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Original Research Article

Effect of Different Carbon Source on Growth of Rhizobium Belonging to Different Region of Chhattisgarh, India

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Bioinoculant, Carbon source, Leguminous and Rhizobium.

A B S T R A C T

Symbiotic bacteria belonging to the genus *Rhizobium* play an important role in the nutrition of leguminous plants by fixing atmospheric nitrogen in the root nodules. Industrial production of *rhizobium* inoculants, it is important to identify inexpensive and easily available sources of nutrients for culture medium. The purpose of study has to develop methods to use low-cost carbon compounds for *rhizobium* inoculants production and the effects of different carbon sources on growth of *Rhizobium*. Six carbon sources viz. dextrose, fructose, lactose, mannitol, sucrose, and starch were tested. These were utilized for the growth of *Rhizobium.* Mannitol was found to be the best carbon source for *Rhizobium* but polysaccharide like starch also favored growth. Most of the strains were unable to utilize sucrose and thus it supported minimum growth. In this study JD3 and JP27 strain were found to be best out of 10 strains which can be further characterized and used for bioinoculant.

Introduction

Rhizobium is an aerobic, gram negative, rods and cocci which belong to family-*Rhizobiaceae* (Dubey, 2008). Symbiotic bacteria belonging to the genus *Rhizobium* play an important role in the nutrition of leguminous plants by fixing atmospheric nitrogen in the root nodules. The ability to form nodules has been found to be highly host specific for different *Rhizobium* species (Fahraeus and Ljunggren, 1968). Nitrogen is amongst the most limiting nutrient for plant growth. It is a constituent of all proteins, nucleic acids and many other biomolecules and it is essential in all living organisms (McCammon and Harvey, 1987). *Rhizobium* encompass a range of bacterial genera, including *Brady rhizobium, Sino rhizobium, Meso rhizobium, Allo rhizobium*,and *Azo rhizobium*,. Symbiotic nitrogen fixation is an important source of nitrogen, and the various legume crops and pasture species often fix as much as 200 to 300 kg nitrogen per hectare (Peoples *et al*., 1995).

Besides biological nitrogen fixation, some strains of *Rhizobium* are also involved in PGPR activity (Deshwal *et al*., 2003). Plant growth promoting rhizobacteria (PGPR) are involved with host plants in mutual interaction. Plant growth promoting rhizobacteria (PGPR) are considered to promote plant growth directly or indirectly, can exhibit a variety of characteristics responsible for influencing plant growth. *Rhizobia* has the dual beneficial effect as a phosphate solubilizer and nitrogen fixer when used as inoculant. Chickpea is an important legume food crop in many developing countries, and substantial research programmers are being carried out to improve its yield, disease resistance and nutritional value (Lauter *et al.* 1981).

Industrial production of *Rhizobial* inoculants, it is important to identify inexpensive and easily available sources of nutrients for culture medium. In such media preparations, a single source of carbon cannot be used for all strains, because *rhizobial* strains of different genera often differ in carbon utilization. Generally, fast growing *rhizobia* can utilize a variety of sugars such as glucose, sucrose, maltose, mannitol is universally used for cultivation both fast and slow-growing *rhizobia* (Stowers, 1985). The present work mainly deals with the effects of different carbon sources on the growth of *Rhizobium* strain.

Materials and Methods

Soil sample was collected from (Simga, Fingeswar, Kawrdha and Bemetara) different region of Chhattisgarh. In this research 10 bacterial strains were isolated in the Devleela Biotech, Anand vihar, Urja Park, V.I.P. Road, Raipur 492001 (C.G.). 1.0g of freshly collected soil was mixed with 9.0 mL of sterile distilled water in autoclaved test tube and serial dilutions were followed. 0.5mL of 10^{-5} dilution was inoculated into sterilized petri plate and containing 20ml of nutrient agar media and incubated at 37^{0}C for 24 hours.

