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Characterization of Arbuscular Mycorrhizal Spores Isolated from Southern Guinea Savanna of Nigeria

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Abstract

The hydrolytic activities (cellulases and pectinases) of some arbuscular mycorrhizal fungi (AMF) from four study sites; namely,- Malete, Bacita, Pategi and Ilorin; in southern Guinea savanna of Nigeria were studied. The DNA, RNA and protein contents of their spores were also quantified. Significant differences were observed in the hydrolytic activities of the AMF isolates. Pectinase activity of Malete AMF was F11, 22 =192.4; ($p < 0.05$); from Ilorin AMF was F13, 26 =1.38; from Bacita AMF was F8, 16 = 7.5; while from Pategi AMF was F10, 20 = 3.64. On the other hand, cellulase activity of Malete AMF was F11, 22 = 1.58; while in Ilorin, it was F 13 26 =2945.21; and in Bacita, it was F 8, 16 = 3849.34 AMF and in soil from Pategi, F 10, 20 = 5.681.23. Variations in DNA, RNA and protein contents of the 13 isolates were also observed. *Scutellospora reticulata* was predominant in all the soils; it had the highest hydrolytic activity and highest DNA, RNA and protein contents followed by *Glomus pansihalos.*

Keywords: arbuscular, mycorrhizal fungi, cellulases, pectinases, DNA, RNA, protein

1. Introduction

The Arbuscular Mycorrhizal association is one of the active and diverse soil biological communities that are highly essential for increasing the sustainability of agricultural systems. The diversity of AMF has significant ecological consequences because individual species or isolates vary in their potential to promote plant growth and adaptation to biotic and abiotic factors. Thus, the composition and dynamics of populations of AMF have a marked impact on the structure and diversity of the associated plant communities, both in natural and agricultural ecosystems (Gange et al., 1990). The correct identification of individual isolates is essential in the analysis of populations of AMF in ecological studies. Genetic, biochemical and functional analysis of selected isolates are required tools in their characterization. For better nutrient management in southern Guinea savanna of Nigeria, less emphasis on use of chemicals and an increase in the use of biological potential such as AMF is required. In our laboratory, AMF from selected soils of southern Guinea savanna of Nigeria were extracted and their morphological characterization was carried out. Thirteen species of AMF: *Scutellospora reticulata, Scutellospora calospora, Scutellospora pellucida, Acalospora laevis, Gigaspora decipien, Gigaspora margarita, Enthrophosphora infrequens, Glomus intraradices, Glomus pansihalos, Glomus tortuosum, Glomus manihotis, Paraglomus brasilianum and Paraglomus occultum* were identified in the soil samples (Olowonihi, 2011).

More information, particularly on the biochemical and molecular characteristics of AMF indigenous to soils of this ecological zone is however needed. This paper thus presents the hydrolytic activities as well as DNA, RNA and protein content of the spores of AMF extracted from soils of southern Guinea savannah of Nigeria. Such information would be useful in controlling the AMF symbiosis through agro biotechnology for a sustainable agricultural environment.

2. Materials and Methods

2.1 Collection of Soil Samples

Soil samples were randomly collected from four locations; Pategi and Bacita soils which are formed over sedimentary rock, Malete and Ilorin soils which are formed over basement complex in southern Guinea savanna of

Nigeria. Using the random sampling method, auger samples were collected from each of the sampling units at 0-15 cm. The soil samples collected from the four study sites were bulked and transported to the laboratory in well labeled polyethylene bags. The core samples passed through 2mm sieve in preparation for analysis haven been air dried for 3 days.

2.2 Extraction of Arbuscular Mycorrhizal Fungi (AMF) Spores

AM spores were extracted using the wet- sieving and decanting /density gradient centrifugation method of Brundrett et al. (1996)**.** Enumeration of spores was done under the dissecting microscope with magnification X40. The number of spores per gram of soil was calculated and recorded.

2.3 Biochemical Characterization of AMF Spores

Isolates were characterized based on the ability of the spores to produce hydrolytic enzymes – cellulases and pectinases in infected tomato plants.

