Isolation and Evaluation of Nitrogen Fixing Bacteria in Tea Soils for the Production of Biofertilizer

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Introduction

The field practices are very important and soil is very significant factor that consider in the agricultural crop production and management. Though each and every field practices are important, the soil fertility management is most significant one. Synthetic and organic fertilizers generally used to provide nutrient elements. Nitrogen is an essential nutrient component that has several beneficial effects on the crop growth. And also, nitrogen is a limiting nutrient which can be identify in the soil in cultivable lands for growth and yield of crops. Several kinds of microorganisms engage with Biological Nitrogen Fixation, *Azospirillum* bacteria is an associative micro aerophilic nitrogen fixer commonly found in association with the rhizosphere and roots of a variety of plants including cereals and grasses. They have the ability to produce usable form of nitrogen for the plants when they functioning in the soil biosphere. The present study was aimed to isolate Azospirillum spp.from tea soils, root parts of rehabilitation grasses in tea plantations and maize and study their nitrogen fixing abilities aiming at applying the Azospirillum as Biofertilizer in Tea lands.

Methodology

This research was carried out at the Microbiology laboratory of Uva Wellassa University, Soil samples and parts of root samples of Mana (*Cymbopogan confertiflorus*), Guatamala (*Tripsacum laxum*), Guinea (*Panicum maximum*) grasses and Maize (*Zea mays*) were collected from the university premises, farmer fields and tea estates closer to Uva Wellassa University, Badulla. Sample collection was done from well grown, healthy plants. All samples were collected in polythene bags to prevent the contamination and water evaporation. Collected soil samples were sieved. 10g of even sized soil samples were mixed with 100ml of distilled water and kept on an orbital shaker for 30 minutes. 1ml of aliquot was taken from the above sample and ten-fold soil dilution series was followed up to10^{-8}.The 0.1ml of aliquot taken from the 10^{-8} soil dilute was inoculated into semi solid medium and also washed solution of roots, leaf parts and distilled wash root parts were inoculated in to test tubes containing Nitrogen free Bromothymole blue (NfB) semisolid media. All the tubes were inoculated at ambient temperature 30 ℃ to 32 $C^0$for 48 hrs and the growth was observed by formation of subsurface white pellicles. The pellicles of each sample were streaked on NfB solid media and incubated at ambient temperature (28 ℃). Purified *Azospirillum* isolates were named as 100 series and stock cultures were prepared for further studies.

Isolated *Azospirillum spp* were examined for identification of their Gram reaction, shape and motility. 0.2 ml of isolated bacterial broth were inoculated into 20 ml of NfB semisolid medium and allowed to incubate at 32 ℃ for 10 days. The efficiency of Nitrogen fixation was determined after10 days using micro kjeldahl analysis.

Results and Discussion

The growth of *Azospirillum* bacteria were identified after 24 hr incubation by the subsurface white pellicle in semisolid N-free medium. Pellicle formation was about 1 to 2 mm below the surface of semisolid medium and light green color medium changed the color into Blue. Pellicles were transferred into NfB solid plates. After colony formation, 10 morphologically distinct *Azospirillum* colonies were purified from Mana, Guatemala, Guinea and Maize plants. Forage grasses and cereals always had a large bacterial population. *Azospirillum* was very

common in soil and in the roots of plants grown in tropical and temperate regions (Baldhani et al., 2005). *Azospirillum* bacteria was successfully isolated from the soil suspension, washed solution of roots and distilled wash roots though washed solution of leaf parts was not successful during this experiment. Isolation of endophytic bacteria was not provided positive results.

Characteristically all the 10 isolates of *Azospirillum* were gram negative, rod shaped and exhibited spiral (Cork Screw) movement when observed using microscope. At the physiological study of metabolism of glucose all the isolates were showed a fermentative result as indicated by acid production and changes the color of pH indicator from green to yellow both in the open (aerobic) and oil covered (anaerobic) tubes.

Nitrogen fixing ability of the Isolated ten *Azospirillum* strains were measured by micro kjeldhal method after 10 days of the incubation period. Mean value of fixed nitrogen content of the three replicates were used to evaluate the nitrogen fixing abilities. Mean value of three replicates are presented in figure 1.

