et al*., [28] who reported that extracellular

enzymes such as proteases are involved in pathogen suppression on plants. The production of proteases by these rhizobacteria has been reported by other researchers [29]. The production of chitinase by rhizobacteria showed their ability to interfere with chitin an insoluble linear polymer of β-1, 4-N-acetyl-glucoseamine, which is the major component of the fungal cell wall of phytopathogenic fungi [20]. Rhizobacteria may not possess all the hydrolytic enzymes, but it may have the ability to produce one metabolite that can disrupt a specific component of the cell wall of *Fusarium oxysporum.* Each of the hydrolytic enzymes can hydrolyze a specific component of the cell wall and this can result in the death of any phytopathogenic fungi. The secretion of chitinase by the rhizobacteria in this study is similar to the work of Kamilova *et al*, [19].

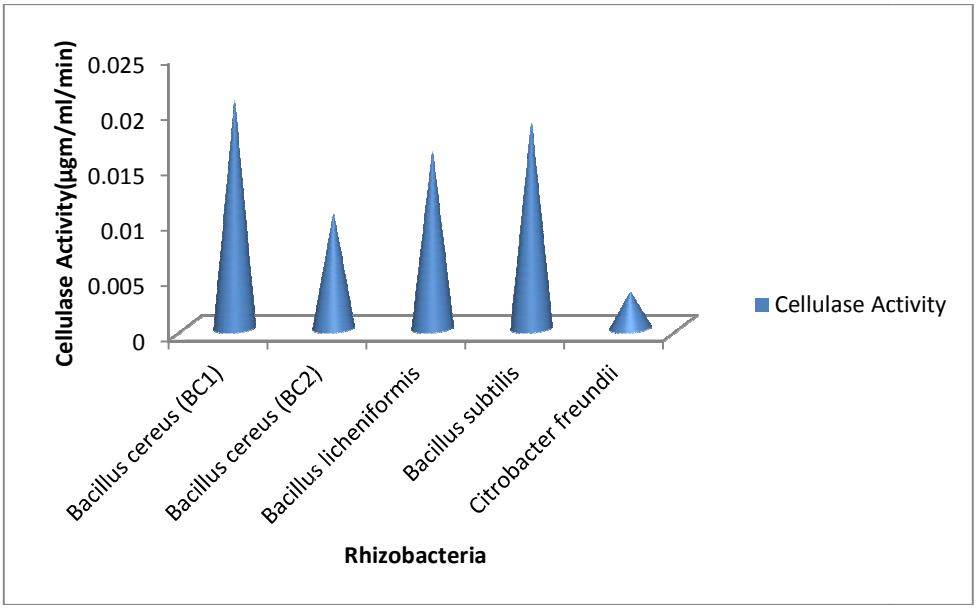

Fig. 1. Cellulase production by isolated rhizobacteria

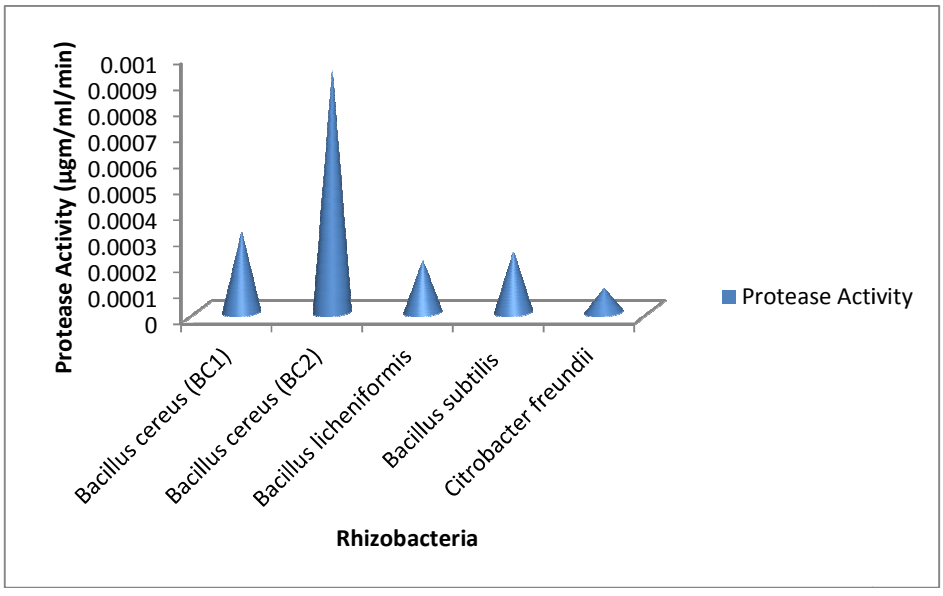

Fig. 2. Protease production by isolated rhizobacteria

Fig. 3 showed the glucanase enzymatic activity for the isolated rhizobacteria. *Bacillus cereus* BaC1 produced the highest Glucanase activity of 0.022525 µgm/ml and followed by *Bacillus subtilis* with 0.020527µgm/ml. lowest activity of 0.005177 µgm/ml. was produced by *Citrobacter freundii.* In disease control management rhizobacteria employed glucanase to breakdown glucans present in the cell wall of phytopathogenic fungi. *Bacillus subtilis* isolated from sugarcane rhizosphere has been reported to produce glucanase that can be effectively used in sugarcane disease management [30].

3.3 Antagonistic Activity

