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Isolation and Mass Multiplication of *Rhizobium* sp. for Nitrogen Enrichment of Vermicompost

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Gram-positive rod shaped aerobic bacteria related to the genus *Rhizobium sp* was isolated from the root nodules of cowpea plant and mass multiplied. The cell count was characterized periodically and the cell suspension was mixed with vermicompost at 1x10^8 cells per ml for 1 gram of vermicompost and the mixture was tested in pot culture studies. Higher productivity of cowpea (*Vigna unguiculata)* was observed.

Keywords: *Rhizobium sp, Eudrilus eugeniae,* Vermicompost, Vigna *unguiculata*.

The role of earthworms in organic matter decomposition, nutrient cycling, and soil structure and plant productivity has been studied by several authors (Lavelle 1988; Scheu and Wolters 1991; Zhang and Schrader 1993). The earthworms assimilate organic waste and discharge beneficial products known as vermicast and vermicompost. The vermicompost is rich in plant nutrients (Tomati *et al.,* 1998; Edwards and Burrows 1988; Bhawalkar 1991). The microbe-plant interaction in the rhizosphere can be beneficial, neutral, variable for plant growth. Rhizobacteria that exert beneficial effects on plant development are termed as plant growth promoting rhizobacteria (Kloepper and Schroth 1978). The term rhizobacteria is used for bacteria that aggressively colonize the rhizosphere (Subba Rao 1999). The present study reveals that the *Rhizobium sp* (Nitrogen fixing bacteria) was isolated from the root nodules of *V. unguiculata*, and that has been mass multiplied and mixed with casts of the earthworm *Eudrilus eugeniae* and that has to be used for the plant growth.

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MATERIAL AND METHODS

Isolation of *Rhizobium sp* from root nodules of cow pea

The root nodules were collected from *V. unguiculata* (L.). *Rhizobium sp*. was isolated from root nodules from following the technique of Vincent (1970). The root nodules were washed in running water to remove gross surface contaminants, surface sterilized in 0.1 percent $HgCl_2$ for 5 to 7 minutes and washed thoroughly with sterile distilled water, using a sterile glass rod the nodules were gently crushed and the exudates was streak plated onto a sterile yeast extract mannitol agar (YEMA) containing 0.025 percent Congo red (Hahn, 1966) and incubated at 28 ± 2°C. The white, translucent, glistering and

elevated colonies were transferred to YEMA slants.

Identification

The organisms were isolated from Congo red agar plates on the basis of colony morphology. Then the above isolates were identified on the basis of staining, motility, Bio-chemical test (Table 1) Identification based on the Bergey's manual of Determinative Bacteriology and molecular sequencing (Smoker and Barnum, 1988) (Weisburg *et al.,* 1991) (Fig. 1).

Table 1. *Rhizobium* identification based on the Bergey's manual of Determinative Bacteriology

S.No.	Characteristics	Results
1	Gram's Reaction	G - ve
2	Cell shape	Rods
3	Cell diameter	0.5-0.9 um
4	Motility in liquid medium	+
5	Grow that 60° C (or) higher	-
6	Acid from glucose	+
7	Denitrification (N_2)	-
8	Grow with 20% (or) Nacl	-
9	Growth in glucose peptone broth	-

Mass multiplication

The selected Rhizobial colony was transferred to conical flask containing sterile liquid medium and kept it for 2 days. This was called as the "Starter Culture." Later starter culture was transferred to mass culture medium flask and kept it for 3-5 days.Cell count was done by normal Haemocytometer (Vincent) 1970 technique.

Pot culture studies

After the count, the spore suspension was mixed with vermicompost at the rate of 1 x 10^8 spores per ml for one gram of vermicompost and the mixture was mixed with soil for pot culture.

RESULT S AND DISCUSSION

The predominant colonies of watery translucent white opaque appearance without absorbing the Congo red were identified as Rhizobium *sp*, using staining, motility, Bio-chemical test and molecular sequencing. *Rhizobium sp* was mass multiplied through shaker flask technique orbitory shaker) and their cell count and viability were observed and recorded. The maximum spore count and viability were observed in 96 hrs. (Table 2).

Rhizobium sp. GGNM 66 Bacteria; Proteobacteria; Alphaproteobacteria;
Rhizobiales; Rhizobiaceae; Rhizobium /Agro bacterium group; *Rhizobium*.
/isolation source="cowpea root nodules"
/note="capable of fixing atmospheric nitrogen"
rRNA <1...>702
/product="16S ribosomal RNA"

ORIGIN

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   1 ccccntggac aaagactgac gntcaggtgn gaaagcgtgg ggagcaaaca ggattagata
  61 ccctggtagt ncacnccgta ancgatgtng acttggaggt tgtgcccnng anncgtggct
 121 tccggannta acgcgttaan ncgaccgcct ggggagtacg nccgcaaggt taaaactcaa
 181 atgaattgac gggggcccgc acaagcggtg gagcatgtgg tntaattnga tgcaacgcga
 241 aganccttac ctactcttga catccagaga actttccaga gatggatngg nnccttcggg
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 361 tcccgcaacg agcgcaaccc ttatccttng tngccagcgg ttaggccggg aactcaaagg
 421 agactgccag tgataaactg gaggaaggtg gggatgacgt caagtcatca tggcccttac
 481 gagtagggct acacacgtgc tacaatggcg catacaaaga gaagcgacct cgcgagagca
 541 agcggacctc ataaagtgcg tcgtagtccg gattggagtc tgcaactnga ctccangaag
 601 tcggaatcgc tagtaatcgt agatcagaat gntacggtga atacgttccc gggccttgta
 661 cacaccgccc gtcacaccat gggagtgggt ngcaaaagaa gt
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Fig 1. Molecular sequencing