Culture media was prepared by using Yeast extract mannitol agar (YEMA) medium. Carbon is one of the vital nutrients for growth of *Rhizobium*. To study the effect of six different carbon sources like dextrose, fructose, lactose, mannitol, starch and sucrose on growth of *Rhizobium*, YEMA broth was prepared by adding 1% each of the carbon source and their effect on *Rhizobium* growth was analyzed. The bacterial cultures were inoculated in test tube and incubated at 30^{0}C± 1°C on a gyro-rotatory shaker (REMI, India) at 200 rpm and reading was taken after 24h, 48h and 72 h. After incubation, absorbance wes measured at 560 nm using a spectrophotometer.

Result and Discussion

Kumari *et al.*, (2009) reported that five *Rhizobium* strains were found to utilize a wide range of carbon sources. Glucose, galactose, arabinose, fructose, Raffinose, Xylose, Mannitol, lactose, Maltose, Sucrose, Starch and Cellulose were tested where Monosaccharide (Glucose, galactose, arabinose, fructose, Raffinose, Xylose) support maximum growth followed by sugar alcohol (Mannitol) whereas In this study six carbon sources were chosen viz. Dextrose, Fructose, Lactose, Mannitol, Starch and Sucrose to observe their effect on growth of *Rhizobium*.

Optical density was measured on double beam UV-Visible spectrophotometer for each carbon source; reading was taken at interval of 24, 48 and 72 hours (Table No. 1 and Figure 1-10). Highest optical density for all strains reported in Mannitol. It determines the growth of bacteria is highest in presence of Mannitol.

Table No. 1 Effect of different carbon source on growth of *Rhizobium.*

Strain No.	Time	Absorbance at 560 nm						
		Control	**Dextrose**	**Fructose**	**Lactose**	**Mannitol**	**Starch**	**Sucrose**
JP 2	24H	0.075	0.176	0.422	0.175	0.314	0.294	0.262
	48H	0.120	0.184	0.486	0.235	**0.687**	**0.526**	0.476
	72H	0.089	0.141	0.451	0.189	0.498	0.355	0.335
JD15	24H	0.072	0.260	0.327	0.154	0.321	0.401	0.213
	48H	0.124	0.324	0.383	0.261	**0.615**	**0.439**	0.246
	72H	0.101	0.270	0.232	0.228	0.468	0.412	0.225
JP3	24H	0.032	0.281	0.469	0.450	0.320	0.354	0.158
	48H	0.075	0.334	**0.501**	0.496	**0.542**	0.382	0.219
	72H	0.070	0.295	0.386	0.395	0.433	0.345	0.201
JD3	24H	0.087	0.228	0.320	0.307	0.358	0.287	0.408
	48H	0.154	0.341	0.489	0.420	**0.648**	**0.612**	0.540
	72H	0.120	0.258	0.460	0.414	0.384	0.249	0.468
JP9	24H	0.105	0.200	0.297	0.347	0.326	0.273	0.273
	48H	0.167	0.237	0.350	**0.420**	**0.589**	0.329	0.329
	72H	0.143	0.139	0.320	0.395	0.427	0.311	0.343
JD9	24H	0.094	0.210	0.235	0.210	0.298	0.284	0.128
	48H	0.157	0.250	0.282	0.253	**0.502**	**0.470**	0.205
	72H	0.146	0.224	0.234	0.186	0.381	0.352	0.154
JP23	24H	0.098	0.215	0.300	0.327	0.287	0.247	0.128
	48H	0.187	0.378	0.277	0.385	**0.493**	**0.438**	0.173
	72H	0.158	0.329	0.325	0.218	0.483	0.397	0.107
JP24	24H	0.065	0.244	0.316	0.313	0.396	0.281	0.154
	48H	0.098	0.298	0.452	0.374	**0.698**	**0.601**	0.245
	72H	0.105	0.143	0.425	0.315	0.512	0.397	0.214
JP26	24H	0.065	0.174	0.166	0.151	0.267	0.330	0.298
	48H	0.098	0.243	0.220	0.191	**0.483**	**0.420**	0.411
	72H	0.105	0.177	0.103	0.117	0.298	0.283	0.227
JP27	24H	0.085	0.245	0.374	0.360	0.384	0.393	0.214
	48H	0.126	0.268	0.444	0.388	**0.574**	**0.522**	0.241
	72H	0.118	0.113	0.365	0.279	0.456	0.426	0.120

