Journal of Advances in Microbiology Research

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2020; 1(2): 72-74

www.microbiojournal.com
Received: 20-04-2020
Accepted: 22-06-2020

Ajayi OO
[1] Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, Nigeria
[2] Soil microbiology unit, International Institute of Tropical Agriculture, Ibadan, Nigeria

Nwodo DC
Department of Microbiology, Faculty of Science, Abia State University, Nigeria

Dianda M
[1] Soil microbiology unit, International Institute of Tropical Agriculture, Ibadan, Nigeria
[2] Laboratoire de microbiologie forestière (INERA/DEF) BP 7047 Ouagadougou 03, Burkina Faso, Niger

Fagade OE
Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, Nigeria

Correspondence
Ajayi OO
[1] Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, Nigeria
[2] Soil microbiology unit, International Institute of Tropical Agriculture, Ibadan, Nigeria

Determination of the number of Rhizobia cells in some soil samples collected in Nigeria and Ghana

Ajayi OO, Nwodo DC, Dianda M and Fagade OE

Abstract
Soil samples from different locations in Nigeria and Ghana were selected these soil samples were used to carry Most probable number test using six-fold serial dilution. Cowpea which allows more than one type of rhizobial to nodulate it was used as the trapping plant. Soils were found to contain 7.9×10^6 cells per gram of soil showing that although they can support the growth of leguminous plants the use of inoculant will enhance the growth and yield of the plants in these soils. It expected that groundnut, Bambara nut and soybeans will also be able to grow successfully on theses soils.

Keywords: Cowpea, rhizobial to nodulate, leguminous

Introduction
The MPN technique is commonly used for estimation of microbial population sizes. It is an old technique that has been used for evaluation of the amount of rhizobia found is soils, innoculants etc. A dilution series is prepared, after which known amounts of consecutively following dilutions (10^{-1}-10^{-6} or 10^{-10}) are applied using pipette into potted surface-sterilized seedlings of the host plant to be tested. The method depends on the pattern of positive (+ve) and negative (-ve) nodulation observed in experimental plants (Simon *et al*., 2014; Bala and Giller; 2006) [12, 3], this is based on the assumption that a single Rhizobium cell (is able or enough) at a given dilution will be infective enough to cause nodulation. Nodulation is the proof of infective Rhizobia; the validity of the test is demonstrated by the absence of nodules on un-inoculated plants; and absence of nodules on inoculated plants is proof of the absence of infective rhizobia (Prevost and Antoun; 2006, Anonymous, 2005) [11, 2]. Previous research has shown that the MPN of rhizobia found in dry regions, hot regions and regions where heavy rainfall is experienced has been shown to be low while in regions with moderate rainfall, the population recorded is high (Chatel and Parker; 1973; Hartel and Alexander; 1984) [4, 5]. In the enumeration for rhizobia using the MPN method, it is highly important to grow or pre-geminate healthy legume host plants that are kept free from external contamination and in controlled environments.
The determination of the quantity and the detection of rhizobia presence in the soil is important for determining the fate of rhizobia on seed seedlings and how long they can remain in the soil and in plant rhizosphere (Thompson and Vincent). Inoculants to be used for MPN tests are expected to contain about 1 x 10^9 cells ml^{-1} (Woomer *et al*., 2011) [15]. Studies which have been carried out in India for legumes under field trials for a period of 15 years show no yield responses to inoculation as a result of the presence of high populations of native nitrogen fixing rhizobia for nodulating cowpeas and common beans in the soil. Various populations for rhizobia determined using MPN include 6×10 0 to1×104 per gram of soil in India (Zahran, 1999) [16], 3.6±2.4 - 4×10^3 cells per gram of soil in Tunisia (Mnasri *et al*., 2001) [8], 1.57×10^3 - 7.6×10^4 cells per gram of soil in Brazil (Andrade *et al*., 2002), 1.16×10^4 – 1.16×10^5 cells per gram of soil in japan (Soe *et al*., 2013; Nutman and Hearne, 1980) [13, 10], log 2.23-5.34 in 21/32 soils in Poland (Martyniuk and Jadwiga, 2008) [6], 3.6±42.4 cells per gram of soil in uncropped soil and 4×10^3 cells per gram of soil in cropped soils in Spain (Simon *et al*., 2014) [12] and 1.1-2.3 × 10^2 in soil in which non legumes were planted (Mwendaa *et al*., 2011) [9] and 0 – 6.1 × 10 in soil collected from areas where trees were planted (Mathus *et al*., 2012) [7] in Kenya.

