Recombination in *Rhizobium leguminosarum*

Transformation in *Rhizobium* has recently attracted the attention of bacterial geneticists[1-5]. Characters like infectivity, cysteine independence, resistance to antibiotics and ability to utilize fructose have been employed as genetic markers, using the transforming principle (DNA) from the donor cells. The present communication deals with recombination in *Rhizobium leguminosarum*, using drug resistant and sensitive strains obtained by ultra-violet irradiation.

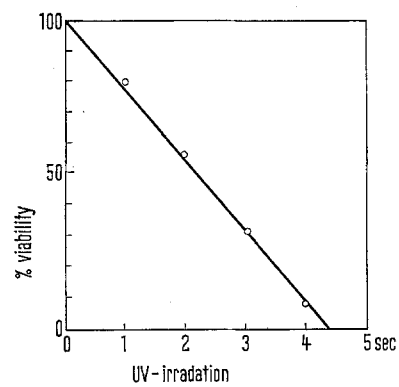
A Hanovia mercury vapour germicidal source with its main output at 2537 Å was used for irradiation. The cells were irradiated at a distance of 30 cm and the dosage was varied by regulating the exposure time. During the irradiation period, the cell suspension was constantly stirred and after fixed intervals of exposure time, it was plated on yeast extract-mannitol agar medium, using the dilution technique.

The Figure shows a linear single hit curve of the viability of *R. leguminosarum*.

By screening the irradiated cells, isolates showing different degrees of sensitiveness and resistance to penicillin and streptomycin were obtained independently. Table I shows the characters of 3 strains used in the present study. All the 3 strains were carried through a series of subculturing in the respective antibiotic media, interpolated by a series of transfers in drug free medium, so as to ensure the stability of the characters.

Mixed inoculation experiments were performed by using together 10 µg streptomycin/ml and 25 µg penicillin/ml. When equal number of cells of S4 and P2 strains were brought together as a mixture and plated after $1^1/_2$ h cellular contact at 30 °C on plates containing both penicillin and streptomycin, growth occurred to some extent. The frequency of such S^+P^+ cells is 4.5×10^{-7} (Table I).

The fact that S^+P^+ cells were obtained from mixed inoculations of the 2 different mutant strains may be due either to recombination through sexual cycle or through back mutations of the individual locus or due to adaptation induced by the drug. Back mutation would involve both the S4 ($P^- \rightarrow P^+$) and P2 ($S^- \rightarrow S^+$) strains. Tests for back mutation were carried out by repeatedly subculturing the S4 and P2 strains in drug free medium for a number of generations and then plating in individual and combined antibiotic media. There was no evidence of back mutation (Table II). The phenomenon of adaptation seems to be ruled out since series of subculturing with and without the drug has not changed the phenotype of either S4 or P2. Phage mediated transfer of genetic material as a possible explanation may also be ruled out, since no plaque formation was observed in the medium of either the pure or mixed bacterial cultures. This leads to the recombination as a possible explanation for this behaviour. The finding of HIGASHI[5] of the sexual cycle in *Rhizobium* lends further support to this conclusion. The calculated frequency of recombination, 4.5×10^{-7}, is comparable to recombinational genes in other microorganisms. Because only 2 loci are involved, it is difficult to determine the location on the genome with reference to each other. This would require more markers to be located and work is in progress in this direction[6].



Survival curve of *Rhizobium leguminosarum*.

Table I. Characters of the strains of *R. leguminosarum* obtained by UV-irradiation

Strain	UV (sec)	Resistance to streptomycin (10 µg/ml)	Resistance to penicillin (25 µg/ml)	Character	Frequency of S^+P^+
W (Wild)	–	+	+	S^+P^+	
S4	2	+	–	S^+P^-	
P2	1	–	+	S^-P^+	
S4 × P2		+	+	S^+P^+	4.5×10^{-7}

Table II. Plate counts of S4 and P2 strains on antibiotic media

	S4 (P^-S^+)	P2 (P^+S^-)
Drug free	31	42
Penicillin (25 µg/ml)	–	40
Streptomycin (10 µg/ml)	30	–
Penicillin + Streptomycin	–	–

Values indicate the number of colonies/plate; average of 10 replications.

Zusammenfassung. Es wird die Rekombinationshäufigkeit bei *Rhizobium leguminosarum* am Beispiel der Streptomycin- und Penicillinresistenz untersucht und eine solche gefunden, die in der Grössenordnung anderer bekannter Rekombinationshäufigkeiten liegt.

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