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Full Length Research Paper

Endophytic Microbial Communities in Maize: Bacterial and Fungal Associations with Grains and Roots

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The study was carried out to determine the microbes of maize grains sourced from five markets in Akungba and Ikare-Akoko, Ondo State, Nigeria. Bacterial and fungal microbes from roots of two maize cultivars DMR-LSR-Y and TZMSR-W were also investigated using the pour plate method. Results showed that grains from Oja Oba had the highest bacterial population of 4.8×10^5 cfu/g, while, the highest fungal load of 4.1×10^3 cfu/g was obtained from Osele market in Ikare. The two maize cultivars showed generally a low fungal count compared to their bacterial counterparts with 1.1×10^5 cfu/g root for a cultivar DMR-LSR-Y and 0.2×10^5 cfu/g root for TZMSR-W. The dry white maize grains showed generally low bacterial and fungal colonizations of 0.2×10^5 and 0.3×10^3 cfu/g respectively when compared to dry and fresh yellow types. Eleven bacteria genera and eight fungal species were isolated and identified from the roots and grains of maize. These include *Cellulomonas, Bacillus, Pseudomonas, Staphylococcus, Micrococcus, Pediococcus, Microbacterium, Azospirillum, Kurtia,* and *Enterobacter, Acremonium zeae, Alternaria alternata, Aspergillus flavus, Aspergillus niger, Colletotrichum graminicola, Fusarium verticillioides, Saccharomyces cerevisiae,* and *Trichoderma koningii.* The study was important in bioprospecting for biological activities and plant growth enhancers.

Key words: Bacterial population, fungal population, endophytes, grains, roots, maize.

INTRODUCTION

Maize is a household food crop and the second most important cereal found throughout Nigeria after sorghum (Abdulrahaman and Kolawole, 2006; Iken and Amusa, 2004). It is also an important crop for the brewing of alcohol coupled with many other traditional uses like pap, tuwo, ice cream, donkunu, akamu, roasted or boiled (Agu et al., 2006). It grows in virtually all soil types (Iken and Amusa, 2004) though with varying degree of yield. Over fifty species are cultivated depending on the region; the species vary in texture, taste, shapes and sizes. The grains contain vitamins A, C and E, carbohydrates, minerals, and about 9% protein (Okoruwa, 1996).

On the other hand, maize is a host to a variety of microorganisms: non-mycorrhizal fungal endophytes (Fisher et al., 1992), natural associations with N_2-fixing bacteria like *Azospirillum* (Christansen-Weniger and Vanderleyden, 1994), *Klebsiella* (Chelius and Triplett, 2000a; Dong et al., 2001), *Pantoea*, *Herbaspirillum* and *Bacillus* (Chelius and Triplett, 2000b; Palus et al., 1996). *Bacillus subtilis, Bacillus megaterium, Bacillus cereus, Bacillus licheniformis, Bacillus anthracis, Bacillus mycoides, Bacillus pumilus* and *Bacillus circulans* were isolated as endopyhtes from 14 maize cultivars; others were *Enterobacter* spp., *Serratia* spp., *Pseudomonas* spp., *Xanthomonas* spp., *Clavibacter* spp. (Gao et al., 2004). McInroy and Kloepper (1995), Chelius and Triplett (2001) and Fisher et al. (1992) found that *Burkholderia* spp, *Enterobacter agglomerans, Klebsiella terrigena, Pseudomonas corrugata, Pseudomonas fluorescens, Pseudomonas marginalis,* and *Vibrio* sp. were the predominant species in maize stems and roots. The objective of the study was to isolate and characterize bacterial and fungal microbes of maize kernels and roots in Akungba, Akoko, Ondo State with a view to screening them later for their biological activities and chemical profile.

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MATERIALS AND METHODS

Collection of maize kernels

Maize kernels were purchased from markets in Akungba and Ikare-Akoko, Ondo State, Nigeria. Fresh and dry samples from different market locations were obtained. A total of fifteen samples were collected between 28th and 30th June, 2009. The local white and yellow varieties were analysed for microbial load. Two other varieties DMR-LSR-Y and TZMSR-W obtained from the International Institute of Tropical Agriculture (IITA), Ibadan were planted. Maize variety DMR-LSR-Y was downy mildew and streak resistant, while variety TZMSR-W is streak resistant (Iken and Amusa, 2004).

