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Isolation and characterization of bacteria involved in nodulation and nitrogen fixation in Yam Bean (*Pachyrhizus erosus*)

S.C. POI AND P. K.PAL

Nodule Research Laboratory, Research Building No. 2, B.C.K.V., Krishiviswavidyalaya 741235, Mohanpur, Nadia, Wast Bengal

Twenty samples of nodule bacteria were isolated from nodules of yam bean (*Pachyrhizus erosus* L. Urban) of diverse origin. Their microbial and chemical characterizations were made. Some of the isolates were found to be fast growers and others were slow growers. The slow grower strains showed higher nitrate reductase and catalaes activities. All the isolates/appeared to be negative to nile blue and congo red tests. The isolates were classified serologically into two distinct groups. The fast growers constituted one sero group while the slow growers appeared to be of another group. All the isolates were able to fix nitrogen symbiotically. The highest nitrogen fixation was observed in case of inoculation with yb–10 isolate. It was suggested that isolate yb–10 may be tried as a suitable inoculant for yam bean cultivation.

Key words: Yam bean, bactaria, nodulation, nitrogen, fixation

INTRODUCTION

Yam bean is root tuber producing leguminous plant. These tubers are thirst quenching and nutritious with good amount of easily digestable starch. The crop is nodulated by *Rhizobium* which can fix nitrogen. Earlier, many workers have classified those symbiotic nitrogen fixing bacteria in two different genera i.e. *Rhizobium* and *Bradyrhizobium* (Elkan, 1984) depending on their growth habit. This experiment has been undertaken for characterization of different isolates of nitrogen fixing nodule bacteria of yam bean and their efficiency in nitrogen fixation with view to use the efficient isolates as seed inoculants in the cultivation of yam bean.

MATERIALS AND METHODS

The nodules were collected from yam bean roots of diverse origin (different zones of West Bengal). The nodules were found mainly in lateral roots. Edible tubers devoloped only on the taproots. The bacteria were isolated from nodules by using a method described earlier by Vincent (1970).

A Total of 20 strains of rhizobia were isolated from nodules of collected samples and their characterizaation was done through standard microbial and biochemical methods (Poi, 1986). The serological test of the isolates was done according to Vincent (1970) and nitrogen fixation was recorded following Jackson (1962).

Nodulation and plant growth were recorded from 75 days old plants by harvesting and washing the root-system in running tap water. The number of nodules, their shape and sizes were recorded. The dry weights of oven-dried plant materials were recorded and nitrogen contents were estimated from individual sampls.

RESULTS

Out of twenty isolates ten isolates (yb = 2, yb = 3, yb = 4, yb = 6, yb = 11, yb = 12, yb = 14, yb = 17, yb = 19 and yb = 20) showed fast growers on YAM and the rest ten slow growers. All the fast growers were positive to nitrate reductase and catalase test, the slow growers were highly positive to nitrate reeductase and catalaes test. Both fast and slow

Table 1. Microbial and biochemical assay of nodular isolates of yam bean

Isolates	Growth on YMA		Nitrate reduction	Catalase test	Litmus milk test		Keto lactase test	Nile blue test	Congored test
	Fast	Slow							
yb - 1	-	+	hP	hP	P	al	-	-	-
yb - 2	+	-	P	P	P	al	-	-	-
yb - 3	+	-	P	P	P	al	-	-	-
yb - 4	+	-	P	P	P	al	-	-	-
yb - 5	-	+	hP	hP	P	al	-	-	-
yb - 6	+	-	P	P	P	al	-	-	-
yb - 7	-	+	hP	hP	P	al	-	-	-
yb - 8	-	+	hP	hP	P	al	-	-	-
yb - 9	-	+	hP	hP	P	al	-	-	-
yb - 10	-	+	hP	hP	P	al	-	-	-
yb - 11	+	-	P	P	P	al	-	-	-
yb - 12	+	-	P	P	P	al	-	-	-
yb - 13	-	+	hP	hP	P	al	-	-	-
yb - 14	+	-	P	P	P	al	-	-	-
yb - 15	-	+	hP	hP	P	al	-	-	-
yb - 16	-	+	hP	hP	P	al	-	-	-
yb - 17	+	-	P	P	P	al	-	-	-
yb - 18	-	+	hP	hP	P	al	-	-	-
yb - 19	+	-	P	P	P	al	-	-	-
yb - 20	+	-	P	P	P	al	-	-	-

