MASS SCALE PRODUCTION OF *RHIZOBIUM* CULTURE

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Experiments were conducted for mass scale production of *Rhizobium* inoculant in different cheap carbon sources and in different concentrations of yeast extracts in the fermentation medium.
Molasses could serve as a superior carbon source for rhizobial growth. Liquid malt extract could be ranked in the list and third one was the medium having sucrose and mannitol in combination.
Neutral Baker's yeast extract solution 200 ml/l or Oxoid yeast extract 500 mg/l and Difco 250 mg/l served as a very suitable substrate for growth.
The modified composition of medium comprised 10 g sucrose and 5 g mannitol and 200 ml yeast extract solution/l and served as a very suitable composition for rapid cell multiplication.

INTRODUCTION

The suitability of culture media for large scale production of *Rhizobium* depends upon the rate of utilization of a carbon source and multiplication rate of bacteria. The extensive information gathered on nutritive requirement of rhizobia has been reviewed by Fred (1932), Wilson (1940) and Allen (1950, 1958). Nearly all rhizobia utilize mono saccharides, alcohol and acids. Starch is not utilized. Certain pentoses such as arabinose and xylose are preferred sources of carbon for *R. japonicum* (Wilson 1940; Graham and Parker 1964). However, the advantage gained from the use of pentoses would probably be offset by their higher cost, particularly since mannitol and sucrose are satisfactory carbon sources. Sucrose, i.e. cane sugar or beet sugar is most commonly used carbon sources; mannitol is sometimes employed in culturing *R. japonicum*, *R. lupini* and the cowpea rhizobia.

Yeast extract — Yeast extract is used as a source of organic nitrogen and growth factors. Maximum growth depends upon adequate supply of low molecular weight amino acids, such as are contained in yeast extract, cornsteep liquor, hydrolyzed casein or soybean protein.

Many cheap commercial carbon sources such as sugar, molasses and malt extract are available in the market. The present paper deals with a study of the comparative growth of *Rhizobium* in indigenous carbon sources as well as in different types of yeast extracts such as Oxoid, Difco and Baker's yeast extract solution.

MATERIALS AND METHODS

Rhizobial strains for groundnut (*Arachis hypogaea*) and soybean were grown in 500 ml conical flasks in the rotary shaker (200 ml in each) and for large scale production in 10-litre glass fermentor containing 7 litres of medium. The temperature was controlled and maintained at 28° ± 2°C and sterile air was sparged in through the liquid medium at the rate of 7 litres of air per 10 litres of medium.

RESULTS

Viable cell counts of *Rhizobium* were taken by dilution plate technique after 7 days incubation and results are given in Tables I and II.

TABLE I

Growth of two strains of Rhizobium *in the shake culture and in* 10*-litre glass fermentor* (7 *days growth*)

Medium (Basal)	Carbon source	g/l	Cell count × 10^8/ml			
			Shake culture		Fermentor	
			Ground nut	Soybean	Soybean	Ground nut
Yeast extract soln. (200 ml/l) + minerals	Mannitol	10	16.0	1.300	1.200	14.4
Yeast extract soln. (200 ml/l) + minerals	Commercial sugar + Mannitol	10 3	18.0	2.500	2.800	21.1
Yeast extract soln. (200 ml/l) + minerals + molasses (cane)	Sucrose	15	120.0	27.000	31.000	150.0
Yeast extract soln. (200 ml/l) + minerals + malt extract (liquid)	Maltose	15	61.0	1.400	1.200	71 0
Yeast extract soln (200 ml/l) + minerals + malt extract (dry)	Maltose	15	2.3	0.026	0.017	3.4

TABLE II

Growth of two strains of Rhizobium *in shake culture as well as in fermentor in different concentrations of yeast extract* (7 *days growth*)

Inoculum : 5 × 10^6/ml (1 ml inoculant/100 ml medium)

Strains	Concentration of yeast extracts														
	Neutral Baker's yeast soln (ml/l)					Yeast extract (Oxoid) (mg/l)					Yeast extract (Difco) (mg/l)				
	200	150	100	50	0	1000	750	500	250	0	500	375	250	125	0
Groundnut															
Shake culture	230	195	142	102	3.0	371	376	249	161	1.2	212	221	172	96	1.3
Fermentor	310	192	172	123	2.0	377	347	256	155	2.1	312	215	163	111	1.4
Soybean															
Shake culture	201	187	131	107	1.5	272	251	237	165	3.3	232	213	195	176	3.1
Fermentor	245	195	158	131	2.1	287	243	242	132	3.1	347	219	201	181	2.2

Cell Count × 10^6

Discussion

The experimental results of shake flask culture as well as the fermentor are given in the Table I. The result showed that molasses could serve as a better substrate for growth of the strains of *Rhizobium*. Liquid malt extract could be ranked next in the list and the third one was the medium having sucrose and mannitol in combination. Molasses contain some organic substances and trace elements which help the growth of rhizobia. The next best medium was malt extract (liquid), the cost of which was prohibitive for large-scale cultures of bacterial cells. The medium with sucrose and mannitol in combination was finally selected on consideration of its moderate cost and suitability for storage as the production of gum served as protective agent for live bacterial cells in liquid medium.

Among the different types of yeast extract, Oxoid yeast served as more superior growth factor for both the strains. Neutral baker's yeast solution could be ranked next in the list and the third one was Difco yeast extract. Neutral baker's yeast was however finally selected as it was the cheapest source for mass culture production.

References

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