Table 3 showed the antifungal activity of rhizobacteria isolates against *F. oxysporum* after five days. All the isolates demonstrated moderate inhibition activity on the pathogen. *Fusarium oxysporum* f. sp. *lycopersici* is economically importance disease agent on tomato. Due to the soil-borne nature of the disease use of chemical methods for the control of disease is rarely successful [9]. Rhizobacteria with antagonistic activity towards plant pathogens play an important role in root growth, development and plant health and are influenced by plant species in their abundance and composition [31]. The use of rhizobacteria for plant disease management is more effective when rhizobacteria is isolated from rhizosphere of the same host plant [9]. In this study it was observed that those isolates that produce higher quantity of enzymes have high inhibitory effects on the mycelium of the test organisms. This is showing evidence that the hydrolytic enzymes play an importance role in the suppression of phytopathogenic fungi. In the growth inhibition, all the isolates demonstrated more inhibitory activity against *F. oxysporum* f. sp*. lycopersici B.* This could be as a result of the two fungi belonging to different strains. It was also noted that all the isolates belonging to *Bacillus* spp exhibited more antagonistic activity as well as higher enzyme secretion in this study when compare with *Citrobacter* spp. Suppression against plant pathogenic fungi exhibited by the rhizobacteria has been reported by other workers; Kamil *et al.,* [31] reported the antifungal activities of *B. licheniformis* against *Fusarium culmorum, Rhizoctonia solani, Macrophomina phasiolina*, *Pythium sp, Alternaria alternata* and *Sclerotium rolfsii*) The ability of *Bacillus subtilis* to suppress *Fusarium* spp growth was reported by Khan *et al.,* [32]. The most commonly studied biocontrol agents were *B. licheniformis* and *B. subtilis* [32]. There is little or no information regarding the antifungal activity of *Citrobacter* spp. The cases of antagonistic activity of *Citrobacter freundii* against *Fusarium* spp has not been reported as at the time of this report.