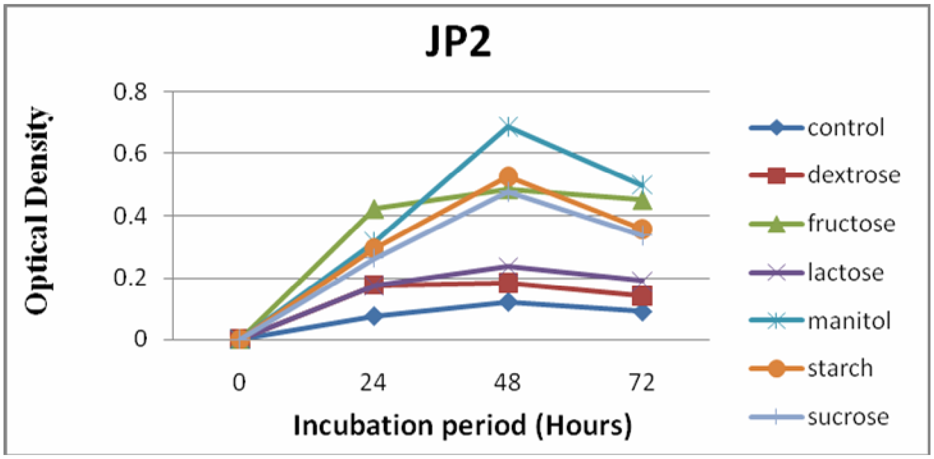

Figure.1 Growth of JP2 strain on different carbon source. (Table No. 1)

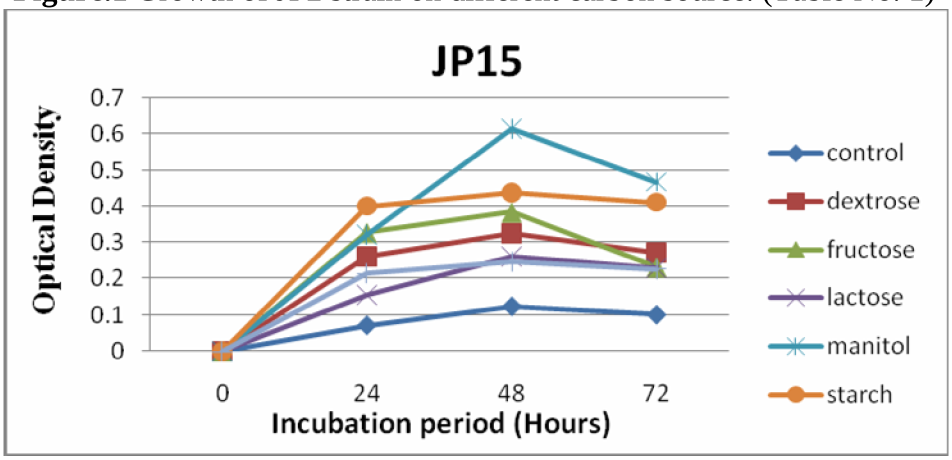

Figure.2 Growth of JD15 strain on different carbon source. (Table No. 1)

