

heartwood of *T. paniculata* Roth⁸. The 3, 3', 4-tri-O-methyl ellagic acid was isolated from the bark and heartwood of *Eugenia maire* A. Cunn⁹. The co-occurrence of these two methyl ethers has been found for the first time in *T. procera*.

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THE UTILITY OF MANGANESE IN LACTOSE MEDIUM TO DIFFERENTIATE RHIZOBIA FROM AGROBACTERIA

AGROBACTERIA are the most common bacterial contaminants which resemble rhizobia and are often confused with them during the routine isolation procedures. However, certain biochemical tests^{1,2} have been proposed earlier to differentiate rhizobia from many species of *Agrobacterium*. In this connection, Clark³ has reported that a modified Bergersen's medium⁴ containing lactose and 20 m.e. Mn²⁺/l could preferentially support the growth of all the species of *Agrobacterium* but exclude strains of clover and medic-rhizobia. In the present investigation, this finding has been verified extensively with tropical rhizobia of diverse origin comprising 115 isolates from *Cicer arietinum*, 12 from *Sesbania bispinosa* and *S. sesban*, and 8 isolates of *Agrobacterium* sp.; the latter was isolated as contaminants during routine isolation of nodule bacteria. All the isolates were inoculated on agar slopes of the following composition: lactose, 5.0 g; KNO₃,

1.0 g; MgSO₄.7H₂O, 0.1 g; Na₂HPO₄ (anhydrous), 0.18 g; agar 12.0 g; FeEDTA (0.25% W/v), 10.0 ml; MnSO₄ (33.5% W/v), 10.0 ml; pH, 6.8 and volume made upto 1000 ml by distilled H₂O. After incubating the cultures for 14 days, at 28° ± 1° C, observations were recorded for visible growth. Infectivity of all the isolates was tested on their homologous hosts according to the method of Wieringa and Bakhuis⁵.

It was interesting to note that all the isolates of rhizobia which nodulated their hosts could not also grow on the manganese lactose medium while the isolates of *Agrobacterium* showed luxuriant growth, thereby lending support to Clark's finding³ and confirming the utility of the test as a diagnostic feature to differentiate *Rhizobium* from *Agrobacterium* (Table I).

TABLE I
Growth of rhizobia and agrobacteria on modified Bergersen's medium containing lactose and 20 m.e. Mn²⁺/l

Strains	Isolated from	No. of strains found positive	
		Growth on lactose-manganese medium	Nodulation on homologous host
<i>Cicer</i> -rhizobia (115)	<i>Cicer arietinum</i>	..	115
<i>Sesbania</i> -rhizobia (10)	<i>Sesbania bispinosa</i>	..	10
<i>Sesbania</i> -rhizobia (2)	<i>S. sesban</i>	..	2
<i>Agrobacterium</i> (5)	<i>C. arietinum</i>	5	..
<i>Agrobacterium</i> (3)	<i>S. bispinosa</i>	3	..

Figures in parenthesis are the number of isolates.

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