Planting of maize varieties

Varieties DMR-LSR-Y and TZMSR-W maize varieties were surface-sterilized according to the modified methods of Adejumo and Orole (2010). Maize seeds were soaked in 3.5% m/v NaOCl for 20 s followed by a 30 s dip in 70% ethanol and two rinses in distilled water, blotted dried and planted in a field at Adekunle Ajasin, University, Akungba.

Isolation of bacterial and fungal isolates from maize seeds

The local maize seeds obtained from the markets were surface-sterilized with 0.8% NaOCl for 2 min followed by a 30 s dip in 70% ethanol and two rinses in distilled water according to the methods of Dietmar et al. (2008). The seeds were then mashed with mortar and pestle to expose the microbes inhabiting them. 1 g of the ground maize seeds was dissolved in 9 ml distilled water and further serial dilution of 10^{-3} was done for fungal, while 10^{-7} for the bacterial colonizers using the pour plate methods. Sabouraud dextrose agar (SDA) and potato dextrose agar (PDA) were used for isolating fungi, while nutrient agar (NA) and MacConkey agar were used for bacteria.

Isolation of endophytic bacterial and fungal isolates from maize roots

At 8 weeks after planting when the tassels started showing, maize plants were randomly uprooted and the roots severed 3 cm above the soil according to the methods of Narisawa et al. (2003), they were labelled and kept for further analysis. The roots were washed with distilled water, surface - sterilized for 2 min with 70% ethanol and 2 min with 0.53% NaOCl (Mejia et al., 2008), rinsed in distilled water and dried. 1 g of the root was weighed, mashed and then the root tissue extract was serially diluted in saline solution (NaOH) at 0.85% (Posada and Vega, 2005). Dilutions of 10^{-3} were made for fungal and 10^{-7} for bacterial isolations from which 1 ml of each sample was placed unto Petri dishes, using the poured plate technique. The culture media used for fungi were PDA and SDA, while NA and MacConkey agar were used for bacterial isolation. The Petri dishes were incubated at 28°C, 48 to 72 h for fungi and 27°C, 48 h for bacteria according to the modified methods of Gaviria (1978) and Zinniel et al. (2002) and was then examined.

Identification of isolates

The morphological characterization of each isolate was first performed by noticing color, size, and colony characteristics (form, margin, and elevation), and Gram staining reaction. The following biochemical tests were used for identification: gelatin liquefaction, citrate utilization, oxidase, catalase, growth at 6.5% sodium chloride, fluorescent pigment production, indole formation, and glucose fermentation. The ability of the isolates to grow at 42°C was also detected (Balows et al., 1992; Krieg et al., 1984) for the identification of the bacteria isolates. Fungal colonies were identified and characterized 72 h after inoculation. Isolates were classified according to the type of colony and the morphology of the spores on fungi, based on the descriptions of Dayan (2004) and other different books and pamphlets.

Statistical analysis

Tukey-Kramer Honestly Significant Difference (HSD) tests were conducted to compare colony counts of maize grains for the different markets and the root of the two maize varieties. The threshold for statistical significance was set at a P value of = 0.05. The analysis was conducted using JMP in Version 5.1; SAS Institute 1992–1998.

RESULTS

Bacterial and fungal population in maize grains

Results of the dry white maize colonization showed the highest bacterial population of 1.5×10^5 cfu/g was obtained at Osele market, followed by Oja Oba (1.2×10^5 cfu/g) and least for Ibaka market with 0.2×10^5 cfu/g (Figure 1). The dry yellow maize grains from Osele market had the highest bacterial count of 3.8×10^5 cfu/g followed by Oja Oba (3×10^5 cfu/g) and lowest for Okusa market with 0.3×10^5 cfu/g. Fresh yellow maize from Oja Oba had the highest count of 4.8×10^5 cfu/g, while the least population was observed in Ibaka market at Akungba with 1.9×10^5 cfu/g. At Osele market, dry white maize fungal population of 3.3×10^3 cfu/g was obtained as the highest, it was followed by 1.3×10^3 cfu/g from Oja Oba and the lowest of 0.3×10^3 cfu/g was from Ibaka market (Figure 2).

