

Improved Growth Medium for Industrial Production of *Rhizobium japonicum* strain Soil-18

Somsak Vangnai, Sawaeng Ruaysungnern and Saipin Porntaveevatana

Department of Soils, Kasetsart University, Bangkok 9

ABSTRACT

A new growth medium for the industrial production of *Rhizobium japonicum* strain Soil-18, effective in fixing nitrogen with soyabean variety S.J. 2, is proposed. It is obtained by substituting some of the components of the conventional yeast extract mannitol medium with materials such as cane sugar and baker's yeast readily available in the country. The new medium is markedly cheaper than the conventional medium.

Yeast-extract mannitol is generally accepted as a traditional medium for growth of *Rhizobium japonicum*. Some components of this medium may not be optimal for the growth of specific strain of *R. japonicum* and it may be quite expensive, especially in the developing countries. The use of low cost materials to substitute these components may lead to an economic production of rhizobial cells, leading to the large-scale production of inoculant.

This study was an attempt to produce cells of *R. japonicum* strain Soil-18 using some low cost materials readily available in the country and to optimize the growth of the rhizobial strain.

Materials and Methods

Rhizobium japonicum strain Soil-18 (1), isolated from root nodule of soybean at Department of Soils (Kasetsart University) and shown to be effective in fixing nitrogen with soybean variety S.J.2, was used.

Media: Medium 1. The yeast-extract mannitol medium was used as a basic medium for growth studies of the bacterium. This medium contained (g/l): mannitol, 10; K_2HPO_4 , 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; yeast extract, 1; $CaCO_3$, 3.0; and distilled water 1 l. The p^H was adjusted to 6.8.

Medium 2. Medium 1 without $CaCO_3$ and with tap water.

Medium 3. Medium 2 without mannitol and with 10 g/l of each of the following organic substances: maltose, sucrose, cane sugar, glucose, and glucolin.

Medium 4. Medium 2 without mannitol and yeast extract but with cane sugar and baker's yeast.

Preparation of cell suspension: Bacteria were grown in 250 ml. Erlenmeyer flasks containing 100 ml. of yeast-extract medium (Medium 1). The flask were incubated at room temperature (approximately 28C) with shaking (150 rpm.). After 36 hours the cells were harvested by centrifugation at 5000 xg. for 15 minutes at 5C and washed with 0.2 M phosphate buffer (p^H 7.2) (except where the effect of K_2HPO_4 was studied, distilled water was used instead of phosphate buffer). Before use, the cells were suspended in the same buffer to give an optical density of 0.2 (380 nm.) on a Bausch and Lomb Spectronic 20 colorimeter.

Cell growth: Cell growth was measured turbidometrically using a Bausch and Lomb Spectronic 20 colorimeter at 380 nm.

Enumeration: Enumeration of bacterial population in the samples was performed by plate count technique, using the yeast-extract medium

and following the most probable number (MPN) technique.

Growth studies: One millilitre of the cell suspension was inoculated into 250 ml. flasks containing 100 ml. of the desired medium. The flasks were incubated at room temperature with shaking. Cell growth was measured turbidometrically at 12 hours interval. At the end of the experiment, cell number was evaluated following the MPN technique.

Results And Discussion

Growth characteristics of *R. japonicum* strain Soil-18: Growth characteristics of the bacterium in Medium 1 and Medium 2 are shown in Fig. 1.

The organism was able to grow rapidly in Medium 1 reaching a maximum of 8.1×10^9 cells/ml. in 36 hours. Growth characteristics in Medium 2 was approximately similar to that of Medium 1 reaching a maximum population of 1.1×10^9 cells/ml. in 36 hours. This indicates that CaCO_3 was not required for *in vitro* growth of *R. japonicum*. It was also found that tap water could be used to substitute distilled water. Medium 2 was therefore used in further studies.

Carbon or energy source effects on growth: Results of growth studies in Medium 3 individually substituting mannitol with maltose, sucrose, cane sugar, glucose and glucolin are presented in Fig. 2.

