

AZOSPIRILLUM MEDIUM (W/0.17% AGAR) (DOUBLE PACK)
TM 638

For cultivation of *Azospirillum* species

Composition

Ingredients	Gms/Ltr.
Part I	
Malic acid	5.00
Agar	1.75
Dipotassium phosphate	0.50
Ferrous sulphate, heptahydrate	0.50
Magnesium sulphate	0.20
Sodium chloride	0.10
Calcium chloride	0.02
Manganese sulphate	0.01
Bromo thymol blue	0.002
Sodium molybdate	0.002
Part II	
Potassium hydroxide	4.00

* Dehydrated powder, hygroscopic in nature, store in a dry place in tightly- sealed containers 25°C and protected from direct Sunlight.

Instructions for Use

Dissolve 8.10gms of Part I in 1000ml of purified water or distilled water. Gently heat to boiling with gentle swirling and dissolve the agar completely. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes. Cool to 45°C and aseptically add small amount of Potassium hydroxide (Part II) dissolved in 10ml of sterilized distilled water for the adjustment of pH in the solution. Pour the medium into the sterile flasks.

Appearance: Light yellow colour, clear to slightly opalescent gel

pH (at 25° C): 6.8 ± 0.2

Principle

AZOSPIRILLUM MEDIUM (W/0.17% AGAR) is used for the cultivation of *Azospirillum* species. *Azospirillum* is abundant in tropical soils where it may have particular potentialities for enhancing plant growth. It occurs also in temperate soils, and positive evidence has been forwarded also for these conditions (Those occur as free-living in soil or in association with the roots of cereal crops, grasses and tuber plants. *Azospirillum* species are plant-associated diazotrophs of the alpha subclass of Proteobacteria. Malic acid is used as the carbon source. *Azospirillum* species grow well in presence of Malic acid and are not overgrown by other nitrogen fixers. Dipotassium phosphate provides buffering effect and other inorganic salt ingredients provide necessary growth nutrients. Agar at 0.17% concentrations provides microaerophilic conditions necessary for nitrogen fixation by *Azospirillum* species. *Azospirillum* can perform denitrification. Under anaerobic conditions in liquid cultures, *Azospirillum* utilizes nitrate, nitrite or nitrous oxide and reduces them either to nitrite, nitrous oxide or molecular nitrogen, depending on the assay conditions and the strain used. The conditions for field experiments with *Azospirillum* and cereals are often too complex to give unequivocal results due to variations in plant material and bacterial

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inoculation. Test a nitrogen fixation and denitrification activities of *Azospirillum* in association with wheat with reproducible results. This system, which allows testing nitrogen fixation and denitrification activities of *Azospirillum* strains in association with cereals within a week. The experiments will confirm that *Azospirillum* performs Nitrogen fixation (C_2H_2 -reduction) in dependence of an association with wheat. In addition, it will be shown for the first time that *heat-Azospirillum* associations reduce nitrate to gaseous nitrogen products, depending on the concentrations of nitrate and oxygen in the assays. The experiments were performed with the type strain, *Azospirillum brasilense*, and with the carotenoid forming strain *Azospirillum brasilense* Cd which was said to be particularly suitable for applications in the field. Other *Azospirillum* strains including nitrogenase negative (nif-) mutants were also investigated for N_2 -fixation (C_2H_2 -reduction) and denitrification (N_2O -formation) properties in the association with wheat in the present investigation. The results will show that activities are variable depending on the *Azospirillum* strain used.

Interpretation

Cultural characteristics observed after inoculating (10^3 CFU / ml), on incubation at 30°C for upto 8 days.

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth
<i>Azospirillum brasilense</i>	29710	10^3	Good - Luxuriant

References

1. Bergey's Manual of Determinative Bacteriology, 9th Ed, Williams R. H., (Eds.), Williams and Wilkins, Maryland, USA. (1994).
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3. Berg RH, Tyler ME, Novick NJ, Vasil V, Vasil JK Biology of *Azospirillum* sugarcane association: enhancement of nitrogenase activity. Appl Environ Microbiol 39: 642 – 649. (1980).
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