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## ***In vitro* Antifungal Activities of Bacteria Associated with Maize Husks and Cobs**

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### **ABSTRACT**

The aim of study was to evaluate the antagonistic activities of bacteria associated with maize husks and cobs against some phytopathogenic and spoilage fungi namely: *Fusarium oxysporum*, *F. solani*, *Colletotrichum lindemuthianum*, *Rhizoctonia solani*, *Phytophthora infestans*, *Aspergillus niger*, *A. flavus* and *Penicillium italicum*. The streak bioassay method was employed in determining the antifungal capabilities of the isolated bacteria. Six bacteria were isolated from maize husks and were identified as *Lactobacillus* sp., *Proteus* sp., *Sporolactobacillus* sp., *Clostridium* sp., *Micrococcus* sp. and *Klebsiella* sp. while *Lactobacillus* sp., *Providencia* sp. and *Bacillus subtilis* were isolated from maize cob. *In vitro* bioassay confirmed that only *B. subtilis* was antagonistic to *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani*, *Phytophthora infestans* and *Penicillium italicum* but was not antagonistic to *Aspergillus niger*, *A. flavus* and *Colletotrichum lindemuthianum*. *B. subtilis* has the greatest inhibitory effect on *R. solani* and least on *F. oxysporum* with percentage inhibition of 89 and 50, respectively on the fifth day. The *B. subtilis* was fungistatic in action. The inhibitory effect of *Bacillus subtilis* was attributed to the production of diffusible antifungal compounds.

**Key words:** Bacteria, antagonistic, fungistatic, diffusible antifungal compounds

### **INTRODUCTION**

Fungal plant pathogens are among the most important factors that cause serious losses to agricultural products every year and they need to be controlled to ensure food, feed and fiber production quantitatively and qualitatively (Heydari and Pessarakli, 2010). A number of different strategies are currently being employed to manage and control plant pests (Bargabus *et al.*, 2002, 2004; Benhamou, 2004). Beyond good agronomic and cultural practices, growers often rely heavily on chemical pesticide application (Daayf *et al.*, 2003). However, the development of resistance to many fungicides in major crop and postharvest pathogens and public concern over pesticide residues in food and the environment (Zafar *et al.*, 2000; Shahid *et al.*, 2003; Heydari, 2007; Heydari *et al.*, 2007) have created interest in alternative methods of disease control. Biocontrol of crop diseases has emerged as a promising option (Khetan, 2001; Daayf *et al.*, 2003). Biopesticides compete favorably with conventional chemical pesticides both in efficacy and cost (Kaya and Lacey, 2007; Omoya *et al.*, 2009). It has been shown that non-pathogenic plant-associated microorganisms generally protect the plant by rapid colonization and thereby exhausting the limited available substrates so that none are available for pathogens to grow (Kageyama and Nelson, 2003). Braun-Kiewnick *et al.* (2000) also reported the work of Kempt and Wolf in which *Erwinia herbicola* isolated from plant family Gramiceae was found to be antagonistic to *Puccinia recondita* and *Fusarium culmorum*. Kokalis-Burelle *et al.* (2003) found that bacteria isolated from various types

of agricultural/municipal wastes suppress different types of soil-borne plant diseases by making plants more vigorous and better able to withstand attack. Bacterial biocontrol agents enhance plant growth and reduce disease by utilizing a number of different mechanisms. These include the production of antibiotics (Rahman *et al.*, 2007) and toxins that reduce pathogen growth and infection potential, competition for infection sites and induction of resistance mechanisms in the plant (Ahl *et al.*, 1986; Silva *et al.*, 2004). This present study was undertaken to determine the antifungal activities of the isolated bacteria on some fungal phytopathogens and spoilage pathogens.