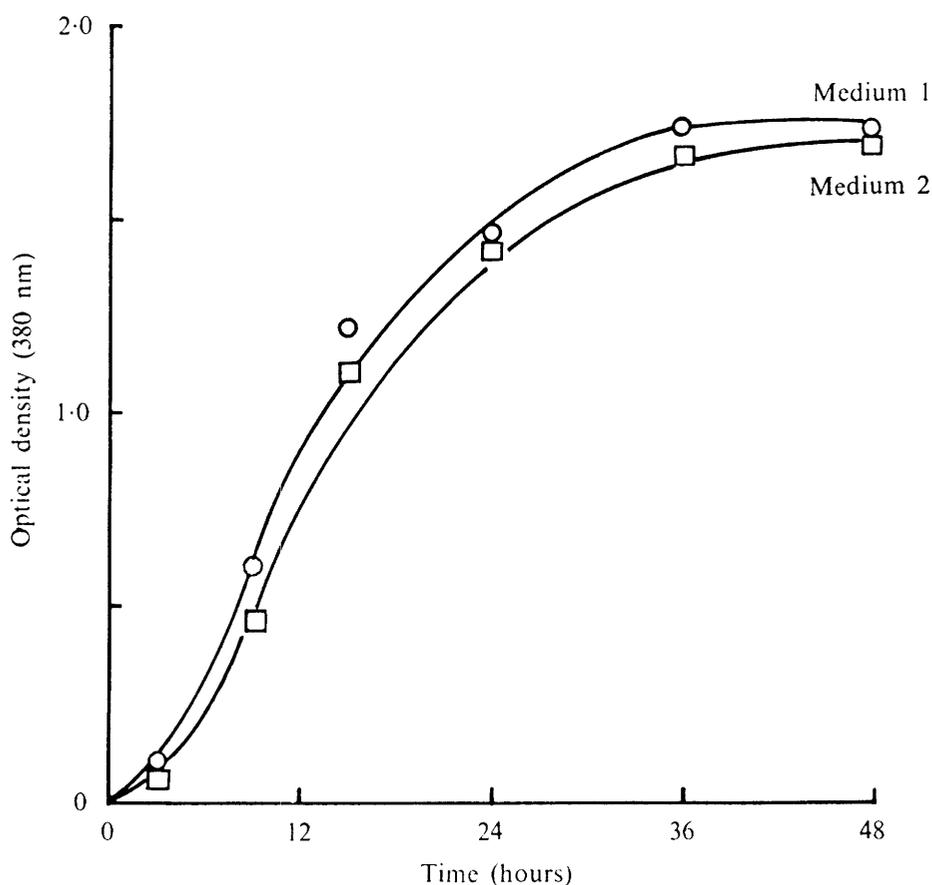


Fig. 1. Growth characteristics in medium 1 and medium 2.

