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Interspecific Transformation in *Rhizobium*

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Summary. Two characters, "Penicillin resistance" and "fructose utilizing ability," were transferred in the recipient *Rhizobium cowpea* (source—Ground nut roots) with the help of the DNA extracted from *Rhizobium phaseoli* culture (source — French bean roots). The recipient culture enters the logarithmic growth phase at the end of 60 min. The percentage transformation frequency is maximum at the beginning of the exponential phase.

Ravin (1961) has listed twenty bacterial species which have been found to be transformable. Interspecific transformation, however, has been reported only in a few microorganisms. Balassa (1963) has studied transformation of three characters—cysteine independence, streptomycin resistance and ability to infect alfalfa host—in *Rhizobium* species. In the genus *Rhizobium* there are a large number of species and their characters are well studied. It is possible to explore the genetic transformation in *Rhizobia* for further studies on Nitrogen fixation and to obtain efficient Nitrogen fixing strains. Studies have therefore been made to find out if interspecific transformation is possible among two Indian species of *Rhizobia* and the data concerning these studies is presented here.

Materials and Methods

I. Organisms. Six cultures of *Rhizobia* were kindly provided by Dr. V. P. Bhide, Agricultural Bacteriologist, Poona, India.

Rhizobial culture	Host
R. phaseoli	*Phaseolus vulgaris* (French bean).
R. leguminosarum	*Pisum sativum* (Pea).
R. (Cowpea group)	*Arachis hypogea* (Ground nut).
R. (Cowpea group)	*Cajanus cajan* (Tur).
R. (Cowpea group)	*Phaseolus aureus* (Mung).
R. (Cowpea group)	*Crotolaria juncea* (Sannhemp).

Four of the cultures as seen here fall in the same group specifically. Cultures were maintained and grown in Balassa's complex medium (Balassa, 1963) or soil extract — yeast extract medium.

II. DNA Extraction and Estimation. Penicillin resistant strain *R. phaseoli* was lysed with Sodium deoxycholate and DNA extracted according to the method described by Marmur (1961). The concentration of extracted DNA was measured at 260 mμ on Beckman's spectrophotometer.

III. Genetic Markers. Two characters, Penicillin resistance and fructose utilization by *Rhizobium* species, were used as genetic markers.

IV. Transformation. A loopful of recipient culture was grown in 100 ml of soil extract broth (1% glucose, 0.1% yeast extract and 20 ml of soil extract in 80 ml of tap water; pH 7.6). The culture was synchronized by two successive transfers in the same medium. From the final flask, 2 ml of cell suspension were withdrawn in a sterile test tube and to this tube was added the Donor DNA solution to give a final concentration of 2 μg/ml. The DNA was allowed to react with the recipient on the shaker at 30° C for 30 min, and at the end of 18 to 24 hours incubation the suspension was plated out (after appropriate dilution in minimal medium containing 0.5% glucose) on soil extract agar (SEA) plates for total count and on SEA supplemented with penicillin for transformants.

The controls included (i) plating out donor DNA solution to confirm the absence of any viable donor cells therein, (ii) incubation of recipient with the recipient DNA and treatment of donor DNA with DNase prior to incubation with recipient to confirm that the transfer of characters is due to donor DNA alone and not due to any other factor obtained during DNA extraction procedure; (iii) plating out the recipient with each set of transformation experiment to confirm its sensitivity to penicillin.

V. Penicillin Acylase. Penicillin acylase was assayed according to the procedure of Batchelor *et al.* (1959).

VI. Penicillin-β-Lactamase. Penicillin-β-lactamase was assayed according to the procedure described by Perret (1954). One unit is defined as the amount of enzyme which degrades 60 units of Penicillin G at 25° C in 1 hour.

VII. Fructokinase. Fructokinase was assayed according to the method described by Hers (1952). One unit is defined as the amount of enzyme which phosphorylates 1 μg of fructose per minute.

