SCREENING OF BACTERIA FROM THE RHIZOSPHERE OF MAIZE FOR THEIR ANTAGONISM AGAINST FUNGAL AND BACTERIAL PHYTOPATHOGENS.

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Abstract: Plant diseases at every occurrence can lead to reduction in crop yield and quality. Hence there is always a need for urgent control measures preferably biological methods of control. In this study, five bacterial strains were isolated from the rhizosphere of maize (*Zea mays)* plant in a farmland in Ago-Iwoye, Ogun State and characterized phenotypically using morphological and biochemical methods. The isolates were screened for their antibacterial and antifungal activities against selected bacterial phytopathogens; *Bacillus subtilis, Xanthomonas axonopodis, Erwinia carotovora* and *Pseudomonas avenae and* fungal phytopathogens; *Aspergillus parasiticus, Ustilago maydis, Rhizoctonia solani, Fusarium verticilliodes* and *Aspergillus flavus*. Results showed that *Bacillus. megaterium* and *Staphylococcus. aureus* were able to inhibit the growth of *A. flavus, F. verticilliodes* and *R. solani* but could not suppress the growth of *A. parasiticus. Proteus vulgaris* was unable to inhibit the growth of any of the tests organisms. *B. megaterium, B. subtilis and B. cereus* were able to inhibit the growth of *E. carotovora, P. avenae* and *X. axonopodis* while *S. aureus* could only suppress the growth of *P. avenae.* This study showed that isolates belonging to the genus *Bacillus* have potentials as biological agents against the selected phytopathogens. It is recommended that a consortium should be developed from these bacteria as a biological control agent against the pathogens.

Key words: Antibacterial activity, antifungal activity, maize, rhizobacteria. Phytopathogens

Introduction

Crop pathogens and pests reduce the yield and quality of agricultural production which leads to economic losses as well as reduction in food security at household, national and global levels (Savary *et al.*, 2019). It has also been reported that soil-borne infections continue to cause considerable crop losses globally in both conventional and organic field production systems in the absence of specific plant-disease management techniques (Khabbaz *et al*., 2014).Although the role of pesticides in plants protection, production of crops of higher quality, reduction of labour, energy in crop production and to optimize yields cannot be overemphasized (Khaing *et al.*, 2021). However, there are detrimental effects accrued to the use these chemicals that can lead to the destruction of the ecosystem as well as threat to human health (Nordiyana *et al*., 2013; Ilusanya *et al*., 2018; Khaing *et al.*, 2021). Therefore, attention has shifted to environmentally friendly alternatives to chemical pesticides one of which is the use of biological control methods (Souza *et al*., 2015).

In its most basic form, biological control refers to the use of any living organism through parasitism, antibiosis, or competition for resources or space to battle or control a particular plant disease or pest (Kohl *et al*., 2019). The control of pathogens by biological control agents in agricultural practices has been adopted on a commercial scale (Souza *et al*., 2015). Microorganisms that actively invade the rhizosphere are known as rhizobacteria. Rhizobacteria are root-colonizing bacteria that collaborate with several plants to grow. Even though certain rhizobacteria include parasitic variants that might have harmful consequences, the word often refers to bacteria that create mutually beneficial relationships (mutualism). Various roots, foliar, and post-harvest diseases of agricultural crops may be controlled by rhizobacteria (Ahemad and Kibret 2014).

Rhizobacteria are now increasingly used as substitutes to agrochemicals due to their ability to supress plant diseases, increase crop quality and yields. (Raaijamaker *et al*., 2009; Khaing *et al.*, 2021). Native or indigenous microorganisms in agricultural plants' rhizospheres can be exploited to control the activities of pathogens in order to prevent plant diseases (Yang *et al*., 2012). These bacteria are use as inoculants in agricultural systems in developed countries because they would significantly reduce the use of chemical fertilizers and pesticides. (Chung *et al*., 2008; Gajbhiye *et al*., 2010).