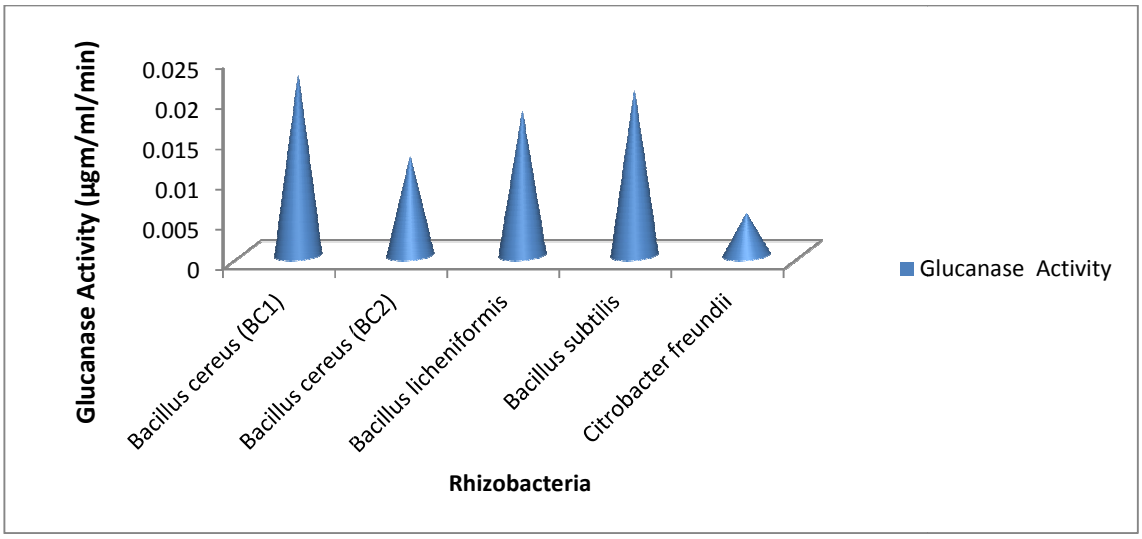

Fig. 3. Glucanase production by isolated rhizobacteria

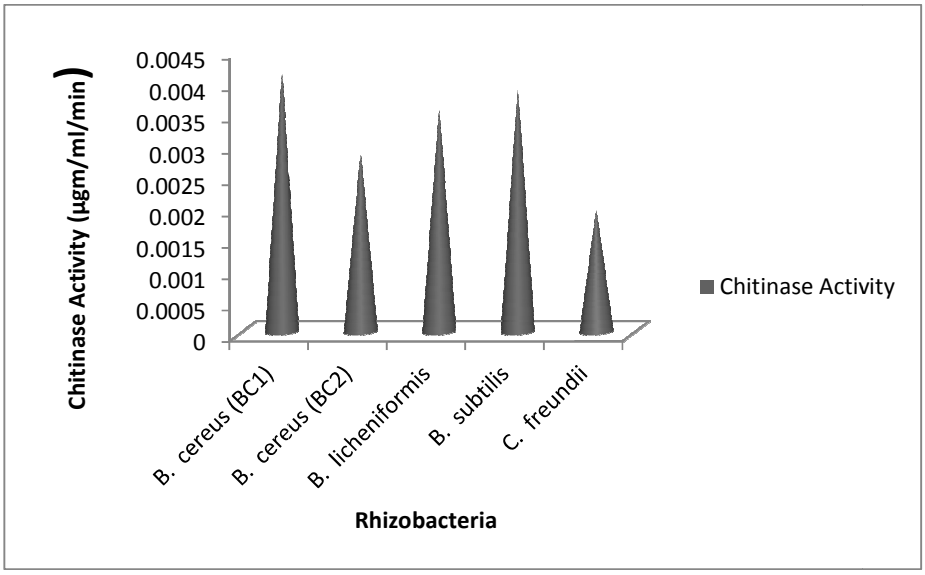

Fig. 4. Chitinase production by isolated rhizobacteria

Table 3. Antifungal activity of bacterial isolates against *F. oxysporum f.sp lycopersici* after five Days

	(%) Mycelia inhibition		
Isolates Code	***F. oxysporum* f.sp. *lycopersici* A**	**Isolates Code**	***F. oxysporum* f.sp. *lycopersici* (B)**
BaC1	21.9	BaC1	33.0
BaC2	27.9	BaC2	22.0
BaL	28.2	BaL	29.5
BaS	17.4	BaS	29.7
CiF	15.9	CiF	23.3
Control	0		0

4. CONCLUSION

Conclusively, our findings showed that the rhizobacteria isolated in this study can be used as a biocontrol agent. The use of these organisms as biocontrol agents would be ecofriendly and are readily available at the rhizosphere of many plants. The use of rhizobacteria in control phytopathogenic fungi is preferred and more effective when rhizobacteria is isolated from rhizosphere of the same host plant. The impact of the rhizobacteria on both plants and soils makes them a good alternative in disease management. Different secondary metabolites (hydrolytic enzymes) secreted by these groups of organisms give them competitive advantage over other soil organisms.