2.3.1 Growth of Tomato Plants and Inoculation Procedures

Tomato (Lycopersicon esculentum) seeds were surface sterilized in NaCl03 for 15 minutes. The surface sterilized seeds were sown in moist filter paper in a petri dish. After 5 days, uniform sized seedlings were transplanted into 300ml capacity pots filled with grey loam soil. The soil (pH 5.6, 2.41% organic matter, mg kg_1 P NaHCO3 -extractable), was steam sterilized at 100^{o}C for 1hr on each day of 3 consecutive days. The spores of each of the 13 AMF isolated from the selected soils of Southern Guinea Savanna zone of Nigeria were used for the study. Tomato seedlings were inoculated with AMF spores immediately after transplanting them into pots. Inoculation was at the rate of 50 spores per seedling. Plants were kept in a controlled –climate glass house. The tomato Plants were harvested after 40days. The root system that has been washed and rinsed several times with sterile distilled water was used for determination of enzymatic activity.

2.3.2 Preparation of Extracts for Enzyme Assays

Roots (10g fresh weight) were pulverized in a mortar with liquid nitrogen and then homogenized in 30mls of 100mM tris-HCl buffer (pH7) plus 0. 02g polyvinyl- polypyriolidone (PVPP), 10mM MgCl2, 10mM NaHCO3, 10mM β- mercarptoethanol, 0. 15mM phenylmethyl sulphonyl fluoride (PMSF) and 0.3% X- 100Triton. Sodium azide 0.03% was added to the solution. The liquid was then filtered through several layers of cheesecloth and centrifuged at 20,000 *rpm* for 20min .The samples were frozen until they were to be used.

2.3.3 Enzyme Assays

Using the viscosity reduction method of Rejon- palomare *et al*. (1996), the extractants were assayed to determine the hydrolytic activities. Carboxymethylcellulose (CMC) and Citrus pectin were used as substrates for cellulases and pectinases respectively. The reduction in viscosity was determined at 0- 30mins intervals.

2.4 DNA, RNA and Protein Quantification

Estimation of DNA, RNA and Protein content of AMF spores were carried out at the molecular biology laboratory of the Department of Biochemistry and Microbiology, University of Fort Hare, Alice, South Africa.

2.4.1 Isolation of Nuclei and Active Chromatin

To isolate nuclei and active chromatin two hundred AMF spores pulverized in a mortar under liquid nitrogen were used. The suspension washed with buffer containing 10mM Tris HCl, pH 7.4, and 150mM NaCl was homogenized in a potter-Elvehjem homogenizer with 9 volume of buffer containing 12% (w/v) sucrose, 10 mM Tris HCl, pH 7.8, 2.5 mM EDTA and 1 mM PMSF. To chelate endogenous Ca2+ and Mg2+ EDTA was included in the buffer. The homogenate was filtered through two layers of cheese cloth and centrifuged at 12000g for 5 min over a sucrose cushion [15% (w/v) sucrose in buffer A (10 mM Tris / HCl, pH 7.8, 10m M NaCl, 1mM PMSF)].

Crude nuclear pellets washed with 12 % (w/v) sucrose in buffer A, and then twice with Triton X-100 (0.2%) in buffer A, followed by pelleting over 15% (w/v) in buffer A. Pellets were further washed with 12% (w/v) sucrose in buffer A to remove traces of Triton X-100. Nuclei were suspended in buffer A (pH 7.0). Nuclei suspended in buffer A (pH 7.0) were incubated at 37^{0} C for 5min. EDTA (2.5 mM final concentration) was added and the solution was placed in ice to stop the reaction. The suspension of nuclei was stirred gently for 15 min and then centrifuged at 12000g for 10 min. The supernatant thus obtained was the active chromatin.

Chromatin was precipitated with 5% trichloroacetic acid. The pellet obtained on centrifugation (12000g, 10min) was washed once with 10% trichloroacetic acid and then with ethanol. Subsequently the pellet was suspended in 5% trichloroacetic acid heated at 95^{0}C. Precipitated proteins were removed by centrifugation (12000g, 10min). DNA, RNA and Protein contents were estimated.

2.4.2 Estimation of DNA and RNA

The colorimetric reaction method of orcinol as described by Endo (1970) was used.