Maize (*Zea mays*) is a stable food crop in Nigeria. Apart from being a diet in many Nigerians homes, it has become a commercial crop used as raw material by many agro based industries (Iken and Amusa, 2004). 10.2 millions of maize are produced annually in Nigeria which makes it the highest producer in Africa (FAO,2018). However, this plant is being infected by a mirage of phtypathogens on the farm which is affecting the production in Nigeria and other developing countries (Iken and Amusa, 2004; Farooq and Bano, 2013). The infestation of maize plants by pathogens can affect the yield and quality of the plant (Czembor *et al*., 2015). The use of chemicals to control pathogens in maize and other plants can have adverse effects on the ecosystem (Ilusanya *et al*., 2018; Khaing *et al.*, 2021). Hence, there is a need to source for other sustainable alternatives to the use of pesticides in disease control of maize plants. This study was therefore carried out to screen indigenous bacteria isolated from the rhizosphere of *Zea mays* (Maize plants) as bio-control agents against selected plants pathogens of maize and other plants.

Materials and Methods

Collection of soil samples

Soil samples were collected randomly at a depth of 0-20cm from the rhizosphere of *Zea mays* plants using sterile hand trowels from farms in Ago-Iwoye, Ogun State, Nigeria. The soils were bulked and the representative composite samples were taken to the

laboratory in pre-sterilized polythene bags for microbial analyses (Wang *et al*., 2018).

Isolation of Rhizobacteria

1 gram of the soil sample was weighed and transferred into 90 ml of distilled autoclaved water. Serial dilution agar plate method was used for further processing of the prepared soil suspension. Suitable dilutions (10^{-6}) were plated on nutrient agar in triplicates. The plates were incubated at 37^{o}C for 24hrs. Distinct colonies were purified by streaking on fresh nutrient agar plates (Aneja, 2002).

Source of phytopathogens

The following fungal phytopathogens were obtained from International Institute of Tropical Agriculture, Ibadan, Nigeria; *Aspergillus flavus, Fusarium verticilliodes, Aspergillus paraciticus, Rhizoctonia solani, and Ustilago maydis* while the bacterial phytopatogens; *Bacillus subtilis, Pseudomonas avenae, Erwinia carotonova, Xanthomonas axonopodis* were collected from the Agricultural Research and Training, (IAR&T), Moor Plantation, Ibadan, Nigeria. The pathogens were resuscitated in peptone water for 24-36 hours before culturing on nutrient agar and potato dextrose agar respectively. Preparation of the agar was carried out according to manufacturer's instructions. The plates were afterwards incubated for 24 hours at 37°C for the bacteria and 72 hours for the fungi at 25°C.

Screening of antibacterial activity of bacterial isolates against bacterial pathogens

Antibacterial activity was screened by agar well diffusion method against selected bacterial phytopathogens as described by Okeke *et al.* (2001). Nutrient agar plates were swabbed (by a sterile wire loop) with 24 hours old broth culture of test bacterial phytopathogens to get a confluent growth. Bores were made by a 6 mm sterile cork borer. Afterwards, 100µl of cell free supernatant of each isolated bacteria was added in triplicate. Plates were placed at room temperature for 24hrs and incubated at 37°C. The relative percentage inhibition of the cell free supernatant of isolated bacteria was calculated using the method of (Kumar *et al.* 2012).

Screening of antifungal activity of bacteria isolates against fungal pathogens

The antifungal activity of the isolated rhizobacteria against the five phytopathogenic fungi was carried out using the dual culture assays on PDA . An agar disc of 3mm in diameter was cut from an actively growing fungal culture and placed in the center of the petri plates containing potato dextrose agar the rhizobacteria was inoculated 2cm from an opposite side of the fungal disc. Plates inoculated with fungal strains and without bacteria were used as control. Incubation was done at 25°C for seven days (Kumar *et al*., 2012).

Results and Discussion

Bacteria isolated from the rhizosphere of maize plant

Five bacterial strains were isolated from the rhizosphere of the *Zea mays* plants and identified phenotypically as; *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Proteus vulgaris* and *Staphylococcus aureus.* Presented in Figure 1 is the percentage frequency of occurrence of the isolates which shows that the dominant genus isolated in his study was *Bacillus* (50%) while the least isolated bacteria was *Proteus* (17%), this findings corroborates that of Kumar *et al*. (2012), that this genus has an advantage over non-spore formers like *Pseudomonas* because they produce spores are durable and resistant to high temperatures and high chemical concentrations. Bacteria similar to those isolated from these studies have also been isolated from earlier studies and investigated as possible biocontrol agents for soil-borne infections (Whipps, 2007; Chung *et al.,* 2008; 2010; Figueroa-Lopez *et al*., 2016).