## MATERIALS AND METHODS

**Isolation and identification of bacterial isolates:** This study was conducted at the Federal University of Technology, Akure, Nigeria in 2008. Maize ears were collected in a local farm at Ibadan, Oyo State, Nigeria. They were then dehusked to facilitate the removal of the grains from the husks in a laminar flow previously sterilized with 70% alcohol in the laboratory. Fifty milliliters of sterile water was then dispensed in a conical flask containing 5 g of maize husk. One milliliter was taken from the content of the conical flask, serially diluted to  $10^{-2}$  and 0.1 mL from each dilution was pour plated using molten nutrient agar (Fluka). Incubation was done at 37°C for 24 h after which the plates were observed. Identification was done using cultural, morphological and biochemical characteristics using the methods described by Holt *et al.* (1994). Elevation, colour and shape were studied on the agar. The cells were Gram stained, tested for motility, sugar fermentation and enzymes such as catalase, urease etc. This process was repeated for maize cob.

**Microorganisms:** The pathogenic fungi used; *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* and *Colletotrichum* species were obtained from the Department of Germ Plasm, International Institute for Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. Also, *Phytophthora infestans*, *Penicillium italicum*, *Aspergillus flavus* and *A. niger* were obtained from the Department of Microbiology, Federal University of Technology, Akure. Both fungal and isolated bacterial cultures were maintained at 4°C by repeated subculturing on potato dextrose agar (Fluka) and nutrient agar (Fluka), respectively.

**Antagonistic assay:** For the antifungal assay, the growing edge of a 5-day old test fungus was aseptically cut and placed at the center of the solidified potato dextrose agar plate. Thereafter, 40 mm streak was made from each of the isolated bacteria 23 mm away from the center of the Petri dish using a 10 mm diameter cork borer. The plates were incubated at 25°C in an inverted position and monitored for 5 days. This was performed in the duplicates. Measurement of the percentage inhibition and intercolony distance was taken daily according to Adetuyi and Cartwright (1985). The control plates contained only the test fungi. This process was repeated all the selected fungi.

## RESULTS

Different species of bacteria were associated with maize husks and cobs. *Lactobacillus* sp., *Proteus* sp., *Sporolactobacillus* sp., *Clostridium* sp., *Micrococcus* sp. and *Klebsiella* sp. while *Lactobacillus* sp., *Providencia* sp. and *Bacillus subtilis* were isolated from maize husk and maize cob, respectively.

The *in vitro* antagonistic bioassay revealed that only *B. subtilis* was inhibitory to *Rhizoctonia solani*, *Fusarium solani*, *F. oxysporum*, *Phytophthora infestans* and

*Penicillium italicum* but not inhibitory to the growth of *A. flavus*, *A. fumigatus* and *Colletotrichum lindemuthianum*. Measurements of the intercolony distance and percentage inhibition of the radial growth of fungi were based on the data accumulated from 24 to 120 h.

The percentage inhibition of *Phytophthora infestans* by *Bacillus subtilis* increased from 48 to 120 h while intercolony distance between them slightly increased from 72 to 120 h (Fig. 1). Also, there was increase in the percentage inhibition of *Rhizoctonia solani* by *B. subtilis* from 24 to 48 h but a slight increase from 48 to 120 h. However, the intercolony distance between these organisms sharply increased from 72 to 120 h (Fig. 2).

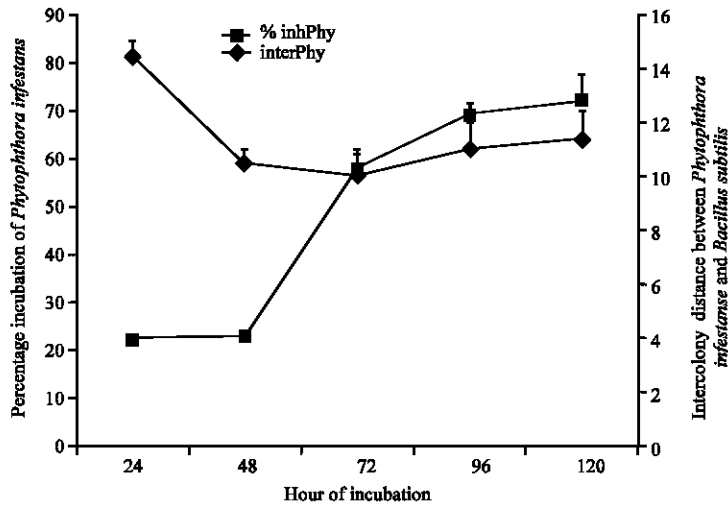


Fig. 1: Inhibition of *Phytophthora infestans* by *Bacillus subtilis* on PDA at 25°C. Values are means of two replicates while vertical bars represent standard error. InhPhy: Percentage inhibition of *Phytophthora infestans* by *B. subtilis*, InterPhy: Intercolony distance between *Phytophthora infestans* and *B. subtilis*