ACKNOWLEDGEMENT

The authors thank Dr O.A Borisade of the Department of Crop, Horticulture and Landscape Design Faculty of Agriculture, Ekiti State University Ado Ekiti Nigeria for providing fungi isolates used in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Barone A, Frusciante L. Molecular marker-assisted selection for resistance to pathogens in tomato. In: Guimaraes EP, Ruane J, Scherf BD, Sonnino A, and Dargie JD ed. Marker- Assisted Selection: Current status and future perspectives in crops, livestock, forestry and fish. Electronic Publishing and Support Branch, Communication Division, FAO, Rome. 2007;151-164.

2. Jones JB, Jones JP, Stall RE and Zitter TA. Compendium of Tomato Disease. American Phytopathological Society Press, St. Paul. 1991;73.
3. Hahn F. Fungal spore detection on tomatoes using spectral Fourier signatures. Biosyst Eng. 2002;81:249–259.
4. Cai G, Rosewich L, Schneider RW, Kistler HC, Davis RM, Elias KS, and Miyao EM. Origin of race 3 of *Fusarium oxysporum* f.sp. *lycopersici* at a single site in California. Ecol Popul Biol. 2003;93:1014–1022.
5. Armstrong GM, Armstrong JK. Formae speciales and races of *Fusarium oxysporum* causing wilt diseases. In: PE Nelson, TA Toussoun, RJ Cook, eds. Fusarium: disease, biology, and taxonomy. University Park, PA, USA: State University Press. 1981;391-399.
6. Anuratha CS and Gnanamanickyam SS. Biological control of bacterial wilt caused by *Pseudomonas solanacearum* in India with antagonistic bacteria. Plant soil. 1990; 124:190-116.
7. Compant S, Duffy B, Nowak J, Clément C, Ait Barka E Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl. Environ. Microbiol. 2005;71:4951-4959.
8. Kloepper JW and Schroth MNl. Plant growth-promoting rhizobacteria on radishes. In: Proceedings of the IVth International Conference on Plant Pathogenic Bacteria. Volume 2. Station de Pathologie Végétale et Phytobactériologie, INRA, Angers, France. 1978;879-882.
9. Weller DM. Biological Control of Soilborne Plant Pathogens in the Rhizosphere with Bacteria. Annual Review of Phytopathology. 1988;26,379-407. DOI:doi.org/10.1146/annurev.py.26.090188.002115
10. Haas D Defago G. Biological control of soilborne pathogens by fluorescent pseudomonads. Nat Rev Microbiol. 2005; 3:307–319.
11. Miller GL. Use of dinitrosacylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959,31(3): 426-428.
12. Ladd JN, and Butler JHA. Short-term assay on soil proteolytic enzyme activity using proteins and dipeptide substrate. Soil Biology and Biochemistry. 1972;4:19-39.
13. Mandels M, Weber J. The production of cellulases and glucanases. Advances in Chemistry. 1969;95:391-413.
14. Sajid SA, Bihar MA. Study of antagonistic capability of *Trichoderma harzianum* isolates against some pathogenic soil borne fungi Agric. Biol. J. N. Am. 2014; 5(1):15-23. DOI:10.5251/abjna.2014.5.1.15.23
15. Killani AS, Abaidoo RC, Akintokun AK, Abiala MA. Antagonistic Effect of Indigenous *Bacillus subtilis* on Root-/Soil-borne Fungal Pathogens of Cowpea. Researcher. 2011;3(3). Available:http://www.sciencepub.net/researcher
16. Gajbhiye A, Rai AR, Meshram SU, Dongre AB. Isolation, evaluation and characterization of Bacillus subtilis from cotton rhizospheriic soil with biocontrol activity against Fusarium oxysporum. Word J Microbiol Biotechnol. 2010;26(7): 1187-94. DOI:10.1007/s11274-009-0287-9
17. Aliu MB and Oyeyiola GP. Rhizosphere Bacterial Flora of Groundnut (Arachis hypogeae)- aensi Advance in Environmental Biology. 2011;5(10):3196-3202
18. Adriana R, Gina H, Bernard RG, Yoav B. Synergism between *Phyllobacterium* sp (N2 fixer) and *Bacillus licheniformis* (P-solubilizer) both from a semiarid mangrove rhizosphere FEMS Microbiology Ecology. 2001;35(2):181-187.
19. Faina K, Johan HJL, Ben L. *Collimonas Fungivorans*, An Unpredicted *In Vitro* But Efficient *In Vivo* Biocontrol Agent For The Suppression of Tomato Foot And Root Rot; Environmental Microbiology. 2007; 9(6):1597–1603.
20. Goswami D, Pithwa S, Dhandhukia P, Thakker JN. Delineating *Kocuria turfanensis* 2M4 as credible PGPR: A novel IAA producing bacteria isolated from saline desert J. Plant Interact. 2014;9:566-576.
21. Ayantola KJ, Fagbohun ED Enzymatic Activity of Rhizobacillus Isolated from Tomato Rhizosphere, Asian Journal of Biochemistry, Genetics and Molecular Biology. 2020;4(3):11-19. DOI:10.9734/AJBGMB/2020/v4i330106
22. Xie G, Su B, Cui Z. Isolation and identification of N2-fixing strains of Bacillus in rice rhizosphere of the Yangtze River