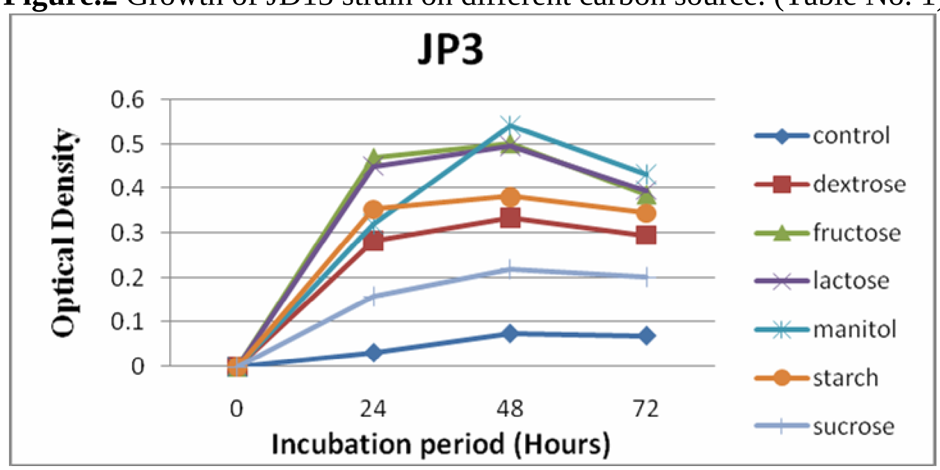

Figure.3 Growth of JP3 strain on different carbon source. (Table No. 1)

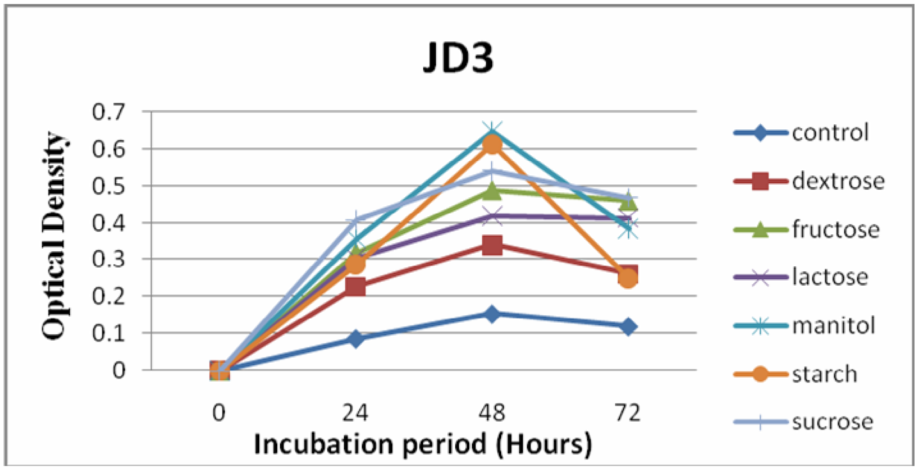

Figure.4 Growth of JD3 strain on different carbon source. (Table No. 1)

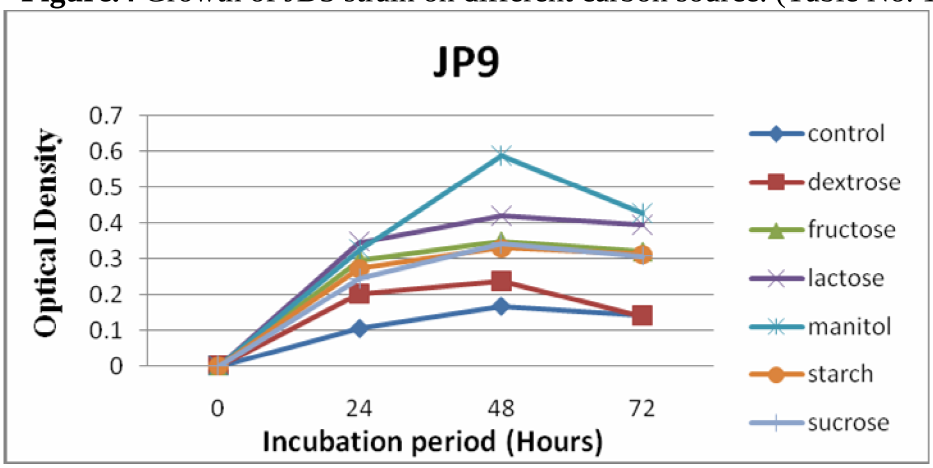

Figure.5 Growth of JP9 strain on different carbon source. (Table No. 1)