Results and Discussion

Out of six different cultures, *R. phaseoli* (source—French bean roots), *R. cowpea* (source—Mung and Sannhemp roots) are resistant to Penicillin whereas *R. leguminosarum* (source—Pea roots) and *R. cowpea* (source—Ground nut, Tur roots) are sensitive to Penicillin as shown in Table 1.

Table 1. *Response of the Rhizobium cultures to varying concentrations of penicillin-G*

Culture	Units of penicillin/ml					
	50	100	250	500	1000	2000
R. phaseoli (French bean)	+	+	+	+	+	+
R. leguminosarum (Pea)	+	−	−	−	−	−
R. (Cowpea group) (Ground-nut)	−	−	−	−	−	−
R. (Cowpea group) (Tur)	−	−	−	−	−	−
R. (Cowpea group) (Mung)	+	+	+	+	+	−
R. (Cowpea group) (Sannhemp)	+	+	+	+	−	−

+ Growth; − No growth.

For transformation experiment, *R. phaseoli* was selected as the donor and *R. cowpea* (Ground nut) as the recipient. (The recipient will hereafter be referred to as *R. cowpea* in this paper.) When sensitive *R. cowpea* culture was treated with DNA extracted from resistant culture *R. phaseoli* the transformants obtained were penicillin resistant as shown in Table 2.

Table 2. *Characters of the donor, the recipient and the transformant*

Culture	Resistance to penicillin	Utilization of fructose
R. phaseoli	+	+
R. cowpea.	−	−
Transformant	+	+

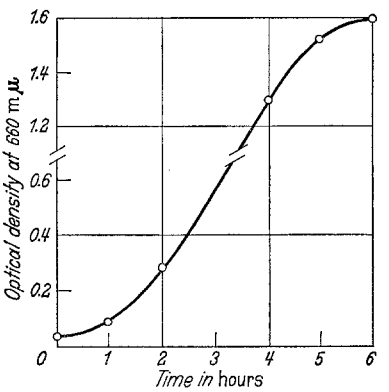

Fig. 1. Growth curve of the recipient culture

Table 3. *Relation between frequency of transformation and age of recipient culture*

Culture removed at (for DNA treatment)	Percentage transformation frequency
20′	0.6
40′	1.0
60′	1.3
80′	1.1
120′	0.95
180′	0.6
240′	0.45

The growth study of the recipient culture revealed that culture enters log phase at the end of 45 to 50 min as indicated in Fig. 1. The percentage transformation frequency was studied at different time intervals. The results listed in Table 3 indicate that, with regard to penicillin resistance character, the recipient culture is maximally competent at the end of 1 hour and the percentage transformation frequency obtained is 1.3. Thus the culture becomes competent when the exponential phase begins. It may be noted that this observation is in good agreement with the one obtained in other *Rhizobial* strains by Balassa (1957).

Table 4
Enzymes in transformants obtained by treatment of R. cowpea with R. phaseoli DNA

Culture	Units of penicillin-β-lactamase	Units of fructokinase
R. phaseoli	82 ± 2	266 ± 21
R. cowpea	0.0	7 ± 3
Transformant	62 ± 3	240 ± 17

During the present study (Table 4) it has been observed that *R. phaseoli* can produce an enzyme which can metabolize fructose and hence enable the culture to grow in presence of fructose as sole carbon source. On the other hand *R. cowpea* is unable to metabolize fructose. It was possible to show that donor culture possessed fructokinase enzyme and after transformation the enzyme appeared in the transformant. The percentage transfer of this enzyme activity into the transformant is 93 to 100 as would normally be expected.

Penicillin is destroyed by bacterial strains which produce Penicillin acylase or penicillin-β-lactamase or both these enzymes. Penicillin acylase was found to be absent in the donor, recipient and transformant. Penicillin-β-lactamase was found to be present in both the donor as well as in the transformant; the percentage transfer of enzyme activity being 75% as compared with the donor.

The significance of interspecific transformation in these *Rhizobial* cultures cannot be assessed at this moment. It may permit one to study the biochemical basis of nitrogen-fixation and also the relationship among the different characters of a strain.

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