Hp = Highly Positive al = Alkaline - = Negative
P = Positive ac = Acidic + = Positive

Table 2. Serogrouping of nodular isolates of yam bean

Isolates	Yam – 1	Yam – 3	Yam – 5	Yam – 9	Yam – 16	Yam – 19
yb - 1	+	-	+	+	-	-
yb - 2	-	+	-	-	+	+
yb - 3	-	+	-	-	+	+
yb - 4	-	+	-	-	+	+
yb - 5	+	-	+	+	-	-
yb - 6	-	+	-	-	+	+
yb - 7	+	-	+	+	-	-
yb - 8	+	-	+	+	-	-
yb - 9	+	-	+	+	-	-
yb - 10	+	-	+	+	-	-
yb - 11	-	+	-	-	+	+
yb - 12	-	+	-	-	+	+
yb - 13	+	-	+	+	-	-
yb - 14	-	+	-	-	+	+
yb - 15	+	-	+	+	-	-
yb - 16	+	-	+	+	-	-
yb - 17	-	+	-	-	+	+
yb - 18	+	-	+	+	-	-
yb - 19	-	+	-	-	+	+
yb - 20	-	+	-	-	+	+

"-" = Negative reaction "+" = Positive reaction

Table 3. Efficacy of the nodule isolates of yam bean in response to nodulation, plant growth and nitrogen fixation

Isolates	Nodule No/ Plant	Nodular fresh wt./ plant (g)	Fresh weight/ plant (g)	Dry weight of nodule/ plant (g)	Dry weight/ plant (g)	Nitrogen% in plant
yb - 1	29	2.3	14.0	1.7	1.4	3.60
yb - 2	20	1.0	10.0	0.1	1.0	3.05
yb - 3	21	1.01	9.9	0.09	0.99	3.03
yb - 4	19	0.99	10.0	0.09	1.01	3.00
yb - 5	30	2.2	13.0	0.17	1.3	3.54
yb - 6	18	1.1	8.9	0.11	0.89	2.90
yb - 7	27	2.3	14.0	0.18	1.4	3.60
yb - 8	29	2.4	14.0	0.19	1.4	3.61
yb - 9	27	2.3	14.0	0.18	1.3	3.59
yb - 10	30	2.5	15.0	0.2	1.5	3.65
yb - 11	19	0.9	9.95	0.09	0.99	2.94
yb - 12	18	0.8	9.75	0.08	0.97	2.92
yb - 13	28	2.4	14.0	0.19	1.4	3.61
yb - 14	19	0.94	9.5	0.09	0.95	2.90
yb - 15	25	2.2	10.0	0.17	0.9	3.56
yb - 16	27	2.3	13.0	0.18	1.3	3.59
yb - 17	18	0.96	9.8	0.08	0.98	2.92
yb - 18	28	2.4	14.0	0.19	1.4	3.62
yb - 19	20	0.98	9.9	0.098	0.99	2.95
yb - 20	18	0.97	9.8	0.096	0.97	2.93
CD at 5%	5.0	0.5	6.0	0.05	0.06	0.15

growing isolates formed serum zone in litmus milk. The fast growing isolates (yb = 2, yb = 3, yb = 4, yb = 6, yb = 11, yb = 12, yb = 14, yb = 17, yb = 19 and yb = 20) formed acid in litmus milk and slow growers exhibited alkaline reaction. All the isolates showed negative reaction to ketolactose, 'Vogesproskauers', starch hydrolysis and gelatin hydrosis, nile blue and congo red tests (Table 1).

Serological test

All the twenty isolates were tested against six antiserum and it was found that the isolates were distributed in two distinct sero-groups, all the fast-growers in one group and the slow growers in another group (Table 2).

Nitrogen fixation

The nitrogen fixation efficiency of the isolates were tested by inoculating the plants with rhizobial strains. Nitrogen fixation differed from isolates to isolates (Table 3). The slow growers were most effective in fixing nitrogen in comparision to the fast growers. The isolates yb = 1, yb = 10, yb = 13 and yb = 18 showed higher rate of nitrogen fixation than the other isolates. In plants the slow growing isolate yb = 10 showed highest nitrogen fixation by about (3.65%).