Materials and methods
Soil sample were collected from three different states in Nigeria which include Zaria in

Kaduna state (Zar), Kano in Kano state (K), Ohaukwu in Ebonyi state (E) and two locations in Ghana which include Zaba zugu (Z) and Nanumba (N) and stored in in a cold room to prevent the soil from drying up and thus ensuring that the microorganisms are kept viable for a long time. 100g of each soil was weighed into 900ml of sterile distilled water mixed thoroughly and used to prepare six dilution series for each soil sample i.e. 10^{-1} to 10^{-6}.

Peat was mixed with sea sand, crushed gravel using the ratio 6:6:1, they were mixed properly until evenly distribution was achieved. The mixture was packed in autoclavable bags and sterilized at 121 $^{\circ}$C and 1.05 kg cm^{-2} for 15 mins. The sterilized mixture was allowed to cool and filled into 250 ml pots (pot sterilization was done using 5% of JIK (or 3.5% w/v sodium hypochlorite) and rinsed thoroughly with sterilized water (twice) allowing for complete removal of the JIK. Sterile seeds (cowpea Tvx 3236) were planted (4 seeds in a pot) in each pot (4 replicates) and allowed to pre-germinate in a screen house.

Table 1: Properties of soil/peat composition for MPN experiment

Properties of peat	Units	Values
Water holding capacity		33.38
Moisture content	%	4.3
pH	-	6.14
Organic Carbon	%	88.8

One week after germination the plants were thinned out to one plant per pot after which they were labelled and inoculated with the different soil sample using four replicates for each sample dilution. They were kept for eight weeks during which the plants were supplied with nutrients weekly which contained sterile water ensure that they were kept moist constantly. At the eighth week they were harvested and presence of nodules were observed for in the plants.

Results

Table 2: Occurrence of nodulation in plant inoculated with soil samples in pot experiment.

Soil source/Dilution	Rep1	Rep2	Rep3	Rep4	No of undulating units
$Z10^{-1}$	+	+	+	+	4
$Z10^{-2}$	+	+	+	+	4
$Z10^{-3}$	+	+	+	+	4
$Z10^{-4}$	+	+	+	+	4
$Z10^{-5}$	+	+	+	+	4
$Z10^{-6}$	+	+	+	+	4
					24
$N10^{-1}$	+	+	+	+	4
$N10^{-2}$	+	+	+	+	4
$N10^{-3}$	+	+	+	+	4
$N10^{-4}$	+	+	+	+	4
$N10^{-5}$	+	+	+	+	4
$N10^{-6}$	+	+	+	+	4
					24
$Zar10^{-1}$	+	+	+	+	4
$Zar10^{-2}$	+	+	+	+	4
$Zar10^{-3}$	+	+	+	+	4
$Zar10^{-4}$	+	+	+	+	4
$Zar10^{-5}$	+	+	+	+	4
$Zar10^{-6}$	+	+	+	+	4
					24
$K10^{-1}$	+	+	+	+	4
$K10^{-2}$	+	+	+	+	4
$K10^{-3}$	+	+	+	+	4
$K10^{-4}$	+	+	+	+	4
$K10^{-5}$	+	+	+	+	4
$K10^{-6}$	+	+	+	+	4
					24
$E10^{-1}$	+	+	+	+	4
$E10^{-2}$	+	+	+	+	4
$E10^{-3}$	+	+	+	+	4
$E10^{-4}$	+	+	+	+	4
$E10^{-5}$	+	+	+	+	4
$E10^{-6}$	+	+	+	+	4
					24

Each soil sample was calculated to contain about approximately 7.9×10^{6} cells per gram of soil.

Discussion

Rhizobia population in these soils were calculated to be 7.9 × 10^{6} using MPN tables and were found to be higher compared to that obtained in Kenya (Mwendaa *et al*., 2011; Mathus *et al*., 2012) [9, 7], Spain (Simon *et al*., 2014) [12] and Tunisia while they were similar to those reported in India (Zahran, 1999) [16] and Brazil (Andrade *et al*., 2002) [1] and lower when compared to the rhizobia population in Japan (Soe *et al*., 2013; Nutman and Hearne, 1980) [13, 10]. These soils showed that they contain enough rhizobia population to support the growth of legumes although we cannot be specific of the type of rhizobia in the soil since cowpea which is promiscuous in nodulation by rhizobia was used in this experiment.