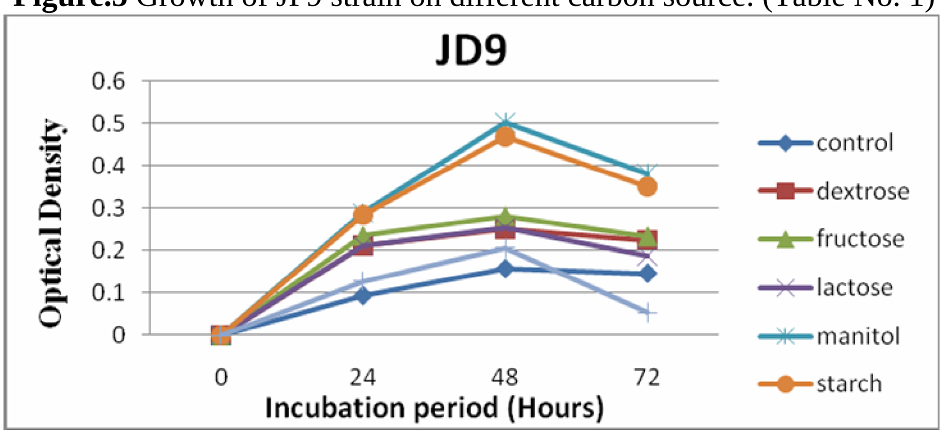

Figure.6 Growth of JD9 strain on different carbon source. (Table No 1)

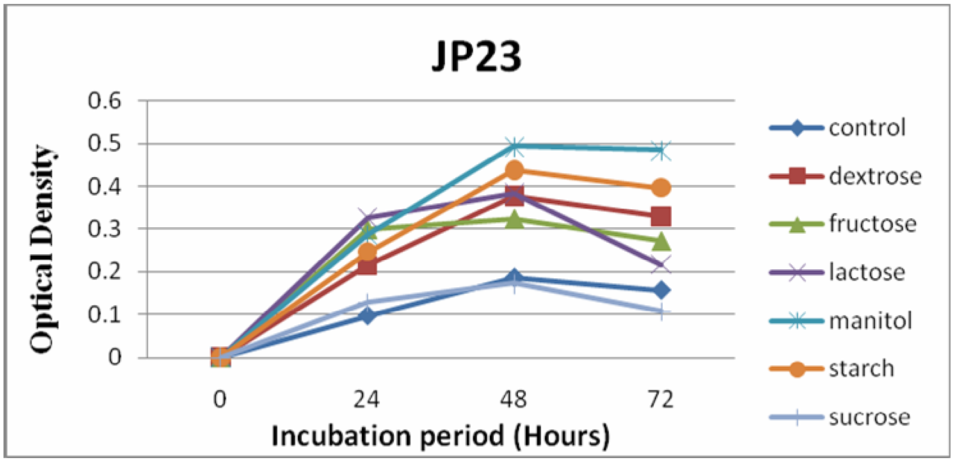

Figure.7 Growth of JP23 strain on different carbon source. (Table No. 1)