The dry yellow maize grains' fungal population was highest at Osele market with 4.1 × 103 cfu/g, then 2.9 × 103 cfu/g from Ibaka market and lowest at Oja Oba with 0.6 × 103 cfu/g. Fresh yellow maize grains had fungal count of 2.2 × 103 cfu/g grain, as the highest from Okusa market and the lowest of 0.4 × 103 cfu/g from Osele. Results showed that dry white maize grains had an average bacterial population of 0.9 × 105 cfu/g, dry yellow grains (2 × 105 cfu/g) and 3 × 105 cfu/g was obtained for fresh yellow maize grains from markets. Generally, the average fungal populations were 1.2 × 103, 2 × 103, and 1.1 × 103 cfu/g for dry white grains, dry yellow maize and fresh yellow maize grains respectively.

Bacterial and fungal population in maize roots

The results observed from maize roots showed that DMR-LSR-Y had a higher bacterial load of 1.1×10^5 cfu/g

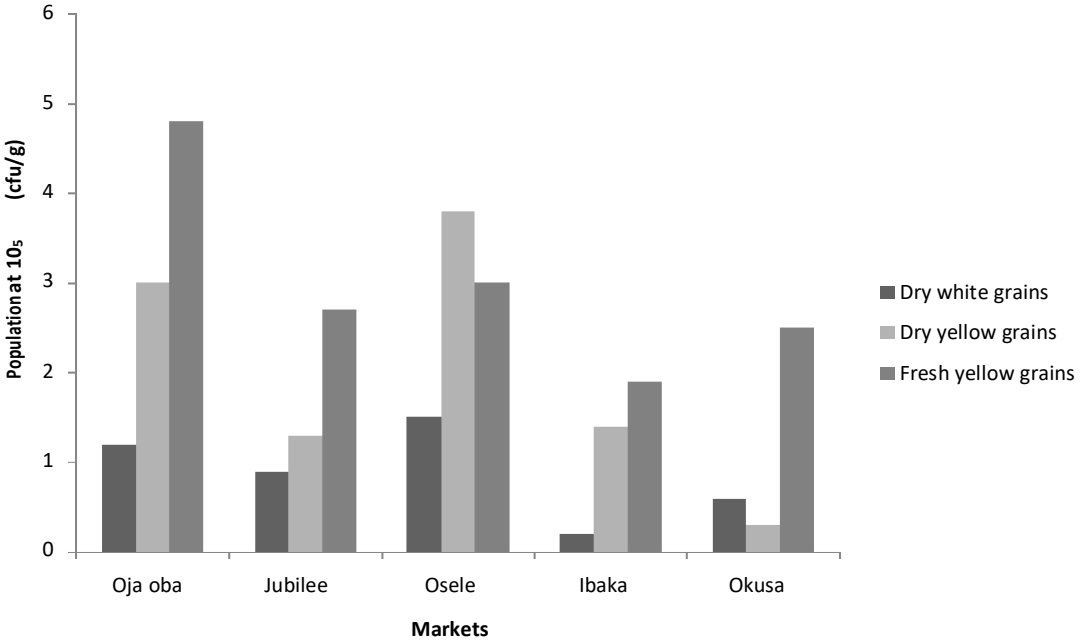

Figure 1. Population of bacteria isolated from maize grains from different markets.