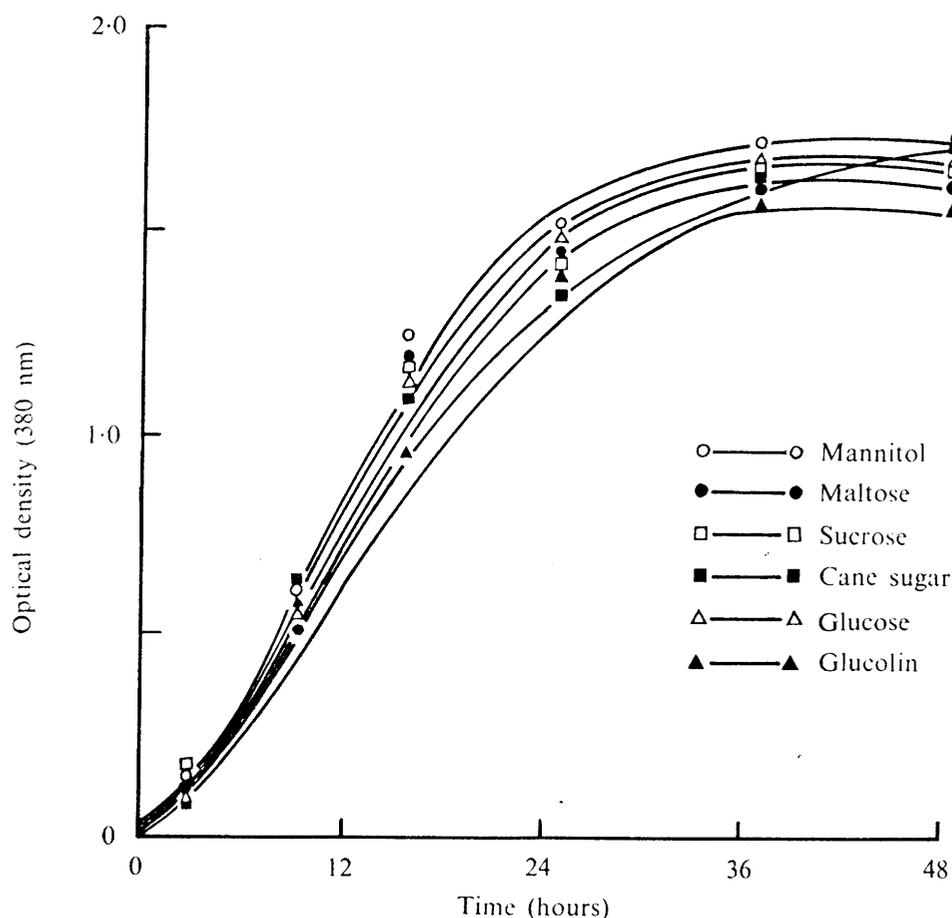


Fig. 2. Effects of mannitol, maltose, sucrose, cane sugar, glucose, and glucolin on growth.

It was noted that maltose, sucrose, cane sugar, glucose, and glucolin were able to support growth as effectively as mannitol. This is in agreement with the observations of Tuzimura and Meguro (4) and Schwinghamer (3) who reported that many types of sugars could be used to substitute mannitol as carbon or energy source for *R. japonicum*. Among the sugars used in this study, cane sugar was the cheapest and constantly available in the country and it was therefore selected for further work.

Further studies were then initiated to evaluate rhizobial growth in the presence of varied levels of cane sugar. The results are shown in Fig. 3. It appeared that best growth was observed with

medium containing cane sugar ranging from 8 to 10 g/l. These results suggested that cane sugar at the concentration of 8 g/l would be sufficient for optimal growth of this strain.

Effects of potassium phosphate and magnesium sulfate levels on growth: Results of growth studies in the media containing varied levels of potassium phosphate and magnesium sulfate are presented in Fig. 4. The results showed that the organism was able to grow best in the presence of 0.1 to 0.2 and 0.01 to 0.2 g/l of potassium phosphate and magnesium sulfate respectively. Consequently, 0.1 g/l potassium phosphate and 0.01 g/l magnesium sulfate was considered to be the optimal concentrations for growth of the rhizobial strain.