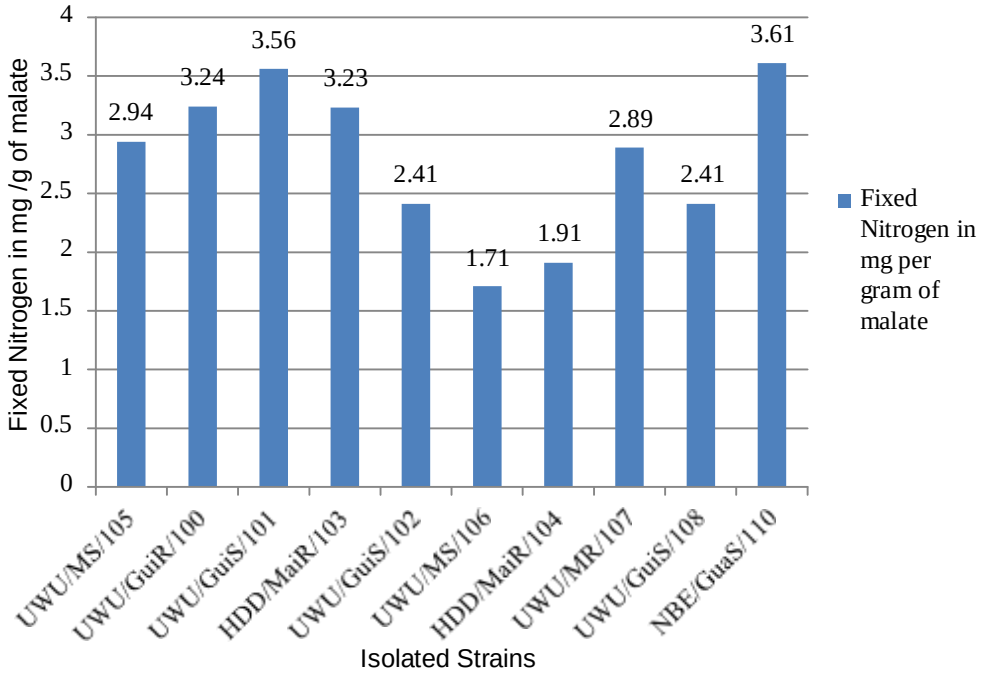

Figure 1. In vitro Nitrogen fixation by *Azospirillum* isolates.

The Nitrogen fixing ability was expressed as mg of nitrogen fixed per gram of carbon source utilized, in here 0.05% malate use as the carbon source. Ten different isolates were undertaken for the in vitro nitrogen fixation. The range of nitrogen fixing ability was from 1.71 to 3.61 mg 'N'/g. Among them, the maximum nitrogen fixing ability (3.61mg'N'/g) was recorded by tea estate, isolated from the rhizosphere of Guatemala grass soil (NBE/ Gua S/110) and minimum (1.71 mg 'N'/g) was recorded in Mana grass soil (UWU/MS/106). Among the 10 isolates, 6 isolates were fixed the highest quantity of nitrogen such as 3.61 mg 'N'/g (NBE/ Gua S/110), 3.56 mg 'N'/g (UWU/GuiS/101),3.24 mg 'N'/g (UWU/Gui R/100), 3.23 mg ' N'/g (HDD/Mai R/103), 2.94 mg 'N'/g (UWU/MS/105) and 2.89 mg 'N'/g (UWU/MR/107).

In the present exploration the total nitrogen fixation ranged from 1 to 4 mg of N per gram of malate. , the greatest nitrogen fixing ability (3.61mg'N'/g) was recorded from Guatemala grass soil (NBE/GuaS/110).These results are nearly similar with the investigation of Sangeeth et al.

(2008) the isolates of *Azospirillum* showed wide variation in their nitrogen fixing abilities ranging from 3.20 to 8 mg N gram of mallic acid. Kanimozhi and Panneerselvam (2010) has found nitrogen fixing ability of 30 isolates from soil samples using micro kjedhal method. They have found that the extent of nitrogen fixation from 3.3 to 15.6 mg of N per gram. The highest nitrogen fixing amount of present investigation, 3.61mg N/g is in the range of other research studies.

Conclusions

Azospirillum bacteria were highly found to be associated with rhizospher of Maize (*Zea mays*) Mana (*Cymbopogan confertiflorus*), Guatamala (*Tripsacum laxum*), and Guinea (*Panicum maximum*) However the isolation from washes solutions of leaves parts was not successful during this experiments. Nitrogen fixing abilities of 10 isolates are in the range of 1.71 mg N/g to 3.61 mg N/g of malate. Highest Nitrogen fixation is recorded by the Guatemala rhizosphere with roots (3.61 mg of N per g of malate).

References

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