Antibacterial activities of bacterial isolates against bacterial phytopathogens

The five bacterial isolates which were *Bacillus megaterium, Bacillus subtilis, Proteus vulgaris, Staphylococcus aureus, and Bacillus cereus* were screened for their antagonistic abilities against the bacterial and fungal phythopathogens. Presented in table 1 is the antagonistic abilities of the rhizobacteria against bacterial phytopatogens, *B. megaterium* and *B. subtilis* were able to inhibit the growths of *E. carotovora, P. avenae and X. axonopodis* while *B. cereus* and *P.vulgaris were antagonistic* towards *P. avenae* and *X. axonopodis.* None of the isolates was able to inhibit the growth of *Bacillus subtilis.* Singh *et al.* (2005) reported that *Bacillus* spp. have high potential in improving the growth of plant and suppress the growth of disease-causing microorganisms because of their abilities to produce hydrolytic enzymes and antibiotics. The presence of a gene encoding enzyme known as barnase in *Bacillus* species as also been attributed to its ability to kill cells that are infected and control the spread of infection such organisms (Aslim *et al.,* 2007). *Bacillus subtilis* can trigger inhibitory mechanisms toward plant parasites and other microorganisms hence the inability of other rhizobacteria to inhibit it growth (Lelliot, 2005; Cucu *et al*.,2019).

Antifungal activities of bacterial isolates

The antagonistic activities of the isolates against the fungal phythopathogens are presented in Table 2. None of the isolates was able to suppress the growth of *Aspergillus parasiticus. B. megaterium* showed the highest level of antagonism against the test pathogens by suppressing the growths of *A. flavus, F. verticilliodes, R. solani, and U. maydis* followed by *S. aureus* which suppressed the mycelial growths *of F. verticilliodes, R. solani and U. maydis. B. cereus and B. subtilis were* able ntagonize the growth *of F. verticilliodes and R.solani. Proteus vulgaris* was not able to inhibit the growth of any of the fungal pathogens. According to a previous research, *Bacillus* species compete for resources and space, synthesize antimicrobial peptides, secrete lytic enzymes, and cause systemic resistance in order to perform antagonistic actions against pathogens (Kang *et al*., 2015). The multiple mode of action in terms of antagonistic capabilities demonstrated by *Staphylococcus aureus* to produce cell-wall degrading enzymes may be a preceding factor as reported by Marra *et al.* (2006) in its ability to also control the mycelial growth of the test organisms. *Proteus vulgaris* was unable to inhibit the mycelial growth of any of the test organisms but it has previously been reported to tolerate and utilize polluting compounds as well as promote plant growth Bursha *et al.* (2007). *Aspergillus parasiticus* was a very difficult test organism to control by the selected test isolates which may largely be due to their inability to disrupt the mycelial growth.

Numerous researches have shown that the use of fungi and bacteria, particularly *Bacillus*, can prevent or stop the growth of phytopathogens (Jangir *et al*.2018; Cucu *et al*. 2019; Karuppiah *et al*. 2019).The ability of the genus *Bacillus* and other rhizosphere bacteria to act as agents of control of pathogens is well documented and attributed to various mechanisms such as synthesis of hydrolytic enzymes, competition for nutrients , colonization of the rhizosphere niche, production of siderophores and antibiotics production (Kumar *et al*.,2018; Rana *et al*.,2019; Lopez *et al*.,2020;Karim *et al*.,2022).

Table 1. Antibacterial activity of bacterial isolates against bacterial phytopathogens.