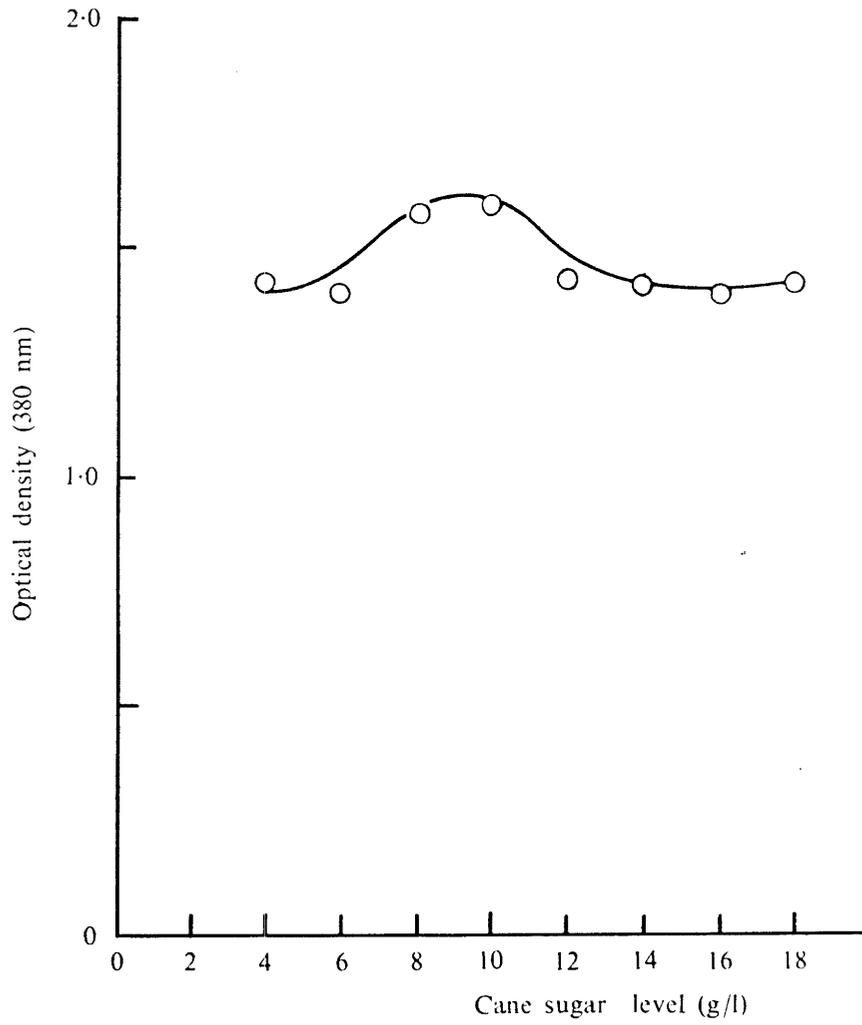


Fig. 3. Effects of cane sugar levels on growth (36 hours after incubation).