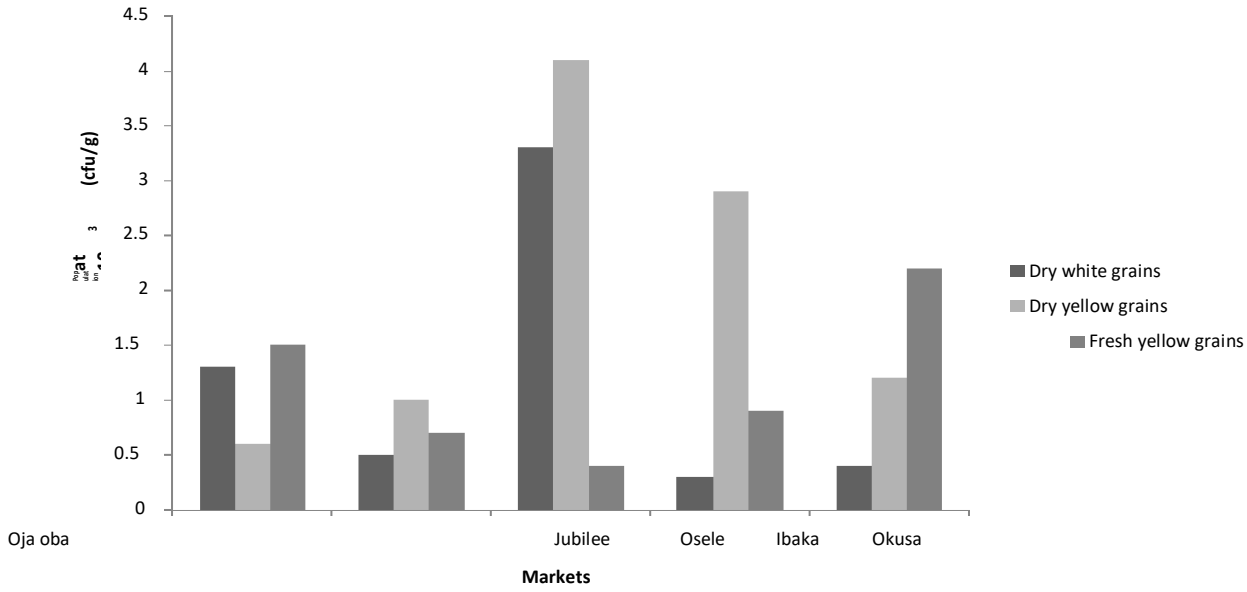

Figure 2. Population of fungi isolated from maize grains from different markets.

root than TZMSR-W with 0.2 × 10^5 cfu/g root (Table 1), and fungal loads of 1.7 × 10^3 and 0.8 × 10^3 cfu/g for DMR-LSR-Y and TZMSR-W respectively.

Microbial isolates from grains and roots of maize

Eight fungal and 11 bacterial species were isolated and identified from the grains and roots of maize samples (Table 2). The three maize grain types were host to *Acremonium zeae, Colletotrichum graminicola, Fusarium verticillioides, Azospirillum* sp., *Bacillus* sp., *Cellulomonas* sp., *Kurtia* sp., *Microbacterium* sp., *Pediococcus* sp., and *Pseudomonas* sp. (Table 2). Among bacterial species *Enterobacter* sp. was the only isolate that was not observed in any of the maize grains sampled. *Saccharomyces cerevisiae*, *Trichoderma koningii*, and

Table 1. Mean bacterial and fungal count of seeds and roots of maize.

	Dry white grains	Dry yellow grains	Fresh yellow grains	DMR-LSR-Y variety	TZMSR-W variety
Bacterial count (cfu/g)	0.9×10^5	2×10^5	3×10^5	1.1×10^5	0.2×10^5
Fungal count (cfu/g)	1.2×10^3	2×10^3	1.1×10^3	1.7×10^3	0.8×10^3

Table 2. Fungal and bacterial isolates from the grains and roots of maize.

Isolates	Maize grains			Roots	
	Dry white grains	Dry yellow grains	Fresh yellow grains	DMR-LSR-Y	TZMSR-W
Fungi					
Acremonium zeae	+	+	+	-	-
Alternaria alternata	+	-	+	+	+
Aspergillus flavus	-	+	+	-	-
Aspergillus niger	+	+	-	-	-
Colletotrichum graminicola	+	+	+	-	-
Fusarium verticillioides	+	+	+	-	+
Saccharomyces cerevisiae	-	-	+	+	+
Trichoderma koningii	-	-	+	+	-
Bacteria					
Azospirillum sp.	+	+	+	+	+
Bacillus sp.	+	+	+	+	+
Cellulomonas sp.	+	+	+	+	+
Enterobacter sp.	-	-	-	+	+
Kurtia sp.	+	+	+	-	-
Microbacterium sp.	+	+	+	-	+
Micrococcus sp.	+	-	-	+	+
Citrobacter sp.	+	+	-	+	+
Pediococcus sp.	+	+	+	-	-
Pseudomonas sp.	+	+	+	+	+
Staphylococcus sp.	-	-	+	+	+

- Isolate absent, +Isolate present.