Isolate code	E. *carotovora* (mm)	*B.subtilis* (mm)	*P. avenae* (mm)	*X. axonopodis* (mm)
MAZ1	40.5	-	34.2	36.1
MAZ2	44.2	-	40.3	38.2
MAZ3	20.0	-	-	27.0
MAZ4	-	-	35.2	-
MAZ5	30	-	-	26.0

Key: MAZ1-*Bacillus megaterium*, MAZ2-*Bacillus subtilis*,MAZ3-*Staphylococcus aureus*, MAZ4-*Bacillus cereus*, MAZ5-*Proteus vulgaris.*

Table 2 Antifungal activity of selected bacterial isolates against fungal pathogens

Isolate code	A. *Flavus* (mm)	A. *Parasiticus* (mm)	*F. Verticilliodes* (mm)	*R. solani* (mm)	*U. Maydis* (mm)
MAZ1	30.1	-	54.2	25.0	22.2
MAZ2	-	-	33	50.0	-
MAZ3	-	-	-	-	-
MAZ4	-	-	51	38.4	25.1
MAZ5	-	-	39.3	25.2	-

Key: MAZ1-*Bacillus megaterium*, MAZ2-*Bacillus subtilis*,MAZ3-*Staphylococcus aureus*, MAZ4-*Bacillus cereus*, MAZ5-*Proteus vulgaris.*

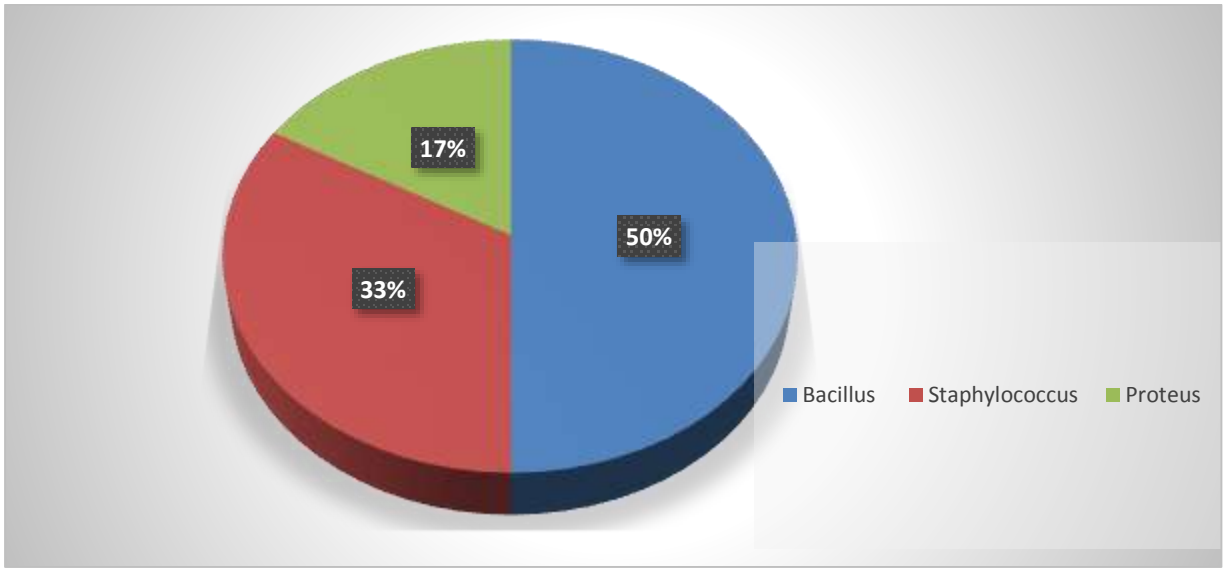

Fig 1. Percentage frequency of occurrence of the bacteria isolated from te rhizospher of maize plant

However, the use of a BCA consortium (biological control agents) has been found to be more effective in suppressing phytopathogens than a single strain (Wong *et al*. 2019). Optimisation of the antagonistic abilities of these rhizospheric isolates as biological control agents can be achieved through the development of consortium from them. (Beneduzi *et al*. 2012; Jangir *et al*. 2018)

Conclusion

Five bacteria isolated from the rhizosphere of *Zea mays* demonstrated various abilities to suppress and inhibit the growths of the selected bacterial and fungal phytopathogens. The bacteria from the genus *Bacillus* identified as *Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis* had the highest potentials as biocontrol agents of the phytopathogens. It is recommended that a consortium can be developed from these bacteria as biological control agent against the pathogens.

References

Ahemad M, Kibret, M. Mechanisms and applications of plant growth promoting rhizobacteria. current perspective. *Journal of King Saud Univ Sci.* 2014; 26(1):1–20.

Aneja KR. Experiments in Microbiology, Plant Pathology, Tissue culture and Mushroom production

technology, *New Age International New Delhi*. 2002.