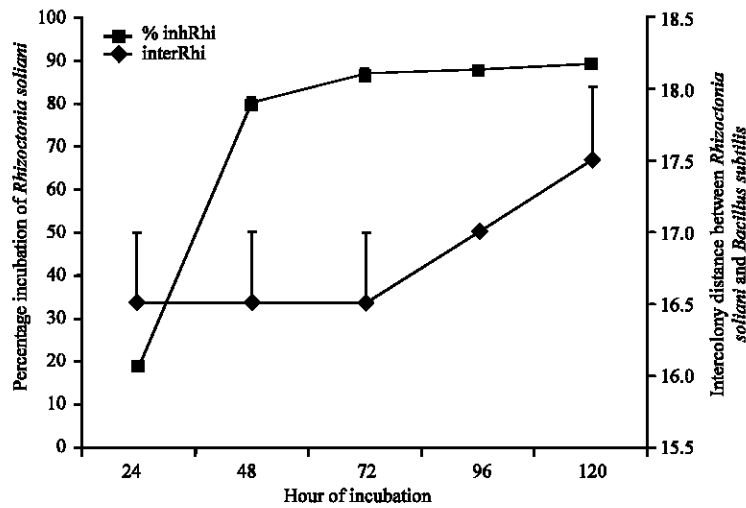


Fig. 2: Inhibition of *Rhizoctonia solani* by *Bacillus subtilis* on PDA at 25°C. Values are means of two replicates while vertical bars represent standard error. InhRhi: Percentage inhibition of *Rhizoctonia solani* by *B. subtilis*, InterRhi: Intercolony distance between *Rhizoctonia solani* and *B. subtilis*

The percentage inhibition of *Fusarium oxysporum* by *Bacillus subtilis* initially decreased from 24 to 48 h before increment from 48 to 120 h. However, after initial decrease of intercolony distance between them, there was stationary phase that was maintained between 48 to 96 h (Fig. 3).

The percentage inhibition of *Penicillium italicum* by *Bacillus subtilis* increased from 24 to 48 h before maintaining almost a stationary phase in its curve. On the other hand, intercolony distance between the two organisms maintained a stationary phase between 24 and 72 h, sharp increase from 72 to 96 h and sharp decrease from 96 to 120 h (Fig. 4).

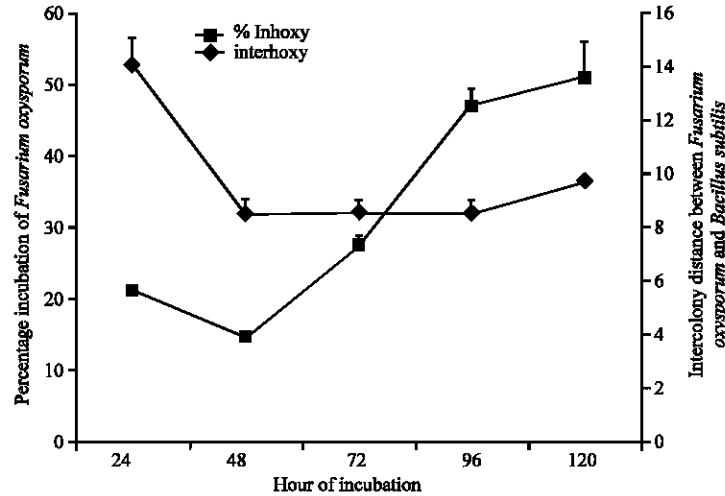


Fig. 3: Inhibition of *Fusarium oxysporum* by *Bacillus subtilis* on PDA at 25°C. Values are means of two replicates while vertical bars represent standard error. Inhoxy: Percentage inhibition of *Fusarium oxysporum* by *B. subtilis*, Interhoxy: Intercolony distance between *Fusarium oxysporum* and *B. subtilis*