DISCUSSION

All the isolates of yam been rhizobia showed differential growth rate on YAM. Elken (1984) considered the fast growers to be *Rhizobium* and the slow grower to be Bradyrhizobium. Gaur *et al.,* (1973) observed that rhizobia of diverse origin were negative to ketolactose test. However, all the isolates tested during present study showed negative reaction to ketolactose test confirming the earlier finding (Poi, 1986). In the present study a negative reaction to Voges proskaur test was found in general. *Rhizobium* may produce nitrite from nitrates. In the present experiment, the isolates showed complete reduction or partial disappearance of nitrate accompanied by appearance of nitrite, amonia and free nitrogen(Conn, 1951). Gaur and Sen (1975), also showed nitrate reduction by

Rhizobium. In the present study, though all the isolates showed reduction of nitrate, a variability was recorded. All the isolates failed to hydrolyze gelatin and starch. It indicates that the isolates did not produce the proteolyses and amylase enzymes necessary for hydrolyzing these substances. Poi and Kabi (1986) also could not find liquifaction of gelatin and starch by any of the rhizobial isolates of *Cicer* sp. Addition of hydrogen peroxide to each of the isolates grown on YAM slants, produced gas bubbles indicating that all the isolates were capable of producing a catalase enzyme. But relative rates of liberation of oxygen by them were not equal. It this respect, isolates yb – 5, yb – 7, yb – 8, yb – 9, yb – 10, yb – 16 showed more catalase activity than the other isolates. Tagieve and Martinosova (1971) observed that the catalase activity of his effective strains were able to reduce nile blue. All of the yam been isolates failed to absorb congored though Gaur and Sen (1975) observed a variation among the rhizobial strains to absorb congored.

The isolated were tested against antisera of yam bean nodule isolates and observed that the twenty isolates were serologically belonged to two distinct groups. It may be mentioned here that a very large number of cicer *Rhizobium* (Poi, 1986) were found to be in twenty serological groupings. The serological grouping depends upon antigenic variation of the isolates. As the yam bean *Rhizobium* isolates were only of two sero groups, the antigenic variation in yam bean rhizobia appeared very conservative. The nitrogen fixation capacities of the isolates were confirmed from oven dried plants samples. It was found that all the isolates were able to fix nitrogen but their rate of N_2- fixing isolate was yb–10 Poi and Kabi (1988) also found that isolates of soybean nodule differ in their N_2- fixation abilities.

It may be concluded from this study that nodulation in yam bean was effected by both *Rhizobium* and *Bradyrhizobium* sp. The isolates not being so wide in both chemical and microbial status, having conservative type of serological grouping and also showing different rates of nitrogen fixation only with relation to their growing habits appeared to be the more advanced in evolutionary sequence. The most efficent isolate (yb–10), however, may be used as the best inoculant for yam bean cultivation.

REFERENCES

Elken, G.H. (1984). Taxonomy and metabolism of rhizobium and its genetic relationship. P-1-38, in Biological nitrogen Fixation ed. M. Alexander, plenum Press, New York.

Jackson, M.L. (1962), *Soil chemical analysis*. Prentic, Hall INC. P–111–82 & 205–206

Conn, H.J. (1951). Routine tests for descriptive chart morphological and biochemical, Manual of methods for pure culture study of bacteria, **healut.** *V. Biotech* Publication.

Gaur, Y.D. and Sen, A.N. (1973). A new approach to the screening of efficient strains of *Rhizobia, Rhizobium, News letter* **18**(1):70.

Gaur, Y.D. and Sen, A.N.and Subbarao, N.S. (1973). Usefulness and limitation of ketolactose test to distinguish *Agrobacterium* and *Rhizobium*. Curr. *Science* **42:** 445–446.

Gaur, Y.D. and Sen, A.N. (1975). Studies of roof nodule bacteria of chickpea *Cicer arietinum* 1: cultural, biochemical characteristics of diagnostic importance. *Rhizobium News Letter.* **20**(2):91.

Poi, S.C. (1986). Biochemical properties of twenty five sero distinct strain specific *Rhizobium* of Bengal gram (*Cicer arietinum* L.) *Environ. & Ecology* **4**(10):44–49.

Poi, S.C. and Kabi, M.C. (1988). Symbiotic effectiveness of different strains of *R. Japanicum* in selecting inoculants for improvement production of soybean (*G. max*). *Environ. & Ecology.* **6**(4):985–987.

Tagieve, V. D. and Martinosova, T. A. (1971). On catalose activity of nodule bactoria of lecurne and peg (Azerbeigani). *Bokl. Acad, Nauk Az sssr.* **27**:88–89

Vincent, J.M. (1970). A manual for practical study of root nodule bacteria. IBP Hand Book No–15, Black Well and Scientific pub (London).

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