Aslim B, Saglam N, Bayal Y. Determination of some properties of Bacillus Isolated from soil. *Turk, J. Biol*. 2007; 26:41- 48.

Beneduzi A, Ambrosini A, Passaglia LMP. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genet Mol Biol*.2012; 35:1044–1051

Camiloa R, Josephw K. Plant growth promotion by Bacillus amyloliquefaciens FZB45 depends on inoculum rate and P-related soil properties. *Biol Fert Soils.* 2010; 46:835-844.

Chung S, Kong H, Buyer JS, Lakshman DK, Lydon J, Kim SD. Isolation and partial characterization of Bacillus subtilis ME488 for suppression of soilborne pathogens of cucumber and pepper. *Appl Microbiol Biotechnol.* 2008; 80(1):115–23.

Cucu MA, Gilardi G, Pugliese M, Gullino ML, Garibaldi A. An assessment of the modulation of the population dynamics of pathogenic *Fusarium oxysporum* f. sp. lycopersici in the tomato rhizosphere by means of the application of *Bacillus subtilis* QST 713, *Trichoderma* sp. TW2 and two composts. *Biol Control.* 2020; 142:104–158.

Czembor E, Stępień Ł, Waśkiewicz A. Effect of environmental factors on *Fusarium* species and associated mycotoxins in maize grain grown in poland. *PLoS ONE*. 2015; 10(7):e0133644.

Farooq U and Bano A .Screening of indigenous bacteria from the rhizosphere of maize (Zea mays L.) for their plant growth promotion and antagonism against fungal and bacterial pathogens.2013;23 (6) 1642-1652.

Figueroa-Lopez AM, Cordero-Ramirez JD, Martinez-Alvarez JC, Lopez-Meyer M, Lizarranga-Sanchez GJ, Felix-Gastelum R, Catro-MartinezC, Maldonado-Mendoza IE. Rhizospheric bacteria of maize with potential for biocontrol of *Fusarium verticilliodes*. *SpringerPlus*. 2016; 5(330): 1-12.

Gajbhiye A, Alok RR, Meshram SU, Dongre AB. Isolation, evaluation and characterization of Bacillus subtilis from cotton rhizospheric soil with biocontrol activity against Fusarium oxysporum. *World J Microbiol Biotechnol.* 2010; 26(7):1187–94.

Gupta VVSR., Rovira AD, Roget DK. Principles and Management of Soil Biological Factors for Sustainable Rainfed Farming Systems. *Rainfed Farming Systems.* 2011; 149-184.

Ilusanya OAF, Banjo OA, Onajobi IB, Bankole SA. Effect of herbicides on physicochemical properties and microorganisms of soil in Ago-Iwoye, Ogun State, Nigeria. *actaSATECH The Journal of Life and Physical Sciences.* 2018; 10 (1) 49-60.

Jangir M, Pathak R, Sharma S, Sharma S. Biocontrol mechanisms of Bacillus sp., isolated from tomato rhizosphere, against Fusarium oxysporum f. sp. lycopersici. *Biol Control.* 2018; 123:60–70

Kang SM, Radhakrishnan R, Lee IJ. Bacillus amyloliquefaciens subsp. plantarum GR53, a potent biocontrol agent resists Rhizoctonia disease on Chinese cabbage through hormonal and 483 antioxidants regulation. *World J Microb Biot.* 2015; 31:1517-1527.

Karim H, Azis AA, Jumadi O. Antagonistic activity and characterizationof indigenous soilisolatesof bacteria and fungi against onion wilt incited by *Fusarium* sp. *Archives of Microbiology*.2022; 204 (68):1-9.

Karuppiah V, Sun J, Li T, Vallikkannu M, Chen J. Co-cultivation of Trichoderma asperellum GDFS1009 and Bacillus amyloliquefaciens 1841 causes diferential gene expression and improvement in the wheat growth and biocontrol activity. *Front Microbiol*. 2019; 10:1068.

Khabbaz SE, Abbasi, PA. Isolation, characterization, and formulation of antagonistic bacteria for the management of seedlings damping-off and root rot disease of cucumber. *Can J Microbio*. 2014;60: 25-33.

