

EFFECT OF VITAMIN AND NITROGEN SOURCE ON THE
GROWTH OF DIFFERENT STRAINS OF *RHIZOBIA*
ISOLATED FROM *CICER ARIETINUM* L.

BY

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The effect of nitrogen source and B-vitamin on growth of rhizobial strains were investigated. The strains showed some variation with regard to utilization of different nitrogen sources. The bacteria were able to utilize all the sources for growth. In four out of six strains the replacement of yeast extract by other nitrogenous compounds had a deteriorating effect on growth. It was also observed that pyridoxine, biotin and thiamine stimulated the growth significantly. Nicotinic acid, riboflavin and vitamin B₁₂ has no stimulatory effect on growth.

INTRODUCTION

Vitamins of B-group have been shown to be essential in minute quantity for growth of many species of *Rhizobium* (Lilly & Leonian, 1945 and Gram, 1963). They are either dependent on or independent of exogenous supply of these vitamins. It is also known that growth of fast growing *Rhizobia* is markedly effected by availability and concentration of nitrogen source in the growth medium (Walker *et. al.*, 1933). Burris and Wilson (1952) have reported that the respiration rate of legume bacteria is increased by certain inorganic nitrogenous salts.

The present study has been aimed to find out the response of B-vitamin and nitrogenous sources on the growth of six strains of *Rhizobium* isolated from *Cicer arietinum* L.

MATERIAL AND METHODS

Effect of nitrogenous sources

The following nitrogenous compounds were added individually to the basal liquid medium at a level of 0.1%.

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Basal medium

Mannitol - 10.00g, K_2HPO_4 - 0.5g, $MgSO_4 \cdot 7H_2O$ - 0.2g, NaCl - 0.1g, Distilled water - 1000ml.

Nitrogenous source

NH_4Cl , KNO_3 , NH_4NO_3 , $(NH_4)_2SO_4$, Asparagin and Cystine.

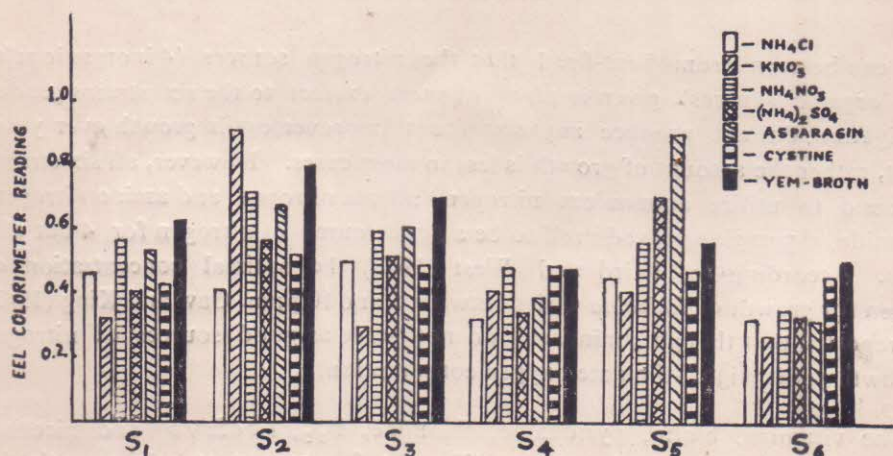
Effect of Vitamin

For determining the vitamin requirement for growth, the isolates were grown in liquid basal medium used for determining the effect of N-sources, with NH_4Cl (0.1%). Three consecutive transfers were made in the basal medium and finally transferred to the basal medium supplemented with the vitamin. The vitamin were added singly at the following levels per 100ml, Pyridoxine - $2\mu g$, Riboflavin - $40\mu g$, Nicotinic acid - $1\mu g$, Thiamine - $1\mu g$, Biotin - $0.1\mu g$, and B_{12} - $1\mu g$.

Growth was measured turbidimetrically after 48 hours at $30^\circ C$.

