Indian Journal of Experimental Biology
Vol. 44, March. 2006, pp. 250-253

Host range nodulation and adaptation in frenchbean rhizobia

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Received 27 June 2005; revised 18 November 2005

The host range nodulation efficiency of four genetically marked frenchbean rhizobial strains (HURR-3, Raj-2, Raj-5 and Raj-6) was studied with five legume hosts namely, frenchbean (*Phageolus vulgaris* L.), pigeonpea [*Cajanus cajan* (L.) Millsp.], mungbean [*Vigna radiata* (L.) Wilezek.], urdbean [*Vigna mungo* (L.) Hepper.] and soybean [*Glycine max* (L.) Merril.]. Except soybean and pigeonpea, all other legume hosts were nodulated by two or more frenchbean rhizobial strains tested. Rhizobia were isolated from nodules produced by strains, HURR-3 and Raj-5, on main (frenchbean) and different (mungbean and urdbean) hosts. There was marked improvement in host range nodulation and nitrogen fixation efficiency of rhizobial strains, HURR-3 and Raj-5, after their isolation from chance nodules on different hosts. This is clearly evident from the ability of such isolates to form nodules on pigeonpea besides mungbean and urdbean, and higher nodulation in all the above three different hosts. The phage-susceptibility pattern and intrinsic antibiotic resistance (used as markers) of the two strains did not change after their passage through different hosts. The results indicate that frenchbean rhizobia had undergone some modification in symbiotic behaviour to adapt to wide host range during their passage through different (alternate?) hosts.

Keywords: Adaptation, Frenchbean rhizobia, Legume hosts, Symbiotic efficiency

It is often observed that when a pulse crop is introduced into an area where it (or any other member of its cross-inoculation group) has not been grown before, nodulation is either absent or sparse. However, on successive cultivation of the pulse crop on the same soil, nodulation gradually improves. Although the increase in population of the native host-specific rhizobia is the main reason for such phenomenon, the possibility of adaptation of different rhizobial species/strains to come into symbiotic association with the new legume host cannot be ruled out. To test the validity of the latter possibility, frenchbean rhizobia isolated from nodules of main host (frenchbean) and chance nodules from different hosts (like mungbean and urdbean) were examined for their difference in symbiotic efficiency with five legumes including the main host. The present investigation has been carried out with the following two objectives: (a) to examine the possibility of nodulation by four genetically marked diverse frenchbean rhizobial strains on five legume hosts, and (b) to observe the difference in behaviour of a *Rhizobium* strain isolated from the main host (frenchbean) and the same strain isolated from the different hosts (mungbean and urdbean) with respect to host range nodulation on subsequent inoculation.

Frenchbean has been found to be nodulated by various fast growing *Rhizobium* species such as *R. leguminosarum*, bv. Phaseoli (non-American species capable of nodulating bean)[1], *R. tropici*[2], *R. etli* (formerly American *R. leguminosarum*, bv. Phaseoli-type I strains)[3], *R. gallicum* and *R. giardinii*[4]. However, the frenchbean rhizobia present in Indian soil have not been categorized under different species. In absence of much idea on species, intrinsic antibiotic resistance[5] and phage sensitivity pattern[6] in addition to colony characteristics on yeast-mannitol agar plate were employed in the present study to illustrate the variation in four rhizobial strains.

The frenchbean rhizobial strains used in the present study include two genetically stable reference strains, HURR-3 and Raj-2[7] and two new isolates, Raj-5 and Raj-7.The bacterial strains were grown and maintained in yeast-mannitol (YM) medium[8]. The colony morphology was studied on yeast-mannitol agar (YMA) plates after 4 days of growth at 28° C (Table 1). Sensitivity of bacterial strains to different phages was studied by putting a drop of high titre phage particles (10^7-10^8 PFU/ml) on the double agar layer containing *Rhizobium*. The lysis of bacteria at the point of addition of phage indicates sensitivity. Four rhizobial strains were marked with two lytic phages, H3V and R2V, in this manner. Intrinsic antibiotic sensitivity of the rhizobial strains was determined from the capacity of the strains to grow on the YM-media containing graded concentrations of two antibiotics, streptomycin and chloramphenicol.