Khaing A, Thent WT, Thioo K, Fu P. Antagonist activity of indigenous Rhizobacteria through biosynthesis of Indole -3-Acetic (1AA), Hydrogen Cyanide (HCN), and siderophores Austin *Journal of Biotechnology and Bioengineering*. 2021; 8(1):1110

Kumar P, Dubey RC, Maheshwari DK. Bacillus Strains Isolated from Rhizosphere Showed Plant Growth Promoting and Antagonistic Activity Against Phytopathogens, *Microbiol. Res*. 2012; 167(8):493-499.

Lelliott RA. The preservation of plant pathogenic bacteria. *Journal of Applied Bacteriology.* 2005; 28: 181-204.

Lopez CG, Castellanos LNM, Oritiz NAF ,Gonzalez JAG. Control of powdery mildew(*Leveillula taurica*) using *Tricoderma asperellum* and *metarhizium anisopliae* in different pepper types. *Biocontrol*. 2019: 64(1);77-89.

Marra R, Ambrosino P, Carbone V, Vinale F, Woo SL. Study of a three-way interaction between *S. aureus*, plant and fungal pathogens using proteomic approach. *Curr Genet.* 2006; 50: 307-321.

Nordiyana N, La Colla P, Tamburini E. Isolation and screening of high efficiency biosurfactantproducing bacteria Pseudomonas sp..*Journal of biochemistry, microbiology and biotechnology*. 2013; 1(1):25-31.

Okeke MI, Iroegbu CU, Eze N, Okole SA, Esimone CO. Evolution of extract of the root of landolphia owerrience for antibacterial activity *J. Ethnopharmacol.* 2001; 78:119-127.

Pereira P, Nesci A, Castillo C, Etcheverry M. Field studies on the relation- ship between Fusarium verticillioides and maize (Zea mays L.): effect of biocontrol agents on fungal infection and toxin content of grains at harvest. *Int. J Agron* 7. 2011.

Raaijamakers JM, Paulitz TC, Albouvette C, Steinberg C, Monne-Loccoz Y. The rhizosphere a playground and battlefield for soilborne pathogens and beneficial microorganisms *Plant soil.* 2009; 321:341-361.

Rana KI, Kour D, Sheikh I, Yadav N, Yadav AN, Kumar V, Saxena AK. Biodiversity of endophytic fungiform diverse niches and their biotechnological applications. In Advances in

endophytic fungal research. 2019; pp 105-144 Springer, Cham

Savary S, Wilocquet L, Pethybridge ST, Esker P, McRobert N, Nelson A. The global burden of pathogens and pests on major food crops. *Nature Ecology and Evolution.* 2019; 3, 430-439.

Solanki MK, Kumar S, Pandey AK, Srivastava S, Singh RK, Kashyap, PL, Srivastava AK, Arora DK. Diversity and antagonistic potential of Bacillus spp. associated to the rhizosphere of tomato for the management of Rhizoctonia solani. *Biocontrol Sci Techn.* 2012; 22:203-217.

Souza RD, Ambrosini A, Passaglia LMP. Plant growth-promoting bacteria as inoculants in agricultural soils. *Gen Mol Biol*. 2015; 38:401–419

Wang X, Wang C, Li Q. Isolation and characterization of antagonistic bacteria with the potential for biocontrol of soilborne wheat diseases. *Journal of Applied Microbiology*. 2018; 125 (6)1868–1880.

Whipps JM. Use of *Pseudomonas vulgaris* as a biocontrol agent. *European Journal of Plant Pathology.* 2007; 121: 323-330.

Wong CKF, Saidi NB, Vadamalai G, Zulperi D. Effect of bioformulations on the biocontrol efcacy, microbial viability and storage stability of a consortium of biocontrol agents against Fusarium wilt of banana. *J Appl Microbiol.* 2019; 127(2):544–555

Yang Q, Xu H, Liu X. Evaluation of biological control agents against Ralstonia wilt on ginger. *Biological Control.* 2012; 62(3):144–151.

Zhan-Bin, Wu Lei-Fei, Liang, Chen, Feng, Yong-Jun, Yuan, Xing-Fang. Isolation, screening and identification of antagonistic downy mildew; endophytic bacteria from cucumber. *Eur J Plant Pathol*. 2013; 137:847-857.