2.4.3 Protein Assay

Bradford (1976) protein assay method of was used. Dye Stock- Coomassie Blue G (100mg) was dissolved in 50ml of methanol. 100ml of 85% H3PO4 was added to the solution which was then diluted to 200ml with water. The final reagent concentrations were 0. 5 mg/ml Coomassie Blue G, 25% methanol, and 42.5 % H3PO4. The solution (dark red, pH 0.01) was stable in a dark bottle at 4^0C. One volume of the dye stock was diluted with 4 volumes of distilled water to prepare the assay reagent. A standard curve was made using bovine serum albumin (BSA) with concentrations 0, 10, 20, 30 40 and 50 µg/ml. Protein assay procedure- six standard solutions (1 ml each) containing 0, 10, 20, 30, 40 and 50 µg/ml was prepared. The spectrophotometer was set to collect the spectra at wave length of 575nm. A 4ml plastic cuvette filled with distilled water was used to blank the spectrophotometer. 0.8 ml of one of the protein standard was added to 0.2 ml dye stock. This was made up to 4 ml with distilled water and mixed gently. The absorbance spectrum at 575nm was recorded. The steps were repeated for each of the protein standard and samples to be assayed.

A graph of absorbance at 575nm versus protein for the protein standards was plotted. The protein concentration of samples was then determined from standard curve.

3. Results and Discussion

3.1 Hydrolytic Activities of Isolates

Some hydrolytic activities of AM fungi isolated from the different study sites are reported in Tables 1&2. Cellulolytic and pectolytic activities were noted to be significantly higher in AMF inoculated plants than the non inoculated control. Ruiz et al. (2011) also reported differential hydrolytic activities in roots of micropropagated *Agave tequilana* Weber var. Blue inoculated with *Glomus intraradices* in comparison with the un- inoculated control. Garmendia et al. (2006) reported hydrolytic activities in pepper roots. infected with *Glomus deserticola (Tr*appe, Bloss and Menge). Garcia- Garrido *et al*. (1992) also reported endoglucanase activities in extracts from spore and external mycelium of *G*. *mosseae* and associated increased hydrolytic activity of the root to AMF infection.

Cellulase, pectinase and xyloglucanase activities have been reported in colonized roots and in external mycelium of AM fungi (Rejon-Palomares et al., 1996). Adriano-Anaya (2006) also reported the the production of cellulases and pectinases by Glomus intraradices both in the root of maize and guinea corn.

In this study, we recorded significant differences in hydrolytic activities of plants infected with varying AMF isolates. This is in agreement with Garcia-Romera et al. (1991), who observed different cellulase and pectinase activities between some Glomus isotypes**.** The variations in the hydrolytic activities of isolates could be an indication of the AMF isolates belonging to different species and their having varying capacities to colonize host tissues. *Scutellospora reticulata* infected plants had the highest cellulolytic and pectolytic activities, followed by *Glomus pansihalos* from the four study sites. The higher hydrolytic enzyme activities of *S. reticulata* and *G. pansihalos* observed, may be a potential mechanism of adaptation of these fungi to colonize plant roots. Garcia-Garrido et al. (2000**)** reported that the cell-wall degrading enzymes, cellulases, hemicellulases and pectinases are implicated in the penetration of roots by beneficial plant microorganisms such as arbuscular mycorrhizal fungi.

3.2 DNA, RNA and Protein Content of Spores

The DNA, RNA and protein content of spores of each isolate differed one from another (Figures 1-3). Ranging from 0.1µg to 0.8µg (DNA), 0.2µg to 1.4µg (RNA) and 1.3µg to 3.5µg (protein content) per 200 spores. Pawlowska, (2005) documented genetic variation within individual and within spore, for ribosomal DNA and protein-coding genes in several species of AMF .According to Parfrey et al. (2008), DNA content varies within individuals throughout life cycles and among individuals within species. Nuclear DNA (nDNA) content determined for two AMF species was reported to be about 0.26 picogram (pg) for Glomus versiforme and 0. 75 picogram (pg) for *Gigaspora margarita* (Bianciotto and Bonfante, 1992). Stommel et al. (2001) reported a mean value of 2 µg/500 spores of Gigaspora rosea spores.

Scutellospora. *reticulata* and *Glomus pansihalos* had the highest quantities of DNA, RNA and protein in the soils studied as shown in Figures. 1-3. This probably explains the high percentage of their spores in the soil. High levels of DNA correlated with large vegetative cells (Kondorosi *et al*., 2000). The biochemical and spore based

characterization in this study has provided a baseline data on the properties of the indigenous AM flora of Southern Guinea savannah zone of Nigeria. This provides a platform for further research work to improve crop production using AM biotechnology.

