BIOMASS PRODUCTION OF RHIZOBIA

BY

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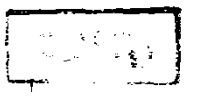

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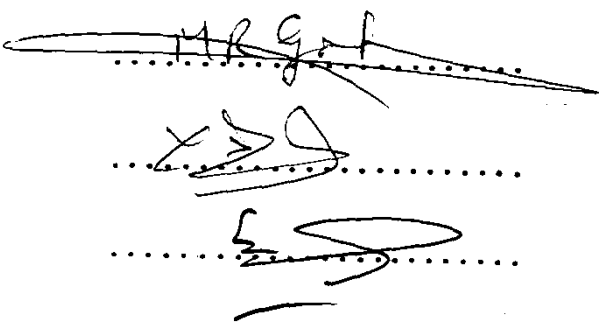

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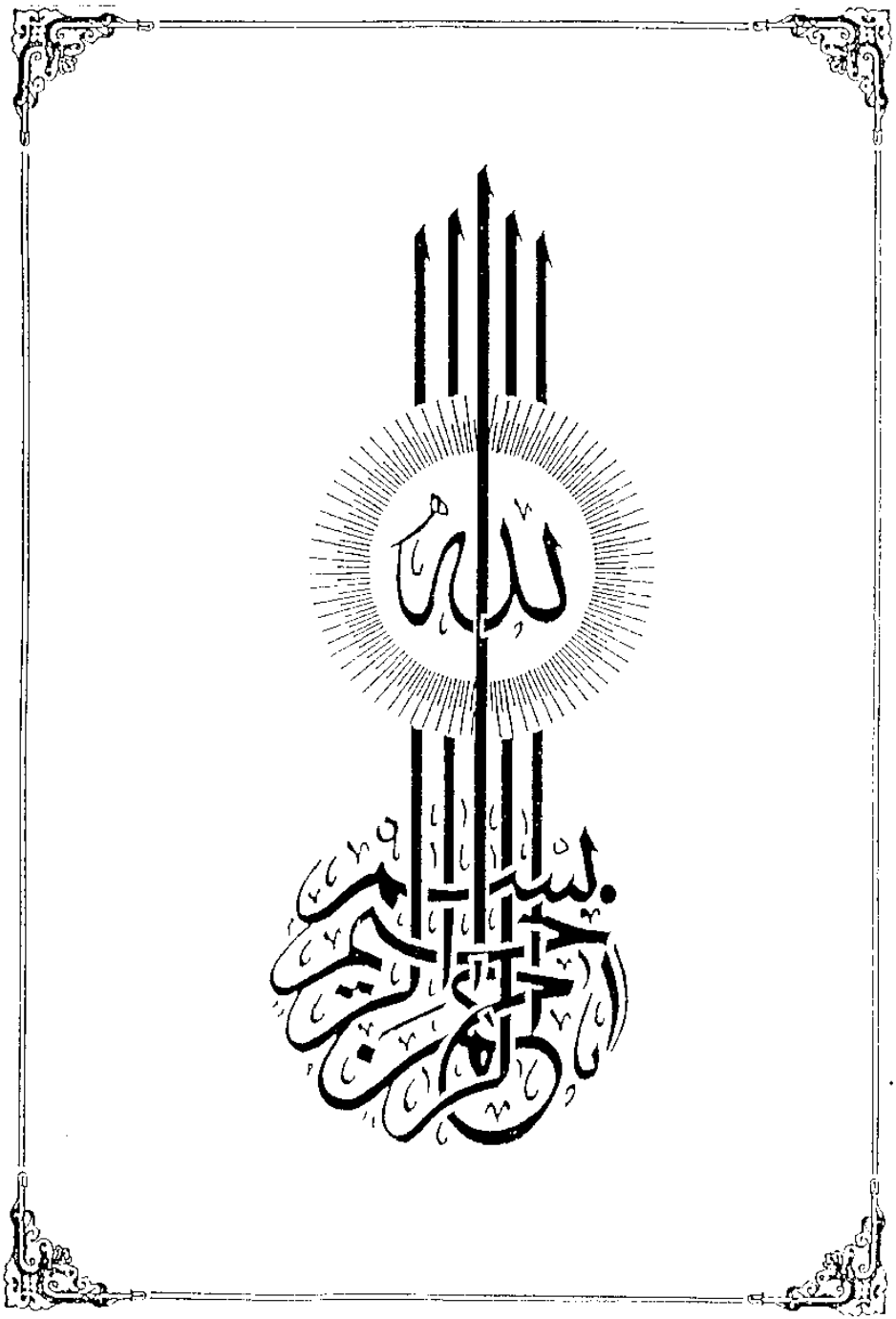

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ARABIC SUMMARY.

INTRODUCTION

INTRODUCTION

Microbial biomass production is utilized in so many important activities. In the field of soil microbiology, microbial biomass are utilized in the production of asymbiotic N_2-fixers such as Azotobacter, blue green algae and symbiotic N_2-fixers that elaborate nitrogen fixation by legumes as soil inoculants.

It is well known that the first step of microbial biomass production is the strain selection. Selected strains of rhizobia which satisfy the requirement, for production (high N_2-fixation efficiency, high infectivity rate, good colonization and persistence in soil) are grown in a suitable medium which should has a suitable pH value and contain the propriate concentration of carbon source, nutrients, growth factors, and should be incubated at the optimum temperature under the normal rate of aeration. The products containing high numbers of viable rhizobial cells assure a good quality of inoculants.