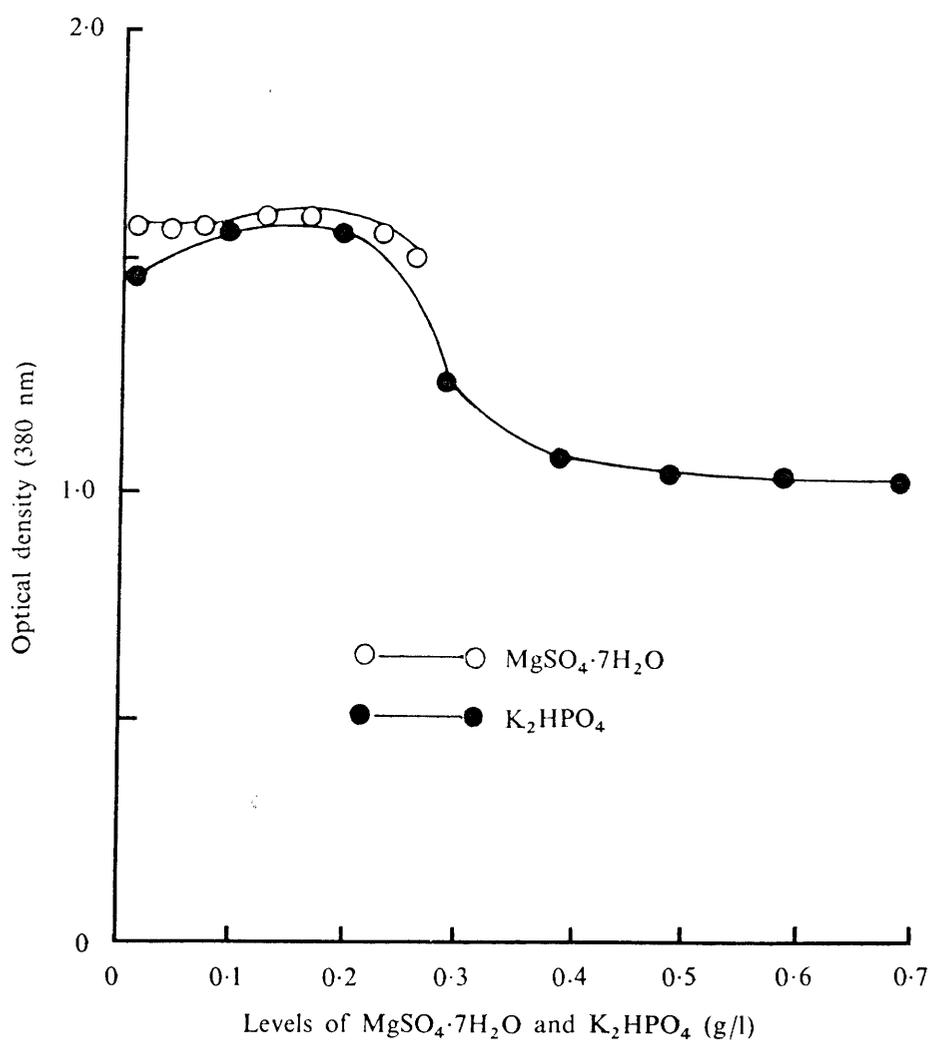


Fig. 4. Effects of levels of magnesium sulfate and potassium phosphate on growth (36 hours after incubation).

Yeast extract and baker's yeast effects on growth: Previous studies (2) revealed that baker's yeast could be used to substitute yeast extract (Difco) without growth reduction. Further growth studies were carried out using Medium 4 containing varied baker's yeast levels. The results are shown in Fig. 5. It is noted that growth was strongly influenced by the presence of baker's yeast. The presence of high concentrations of baker's yeast (more than 1 g/l), however, resulted in the accumulation

of slimy materials. It is, therefore, advisable to use baker's yeast at a concentration of 1 g/l.

According to the results of these studies, a new medium for cell production of *R. japonicum* strain Soil-18 is proposed. This medium contains (g/l); cane sugar 8; K₂HPO₄, 0.1; MgSO₄·7H₂O, 0.02; NaCl, 0.1; baker's yeast, 1.0; and tap water 1 l. pH adjusted to 6.8. The cell yield, after 36 hours was approximately equal to that obtained from the yeast extract medium (8.1×10^9 cells/ml.). The new medium, however, is