Staphylococcus sp. were absent in both the white and yellow dry maize grains. At Okusa and Oja Oba markets, *Microbacterium* sp. was not isolated. However, *Cellulomonas* sp. and *Kurtia* sp. were present in all the samples taken. Roots of maize varieties DMR-LSR-Y and TZMSR-W were not colonized by six of the isolates identified (Table 2). The roots of TZMSR-W were colonized by twelve microbial species, while fresh yellow maize grains from the markets was colonized by 15 different microbial isolates (Table 2).

DISCUSSION

Oja Oba market had the highest bacterial load. It is a big cosmopolitan market, bordered and dotted by residential buildings that mostly have poor and improper waste disposal systems. These and other unsanitary acts of the marketers may explain the high incidence of bacterial load and presence of coliform bacteria (*Enterobacter* sp. and *Citrobacter* sp.). However, the presence of the coliforms does not translate to unsafe environment for business transaction. Colonization by microbes was higher in both the roots and the grains when compared to the white maize grains and roots possibly because the yellow maize types may have better root deposits and exudates (secondary metabolites), which may explain the high incidence of microbial community (Ching-Hong and David, 2000). Other factors that may account for the high differences observed include nutritional status of the varieties, soil structure, micro nutrient status of the soil, root morphology and physiology caused by diurnal

variations, root ageing and root emergence (Sullivan, 2004).

The presence of *Bacillus* sp. and *Pseudomonas* sp. in the samples taken were corroborated by the work of Figueiredo et al. (2009) and Rai et al. (2007) that isolated these bacteria species from stem and kernels of maize which is a normal microbial of maize plant. Franz et al. (2006) in an earlier study isolated a *Pediococcus* sp. from maize grain. *Azospirillum* sp. and *Cellulomonas* sp. observed in the planted cultivars and fresh maize seeds are known to be synergistic in nature. The association of *Azospirillum* sp. with other microbial entities were shown to bring about improved biomass production through increased availability of biologically fixed nitrogen (Baldani et al., 2002; Boddey, 1995). *Bacillus* sp., *Microbacterium* sp., *Micrococcus* sp., *Pseudomonas* sp. and *Staphylococcus* sp. observed in the roots and grains of maize have been reported to have beneficial roles in plant life as endophytes: bacteria and fungi that enter plant parts and establish lifelong relationship with the host without harm (Ajcann, 2007). While the endophytes are mutualistic to the maize host, further studies are needed to elucidate the mode of action of those organisms not yet classified. *F. verticillioides* is a common pathogen of maize, known to cause seedling blight, seed and stem rot and toxic metabolites such as fumonisin and moniliformin (Adejumo et al., 2007). *F. verticillioides* was observed to be common in all the tested samples, except variety DMR-LSR-Y. This confirmed the earlier report that the fungus was frequently encountered as an asymptomatic maize endophyte. This species is globally distributed with a wide host range, including sorghum, millet, and sugarcane. *F. verticillioides* was the most frequently isolated microbial of Nigerian maize (Adejumo et al., 2007).

The results also led to the inference that the microbial incidence of bacteria and fungi from maize grain must have been the residual microbial load after harvesting, while the large colony forming units observed for the roots must have passed through the soil solution. Results from this culture dependent and independent approach are complementary and could be a basis for future studies on ascertaining the associated bacteria and fungi of maize by metagenomic and functional metagenomic analyses (Hardoim et al., 2008; Pereira et al., 2011). The knowledge from this study will facilitate the search for bacteria and fungi, capable of exerting antagonism to pathogenic infections, or the detection of biological plant growth enhancers.

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