Table 2. Optimization of cell count and viability of *Rhizobium sp*

S. No.	Incubation period(Hrs)	Cell count 1×10^8/ml	Viability %
1.	72	2.71	80
2.	96	4.08	More than 90
3.	120	4.92	Less than 90

The cell count and viability of *Rhizobium sp* were optimized. Thus the *Rhizobium sp* incubated for 96 hrs shows more viability than others.

The cell count and viability of *Rhizobium sp* were optimized. Thus the *Rhizobium sp* incubated for 96 hrs shows the more viability than others. There is clear cut difference in shoot length, shoot weight, pod length and number of pods between the control, vermicompost and vermicompost along with *Rhizobium* sp.

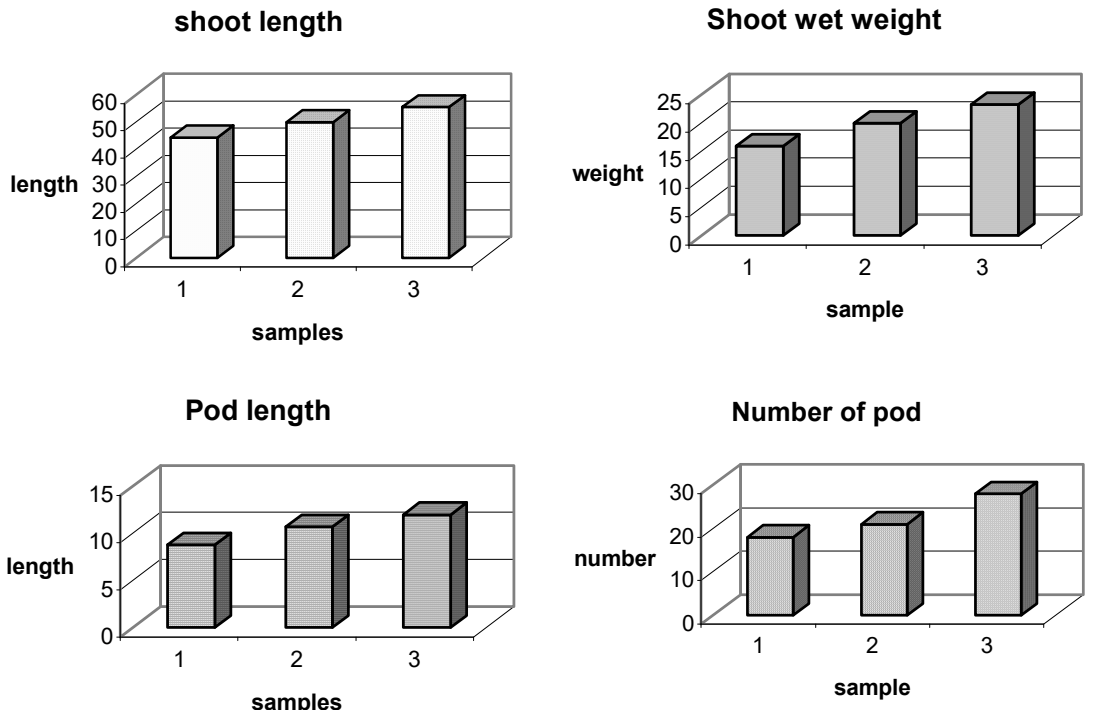

Fig 2. Growth performance and yield in Cow-pea (*Vigna unguiculta*) in different organic formulations (1. control, 2. vermicompost and 3. vermicompost with *Rhizobium .sp*)

Earthworms are responsible for translocation of the accumulated organic debris from the soil surface to the subsurface layers and during this process much of organic materials were indigested, macerated and excreted due to the presence of microbes. Macronutrients such as nitrogen, phosphorous and potassium content also increased significantly in the vermicompost having *Rhizobium sp.* Graff (1971) reported that the excreta had considerably more nitrogen in casts than the surrounding soil. Hence the increase of nitrogen and other nutrients in the present study may be due to nitrogen fixing bacteria (*Rhizobium sp)* and also by the addition of mucoprotiens secreted from the body wall of the earthworms. Rhizobial activities result in release of mineral

nitrogen in ammonium form during composting and in nitrate form that is readily available for plant growth. *Rhizobia* are symbiotic bacterial partners forming nitrogen-fixing nodules on legumes. These bacteria share characteristics with plant growth promoting rhizobacteria (PGPR). Nodule inducing bacteria, like other PGPR, are capable of colonizing the roots of non-legumes and produce phytohormones, siderophores and HCN. They also exhibit antagonistic effects towards many plant pathogenic fungi. From the above results, it is evident that maximum growth performance and the yield of a cowpea were observed in the *Rhizobium sp* enriched vermicompost than in the control.

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