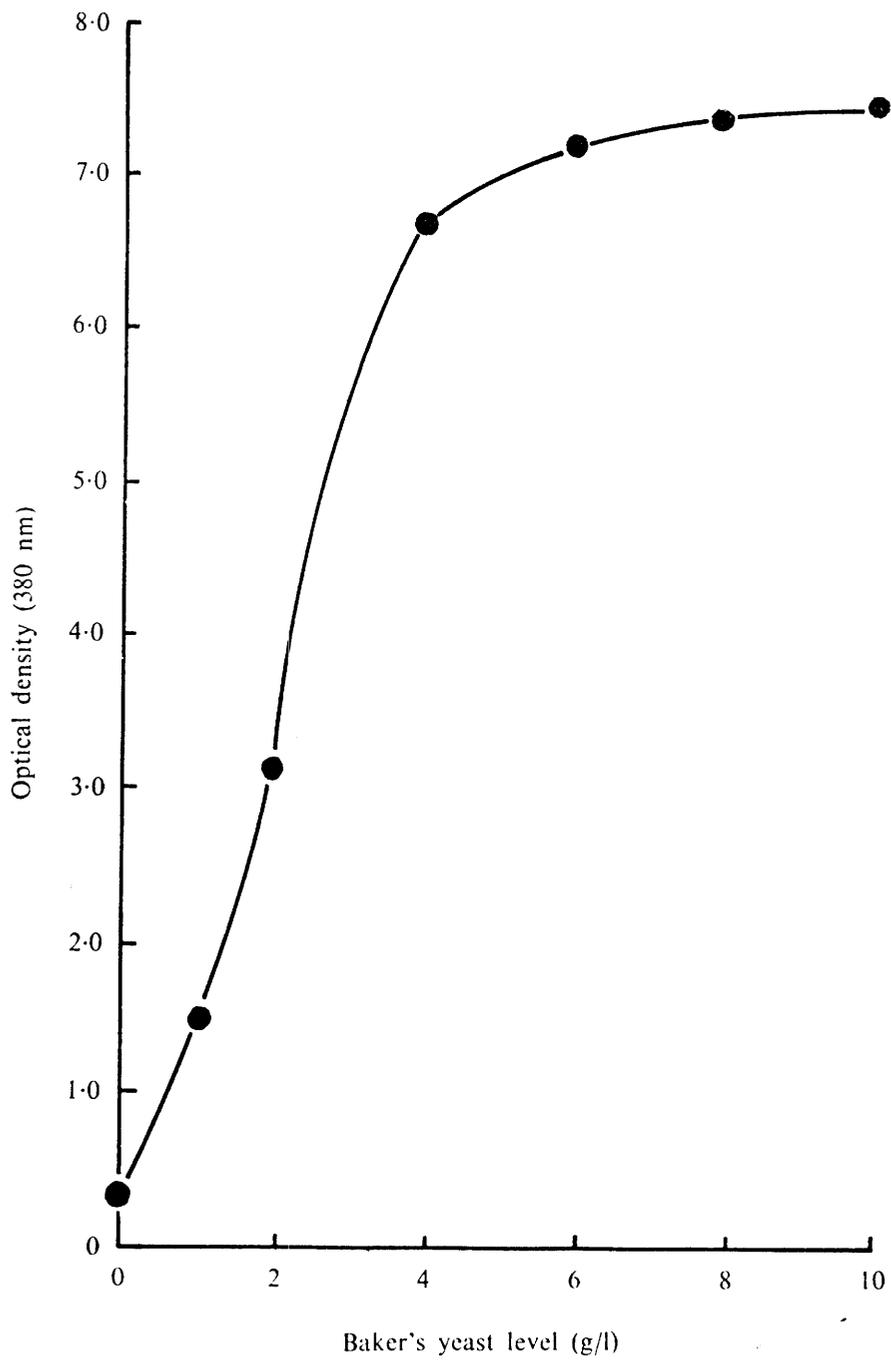


Fig. 5. Effects of baker's yeast levels on growth (36 hours after incubation).

much cheaper; the cost of yeast extract medium and the new medium are 4.21 and 0.55 Baht/l (20 Baht = 1 US dollar) respectively. This indicates that, by using the new medium, the production cost can be reduced by about 87 percent.

Literature Cited

1. MANESAWAT, S. and S. VANGNAI. 1973. A Rhizobium strain capable of effectively fixing nitrogen with soybean S.J. 2. Agric. Jour. (Thai), 5 : 217 : 220
2. RUAYSUNGNERN, S. and S. VANGNAI. 1974. Vangnai Et Al : Rhizobium japonicum Effects of different carbon and nitrogen sources on growth and effectiveness of *Rhizobium japonicum* strain Soil-18. 14th Conf. Agri. Biol. Sci. Bangkok, Thailand: 48.
3. SCHWINGHAMER, E.A. 1960. Studies on induced variation in the rhizobia. 1. Defined media and nodulation test techniques. Aust. J. Agric. Res. 8: 349-352.
4. TUZIMURA, K. and H. MEGURO. 1968. Respiration substrate of Rhizobium in the nodules. J. Biochem., 47: 391.