It is expected that any of groundnut, Bambara nut and soybean will likely grow on these soils since cowpea has been reported to allow nodulation with rhizobia obtained from these legumes (Simon *et al*., 2014) [12]. If the soil is supported by the use of inoculant it will enhance the growth of legumes immensely.

Conclusion
From the experiments, all the soils have shown that they contain substantial amount of rhizobia to enable them support the growth of legumes but if supported by the use of inoculants better yields of crop will be obtained.

Conflict of Interest
Not available

Financial Support
Not available

Reference
1. Andrade D, Murphy PJ, Giller KE. The diversity of phaseolus-nodulating Rhizobial populations is altered by liming of acid soils planted with *Phaseolus vulgaris* L in Brazil. Applied and environmental microbiology. 2002;68:4025-4034.
2. Anonymous. Methods for testing legume in inoculant and pre-inoculated seed products, fertilizer Acts section 23, Regulations Canadian food Inspection Agency Canada; c2005.
3. Bala A, Giller KE. Relationship between Rhizobial Diversity and Host legumes Nodulation and Nitrogen Fization in tropical ecosystem. Nutrient cycling in Aroecosystems. 2006;76:319-330.
4. Chatel D, Parker C. Survival of field-grown Rhizobia over the dry summer period in western Australia. Soil biology and Biochemistry. 1973;5:415-423.
5. Hartel PG, Alexander M. Temperature and Desiccation tolerance of cowpea Rhizobia Canadian Journal of microbiology. 1984;30:820-823.
6. Martyniuk S, Jadwiga O. Population of Rhizobia in some polish soils not planted with legumes. Ekologija. 2008;54:165-168.
7. Mathu S, Herrmann L, Pyers P, Matiru V, Mwirichia R, Lesueur D. Potential of indigenous Bradhyrhizobia versus commercial inoculants to improve cowpea (*Vigna unguiculata* L walp) and Green grain (*Vigna radiate* L. wilczek) yield in Kenya. Soil science and plant nutrition. 2012;58:750-763.
8. Mnasri B, Tajini F, Trabelsi M, Aouani ME, Mhamdi R. *Rhizobium gallicum* as an efficient symbiont for beans cultivation. Agronomy for sustainable development. 2001;27;331-336.
9. Mwendaa G, Karanjac N, Bogaa H, Kahindib J, Muigaia A, Odeed D. Abundance and Diversity of Legume Nodulating Rhizobia in Soils of Embu District, Kenya. Tropical and Subtropical Agroecosystems. 2011;13:1-10.
10. Nutman P, Hearne R. Persistence of nodule bacteria in soil under long term cereal cultivation Rothamsted experimental station report for 1979. 1980;2:77-90.
11. Prevost D, Antoun H. Root nodule bacteria and symbiotic Nitrogen fixation (chapter 31). Taylor & Francis Group, LLC; c2006.
12. Simon Z, Mtei K, Gessesse A, Nkakidemi PA. Isolation and characterization of Nitrogen fixing Rhizobia from cultivated and uncultivated soils of Northern Tanzania. American Journal of plant science. 2014;5:4050-4067.
13. Soe KM, Yamakawa T. Low density co-inoculation of Myanmar *Bradyrhizobium* yuanmingense MAS34 and *Streptomyces griseoflavus* P4 to enhance symbiosis and seed yield in soybean varieties. American Journal of plant science. 2013;4:1879-1892.
14. Vincent JM, Thompson JA. Methods of detection and estimation of Rhizobia in soil. Plant and soil. 1970;1:72-77.
15. Woomer PL, Karanja N, Stanley M. A revised manual for rhizobium methods and standard protocols; c2011. (www.n2africa.org).
16. Zahran HH. Rhizobium legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and molecular biology reviews. 1999;63:968-989.

How to Cite This Article
Ajayi OO, Nwodo DC, Dianda M, Fagade OE. Determination of the number of Rhizobia cells in some soil samples collected in Nigeria and Ghana. Journal of Advances in Microbiology Research. 2020; 1(2):72-74.