RESULTS

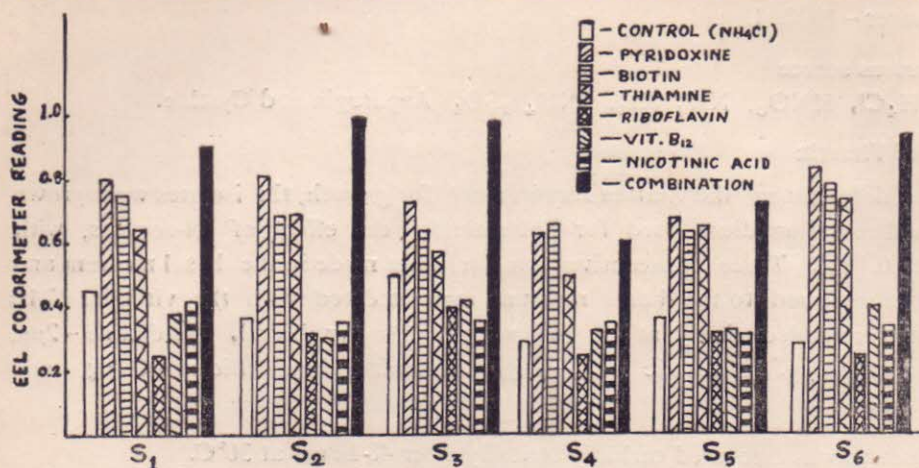
The results presented in Text-fig. 1 show clearly that addition of nitrogen, either in organic or inorganic forms to the *Yem-broth* replacing yeast extract has no significant effect on enhancement of growth for most of the strains when



Text-fig. 1. Graph showing the effect of nitrogen sources on the growth of the bacterial strains.

compared with control. In fact, the tried nitrogen sources tend to produce an inhibitory influence. Exceptions to this general finding are provided by KNO_3 in case of S_2 and Asparagin and $(NH_4)_2SO_4$ in case of S_5 .

The results shown in Text-fig. 2 reveal that the B-vitamins are not essential for growth, but three of them, pyridoxine, biotin and thiamine induce considerable



Text-fig. 2. Graph showing the effect of vitamins on the growth of bacterial strains.

enhancement of growth. Addition of all the tested vitamins together in the growth media results in further increase in growth.

DISCUSSION

It can be seen from Text-fig. 1 that the nitrogen sources (4 inorganic salts and 2 organic sources) given in place of yeast extract to the six strains under investigation, do not produce any significant improvement in growth over yeast extract, rather the amount of growth is less in most cases. However, all six strains are found to utilize ammonium nitrogen, nitrate nitrogen and amino nitrogen. The amide, asparagine proved itself to be a good source of nitrogen for 4 out of 6 strains. According to Laird and West (1938) the optimal concentration of nitrogen for growth of *Rhizobia* varies between 5 and 10 ppm. Gaw and King (1943) have reported that though amino acid do not serve as good sources of nitrogen for growth yet it might stimulate oxygen consumption.

The vitamins, biotin, pyridoxine, thiamine, B₁₂, riboflavin and nicotinic acid, which are offered to the strains (Text-fig. 2) are not strictly essential for growth but biotin, pyridoxine and thiamine have promoting effect on growth of all the strains. The strains differ among themselves when the effects of pyridoxine, thiamine, and biotin are compared. A flexibility in vitamin requirement, as noted, may not be totally unexpected. It has been shown by West & Wilson (1939) that fast growing *Rhizobia* usually respond to addition of biotin while slow growing strains of cowpea and soybean *Rhizobia* are not stimulated. Graham (1963) has

pointed out that *R. trifolii*, *R. leguminosarum* and *R. phaseoli* require in most cases thiamine, biotin and Ca-pantothenate while *R. meliloti* and slow growing *Rhizobia* respond to biotin but not to other B-vitamins.

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