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Nodulation test was carried out for frenchbean (cv. Contender), pigeonpea (cv. T-21), mungbean (cv. Malviya Jyoti), urdbean (cv. T-9) and soybean (cv. JS-335). Legume seeds were surface-sterilized with acidified $HgCl_2$ (0.2%) for 2-5 minutes (depending on the size of seed) and then thoroughly rinsed with sterile distilled water. The seeds were plated for germination while partially submerged in water agar. After germination, seed coats were gently removed by sterilized forceps inside laminar flow cabinet and seedlings were transferred to plant growth tubes containing nutrient agar slant (Thornton's media[8]). Narrow plant growth tubes (25 mm × 200 mm) containing 30 ml nutrient agar were used for mung and urd, while wide plant growth tubes (38 mm × 200 mm) containing 60 ml nutrient agar were used for frenchbean, pigeonpea and soybean. The seedlings in each tube were inoculated with 0.5 ml log phase culture of respective rhizobial strains. Plants were allowed to grow in culture room under proper light and temperature conditions (at 26°±2°C with 14/10-hr light/dark cycle). Observations on the number of nodules per plant, nodule fresh weight, nitrogenase activity[9] and total plant dry weight were recorded at 45 days after inoculation (DAI). Standard statistical procedures were followed for the analysis of data. Square root transformations have been made in cases with one or more zero values in the data for analysis.

The four rhizobial strains differed from each other with respect to size, colour and consistency of colony (Table 1). Strain HURR-3 was sensitive to phage H3V while Raj-2 and Raj-7 were sensitive to phage R2V. On the other hand, Raj-5 was resistant to both the phages. The intrinsic antibiotic resistance level (streptomycin and chloramphenicol) also varied in the four strains.

Out of the five hosts used to test the host range nodulation of four frenchbean rhizobial strains, soybean (cv. JS 335) and pigeonpea (cv. T-21) were not nodulated by any strain. The efficacy of the four strains with frenchbean (cv. Contender), mungbean (cv. Malviya Jyoti) and urdbean (cv. T-9) differed as evident frovfm the data on nodules per plant, nodule fresh weight, acetylene reduction activity (ARA) and plant dry weight (Table 2). As expected, the general performance of the rhizobial strains tested was much better with frenchbean as compared to other legume hosts. Mungbean was nodulated by two frenchbean rhizobial strains (HURR-3 and Raj-7) while urdbean was nodulated by three strains (HURR-3, Raj-5 and Raj-7). With frenchbean, maximum nodulation and ARA were exhibited by Raj-2 followed by HURR-3. Ironically, while HURR-3 resulted satisfactory nodulation in mungbean and urdbean, Raj-2 failed to form any nodule in any of these two hosts. Raj-7 was symbiotically compatible with both mungbean and urdbean while Raj-5 was found to be incompatible with mungbean. All these observations can be attributed to intrinsic characteristics of both host plant and bacteria[10].

Variation in the same rhizobial strain after isolation from different hosts was also observed (Table 3). Strain HURR-3, depending on the host from which it is isolated, showed significantly different result with respect to nodulation and ARA activity in the main (frenchbean) and different hosts (mungbean and urdbean). When isolated from frenchbean nodules, it produced maximum nodule number (53) and showed maximum symbiotic activity only in case of frenchbean. In contrast, HURR-3, derived from nodules of mungbean and urdbean, exhibited lower symbiotic activity with frenchbean (16 and 26 nodules/plant, respectively) but much higher symbiotic effectiveness with mungbean and urdbean (compared to the same strain isolated from frenchbean) and started forming effective nodules in pigeonpea. Raj-5, derived from frenchbean and urdbean, also exhibited similar result as in HURR-3; Raj-5 from frenchbean showing higher nodulation and ARA activity (4.33 μM C_2H_2 reduced/hr/plant) in frenchbean only but much lower