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References

Adriano-Anaya, M. L., Solis-Dominguez, F., Gavito-Pardo, M. E., & Salvador-Figueroa, M. (2006). Agronomical and environmental factors influence root colonization, sporulation and diversity of arbuscular mycorrhizal fungi at a specific phenological stage of Banana trees. *J. Agron., 5*(1), 11-15.

Bianciotto, V., & Bonfante, P. (1992). Quantification of the nuclear DNA content of two arbuscular mycorrhizal fungi. *Mycol Res, 96*(12), 1071-1076. http://dx.doi.org/10.1016/S0953-7562 (09) 80118-4

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1-2), 248-254. http://dx.doi.org/10.1016/0003-2697(76)90527-3

Brundrett, M., Bougher, N., Dell, B., Grove, T., & Malajczuk, N. (1996). Working with mycorrhizas in forestry and agriculture. *Canberra: Australian Centre for international Agricultural Research Monograph*, *32,* 374.

Endo, Y. (1970). A simultaneous estimation method of DNA and RNA by the orcinol reaction and study on the reaction mechanism. *J. Biochem (Tokyo), 67*, 629-633.

Gange, A. C., Brown, V. K., & Farmer, L. M. (1990). A test of mycorrhizal benefit in an early successional plant community. *New Phytologist, 115*(1), 85- 91. http://dx.doi.org/10.1111/j.1469-8137.1990.tb00925.x

Garcia-Garrido, J. M., Garcia-Romera, I., & Ocampo, J. A. (1992). Cellulase production by vesicular arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol and Gerd) Gerd and Trappe. *New Phytologist, 121*(2), 221-226.

Garcia-Garrido, J. M., Tribak, M., Rejon-Palomare, A., Ocampo, J. A., & Garcia-Romera, I. (2000). Hydrolitic enzymes and ability of arbuscular mycorrhizal fungi to colonize roots. *Journal of experimental Botany, 51*(349), 1443-1448. http://dx.doi.org/10.1093/jexbot/51.349.1444

Garcia-Romera, I., Garcia-Garrido, J. M., & Ocampo, J. A. (1991). Pectinase activity in vesicular- arbuscular mycorrhiza during colonization of lettuce. *Symbiosis, 12,* 189-198**.**

Garmendia, I., Aguirreolea, J., & Goicoechea, N. (2006). Defence-related Enzymes in Pepper Roots During Interactions with Arbuscular Mycorrhizal Fungi and/or Verticillium dahlia. *Biocontrol, 55*(3), 293-310.

Kondorosi, E., Roudier, F., & Gendreau, E. (2000). Plant cell-size control: growing by ploi *Cuee opin. Plant Biol., 3*(6), 488-492. http://dx.doi.org/10.1016/S1369-5266(00)00118-7

Olowonihi, E. T. (2011). Studies on Arbuscular mycorrhizal fungi in selected soils of Southern Guinea Savanna of Nigeria. (Ph.D Thesis)*,* University of Ilorin. Nigeria.

Parfrey, l. W., Lahr, D. J. G., & Katz, L. A. (2008). The Dynamic nature of Eukaryotic genomes. *Mol. Biol. Evol., 25*(4), 787-794. http://dx.doi.org/10.1093/molbev/msn032

Pawlowska, T. E. (2005). Genetic processes inarbuscular mycorrhizal fungi. *FEMS Microbiol Lett., 125*, 185-192.

Rejon-Palomares, A., Garcia-Garrido, J. M., Ocampo, J. A., & Garcia-Romera, I. (1996). Presence of xyloglucan-hydrolyzing glucanases (xyloglucanases) in arbuscular mycorrhizal symbiosis. *Symbiosis, 21*, 249-261.

Ruiz, S., Adriano, L., Ovando, I., Navarro, C., & Salvador, M. (2011). Biofertilization of micropropagated *Agave tequilana*: Effect on plant growth and production of hydrolytic enzymes. *African Journal of Biotechnology, 10*(47), 9623-9630.

Stommel, M., Mann, P., & Franken P. (2001). Est-library construction using spore RNA of the arbuscular mycorrhizal fungus Gigaspora rosea. *Mycorrhiza, 10*(6), 281-285.