Although rhizobia grow satisfactorily in still culture with large surface area, more rapid growth and higher cell yields can be obtained with vigorous aeration in fermentors varied from elaborate large

capacity of industrial fermentors of 1000-2000 L (Date, 1965) to simple flasks of 1-5 L capacity or drums (10-100 L).

The present work was focused on selecting an effecient Rhizobium japonicum strain in cell productivity, by estimating the specific growth rates, doubling time and productivity of different strains. Influence of different environmental and nutritional conditions on the growth of selected strain was determined. The effect of inoculum with the selected strain on the growth and nodulation of soybean plants was also evaluated.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Biomass production of legume inoculants :

Rhizobial biomass production includes three main stages, namely (a) selection and testing of rhizobia strains, (b) preparations of the inoculants, including selection, preparation of the carrier and mass culturing of rhizobia, and (c) controlling the quality of the inoculant produced.

Selection of rhizobia strains :

The selection of rhizobia strains is the most important step in inoculant production. Screening of rhizobia strains following isolation and confirmation of purity is generally aimed at selecting for high infectivity and N_2-fixation efficiency.

This is done by testing their inoculating and N_2-fixing ability on their hosts and growing the host plants in a nitrogen-free substrate in jars or tubes under greenhouse or growth chamber conditions for a period of 4 to 6 weeks and measuring dry weight or total nitrogen content as affected by the inoculation with the tested organism (Vincent, 1970).

Other investigators (Roughly, 1970, 1976, 1977 ; Date 1974, 1976, and Date & Roughly, 1977) reported

that selection of suitable strains of a Rhizobium species is dependent upon many criteria - a single strain or more than one strain for a particular cultivar or a group of cultivars or crops. The selected strain is grown for three to four days on YEM agar slant depending on the fast or slow growing mature of the strain. The culture is tested for purity by well-known tests and transferred to large flasks containing sterile solid or liquid medium for four to nine days. This is called 'Starter culture' which is transferred to a seed tank fermentor and and incubated for four to nine days. By about this time, a large quantity of liquid broth is formulated in the fermentor (the size depending upon the requirements), pH adjusted to 6.5 to 7 with KOH or H_2SO_4 and sterilized. After cooling to 30°C, inoculum from the seed tank fermentor is transferred aseptically to the production fermentor at the rate of 1 per cent by volume.

Burton (1975) used another method of screening good N_2-fixing strains of rhizobia for their competitiveness with highly infective poor nitrogen-fixing strains in soils. Seed inoculated with a peat-base inoculum of the test strain are planted in sand impre-

gnated with a massive inocula of the native ineffective rhizobia. Dry weight and total N content of plants are measured after a growth period of 5 to 6 weeks. The investigator found a positive correlation between infectivity in growth chamber experiments and in actual field tests. Strains which prove competitive in these growth chamber tests have usually proved very competitive under actual field conditions also.

Imshenetskii et al (1976) stated that the aim of selecting nodule bacteria [Rhizobium meliloti and R. trifolii] is to obtain stable and genetically pure strains with elevated effectiveness in symbiosis with bean plants, sufficient viability and competing ability and long survival in soil. Methods suggested to solve this task were selection using mutagenic factors, genetic markers, production of genetically homogenous material, and determination of the degree of persistance in mutants.

Balasundaram et al (1977) studied the nodulation and shoot nitrogen of 2 varieties of soybean (Glycine max cv. Bragg and Geduld) with 20 strains of Rhizobium japonicum. A number of cultural characteristics of the strains in isolation to the symbiotic

system were also studied. A stepwise selection method was employed for detecting efficient cultures through the cultural characteristics which showed association with the steps in the symobitic system. Nodulation of one variety was found to be associated with the dehydrogenase activity and the growth of microbes in the medium containing soil extract; the nodulation of another variety showed association with the growth in the media containing asparagine and tryptophane. The shoot N of 1 nodulated cultivar correlated with the microbial growth in Elkan's medium (containing serine and glucose) the shoot N of the other nodulating variety correlated with the growth of the cultures in the medium containing aspartic acid.

Rhizobia strains to be used for preparation of legume inoculants should go through two or three stages of evaluation namely : (a) screening for effectiveness in nitrogen fixation, (b) assay of selected effective strains for their N_2-fixing ability under field conditions which includes competition with native rhizobia for nodules sites, persistence and colonising abilities in the soil, and (c) when necessary, coverage of other aspects such as pH, pesticide tolerance, survival in culture and on seed. Controlled environment facilities provide reliable comparative information of the ability

of a large members of strains to fix nitrogen, but are not suitable for the second and the third stages.

Field trials, on the other hand, can evaluate only a limited number of strains because of demands on time, labour and facilities. In special circumstances, glasshouse triats in pure culture (Johnson and Means, 1964) can be used to forecast field performance (Caldwell, 1969).

Strains of R. trifolii and R. meliloti were tested by Lorkiewicz, et al (1978) for their asymbiotic N_2-fixation ability. From among 10 tested strains 2 R. trifolii and 1 R. meliloti expressed nitrogenase activity within the range of 1.3-9.3 nμ C_2H_4/h per mg protein. Asymbiotic N_2 fixation was affected by the composition of the medium.

Mass culturing of rhizobia :

Selected strains of rhizobia which satisfy the requirements for production of inoculants (e.g. high N_2-fixation efficiency, high infectivity rate, good colonization and persistence in soil) are grown in the suitable media which should contain the appropriate concentration of carbon source, nutrients, growth factors and should be incubated at the optimum