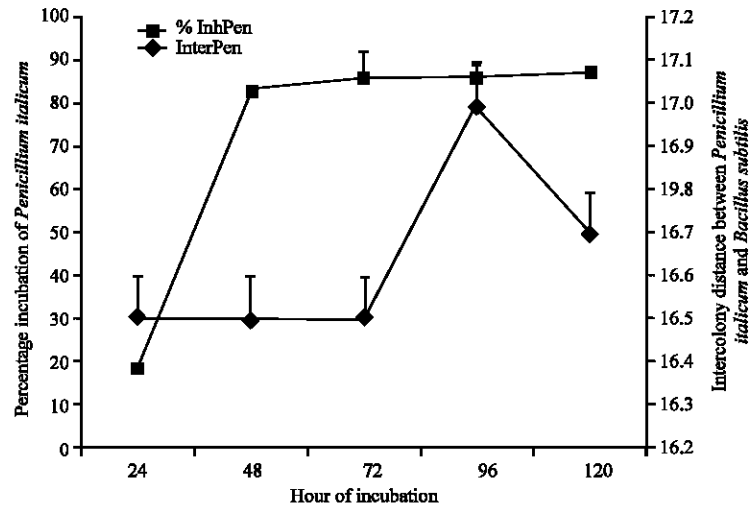


Fig. 4: Inhibition of *Penicillium italicum* by *Bacillus subtilis* on PDA at 25°C. Values are means of two replicates while vertical bars represent standard error. InhPen: Percentage inhibition of *P. italicum* by *B. subtilis*, InterPen: Intercolony distance between *P. italicum* and *B. subtilis*

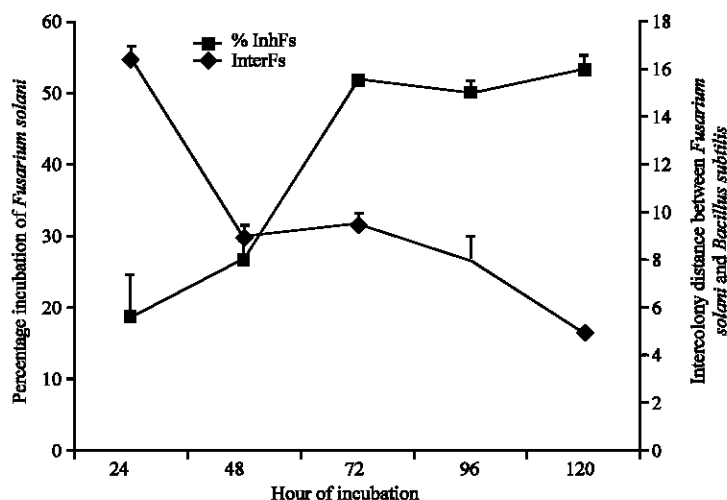


Fig. 5: Inhibition of *Fusarium solani* by *Bacillus subtilis* on PDA at 25°C. Values are means of two replicates while vertical bars represent standard error. InhFs: Percentage inhibition of *Fusarium solani* by *B. subtilis*, InterF's: Intercolony distance between *Fusarium solani* and *B. subtilis*

The percentage inhibition of *Fusarium solani* by *B. subtilis* increased sharply from 24 to 72 h and 96 to 120 h while the intercolony distance decreased from 24 to 120 h (except from 48 to 72 h) (Fig. 5). In all the figures, the highest percentage inhibition of *P. infestans*, *R. solani*, *F. oxysporum*, *P. italicum* and *F. solani* by *B. subtilis* was on 120 days. The highest intercolony distances between *B. subtilis* and *P. infestans*, *B. subtilis* and *F. oxysporum*, *Bacillus subtilis* and *F. solani* were at 24 h. The highest intercolony distance between *B. subtilis* and *R. solani* was recorded at 120 h while that of *B. subtilis* and *P. italicum* was at 96 h. The result of this investigation revealed that *B. subtilis* was most antagonistic to *R. solani* and least to *F. oxysporum* with percentage inhibition of 89 and 50 at 120 h, respectively.

