# Evaluation of Nitrogen Fixation Ability of *Azospirillum spp.* associated with Sugarcane, Maize and Guinea Grass

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## Introduction

Biological nitrogen fixation is gaining importance in current agriculture because of people's concern on the environmental and soil heath that are caused by the continuous use of nitrogenous fertilizers and need for improved sustainable productivity. From several earlier studies, it has been shown that,Azospirillum is an important free-living, N fixing bacteria which make a symbiotic association on roots of different crops. Nitrogen fixation by Azospirillum spp. in association with grasses and other plants has been examined when it is observed in preparation of a biofertilizer.

Biofertilizers are defined as preparations containing living or latent cells of efficient strains of microorganisms that help crop plants to uptake of nutrients by their interactions in the rhizosphere, when applied through seeds or to soil treatments. The present study was conducted to isolate the Azospirillum spp., to evaluate the nitrogen fixation abilities of isolates and to assess the performance of inoculated plants.

## Methodology

The study was carried out at the microbiology laboratory of Uva WellassaUniversity. Samples of parts of roots and leaves of sugarcane, maize and guinea grass were collected from the farmer's fields. Sample collection was done from well grown, healthy plants. From the collected samples, washed solutions of the roots and leaves and surface sterilized 1 cm long root parts were inoculated in test tube containing Nitrogen free Dobereiner's (NfD) (Biofertilizer Manual, 2006) semi-solid media. All the tubes were incubated at ambient temperature (28 oC) for 48 h and observed the growth by formation of pellicles. The pellicles were by streaking on NfD solid media and incubated at ambient temperature (28 oC) and stock cultures of purified Azospirillumspp. were prepared and isolates were named UWU K 300 series.

Isolated Azospirillum strains were further examined for their Gram-reaction, shape and motility. The efficiency of nitrogen fixation of Azospirillum isolates were made in semisolid NfDmedium. The isolates were inoculated in 100 ml of semisolid NfD medium and incubated at 32oC. After 10 days of incubation, the total amount of nitrogen fixation was determined by kjeldahl analysis.

## Results and Discussion

In the present study, for the isolation of Azospirillum spp., NfD semi-solid medium was used. After 24 h of incubation, the NfD semi-solid medium showed white colored pellicle. Appearance of pellicle formation on NfD semi-solid medium indicated successful growth of Azospirillum.

Although the sterilized root bits and washed solutions of roots and leaves were used for the isolation in the semi-solid N-free medium, there is no growth of Azospirillum spp. from washed solution of leaves parts. This indicated that, the Azospirillum population of leaves

of sugarcane, maize and guinea grass is lower when compare with the roots of the same plant.

To date, diverse nitrogen- fixing bacteria, including Azospirillum, have been isolated from the roots of numerous wild and cultivated grasses grown in tropical, subtropical and temperate regions all over the world. Those isolates formed a fine sub-surface white pellicle in nitrogen free malate medium within 24h (Jolly et al., 2010).

Isolated Azospirillum strains were further examined for their Gram-reaction, shape and motility. Characteristically all the isolates were Gram negative, rod shaped and exhibited spiral (CorkScrew) movement when observed using the hanging drop technique. All the isolates were showed a fermentative result as indicated by acid production in both the open (aerobic) and oil covered (anaerobic) tubes. Azospirillum spp. are normally Gram negative but Gram variability has been observed in A. brasilense (Tarrandet al., 1978).

The total nitrogen fixed by the different isolates was measured by kjeldahl method and the mean values of three replications are presented in Figure 1.

The total N fixed was expressed as mg of nitrogen fixed per gram of carbon source utilized. The total N fixation (in vitro) by 10 isolates (UWU K 300, UWU K 301, UWU K 302, UWU K 303, UWU K 304, UWU K 305, UWU K 306, UWU K 307, UWU K 308 and UWU K 309) werebelow 2 mg of N per gram of malate. The nitrogen fixed by all the isolates ranged between 1 to 2 mg per gram of malate. The isolate UWU K 300 that is isolated from the surface sterilized root bits of sugarcane, exhibited the highest nitrogen fixing ability (1.35 mg of N per gram of malate) among the native isolates.

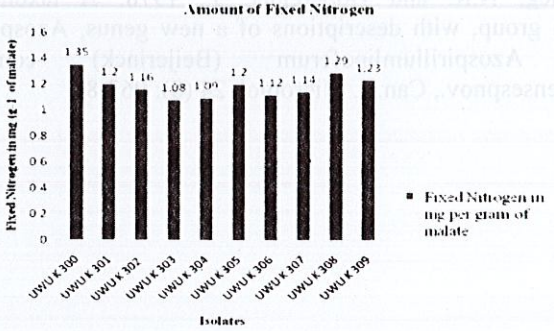


Figure 1: In vitro nitrogen fixation by Azospirillum isolates.

Though the reports of nitrogen fixing efficiency of Azospirillum strains isolated from plants ranged from as low as 2.00 mg of N g-1 of substrate (Jolly et al., 2010) to as high as 25 mg of N g-1 of substrate (Purushothaman and Govindarajan, 1986), during this investigation it was lower than 2.00 mg of N g-1 of substrate.

## Conclusion

Surface sterilized root parts and washed solutions of roots of sugarcane, maize and guinea grass can be considered as a good source for isolation of Azospirillum, than the washed solutions of leaves of the same plants.

The ability of these isolates to fix atmospheric nitrogen ranged from 1.08 to 1.35 mg of N per g of malate.

Highest nitrogen fixation was recorded from surface sterilized sugarcane root parts (1.35 mg of N per g of malate).

Further research studies and field trials are needed for the recommendation of Azospirillum inoculants as a biofertilizer.

## References

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