Table 1— Distinguishing features of four frenchbean rhizobial strains

Rhizobial strain	Colony characteristics on YMA	Sensitivity to phage (ϕ)		Intrinsic antibiotic resistance level ($\mu g.ml^{-1}$)	
		ϕ H3V	ϕ R2V	Streptomycin	Chloramphenicol
HURR-3	White mucoid colonies, 2.5± 0.2 mm diam*	S	R	10	20
Raj-2	White firm with little gum, 2.0+ 0.3mm diam	R	S	10	30
Raj-5	Milky dome-shaped, 4.0+0.2mm diam	R	R	50	100
Raj-7	Watery translucent, 3.5+0.2mm diam	R	S	10	50

* Colony diameter after 5 days of incubation; R= Resistant, S= Susceptible

nodulation in mungbean and urdbean as compared to Raj-5 from urdbean. Raj-5, derived from urdbean, was also able to form nodules in pigeonpea. It is interesting to note that the same frenchbean rhizobial strains, after getting adapted to different hosts, showed less symbiotic effectiveness with their main host (frenchbean). This indicates that widening of the host range of rhizobial strains resulted in lower affinity/efficiency with the original host. Rhizobial strains, HURR-3 and Raj-5 isolated from nodules of mungbean, urdbean and pigeonpea, exhibited similar phage susceptibility pattern (as tested with ϕ H3V and ϕ R2V) and intrinsic antibiotic resistance (tested with streptomycin and chloramphenicol) as their original parental strains.

The present results indicate that during passage of the two frenchbean rhizobial strains through the different (alternate?) hosts, some change may have occurred in them rendering them capable of broadening their host range. Similarity in phage susceptibility pattern and intrinsic antibiotic resistance level of the same rhizobial strain isolated from different hosts in-

Table 2— Symbiotic efficiency of Frenchbean rhizobial strains with different legume hosts

Rhizobial strains	Frenchbean cv. Contender				Mungbean cv. Malviya Jyoti				Urdbean cv.T-9			
	Ch.1*	Ch.2	Ch.3	Ch.4	Ch.1	Ch.2	Ch.3	Ch.4	Ch.1	Ch.2	Ch.3	Ch.4
Control	0.0	0.0	0.0	750	0.0	0.0	0.0	320	0.0	0.0	0.0	305
(Uninoculated)	15.3	180	3.52	1000	3.0	10.0	0.55	350	6.0	15.0	0.92	330
HURR-3					(1.87)	(3.24)	(1.02)		(2.55)	(3.94)	(1.19)	
Raj-2	17.6	205	3.96	1050	0.0	0.0	0.0	330	0.0	0.0	0.0	305
					(0.71)	(0.71)	(0.71)		(0.71)	(0.71)	(0.71)	
Raj-5	10.8	110	2.80	900	0.0	0.0	0.0	325	2.0	10.0	0.56	315
					(0.71)	(0.71)	(0.71)		(1.58)	(3.24)	(1.03)	
Raj-7	13.4	175	3.15	950	2.0	8.0	0.42	335	2.0	10.0	0.48	320
					(1.58)	(2.92)	(0.96)		(1.58)	(3.24)	(0.99)	
Mean	14.27	167.50	3.36	975	1.25	4.5	0.24	335	2.50	6.25	0.49	317
					(1.22)	(1.89)	(0.85)		(1.61)	(2.78)	(0.99)	
SEM	0.80	8.31	0.10	23.11	0.06	0.08	0.02	5.69	0.05	0.11	0.03	9.8
CD (P=0.01)	3.31	34.34	0.43	95.45	0.24	0.33	0.10	23.51	0.22	0.45	0.11	40.46
CV (%)	12.60	11.10	6.90	5.30	10.60	9.50	6.30	3.80	7.30	8.70	5.90	6.90