Uncolonized zone was maintained throughout between *B. subtilis* and the fungi suggesting that diffusible toxins of bacterial origin were responsible for the antagonism shown by the restriction of the mycelial growth. Seven days and even beyond this time, a 10 mm plug of the fungi were cultured on potato dextrose agar and it was noticed that the antagonist was not completely lethal to the growth of the fungi but only affected mycelial formation in the bioassay.

## DISCUSSION

Various bacteria have found to be associated with agricultural systems. The results of this investigation showed that *Lactobacillus* sp., *Proteus* sp., *Sporolactobacillus* sp., *Clostridium* sp., *Micrococcus* sp. and *Klebsiella* sp. while *Lactobacillus* sp., *Providencia* sp. and *Bacillus subtilis*. This was supported by the findings of Kokalis-Burelle *et al.* (2003). The *in-vitro* bioassay of the current investigation revealed that only *B. subtilis* out of the nine bacteria isolated from maize husks and cobs was inhibitory to mycelial formation of *R. solani*, *P. italicum*, *P. infestans*, *F. solani* and *F. oxysporum*. However, the degree of inhibition varied as shown in Fig. 1-5. The streak bioassays resulted in deformation of the test fungi. Clear zones of inhibition were maintained throughout the bioassay. Percentage inhibition increased to 89, 88, >63, 55 and 50% after 120 h of incubation of *B. subtilis* against *R. solani*, *P. italicum*, *P. infestans*, *F. solani* and *F. oxysporum*, respectively. Antagonism is known to be mediated by a variety of compounds of microbial origin, e.g., bacteriocins, enzymes, toxic substances, volatiles and others (Chaurasia *et al.*,

2005). Previous investigation by Chaurasia *et al.* (2005) has revealed that *B. subtilis* was inhibitory to radial growth of *Alternaria alternata*, *Cladosporium oxysporum*, *F. oxysporum*, *Paecilomyces lilicanus*, *P. variotii* and *Pythium afertile*. These pathogenic fungi are the causative agents of leaf spot and leaf blight, fruit and crop rot, wilt and rots, damping off of seedlings and blight, respectively. *Bacillus subtilis* are known for their antifungal properties, hence their importance in the biological control of plant and animal diseases (Whipps, 2001). Their antagonistic potentials may also be due to the production of endospores, diffusible and volatile toxins (Ryu *et al.*, 2003; Chaurasia *et al.*, 2005) and effectiveness in colonization of plant roots (Mahaffee and Backman, 1993). It has been discovered that *B. subtilis* directs antagonism to pathogens through antibiosis and competition for resources and indirect stimulation of plant growth by promoting nodulation and secreting growth factor (Brannen and Kenney, 1997; Kokalis-Burelle *et al.*, 2003).

*Bacillus subtilis* was not inhibitory to the growth of *A. flavus*, *A. fumigatus* and *Colletotrichum lindemuthianum*. This may be attributed to the fact the fungi are capable of producing enzymes that could detoxify the antibiotic produced by *B. subtilis*. The present study revealed that diffusible compounds produced by *B. subtilis* an isolate of maize cob induced morphological abnormalities in the fungal structures and may be found useful in suppressing soil-borne pathogens.

## CONCLUSION

In conclusion, the use of bacteria as biocontrol agent of both phytopathogens and spoilage pathogens of fungi origin is essential because they can provide short and occasionally, long term control. It affords minimal disturbance to non-target species and to the environment. Moreover, it is cheaper than chemical pesticides. This preliminary study has shown that *Bacillus subtilis* isolated from maize cob which is regarded as a waste can be used as a biocontrol agent.

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