Table 1. Production of Cellulase enzyme by AM Fungi

	Enzyme activity (U/ml)			
AMF Species	**Ilorin**	**Malete**	**Pategi**	**Bacita**
P. brasilianum	6.67kl	27.24g	6.97j	-
G.intraradices	24.99e	-	-	28.17e
S. reticulata	59.63a	60.57a	62.42a	55.55a
S.calospora	6.89k	16.37j	-	-
Gi. margarita	3.54m	18.32i	23.12g	-
E.infrequens	20.66f	34.71f	30.18f	24.01f
P. occultum	17.57g	-	40.64c	31.03d
S.pellucida	37.0c	45.42c	43.04d	38.6c
A.laevis	29.42d	37.61e	38.68e	9.09h
G.pansihalos	46.98b	52.50b	54.58b	46.18b
G.tortuosum	12.52i	8.01k	16.44h	15.52g
G.manihotis	9.79j	42.57d	12.0li	-
Gi.decipien	13.98h	25.57h	-	-
No Inoculum	1.26n	1.25l	1.28k	1.27i
Sed	**0.4495**	**0.2026**	**0.3717**	**0.3993**
Lsd	**0.9239**	**0.4202**	**0.7753**	**0.8465**

Means followed by the same letter along the column are not significantly different at 5% level of probability using least significant difference (LSD) test.

Table 2. Production of Pectinase enzyme by AM Fungi

	Enzyme activity (U/ml)			
AMF Species	**Ilorin**	**Malete**	**Pategi**	**Bacita**
P. brasilianum	26.81l	37.51f	24.58g	-
G.intraradices	51.38e	-	-	40.3gh
S. reticulata	96.56a	94.22a	98.93a	95.20a
S.calospora	33.99k	3.74j	-	-
Gi. margarita	20.47m	3.69gh	36.55f	-
E.infrequens	46.52f	62.65c	50.91d	60.8d
P. occultum	44.66g	-	13.6h	70.3c
S.pellucida	70.67c	16.34h	62.47c	59.5e
A.laevis	55.47d	42.73e	44.28e	40.80g
G.pansihalos	80.00b	77.80b	76.62b	81.00b
G.tortuosum	42.74h	9.35i	-	42.4ef
G.manihotis	36.43j	51.42d	8.62j	-
Gi.decipien	42.04i	19.01g	-	-
No Inoculum	10.58n	1.49j	1.82k	3.40i
Sed	**0.2790**	**3.112**	**0.2303**	**13.93**
Lsd	**0.5736**	**6.454**	**0.4804**	**29.52**

Means followed by the same letter along the column are not significantly different at 5% level of probability using least significant difference (LSD) test.