Valley Acta Microbiologica Sinica. 1998; 38(6):480-483.
23. Hafiza MS, Ghulam ZJ, Idrees AN, Mehwish I, Muhammad I. Isolation and Characterization of Phosphate-Solubilizing Bacteria from Rhizosphere Soil. 2014; 27(6):4248-4255 DOI:https://doi.org/10.5504/BBEQ.2013.0091
24. Omar SA. The role of rock-phosphate-solubilizing fungi and vesicular-arbuscular-mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J Microb Biot. 1998;14:211–218.
25. Salma M, Mirza MS, Humera AA, Kauser M, Asma M, Samina M. Microbial diversity and metagenomic analysis of the rhizosphere of para grass (Urochloamutica) growing under saline conditions. Pakistan Journal of Botany. 2016;48(2):779-791.
26. Jadhav HP, Shaikh, SS, Sayyed RZ. Role of Hydrolytic Enzymes of Rhizoflora in Biocontrol of Fungal Phytopathogens: An Overview. 2017:183-203. DOI:10.1007/978-981-10-4862-3_9
27. Zerihun T, Birhanu G, Genene T, Adey F, Solomon C, Tesfaye A, Fasil A. Isolation and Biochemical Characterization of Plant Growth Promoting (PGP) Bacteria Colonizing The Rhizosphere of Tef Crop During the Seedling Stage. Biomed J Sci & Tech Res. 2019;14(2):1-13.
28. Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, and Hallmann J. Endophytic and ectophytic potato associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol. Ecol. 2005;51,215–229. DOI:10.1016/j.femsec.2004.08.006.
29. Lian LH, Tian BY, Xiong R, Zhu MZ, Xu J, Zhang KQ. 2007 Proteases from Bacillus; a new insight into the mechanism of action for rhizobacteria suppression of nematode populations. Lett Appl Microbiol. 45(3):262-9. DOI:10.1111/j.1472-765x.2007.02184.x.
30. Zerihun T, Mohammed Y, Dagne B, Solomon C, Fasil, A. Characterization and Identification of Native Plant Growth-Promoting Bacteria Colonizing Tef (Eragrostis Tef) Rhizosphere During the Flowering Stage for A Production of Bio Inoculants. Biomed J Sci & Tech Res. 2019;22(2):16444-16456. DOI:10.26717/BJSTR.2019.22.003710
31. Karimi, K. Amini, J., Harighi, B., Bahramnejad, B. Evaluation of biocontrol potential of *Pseudomonas* and *Bacillus* spp. against *Fusarium* wilt of chickpea. Australian Journal of Crop Science. 2012; 6(4):695-703.
32. Khan N, Martínez-Hidalgo P, Ice TA, Maymon M, Humm EA, Nejat N, Sanders ER, Kaplan D and Hirsch AM Antifungal Activity of *Bacillus* Species Against *Fusarium* and Analysis of the Potential Mechanisms Used in Biocontrol. Front. Microbiol. 2018; 9:23-63. DOI:10.3389/fmicb.2018.02363

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63291