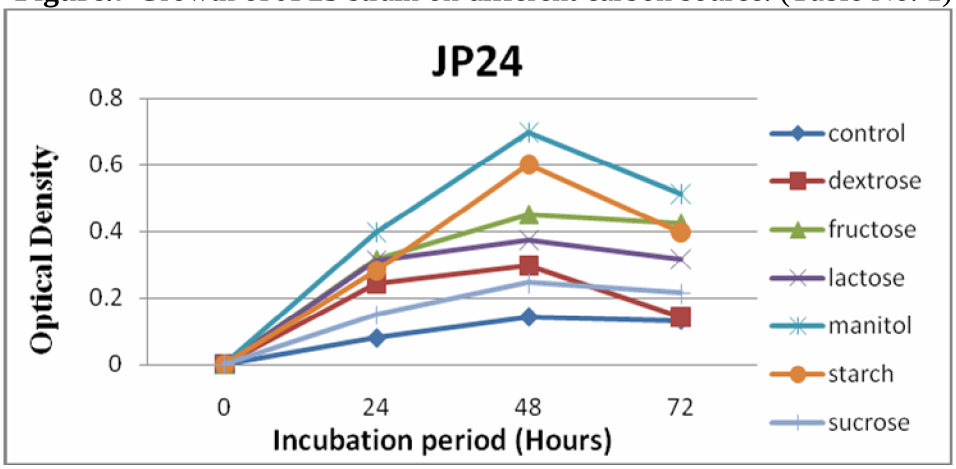

Figure.8 Growth of JP24 strain on different carbon source. (Table No. 1)

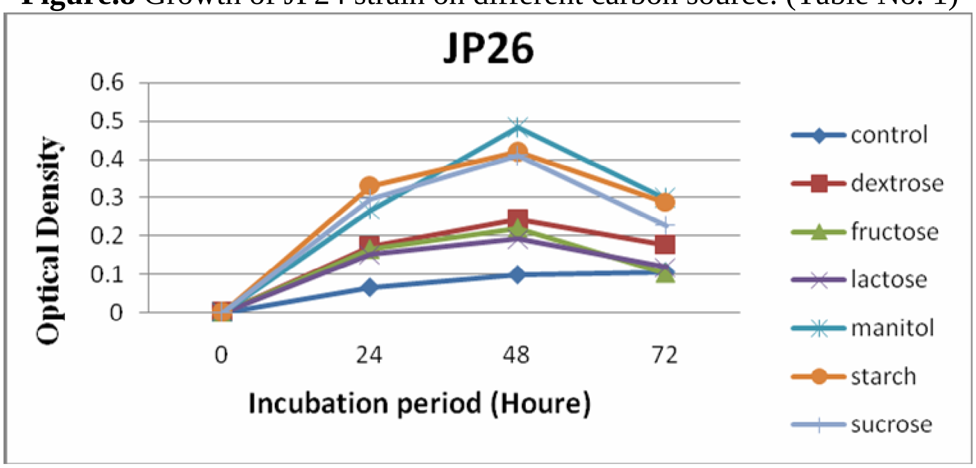

Figure.9 Growth of JP26 strain on different carbon source. (Table No. 1)

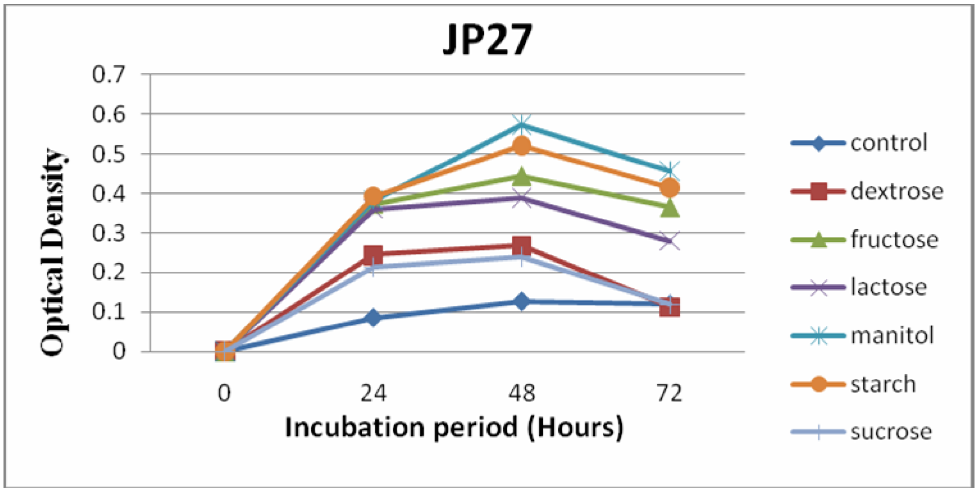

Figure.10 Growth of JP27 strain on different carbon source. (Table No. 1)

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