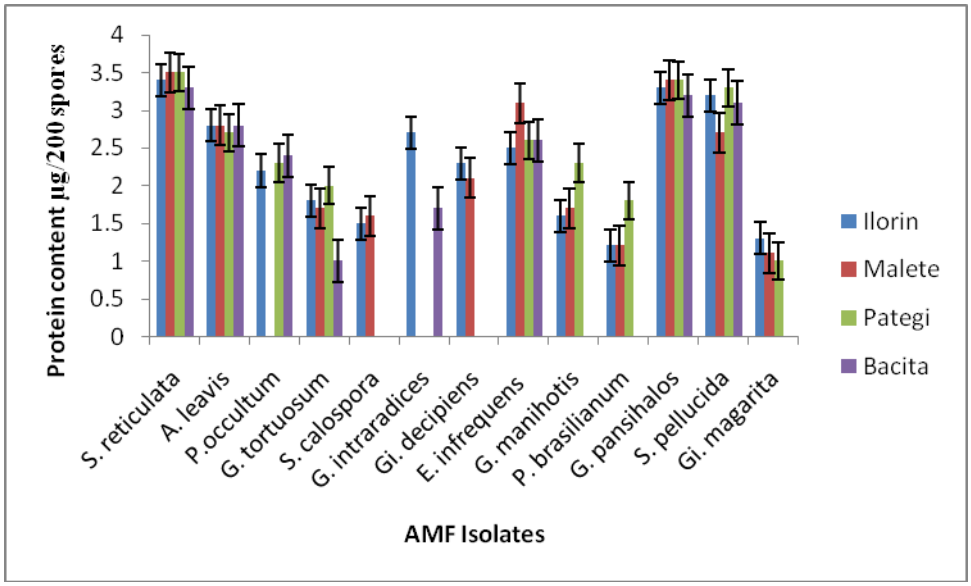

Figure 1. Protein contents of AMF isolates. Bars are standard error

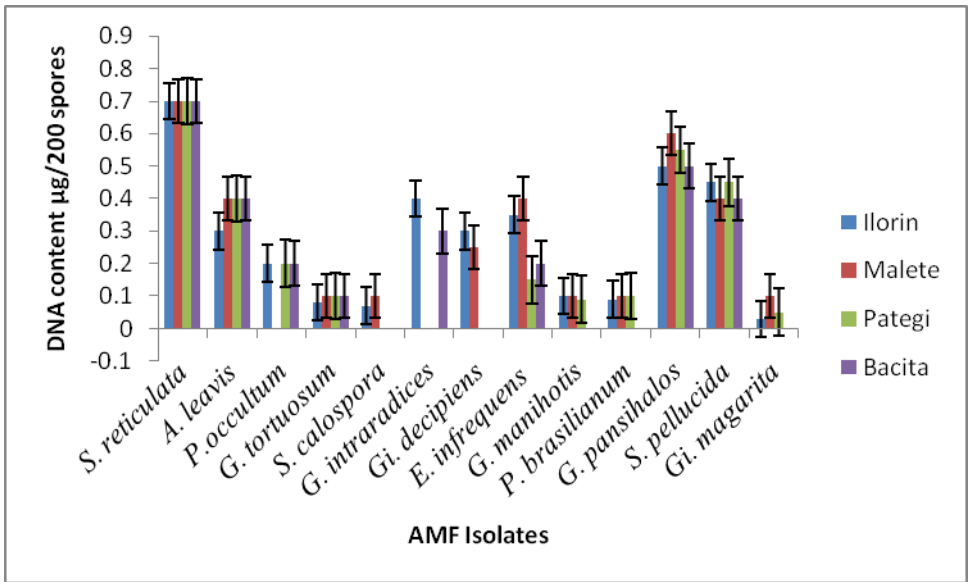

Figure 2. DNA Contents of AMF Isolates. Bars are standard error

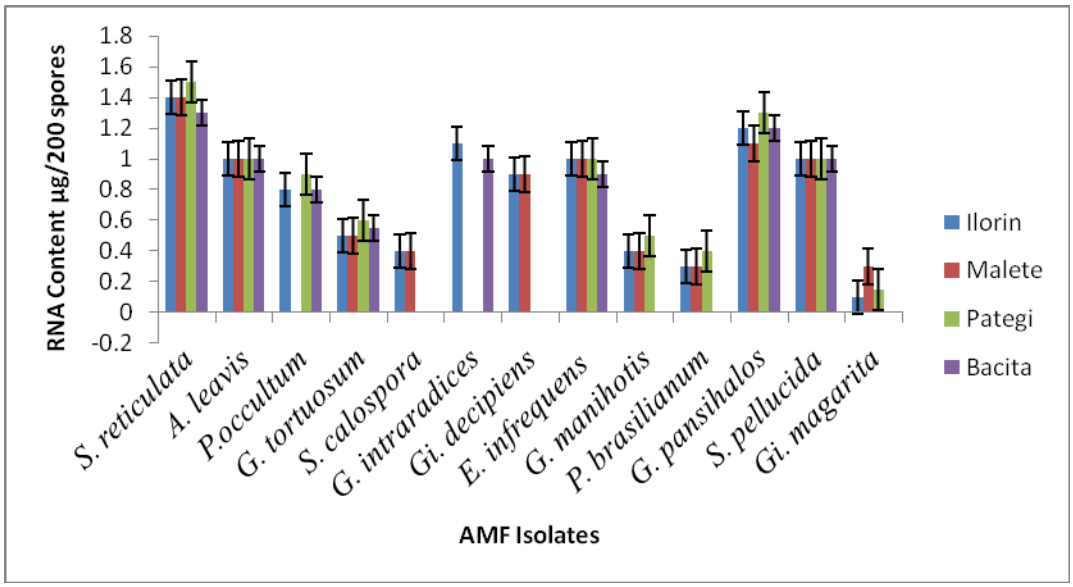

Figure 3. RNA contents of AMF isolates. Bars are standard error