*Ch.1, 2, 3 and 4 refer to the characters, nodules/plant, nodule fresh weight (mg)/plant, acetylene reduction activity (μ mol)/hr/plant and total plant dry weight (mg)/plant, respectively.
Figures in parentheses denote square root transformation values (for statistical analysis). Data of uninoculated control has not been included in the statistical analysis.
None of the frenchbean rhizobial strains produced nodules on pigeonpea and soybean.

Table 3— Changes in symbiotic efficiency of HURR-3 and Raj-5 after passage through different hosts

Rhizobial strain (isolated from)	Frenchbean cv.Contender				Pigeonpea cv.T-21				Mungbean cv. Malviya Jyoti				Urdbean cv.T-9			
	Ch.1*	Ch.2	Ch.3	Ch.4	Ch.1	Ch.2	Ch.3	Ch.4	Ch.1	Ch.2	Ch.3	Ch.4	Ch.1	Ch.2	Ch.3	Ch.4
HURR-3	53	350	4.16	1260	0.0	0.0	0.0	320	3.0	10	0.52	340	2.0	10	0.45	325
(Frenchbean)					(0.71)	(0.71)	(0.71)		(1.87)	(3.24)	(1.01)					
HURR-3	16	94	2.82	1120	4.0	30	1.21	430	17.0	49	1.18	485	12.0	38	1.12	465
(Mungbean)					(2.12)	(5.22)	(1.31)		(4.18)	(7.04)	(1.30)					
HURR-3	26	105	3.25	1075	3.0	25	1.12	420	18.0	55	1.25	475	14.0	50	1.22	475
(Urdbean)					(1.87)	(5.05)	(1.27)		(4.30)	(7.45)	(1.32)					
Raj-5	38	270	4.33	1340	0.0	0.0	0.0	340	0.0	0.0	0.0	320	4.0	10	0.50	310
(Frenchbean)					(0.71)	(0.71)	(0.71)		(0.71)	(0.71)	(0.71)					
Raj-5	24	134	3.35	1100	7.0	42	1.83	460	2.0	10	0.42	350	22.0	63	1.82	510
(Urdbean)					(2.74)	(6.52)	(1.53)		(1.58)	(3.24)	(0.96)					
Mean	31.4	190.6	3.58	1179	2.8	19.4	0.832	394	8.0	24.8	0.674	394	10.8	34.2	1.02	417
					(1.63)	(3.70)	(1.11)		(2.53)	(4.34)	(1.06)					
SEM	1.90	9.15	0.11	18.27	0.08	0.16	0.28	7.09	0.11	0.16	0.03	9.65	0.48	1.33	0.03	5.84
CD (P=0.01)	7.92	38.12	0.47	76.15	0.36	0.67	1.18	29.55	0.47	0.68	0.11	40.22	2.00	5.56	0.11	24.33
Cv(%)	12.1	9.6	6.3	3.1	10.5	8.7	5.1	3.60	8.90	7.50	5.20	4.90	8.90	7.80	5.30	2.80

*Ch.1, 2,3 and 4 refer to the characters, nodules/plant, nodule fresh weight (mg)/plant, acetylene reduction activity (μ mol)/hr/plant and total plant dry weight (mg)/plant, respectively.
Figures in parentheses denote square root transformation values (for statistical analysis).
Nodulation was not observed in soybean.

dicated modification of only the bacterial properties associated with host range nodulation. These observations do establish the possibility of adaptation of native rhizobial strains to nodulate a new legume host that has been introduced for the first time. The ecological significance of the present investigation lies in the fact that the so-called host-restricted rhizobial strains may undergo modification when different legumes are cultivated in cropping sequence.

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